PROCEEDINGS OF V. INTERNATIONAL AGRICULTURAL, BIOLOGICAL, LIFE SCIENCE CONFERENCE AGBIOL 2023

18-20 SEPTEMBER 2023

EDIRNE, TURKEY

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WELCOME NOTES

You are welcome to our V. 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.

There is a worldwide participation from 33 countries 833 papers contributed by over 2000 authors with 522 oral and 311 poster presentations in AGBIOL 2023.

The AGBIOL 2023 will be normal participation as well as with online participation in Trakya University Balkan Congress Center in Edirne, Turkey on 18-20 September, 2023. The program will include 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 will be published in electronic book and will be distributed to the participants on flash memory stick as well as by e mail for online participants. All the e-posters should be prepared in electronic form and then submit to via the conference e mail and will exhibit 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 will be able to access all the normal and virtual presentation talks in each session, as well as to visit the virtual poster hall via preliminary provided participant ID and codes. The selected ABSTRACTs will be published in the Conference ABSTRACT and Proceedings Book. Participants might send us their full papers, which based on their preferences will be 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.

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CONTENTS

SUSTAINABILITY ASSESSMENT OF ANIMAL HUSBANDRY IN TURKEY

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ABSTRACT

This study covers analysis of the sustainability of the animal husbandry activities in Edirne and Kastamonu provinces of Turkey through a thermodynamic sustainability assessment technique, emergy analysis (EA). By classifying energy and material flows as renewable, non-renewable and purchased from economy, EA defines metrics to evaluate the sustainability of a system. This metrics provide insight about evaluated system's renewability, environmental loading and dependence on external inputs. In analyzed systems, 3 breeders raise both cows and sheep; 3 breeders raise cows, only. All animal breeding systems we analyzed are found to have renewability lower than 20%, environmental loading ratio (ELR) of higher than 2 and environmental sustainability index (ESI) of lower than 1. Consequently, they are determined to be unsustainable irrespective of the location of husbandry activities. Purchased animal feed is determined to be the main factor behind the systems' unsustainability. Integration of animal breeding with feed crop cultivation and increasing the ratio of farmer grown food in diets of animals can enhance the sustainability performance of animal husbandry systems.

Keywords: Emergy analysis, system renewability, environmental loading, sustainable animal husbandry, system integration

INTRODUCTION

26% of global greenhouse gas (GHG) emissions and 70% of freshwater use are created by food production. 96% of all mammal biomass excluding humans is livestock and 71% of all bird biomass is poultry livestock (Ritchie and Roser, 2022). This shows the extent of increase in animal-sourced-nutrition production due to human population growth. Hence, performing animal rearing activities in a sustainable manner has the upmost importance in today's world.

Emergy analysis is a thermodynamic sustainability assessment tool that provides insights about a systems renewability, environmental loading and dependence on external resources (Odum, 1996). Biological systems are interconnected through an "energy hierarchy" (Brown and Ulgiati, 2004; Hau and Bakshi, 2004). Hence, the interconnected nature and sustainability of these systems can be analyzed thermodynamically through EA.

EA is widely utilized in sustainability assessment of animal rearing activities as in works of He et al., 2019, Zhang et al., 2007 and Wang et al., 2015. However, studies evaluating sustainability of Turkish husbandry sector through EA are not available. Hence, this work evaluates and compares the sustainability of 6 animal husbandry systems in Edirne and Kastamonu provinces of Turkey.

MATERIAL AND METHOD

Background

Table 1 lists properties of 6 husbandry systems analyzed in this work. Performing research in two different locations enables system comparisons and related recommendations.

Table 1: Location, animal number and type, feeding structure and product characteristics of evaluated animal breeding systems.

System	Location	Animal Number	Feed Type	Products
Husbandry 1	Edirne/ Uzunköprü	100 cows	Purchased + Self-grown	18-20 L milk/animal + 25 male calf/year
Husbandry 2	Edirne/ Merkez	25 cows + 3 sheep	Purchased + Self-grown	15L milk/animal + 5 male calf/year + 60 kg sheep meat/year
Husbandry 3	Edirne/ Merkez	11 cows	Purchased + Self-grown	20 L milk/animal + 5 male calf/year
Husbandry 4	Kastamonu/ Tosya	110 cows + 60 sheep	Purchased	400 kg meat/cow + 30 kg meat/sheep
Husbandry 5	Kastamonu/ Tosya	22 cows + 2 sheep	Purchased + Self-grown	10 L milk/animal + 10 male calf/year.
Husbandry 6	Kastamonu/ Tosya	65 cows	Purchased + Self-grown	15L milk/animal + 15 male calf/year

Emergy Analysis (EA)

Methodologically, EA includes the steps of drawing energy systems diagram (a pictorial model of the system), emergy evaluation table formation (an inventory for inputs and outputs) and the calculation of emergy indicators (Odum, 1996; Brown and Ulgiati, 2004).

Figure 1 shows the energy systems diagram (ESD) for an animal rearing system in Edirne. Here, sun wind and rain are renewable inputs that are provided by the natural environment. Water is the non-renewable local input that is under storage category. It is classified as non-renewable since the groundwater levels are declining in both research locations. Inputs that are exchanged from economy are classified as purchased inputs (Odum,1996).

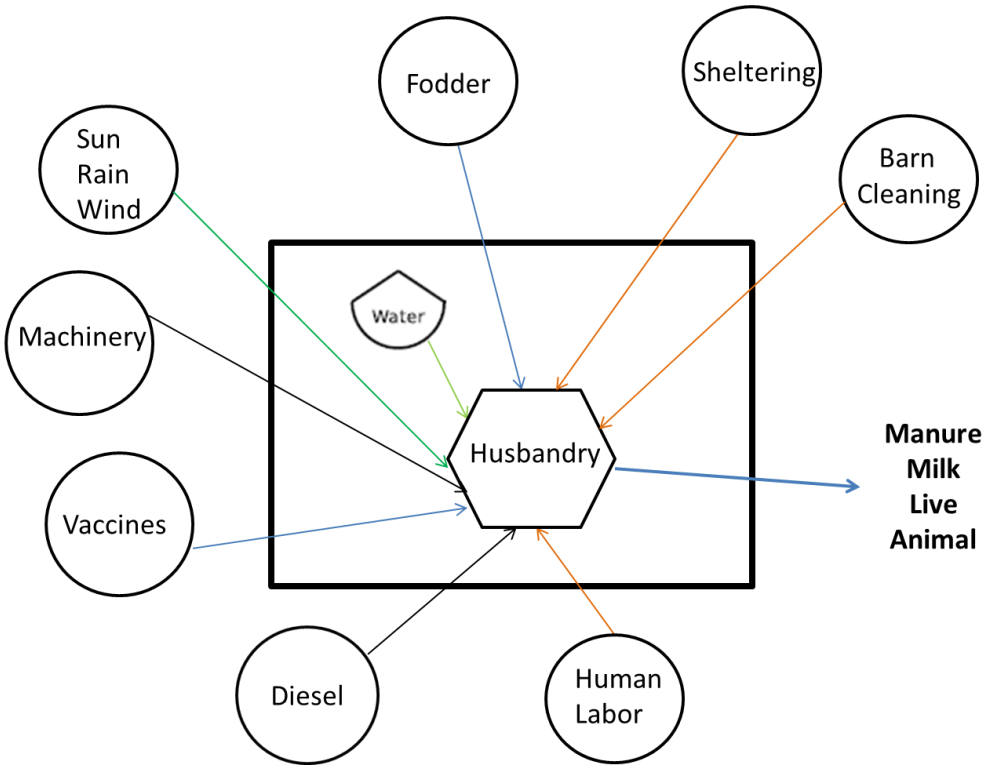

Figure 1: Energy systems diagram for and animal rearing system in Edirne.

Emergy evaluation table (EET) is a listing containing all the energy and material inputs to and the outputs from the system under study. EET is formed based on the determined analysis boundary and the model drawn in the ESD (Odum, 1996).

Calculation of emergy indicators in EA is based on the classification of emergy flows as local renewable (R), non-renewable (N) and purchased (P) (Ulgiati et al, 2010).

The calculation of the emergy indicators is presented in equations 1-6 mathematically.

$$\text{Emergy Yield } (Y) = R + N + P \quad (1)$$

$$\text{Renewability} = \frac{R}{Y} \quad (2)$$

$$\text{EmergyYield Ratio (EYR)} = \frac{Y}{P} \quad (3)$$

$$\text{Environmental Loading Ratio (ELR)} = \frac{(N+P)}{R} \quad (4)$$

$$\text{Environmental Investment Ratio(EIR)} = \frac{P}{(R+N)} \quad (5)$$

$$\text{Environmental Sustainability Index (ESI)} = \frac{EYR}{ELR} \quad (6)$$

Systems having renewability of lower than 20%, EYR of lower than 4, ELR of higher than 2 and ESI of lower than 1 are classified as unsustainable systems. If these systems are improved, they can evolve into being in transition state or sustainable in terms of their sustainability status (Chen et al., 2017). Further information on EA can be found in Kursun and Bakshi (2016).

RESULTS AND DISCUSSION

Figure 2 shows renewability of animal breeding systems evaluated. Out of 6 breeders, 3 have both cow and sheep and 3 breeders solely raise cows. In cow breeding systems renewability changes between 0.42% and 15.9%. In case of sheep, this change is between 1.69 % and 12.1%. The main factor affecting renewability of cow systems is how much of the feed is grown by the farmer. As the integration level of cow rearing and feed crop cultivation systems (feed crops are fertilized with animal manure) increase, renewability of cow rearing increases. The same inclination is also true for sheep rearing, as the portion of farmer grown feed or grazing increases, sheep rearing systems become more renewable.

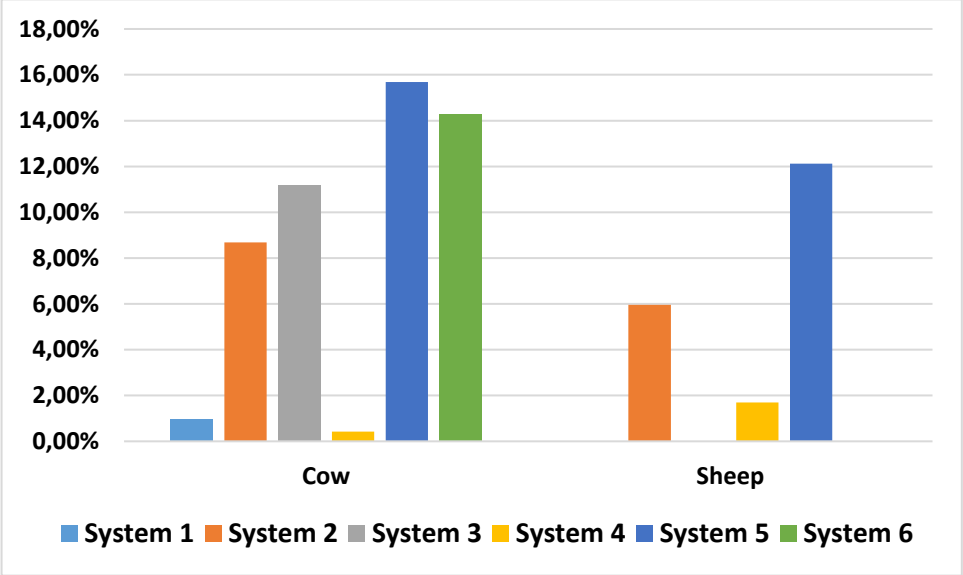

Figure 2: Renewability values for evaluated animal rearing systems.

Figure 3 shows emergy yield ratio (EYR) of animal breeding systems evaluated. In cow breeding systems EYR changes between 1.10 and 1.26. In case of sheep, this change is between 1.02 and 1.17. System renewability and EYR go hand in hand. As renewability increases, system EYR also increases. Here, again how much of the feed is grown by the farmer and level of integration of cow rearing and feed crop cultivation systems are the determining factors that increase EYR value (preferred). For sheep cases as the portion of farmer grown feed or grazing increases, sheep rearing EYR increases.

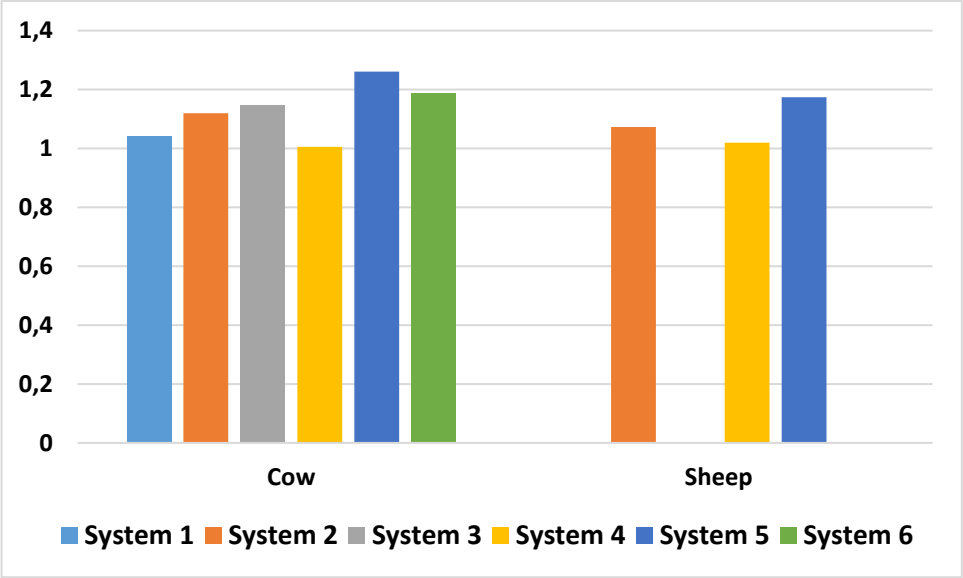

Figure 3: Emergy yield ratio (EYR) values for evaluated animal rearing systems.

Figure 4 shows environmental loading ratio (ELR) of animal breeding systems evaluated. In cow breeding systems ELR changes between 5.33 and 238. In case of sheep, this change is between 7.22 and 58.2. Being purchased feed the largest emergy input to the system followed by non-renewable water create the environmental loading in cow breeding. For sheep, mainly purchased animal feed is responsible from this impact. Due to grazing, sheep breeding generally has lower environmental loading than cow breeding.

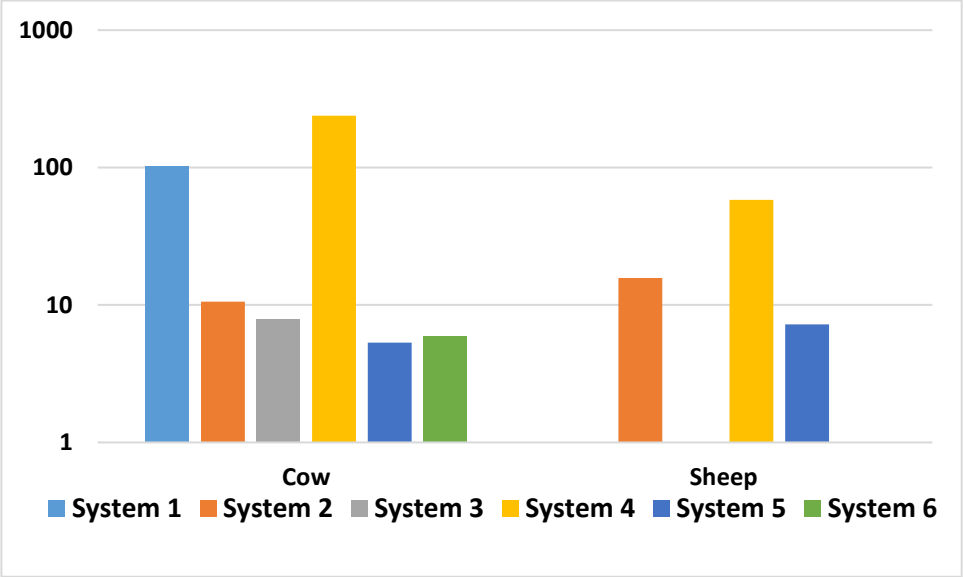

Figure 4: Environmental loading ratio (ELR) values for evaluated animal rearing systems.

Figure 5 shows environmental investment ratio (EIR) of animal breeding systems evaluated. In cow breeding systems EIR changes between 5.36 and 197. In case of sheep, this change is between 5.91 and 13.7. For EIR, dominance of purchased animal feed is the main reason behind high EIR values both for cow and sheep breeding.

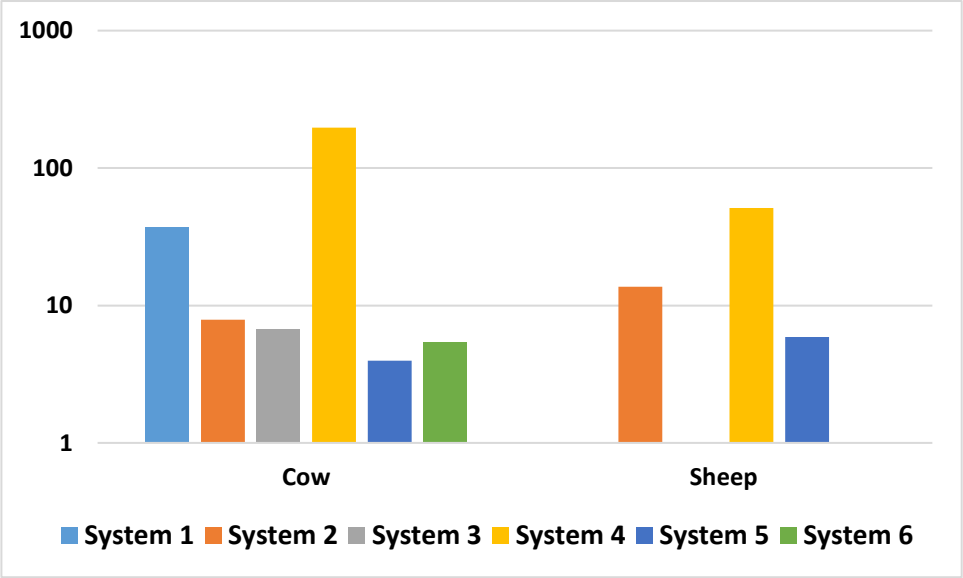

Figure 5: Environmental investment ratio (EIR) values for evaluated animal rearing systems.

Figure 5 shows environmental sustainability index (ESI) of animal breeding systems evaluated. In cow breeding systems ESI changes between 0.01 and 0.24. In case of sheep, this change is between 0.02 and 0.16. ESI is the ratio of EYR to ELR, hence it represents production per environmental loading. Low ESI values obtained both for cow and sheep breeding show that all of the animal breeding systems analyzed are unsustainable.

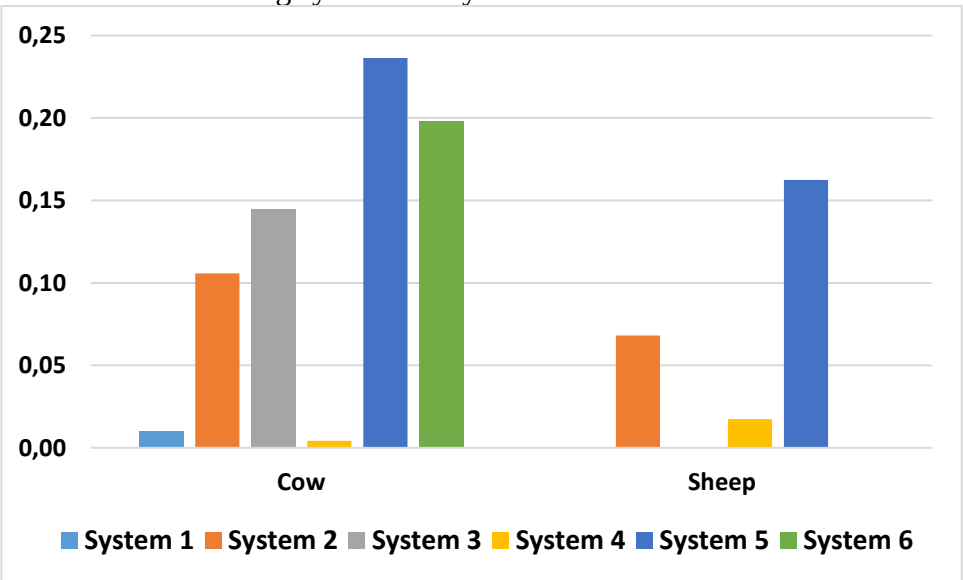

Figure 6: Environmental sustainability index (ESI) values for evaluated animal rearing systems.

CONCLUSIONS

All animal breeding systems studied in this work has renewability lower than 20%, ELR higher than 2 and ESI lower than 1. Consequently, they are found to be unsustainable. Purchased animal feed is determined to be the main factor behind the systems' unsustainability. Integration of animal breeding systems with feed crop cultivation and increasing the ratio of farmer grown food in diets of animals can enhance sustainability performance of animal breeding systems.

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ECO-DENDROMETROC STUDY OF PINUS HALEPENSIS MILL. IN THE FOREST OF TERNI (WESTERN ALGERIA)

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Abstract

A precise knowledge of existing forest resources, as well as their evolution, should focus essentially on the floristic composition and on the structure of valuable species. This study aims to evaluate the productivity of Pinus halepensis and its evolution in the forest of Terni Monts Tlemcen (Algeria). For this study, four plots were randomly selected. Stationary data and dendrometric measurements were carried out on all the trees of each plot in order to assess the floristic diversity of this forest. The inventories carried out on 368 trees made it possible to determine 7 species distributed in 6 genera and 4 families of which the Fabaceae is the dominant one. The average values of the highest diversity indices are H' (1.3) and D (0.60). The highest percentage of Aleppo pine is 29% in plot 3. The average density of Aleppo pine stems is 75 individuals/ha, representing an average basal area of 0.84 m2/ha and an average volume of 5.12 m3/ha. These results will enable the decision maker and concession holders to implement a better sustainable forest management strategy.

Keywords: forest, Pinus halepensis, diversity, dendrometric, management strategy.

INTRODUCTION

Algeria is the largest country on the African continent after Sudan. This geographical location gives it a particular climatic and ecological diversity. This vast territory is very diversified by its climate, its relief, its soils and its natural vegetation (Letreuch Belarouci, 1995). Algeria has one of the most diverse and original flora of the Mediterranean basin where it has 3139 species divided into 150 families. The Aleppo pine (Pinus halepensis Mill.) is considered as a main and essential component of the Mediterranean forest and represents a high value forest capital by the majority of countries around the Mediterranean and more particularly in Algeria (Boudy, 1950 ; Nahal,1962).

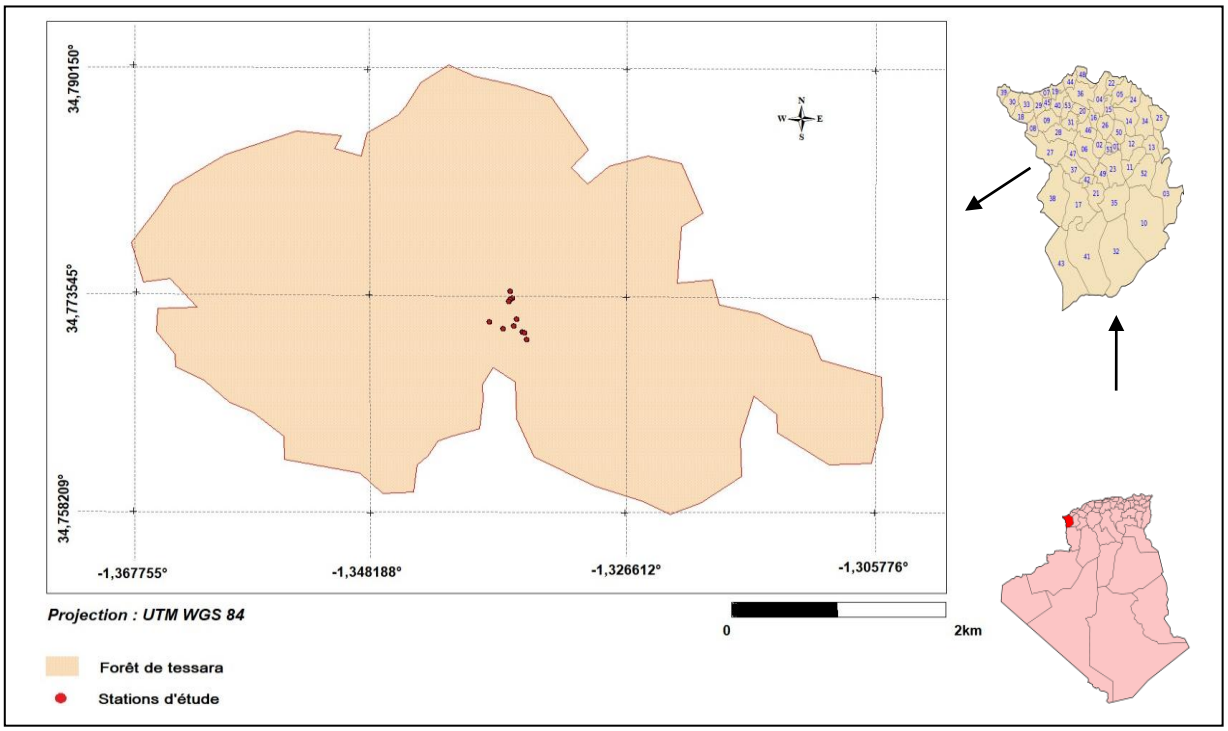
In Algeria, the Aleppo pine covers 35% of the wooded areas in the north, i.e. around 850,000 ha. It forms significant forests with variable ecological values (Bentouati and Baritea, 2006 ; Guit et al., 2015). It is largely located in its natural state in the eastern and central regions of the country, mainly on the Atlas, Tellian and Saharan mountains (Guit et al., 2015). This species, which is present in all bioclimatic stages, from the coast to the Saharan Atlas, finds its optimum growth mainly in semi-arid zones (Kadik, 2005 ; Djerrad, 2016). Its great plasticity and robust temperament have made it a pioneering species for major reforestation (Quèzel, 2000 ; Kadik, 2005 ; Guit et al., 2015 ; Djerrad, 2016). Aleppo pine wood can be used, after removal of the resin, for the manufacture of paper pulp (Nahal, 1962 ;Soltani, 2016) Pine buds, very resinous, also have a medicinal use, as balsamics and diuretics, transformed in particular into syrups and lozenges (Zenzen, 2016). Our work consists in inventorying the stand of Pinus halepensis in the forest of Tessera Mramet region of Terni Monts de Tlemcen (Algeria) using dendrometric measurements. Knowledge of the dendrometric parameters of forest species in

plantations is an important element for decision-making, particularly those relating to management interventions for this species and to enhance it.

MATERIALS AND METHODS

Presentation of the study area

The Tlemcen region is located in the west of the country, it falls under the semi-arid bioclimatic stage with cold winters. The forest of Tessera Mramet region of Terni is located between the coordinates (34.758209°/ 34.773545°) of north latitude and
(-1.326612°/ -1.348188°) of west longitude (Figure 1). It extends over an area of 1379 ha, consists entirely of maquis of which 647 ha are dense and 732 ha are clear (B.N.E.D.E.R, 2008).



Data gathering

The dendrometric characterization is carried out by means of a forest inventory on the stands of 4 square plots of 0.09 ha in the study area where station and dendrometric data are collected at the level of each sampling unit. Inside each of them, the diameter at breast height (dbh) and the total height are measured for each individual. Height is the most important characteristic for measuring or estimating volume. The study of the heights makes it possible to appreciate the fertility of the stations. The inventory of regeneration to concern woody plants with a diameter of less than 5 cm.

Data processing

The data collected is processed and the parameters concerned are:
- Basal area is a good indicator of site richness. It is calculated by the following ratio:

$$g = c2/4\pi \text{ (c: circumference at 1.30m).}$$

- The total basal area is the sum of the cross-sections at 1.30m from the ground of all the trees in the stand, it is expressed in square meters, reduced to the hectare (Rondeux,1999).

- The density corresponds to the number of trees on a given surface per hectare (N/ha).

$$N=ni/s$$

ni: number of trees in a plot; s: area of the plot in ha.

- Average height is used to calculate the productivity and the average volume m3/ha of the Aleppo pine stand. Their uses are becoming increasingly widespread in the practice of the forestry profession (Lecomte, 2008). The arithmetic mean height of the stand is determined by the following mathematical equation:

$$H= \Sigma\ hi/Nt$$

H: average height (m); hi: total height of a tree; Nt: Number of trees measured.

- Diversity indices used for the analysis of the state of each plot:

▪ Species richness

One of the first indices of diversity is species richness (SR). This index assesses the number of tree species in the stand (Parde and Bouchon, 1988). Although it makes it possible to distinguish diversity according to the number of species, it does not give any information on the weight of each species in the mixture (Gonçalves et al., 2010).

▪Shannon index

Shannon Index (H') is an example of a distance-independent algorithm (Shannon, 1948). This index is undoubtedly the index most used to describe the diversity of species. It makes it possible to express diversity by taking into account the number of species and the abundance of individuals within each of these species. Thus, a community dominated by a single species will have a lower coefficient than a community in which all species are codominant (Grall and Coïc, 2006). If only one species is recorded in the plot, the Shannon H' index is equal to zero. For k species with equal proportions, H' corresponds to ln (Pi) (Keren et al., 2020). It derives from information theory and measures the entropy of a sample, or the "saturation" of the community. The index (H') is given by the formula:

$$H' = \sum_{i-1}^{RS} Pi\ ln\ Pi$$

Pi: ni/N; RS: total number of species; ni: number of individuals of a species in the sample; N: total number of individuals of all species in the sample.

▪ Equity Index

The evenness index (E) is the ratio between the calculated diversity H' and the maximum theoretical diversity H'max which is represented by the log2 of the total richness S (Blondel, 1979). This index varies from zero to 1 (Barbero et al., 1987). An equitability equal to 1 corresponds to a community whose numbers are perfectly evenly distributed between the species, i.e. where all the species have the same number of individuals. Evenness is 0 when a single species dominates. Thus, equitability takes into account the potential absolute diversity

of the community represented by H'max, thus reflecting the capacity of the system to support S species represented with equivalent proportions. This index measures equitability in relation to a theoretical equal distribution for all species:

$$E = H'/H'max$$

H'max: log S (number of species)

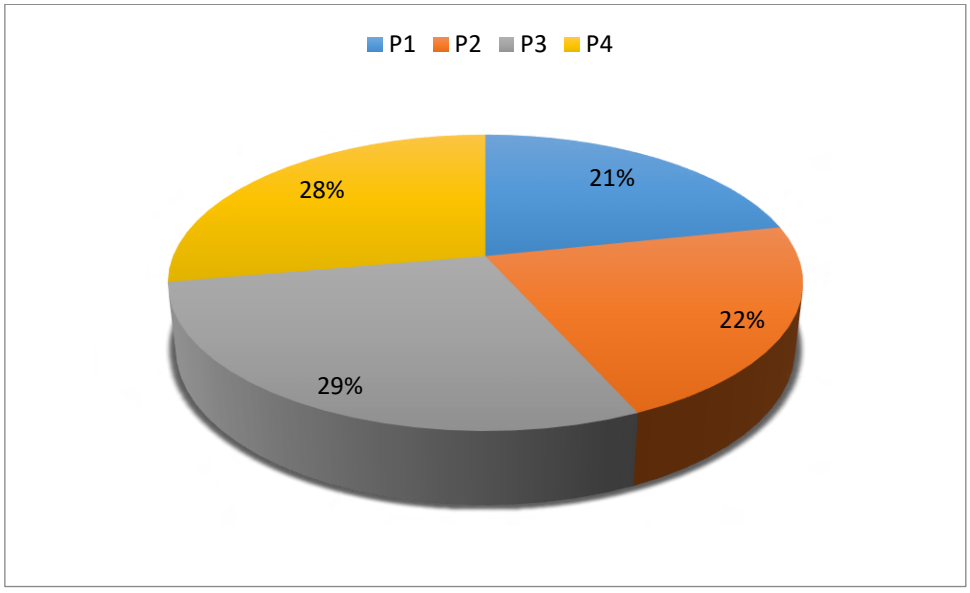
▪Simpson's diversity index (D) is obtained by the formula:

$$D = \Sigma fi2 \text{ and } fi = ni/N$$

Ni: number of individuals of the given species; N: total number of individuals.
The maximum diversity being represented by the value 1, and the minimum diversity by the value zero (Rita, 2000).

RESULTS AND DISCUSSION

The inventory carried out at the level of the study forest made it possible to determine 7 species (Quercus ilex, Pinus halepensis, Juniperus oxycedrus, Cupressus sempervirens, Abies alba, Rosa canina, Quercus canariensis) divided into 6 genera and grouped into 4 families. Holm oak is the dominant woody species. The most represented families are the Fagaceae followed by the Cupressaceae. The results of this study showed that the Aleppo pine stands have percentages that vary from 21% in plot 1 to 29% in plot 3 (Figure 2).



Figures 2. Aleppo pine frequency from four plots.

The average values of species richness, Shannon diversity index, and Piélou evenness are presented in Table 1.

Table 1. Diversity indices.

Plots	RS	H'	E	D
P1	43	0.9	0.43	0.23
P2	81	1.3	0.32	0.29
P3	50	1	0.41	0.25
P4	194	0.72	0.6	0.13
Average	92	0.98	0.13	0.225

According to the previous results, the studied forest massif is not very diversified. The Shannon index varies from 0.72 to 1.3. The values of the diversity index of Simpson are variable, its minimum is recorded for P2 (0.32) and its maximum for P4 (0.60), this difference explains that the floristic diversity is low in this forest where the most dominant species takes the high potential. Evenness tends towards 0, which explains the dominance of a single species (holm oak). We can conclude from these data that this forest is in a state of weakness and very advanced degradation due to the lack of species caused mainly by anthropogenic pressure and the impact of climate change which has been in place for a long time.

According to the results in Table 2, the average density of Aleppo pine at stand level is 75 individuals/ha with an average basal area of 0.84 m^2/ha and a volume of 2.66 m^3.

Table 2. dendrometric parameters of the study plots.

Parameters	P1	P2	P3	P4	Average
N/ha	33	55	44	167	75
G (m^2/ha)	0.68	0.18	0.39	2.12	0.84
D (cm)	16.24	6.05	10.35	13.2	11.46
H (m)	7.06	3.97	5.62	6.04	5.67
V (m^3)	2.49	0.37	1.13	6.65	2.66

The analysis of the average diameter and height, which are respectively 11.45cm and 5.67m, indicates that the Aleppo pine stand in this forest is dominated by young individuals. The analysis of the dendrometric parameters shows that the plots are characterized by a low density due to the increase in competition between the trees, especially in relation to environmental resources. The average density of Aleppo pine is 75 individuals/ha. This density is lower compared to that of the forest of Hamimet (Oum El Bouaghi), which is around 378 individuals/ha (Yahi et al., 2021) and that of Chettaba (Constantine) which is 442 individuals/ha (Rached-Kanouni et al.,2020). Structural characteristics are major indicators for measuring the qualitative and quantitative evolution of forest stands (Oosterhoon and Kappelle, 2000). The population density of Aleppo pine in the Terni forest is low compared to other forests in Algeria. The difference in stand densities could be related to the ecological characteristics of the study environments, including soil types, topography, climate, cover and especially the altitudinal gradient (Rabiou et al., 2015 ; Rached-Kanouni et al.,2020). We note that this pine is in good condition, with the absence of insect attacks such as the pine processionary caterpillar.

Conclusion

The national forest of Tessera Mramet Terni Monts Tlemcen is considered as a forest area not very rich in terms of biological diversity, it is in the form of matorral. This forest is composed mainly of holm oak which is currently in a much degraded state by fires, anthropogenic pressures. It is also composed of Aleppo pine which is a very interesting reforestation species in terms of wood production, soil protection and the development of other products. The

objective of this study is to know the current state of the Aleppo pine in the national forest of Tessera Mramet. The results show that the density, the specific richness, the basal area and the volume of Aleppo pine are low in this forest. It is important to take these results into consideration for the proposal of a management plan necessary for the management of this stand, in particular through silvicultural work for improvements and the protection of this stand against environmental problems.

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DETERMINATION OF SOME WILD PLANT SPECIES CONSUMED AS VEGETABLES IN FETHİYE (MUĞLA)

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ABSTRACT

Some plants that spread spontaneously in nature and are not cultivated are important in terms of human nutrition and their contribution to the economy locally. There are many plant species that grow naturally in Fethiye and its surrounding vegetation and are considered as vegetables. In this study, the plants considered as vegetables, the general characteristics and consumption patterns of these plants were determined by conducting field studies in Fethiye and its surroundings. In the region, 27 plant species belonging to 15 families, which are most evaluated as vegetables, were identified. The families with the highest number of species are Brassicaceae, Polygonaceae and Amaranthaceae. Seasonally, these plants are collected by the public, mostly in the spring, they are sold in the markets and consumed. Some of these plants are dried, frozen, canned, but most of them are consumed raw. Determining the wild plant diversity adapted to the natural environment will provide important benefits in the studies on plant breeding in the future.

Keywords: Fethiye, vegetable, wild plants

INTRODUCTION

Turkiye is one of the countries rich in plant diversity. Turkiye's geographical structure and different ecological conditions are the most important factors of this diversity. Turkiye is the primary or secondary homeland of many plant species. Wild relatives of many cultivated plant species have found wide distribution. Changes in climate and topography in Turkiye have also led to an increase in habitat types and thus the rate of endemism. Turkiye has 9.222 plant species and a total of 12.006 taxa, with 2.981 endemic species and 3.778 endemic taxa. Until 2011, the number of taxa in the flora of Turkiye increased to 12.755 (Kayıkçı et al., 2012). Studies to determine this genetic resource richness of Turkiye are increasing rapidly.

Since ancient times, human beings have benefited from the plants around them in various ways. Since ancient times, some plant species have grown spontaneously in nature and have been used as food by humans. Humans have collected and consumed plants as food throughout history (Turner et al., 2011). These plants, which grow in the natural environment and are not exposed to any human intervention, have also become important in terms of their medicinal effects (Sekeroğlu et al., 2005). However, lifestyle changes have led to reductions in the collection and use of wild plants. This situation causes a decrease in traditional knowledge about wild plants considered as vegetables (Luczaj et al., 2012; Menendez-Baceta et al., 2017).

In Turkiye, which has a rich plant diversity, many wild plant species are collected and consumed as vegetables by the public. Awareness of plants considered as vegetables in the world and in Turkiye has increased recently. It is important to maintain the plant collection traditions related to the plants considered as vegetables in order to ensure food safety and sustainability. The names and characteristics of wild plants, which are considered as vegetables, have been transferred from generation to generation through oral and written sources.

Wild plants considered as vegetables are an important part of the Mediterranean food culture (Łuczaj and Pieroni, 2016). Muğla city is very rich in terms of endemic plants as well as having a high plant diversity. There are 1219 taxa and 1164 species within the provincial borders of Muğla. Of these, 238 of the species are endemic (Davis et al., 1965-1985; Güner et al., 2000). There is an important wealth of information in terms of ethnobotany in the province of Muğla. However, factors such as migration, developing technologies, and changes in people's diets cause a decrease in the knowledge of new generations about wild plants growing in nature. For this reason, it is important to determine the plant varieties that are considered as vegetables and their consumption patterns. This study was carried out with the aim of determining the plants that are widely distributed in Fethiye (Muğla) and its surroundings and considered as the most consumed vegetable.

MATERIAL AND METHOD

This work was carried out in 2023 in Fethiye district of Muğla province, which is located in the Aegean Region of Turkiye, and in the surrounding districts. Scanning was carried out in the area between 0 and 2000 meters altitude, including the coastal and highland regions. In addition, information on the subject was obtained from people who lived in that region for a long time. The types of plants consumed as vegetables in the place where they are found, the local names given to the plant and the way they are consumed are recorded.

RESULTS AND DISCUSSION

The list of plants identified from the research area is given in alphabetical family order.

Amaranthaceae

Amaranthaceae family is a crowded family in terms of the number of genera. Many species belonging to the family are widely produced as vegetables or are harvested seasonally from their growing areas. It is mostly seen in degraded lands, near wetlands, by the roadside and sometimes in vacant fields. It is common in Africa, Asia, Europe, North and South America (Gelin et al., 2003). Amaranthus sp. about 10 of the species in the genus are considered as vegetables, cereals and ornamental plants. In Fethiye and its surroundings, this family member is one of the most common types evaluated as 3 types of vegetables. *Amaranthus retroflexus* L. (horozibiği) is now grown in some regions for its vegetable value. Young leaves are consumed raw or cooked. *Chenopodium album* L. (sirken) is distributed in destroyed lands, seaside, roadside and fields. This plant, which shows a wide distribution area all over the world, grows especially in agricultural lands in and around Fethiye. Its leaves are consumed raw and cooked. *A. retroflexus* and *C. album* are also fast-growing weeds in agricultural areas and are subject to

agricultural struggle in aquaculture. *Salicornia europaea* L. (deniz börülcesi) is distributed along the sea coasts of the European continent. This plant, which is consumed raw or cooked, has found a growing area on the coast of Fethiye.

Amaryllidaceae

Most of the species belonging to the family consist of perennial, rhizome, tuber and bulbous plants. Leaves are located at the base or stem. There are onions, garlic and leeks among the cultivated vegetable species belonging to this family. There are also important ornamental plants belonging to this family. Many sulfur compounds in *Allium* species show antimicrobial (Casella et al., 2013), and immunomodulatory (Kumar and Brijeshlata, 2012) activities. There are plants belonging to this family, which are considered to be vegetables that spread spontaneously in nature. *Allium subhirsutum* L. (körmen), one of the plant species considered as a vegetable belonging to this family in and around Fethiye, is an important plant species with important medicinal properties. It is seen especially in high areas on bushes, stony, rocky and slopes.

Apiaceae

It is a family characterized by species that are usually combined umbrella or rarely umbrella. There are many types of vegetables grown in this family. The Apiaceae family includes many vegetable products rich in flavonoids, carotenoids, vitamins and minerals (Que et al., 2019). Carrots, parsley, dill and celery are the most important vegetables of this family. Among the members of this family, *Conium maculatum* L. (baldıran) and *Oenanthe pimpinelloides* L. (kazayağı) are among the wild plants that are considered as vegetables and spread around Fethiye. *C. maculatum* is distributed around forests and wetlands. The leaves are consumed by cooking. *O. pimpinelloides* also grows on the edge of marshes and wetlands. Some species of the genus *Oenanthe* are poisonous.

Araceae

Members of this family are usually rhizome and tuberous perennial plants. There are important ornamental plants (*Arum* sp., *Dracunculus* sp.) in the family. Many species of this family are toxic to humans. In the southern parts of Turkiye, *Colocasia esculenta* (Gölevez) is a plant species that is both cultivated and self-propagating in nature and considered as a root vegetable collected by the public. Some species belonging to the subfamily Lemnoideae, which are also in this family, are consumed by humans as vegetables in some parts of the world. It includes plant species that are likely to have significant vegetable potential for Turkiye (Coşkun, 2022). Members of this subfamily include aquatic plants that make up the smallest flowering plants in the world. Duckweed species have been observed in some aquatic areas (Girdev plateau) belonging to Fethiye and its surroundings. This plant, which is consumed as an important vegetable in some parts of the world, grows naturally in the region.

Asparagaceae

It is a family that includes perennial, rhizome, tuberous or bulbous species. Leaves can be at the base or on the stem. These family members include important vegetables and ornamental plants. *Asparagus officinalis* is a vegetable species cultivated in significant quantities in the world. *Asparagus acutifolius*, on the other hand, spreads naturally in a large part of Turkiye. In addition, *A acutifolius* is used as a diuretic and antineuralgic in traditional medical treatments (Marc et al., 2008; Fenga et al., 2016). In Fethiye and its surroundings, *A*

acutifolius has found a distribution area in nature. It is collected by the people in Fethiye and its surroundings and sold in village markets and is generally consumed by frying or boiling.

Brassicaceae

The Brassicaceae family consists of annual, biennial or perennial herbaceous plants, shrubs and small trees. The Brasicaceae family can be found almost everywhere in the world. There are many field and garden plant species in this family. It is one of the plant families with the highest number of species in the Flora of Turkiye. The Brassicaceae family is represented by approximately 676 taxa in Turkiye (Güner et al., 2012). Cabbage, cauliflower, broccoli, radish, arugula and cress are the economically valuable vegetable species in this family. In this family, there are many plant species that grow spontaneously in nature and these species are consumed as vegetables. In Fethiye and its surroundings, *Nasturtium officinale* R.Br. (su teresi), *Raphanus raphanistrum* L. (turp otu-hardal), *Sinapis arvensis* L. (hardal otu) and *Capsella bursa-pastoris* (L.) Medik. (çoban çantası-kuş tırnağı) are important species considered as vegetables. *N. officinale* is a semi-aquatic plant and a vegetable of high medicinal importance. This species is cultivated in large areas in many parts of the world. In Turkiye, it spreads especially in regions close to clean water resources. It grows in high altitude plateaus (Girdev plateau etc.) in and around Fethiye. The leaves are consumed raw or cooked. *R. raphanistrum* is widespread in Fethiye and its surroundings, as in many regions of Turkiye. This species is also an invasive species and has the capacity to grow in large areas. *S. arvensis* has been observed on roadsides and damaged areas in this region. It is a type of plant that is consumed by adding young leaves to salads. *C. bursa–pastoris* has hamostatic properties, it is consumed raw or boiled.

Caryophyllaceae

This family is widespread in most of the world and has a large number of species. This family consists of about 700 species found in the northern temperature regions, Africa and South America (Melzheimer, 1988). Popular ornamental plants have an important place in this family. Dianthus sp and Silenesp genera are popular ornamental plants in this family. *Silene vulgaris* (Moench) Garcke (gıyısgan), which has found a distribution area in Fethiye and its surroundings, can be seen in meadows, slopes and bushes. Fresh leaves are added to salads, while mature leaves are boiled or fried. Another wild plant that can be considered as another vegetable belonging to the Caryophyllaceae family in this region is *Stelleria media* (L.) Vill. This plant species can be consumed raw and has a very delicious taste.

Compositae

It is generally herbaceous, with very few shrubs, trees, and woody wrapping plants. Flowers are in head or capitulum states. It is one of the richest families in terms of the number of species in the Flora of Turkiye and there are approximately 1209 species (Özhatay and Kültür, 2006; Doğan, 2007). This family has important species spreading all over the world. Field and garden crops with economic value such as lettuce, sunflower, artichoke belong to this family. In addition, many medicinal plants such as yarrow, chamomile, calendula are members of this family. This family member also includes species that are not cultivated, grow spontaneously in nature and are consumed as vegetables. It has been observed that *Taraxacum* sp. (hindiba) and *Tragopagon* sp. (Tekesakali) are found in Fethiye and its surroundings. The fresh leaves of these plants are consumed by adding them to salads.

Dioscoreaceae

Plants belonging to this family are in climbing and winding formations. There are plants used as food and ornamental in this family. It also includes starchy plant species such as *Dioscoreales rotundata*, whose tubers are edible. There are 350-400 species in the genus Dioscorea (Caddick et al., 2002). *Dioscorea communis* (L.) Caddick & Wilkin (acı ot) plants, which are considered as vegetables belonging to this family, were observed in Fethiye and its surroundings. Young shoots of this plant species can be consumed and used in making pastries. The tubers of this plant are known to be poisonous (Yesil and Inal, 2021).

Lamiaceae

It spreads almost all over the world, except for the cold polar regions (Abdelhalima and Hanrahan, 2021). Family members include important medicinal and aromatic plants. Due to the essential oil content of this family members, it is included in the composition of cosmetic products and is used in the preparation of various foods and beverages. Mint, thyme, sage, lavender, rosemary and basil are important species in this family. It has been observed that the *Mentha pulegium* L. (yarpuz-narpız) plant belonging to this family can naturally find a distribution area, especially on the edges of wetlands, in the lands in Fethiye and its surroundings. The leaves and fresh shoots of this plant are used as vegetables and spices.

Malvaceae

It is a family of mostly grasses and rarely shrubs and trees. It is important in terms of vegetable, ornamental and medicinal plant species. Many Malva species are used as a vegetable or herbal medicine (Pandy, 2006). Famous plant species such as linden, cotton, cocoa, marshmallow, okra are included in this family. There are plants that are considered to be vegetables that grow naturally in this family, which spreads in most of the world. *Malva nicaeensis* (ilmik otu) and *Malva sylvestris* L. (ebegümeci) are distributed in and around Fethiye. It has been widely observed in the area in fields, devastated lands and roadsides. These plants are collected from nature and their aboveground organs are consumed fresh or cooked.

Papaveraceae

Turkiye is a gene center for *Papaver sp*. species as well as for many plants (Kapoor 1995). There are species with distinctive crown and bowl in the family. The flowers are usually showy and spread throughout much of the world. This family includes many popular ornamental plants (*Dicentra spectabilis*; *Hunnemannia fumariifolia*; *Meconopsis betonicifolia*). Papaver rhoeas L (poppy), which is in the family and spreads naturally in Fethiye, is considered as a vegetable. Before blooming in early spring in the region, the above-ground parts are boiled and roasted and consumed as food. *Papaver rhoeas* is used in the form of syrup as cough suppressant, sedative, sugar reducer, pain reliever, expectorant and sweat remover, and its leaves are consumed as salad or roasted (Duke, 1973; Akın, 2011).

Polygonaceae

The family includes climbers, grasses and shrubs distributed throughout the world, especially in temperate regions of the northern hemisphere. The family includes species grown as food (*Fagopyrum esculentum*) and ornamental plants (*Fallopia sachalinensis*). Within this family, there are species used as food (Özudogru et al., 2011) and for traditional medicine (Howes and Perry, 2011). In this family, the number of species consumed as a self-growing vegetable in nature is also high. *Rumex acetosella* L. (kuzukulağı), *Rumex crispus* L., *Rumex patientia* L. (labada) and *Polygonum aviculare* L. (kuş ekmeği) in and around Fethiye are

important plant species in this context. They is consumed raw and cooked, *R. patientia*'s leaves are usually cooked, and *P. aviculare* is consumed by cooking aboveground organs. The leaves of this species contain oxalic acid and can be consumed raw or cooked. It is added to salads because of its sour taste.

Portuguese

It is a family of annual or perennial herbs and shrubs. It is widespread in most of the world. There are plants considered as ornamental plants (*Portulaca grandiflora*) and vegetables (*Portulaca oleracea*) in this family. This is a plant rich in important phytochemicals with medicinal and nutritional properties (Uddin et al., 2014; Zhou et al., 2015). *P. oleraceae* (semizotu), which is considered to be an important vegetable in terms of Omega-3, is distributed in Fethiye and its surroundings. It is consumed fresh or roasted in salads.

Urticaceae

Members of the Urticaceae family consist of herbs and shrubs. Plants of this family are widespread in most of the world. One of the most important species of this family is *Urtica dioica* L. (ısırgan otu). Stinging nettles are easy to digest and highly nutritious. The leaves are rich in iron, vitamins A and C (Allardice, 1993), essential amino acids (West, 2000) and essential fixed fatty acids (Guil-Guerrero et al., 2003). This plant has found a wide distribution area in the geography of Turkiye. It is a plant that can grow in wetlands, destroyed lands and forest borders in and around Fethiye. It is an important plant species that is used as a vegetable for cooking, used in pastries, dried and used as a tea.

In this study, the plants considered as vegetables were determined in 15 families. The families with the highest number of species are Brassicaceae, Polygonaceae and Amaranthaceae. Aksakal and Yusuf (2008) determined that the species used for food purposes mostly belong to the families of Lamiaceae, Rosaceae, Apiaceae and Asteraceae. Korkmaz and Karakurt (2015) stated that Rosaceae, Asteraceae, Apiaceae, Lamiaceae, Chenopodiaceae families ranked in the top five in terms of the number of natural plant taxa used as food by the people living in Kelkit (Gümüşhane) region.

In this study, it was determined that 27 plant species wer considered as the most vegetable in Fethiye and its surroundings. Akan et al (2008) determined that 33 of 299 taxa were used as food in their study to determine the ethnobotanical characteristics of Mount Arat and its surroundings (Birecik-Sanlıurfa). Yapıcı et al (2009), ethnobotanical characteristics and local names of some plants identified from Kurtalan (Siirt) district were investigated. They identified 34 ethnobotanical features. Some ethnobotanical studies have been carried out near the region. In the study conducted in Köyceğiz district, 154 plants (Uysal, 2008), in the study conducted in Marmaris district, 95 taxa (Gürdal and Kültür, 2013) and in the study conducted in Ortaca district, 80 plant species were identified (Kazan, 2007). In these studies, it has been detected in medicinal and aromatic plants as well as for use as food. In this study, information about the species used as a vegetable is given. If other field crops used as food are included, the number of these species will increase even more.

Some plant species considered as vegetables determined in Fethiye and its surroundings have also been detected in other parts of Turkiye (Alpaslan, 2004; Akgünlü, 2012). However, the local names of the determined plant species may vary from region to region. While plant species such as Urtica dioica are usually given the same local name (nettle), there may be significant local name variation in some other plants. Consumption patterns of plant species

identified in this study may differ from each other. Satil et al. (2007) reported that the plants used for food purposes in and around Madra Mountain are consumed raw as well as cooked. Kadıoğlu et al. (2016) reported that wild plant species consumed as vegetables in Erzurum and Erzincan provinces are consumed for a long time by drying, pickling, freezing or preserving as canned, as well as consumed fresh by the local people. Koca et al. (2011) reported in their study in Samsun and its surroundings that some of the wild plants grown are consumed raw or cooked fresh, as well as some of them are consumed by freezing, drying, brine or processing. Wild plant species that are considered as vegetables in Fethiye and its surroundings are mostly consumed raw, but some of them are also consumed by boiling and frying (*A. acutifolius*). Apart from this, some plant species can be consumed for a long time by drying, pickling, freezing or preserving in canned form.

In the Fethiye region, people evaluate some herbaceous plants in every season. The most important form of evaluation is to consume it as a vegetable in various ways. Local people have known these plants for a long time and used them for food and medicinal purposes. These plants are collected and consumed by the public, as well as being sold in the neighborhood markets. Fethiye and its surroundings have been under intense pressure in terms of plant gene resources in recent years due to factors such as agricultural practices, tourism and population growth. However, there is a high interest in plants, which are considered vegetables due to their nutritional regime. However, changes in diet and other factors may cause these plants to be forgotten over time. However, there may be a gradual loss of traditional knowledge about these plants as the intergenerational transmission of knowledge declines. For this reason, it is important to determine the plant varieties that are considered as vegetables and their consumption patterns. In this study, plants that are considered as vegetables, which find a distribution area in Fethiye (Muğla) and its surroundings, were determined. Determining the wild plant diversity adapted to the natural environment will provide important benefits in the studies on plant breeding in the future.

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RATIONAL USE OF PASTURES USING REMOTE SENSING ON THE LANDS OF NORTHERN KAZAKHSTAN

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Intensive grazing leads to degradation of pastures and, accordingly, to a shortage of pasture feed for animals. With the haphazard use of forage lands, they are trampled and the coefficient of their use decreases, the botanical composition of phytocenoses is depleted and valuable crops fall out of its composition. To maintain pasture productivity, it is necessary to develop a pasture resource management system to control pasture capacity and grazing duration. The article shows the results of studies of corral grazing of beef cattle, using remote sensing of the land in Northern Kazakhstan.

Keywords: pastures, paddock grazing, pasture capacity, pasture turnover.

Introduction.

The potential productivity of the pasture lands of the Republic of Kazakhstan, which make up about 70% of its entire territory, reaches 25 and more million tons of fodder units. The largest areas of pastures are concentrated in the western, central and eastern regions of the country, and the smallest areas of pastures are concentrated in the northern regions due to the plowing of land for agricultural crops.

However, it should be noted that the natural forage lands of the republic and the region are used insufficiently and unevenly. Their unsystematic exploitation has led to the fact that at present the yield of pastures and hayfields has decreased almost everywhere, the area of degraded lands has increased, 48 million hectares of 188 million hectares of natural forage lands are subject to degradation, of which 27 million hectares are downed. As a result, the productivity of hayfields and pastures is significantly lower than the potential. The average productivity of pastures in the steppe and forest-steppe zones does not exceed 5 centners per hectare of pasture mass.

At the same time, only 30% of all pastures in the country are used for grazing, since most of the pastures are not provided with reservoirs. All these factors require new approaches in the use of forage lands. In this regard, the purpose of these studies was the organization of corral grazing for the rational use of pastures using remote sensing of the land in one of the farms of Northern Kazakhstan.

Materials and methods

The study was conducted by LLP "North Kazakhstan Agricultural Experimental Station" (54°12'45.0" N 69°30'50.1"E), located in Akkayyn district of North Kazakhstan region. For the experiment, natural forage lands and beef cattle of the Kazakh white-headed breed in the amount of up to 60 heads were selected. From the total area of the pastures of the farm, a separate experimental pasture with an area of 70 hectares, with 7 paddocks of an average of 10 hectares, was selected for the organization of grazing by a corral (portion) method (Fig.1). The paddocks were divided into the shape of a petal with a separate single outlet to the watering

hole. After the organization of the pasture territory, the animals were grazed alternately in the paddocks during the pasture period.

When selecting and defining the boundaries of the experimental pasture site, the following was carried out:

1) collection of information using digital technologies: land and cartographic maps, identification numbers of farm land plots in the AIS GZK system.
2) superimposing coordinates on the map and processing satellite images using ArcGIS.
3) Fixing the boundaries of pastures and contours using the Garmin Montana 610 GPS navigator using GPS/GLONASS satellite data.
4) Calculation of the required area for 60 heads of cattle and the number of paddocks, the number of days for grazing for the entire pasture period.

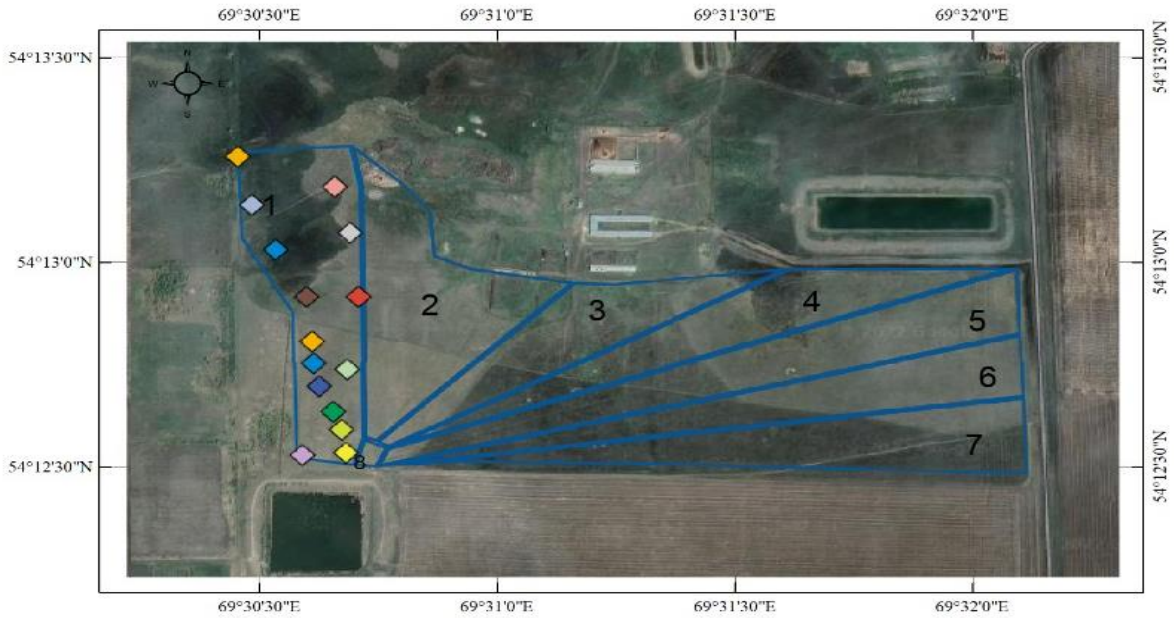

Figure 1. Pasture turnover scheme

The total area and paddocks of the pasture were fenced with electric fences with a battery power system and solar panels.

After the organization of the pasture territories, the animals were grazed alternately in the paddocks during the pasture period. During this period, aboveground records and observations of the dynamics of the botanical composition, the projective cover, plant height, yield, before and after the animals were pitted in the paddocks were carried out.

Analysis of the botanical composition. To determine the botanical composition of the herbage, samples were taken from $1m^2$ of the area. The selected samples were weighed and divided according to the botanical composition. The botanical description of the herbage was carried out according to the determinants [1, 2] and the method of botanical weight analysis of hay and pasture feed samples [3].

Projective coverage (PC) is determined by the Ramensky method, with the help of a mesh superimposed on a plot of $1m^2$, empty spaces are counted, which are measured by cells. Then the number of empty cells (Ec) is divided by the total number of cells (Tc) and multiplied by 100, % of empty cells (n) are obtained: n = Ec / Tc * 100, then the projective cover is determined by the following formula:

PC=100-n

Determination of pasture productivity. Productivity accounting on pastures was carried out by the seasonal sloping method, in each contour on 10 accounting sites with a size of at least 2.5 m^2 (1x2.5 m) each at a height of 5-6 cm from the ground. An average sample was also taken to determine the absolutely dry substance and then the dry mass yield from 1 ha.

Determination of pasture capacity and load. The actual load for 1 conditional head cattle (A, ha) is the actual pasture area for one head or the need for pasture area, determined by the formula: $A = f/p$; where f is the need of animals for pasture feed during the pasture period; p is the productivity of the pasture during the entire pasture season. The load on 1 ha of pastures (pasture capacity or C, conditional heads) is the number of animals that can be grazed on 1 ha without damage to pasture ecosystems, determined by the formula: C=Y/(K*D) ; where C is the permissible load on 1 ha of pastures (heads), Y is the yield of green feed or dry mass eaten during the pasture period (kg or feed units), K is the daily need for green feed or dry mass per head of cattle (kg, feed units), D – duration of pasture use (day) [4].

Research results and discussion.

The soil cover of the experimental pasture site is characterized by a low humus content, an average nitrogen content and a low phosphorus content, and in terms of exchange potassium it belongs to a high group, in terms of volumetric weight it belongs to a medium-density group. The degree of acidity of the soil is neutral.

The botanical composition was represented by a typical mixed-grass vegetation with a predominance on individual contours of wormwood (Fig.3), height from 15.7 to 33.3 cm, with a projective coverage from 55.0 to 97.7% and a seasonal yield from 5-6 centners per hectare with a content of 5.58 to 33.59 g of digestible protein in 1 kg of pasture mass, fodder units from 0.20 to 0.55 k units, exchange energy from 2.7 to 6.2 MJ. Thus, according to the soil and botanical characteristics, this site is a typical pasture area of the steppe zone of the northern regions of the republic, the areas of which occupy more than 70% of the total pasture area of the region.

The height of plants varied from 11.5 to 18.6 cm before the first grazing, NDVI indicators were at the level of 0.24-0.39, from 11.7 to 17.6 cm before the second grazing, NDVI from 0.31 to 0.48 (Table 1).

Table 1 – Dynamics of the height of the grass stand and NDVI by the periods of grazing of the paddocks

Paddocks	Projective coverage, %			NDVI		
	1- grazing	2- grazing	+/- between 1-st and 2-nd grazing	1- grazing	2- grazing	+/- between 1-st and 2-nd grazing
1	11,5	17,6	+6,1	0,26	0,32	+0,06
2	14,8	13,7	-1,1	0,38	0,35	-0,03
3	13,6	14	+0,4	0,39	0,36	-0,03
4	18,6	17,6	-1,0	0,24	0,48	+0,24
5	14,5	15,5	+1,0	0,32	0,39	+0,07
6	14,9	11, 7	-3,2	0,31	0,35	+0,04
7	14	14,9	+0,9	0,29	0,31	+0,02

The productivity of pastures during the second grazing was higher than during the first. This is due to the rainfall in July, which was 12 mm higher than the annual average and helped the herbage to recover. Whereas at the first grazing in May, the amount of rainfall was 3-4 times lower than the average long-term indicators (Table 3).

Table 3 – Pasture productivity by paddocks

Paddocks	Productivity, t/ha		
	1 – grazing	2 - grazing	+/- between 1-st and 2-nd grazing
1	0,49	1,13	+0,64
2	1,49	1,26	-0,23
3	1,27	1,12	-0,15
4	1,18	2,15	+0,97
5	1,24	1,92	+0,68
6	1,08	0,86	-0,22
7	1	0,6	-0,4
LSD	0,36	0,29	

For example, in paddocks No. 1, 4, 5, pasture productivity increased by 0.64, 0.97, 0.68 t/kg. And also, on the whole, productivity in all pasture paddocks improved on average by 1.29 t/ha.

Subsequently, after data collection, the required area for 60 heads of cattle of the meat direction of the Kazakh White-headed breed was calculated and the number of days for grazing for each paddock for the entire pasture period was determined (Table 4).

Table 4 – Calculation of the load and duration of grazing for each paddock

Paddocks	grazing duration	area	pasture mass yield, t	Actual load, head/ha	Pasture area for one head, ha
1 – grazing					
1	7	9,94	0,49	1,69	0,59
2	8	9,26	1,49	4,50	0,22
3	5	9,57	1,27	6,13	0,16
4	7	9,52	1,18	4,07	0,25
5	6	9,7	1,24	4,99	0,20
6	5	9,68	1,08	5,21	0,19
7	5	9,46	1	4,83	0,21
2– grazing					
1	5	9,94	1,14	5,50	0,18
2	5	9,26	1,27	6,13	0,16
3	5	9,57	1,12	5,41	0,18
4	9	9,52	2,15	5,77	0,17
5	8	9,7	1,92	5,79	0,17
6	5	9,68	0,82	3,96	0,25
7	4	9,46	0,6	3,62	0,28

Thus, during the first grazing, the cattle were grazed on average for about 6 days on each paddock, for a total of 43 days there was completely one cycle for all paddocks.The period of the first grazing also depended on the type and condition of the pasture herbage. Pasture grass should be inserted during the period of its greatest nutritional tillering, earing-budding, and it is necessary to finish the grazing before the beginning of flowering, when the grasses begin to roughen.

After collecting all the data, a grazing schedule was compiled on the basis of calculations at each paddock (Table 5).

Table 5 - Periods of use of paddocks

Usage periods	Paddocks pastures						
	1	2	3	4	5	6	7
17.05-25.05	B1						
25.05-02.06		B1					
02.06-09.06			B1				
09.06.-15.06				B1			
15.06-23.06					B1		
23.06-1.07						B1	
1.07-11.07							B1
11.07-19.07	B2						
19.07-28.07		B2					
28.07-5.08.			B2				
5.08 -14.08.				B2			
14.08-22.08					B2		
22.08-27.08						B2	
27.08-01.09							B2

*Note: B1, B2...- the sequence of cattle grazing on corral plots

Thus, the livestock, which is consistently drove according to the developed schedule, on average after 6 days of grazing from the previous site to the next, during the first cycle of 43 days, completely passes the entire area of the experimental site allocated for herd grazing, and returns to the initial 1st paddock. The time before re–grazing corresponds to the time for the restoration of the herbage according to agrotechnical standards - 40-50 days. From day 44, the second cycle begins from the 1st paddock. Under favorable weather conditions, the grazing time can be extended.

In our research, with the help of the organization of paddock grazing based on calculations of pasture load, we were able to avoid degradation of pastures without loss of fatness of animals. The organization of cattle grazing for the rational use of pastures is an important factor for preventing the degradation of pastures, since cattle usually spend less time in places far from water, and also do not graze on steep slopes [5].

Conclusion. Thus, when organizing paddock grazing for the rational use of pastures in “North Kazakhstan Agricultural Experimental Station” LLP, a typical pasture site was selected by geobotanical surveys, the required area for 60 heads of beef cattle of the Kazakh white-headed breed was calculated. The average grazing area of one head of beef cattle in “North Kazakhstan Agricultural Experimental Station” LLP, which amounted to 0.22 head/ha, as well as the number of consistently used paddocks in the section – 7, with the exception of the possibility of complete grazing, which will allow the farm to reduce costs for this period and increase the use of pasture productivity, is justified.

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THE ROLE OF DNA METHYLATION IN SHEEP' EMBRYONIC DEVELOPMENT

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ABSTRACT

DNA methylation is one of the epigenetic modifications of the genome, the essence of which is the attachment of a methyl group to nitrogenous bases. They are assumed to CpG islands play an important role in the regulation of gene expression in sheep, coming to regulatory elements of genes.

The interest are processes of methylation and demethylation. The methylation process always depends on the work of enzymatic complexes and is very precisely regulated. The methylation process largely depends on the functioning of enzymes. On other hand, demethylation can be performed not only by enzymatic complexes, but also during DNA replication. So, the maintenance of DNA methylation is more important. Changes in methylation patterns are linked with gene expression and observed during embryonic development.

Keywords: methylation, sheep, gene expression embryogenesis

INTRODUCTION

The crucial DNA modification with significant effects on gene expression is the process methylation. There are the various forms of DNA methylation, but cytosine methylation is the most frequent in eukaryotic cells. It is an epigenetic mechanism in which the methyl group is transferred to the fifth carbon of cytosine and lead to formation a molecule of 5-methylcytosine.It is catalyzed by the DNMT methyltransferase family (Fig. 1), represent a three family groups,numbered in order of their discovery (Lyko, 2018; Bestor, 2000). These enzymes serve the twounic processes of DNA methylation - the establishment of DNA methylation state by de novomethylation and, thereafter, the maintenance of those states by repication (Okano et al., 1999).DNA methyltransferase 1 (DNMT1) is actively involved in the maintenance of DNA methylation patterns. Passive demethylation occurs after each cellular division in the absenceof functional DNMT1. DNMT3A and DNMT3B are included in the establishment of de novoDNA methylation. DNA methyltransferase 3L (DNMT3L) interacts and stimulates activity of DNMT3A and DNMT3B. Ten-eleven Translocation dioxygenases enzymes are helping (TET)active demethylation as oxidize 5-methylcytosine (m5C) to 5-hydroxymethylcytosine (hm5C),5-formylcytosine (f5C) and 5-carboxylcytosine (ca5C), (Lyko, 2018). The structural andfunctional identities of cells throughout cell division are defined from DNA methylation

patterns (Moore et al., 2013).

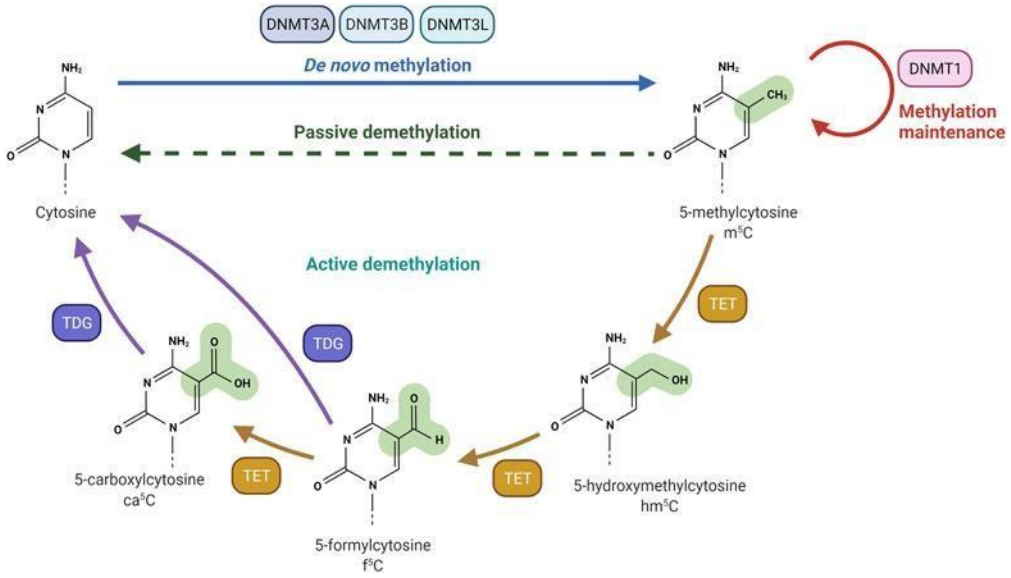


Figure 1. DNA methylation and demethylation machinery

The mammalian genome contains an extremely high burden of sequences. The gene regulation is highly sensitive to the methylation status of these so-called CpG islands. The genome of domestic sheep (*Ovis aries*) contains approximately 28 million CpG dinucleotides, which are unevenly distributed, such each can be in a methylated or unmethylated form. Their diploid number is 54, with 26 pairs of autosomes and two sex chromosomes (Jin, et al., 2011). More than 78% of DNA methylation occurs in cytosines which are located in the CpG dinucleotide in cells. The main of functions of this modification are genomic imprinting, inactivation of X-chromosome, regulation of gene expression, maintenance of epigenetic records or embryonic development (Sun et al., 2021).

During development in mammals, there are two periods of genome-wide DNA methylation reprogramming (Reik & Walter, 2001). The first period is during primordial germ cell differentiation and second period is during preimplantation development. To what extent this important regulatory mechanism operates in early germ cell development and differentiation in specie Ovis aries has not yet been clarified. This knowledge will influence the understanding of the DNA methylation process, its impact on the stability of the genome and reproductive biology in particular.

The aim of this report was to review the current understanding of the mechanisms of DNA methylation and demethylation in sheep and the role of methylation in the regulation ofgene expression and embryo development.

RESULT AND DISCUSSION

Regulation and functions during Development of gene transcription

Epigenetic regulation of transcription through DNA methylation can occur via two mechanisms. One of this, is based on the steric hindrance of methyl groups to the interaction oftranscription factors with DNA. The other mechanism is based on indirect involvement of proteins, which inhibit DNA binding to transcription factors (Loaeza-Loaeza et al., 2020). It isconsidered that approximately 40% of the genes encoding proteins contain CpG islands in

the promoter regions. CpG dinucleotides can facilitate the binding of transcription factors to DNA,thereby supporting gene expression (Hartl et al., 2019).

DNA methylation has long been thought to affect gene transcription alone. However, ithas been found recently that attachment of the methyl group to cytosine may increase diversity

of mRNAs and their protein products, by modulation of the rate of operation of RNA polymerase II (Shayevitch et al., 2018; Jensen et al., 2023). One of the common features of CpG islands is that they contain fewer nucleosomes than other regions of DNA, and this enhancing gene expression (Moore et al., 2013).

The literatuire analysis suggests that methylation does not have a suppressive effect on gene expression, as the activity of some genes appears to be independent of methylation. Research has also shown both positive and negative correlations between tissue-specific methylation and gene expression.

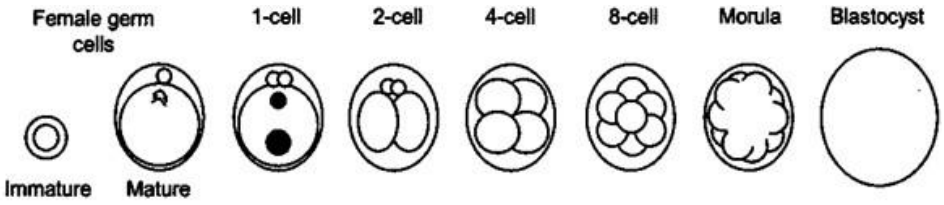
DNA methylation during oocyte maturation is essential for viability of the embryos. The development of a mammalian embryo depends on proper epigenetic modifications and resources provided by the oocyte. The ability of oocytes to synthesize and store sufficient quantities of maternal factors, such as Dnmt1, Dnmt3a, and Tet3 mRNA, during maturation, islinked with global methylation and demethylation during oocyte maturation and respectively early embryonic development. Epigenetic mechanisms, specifically DNA methylation dynamics, have been implicated in the capacity of develpenting oocytes. If DNA methylation defects occur in this phase may occur problems in the pregnancy (Liang et al., 2012).

During germ cell development, erasure of imprinted methylation is followed by temporally asynchronous reacquisition of sex-specific imprints. This restoration of methylation marks requires the activity of de novo MTases. The hypomethylated status of imprinted genes in embryos derived from transplanted Dnmt3a–/– Dnmt3b+/– ovaries suggested a possible role for these MTases in de novo imprint methylation (Hata et al., 2006). Methylation analysis of these embryos revealed severely hypomethylated imprinted genes, suggesting an essential role for Dnmt3a in the establishment of maternal methylation imprints during female germ cell development. The methylation of paternally methylated imprinted gene H19 and minor satellite sequences, appeared normal in these embryos. Likewise, spermatogonia from mutant males also showed hypomethylation of imprinted target sequences. That suggest that Dnmt3a is required by both male and female germ cells for the de novo methylation of imprinted genes during gametogenesis.

The methylation targets of Dnmt3L are identical to those of Dnmt3a, with spermatogenesis was also perturbed in Dnmt3L null males, and germ cells showed impaired denovo methylation of the essencial gene H19. Interestingly in this case is that Dnmt3L-deficientspermatocytes display severe meiotic defects (Bourc'his and Bestor, 2004). This reinforces the critical role for maintenance of DNA methylation in chromosoms stability. Dnmt3a and 3b havebeen shown to be required for aintenance of methylation. About Dnmt1, it remains active following replication, and may achieve this postreplication methylation in only with Dnmt3a and/or Dnmt3b (Liang et al., 2002; Rhee et al., 2002). These results suggest a mutual interrelatedness of the MTases to create and maintain distinctive patterns and levels of DNA methylation.

Dnmt1o is actively transcribed and translated during oocyte growth and maturation. In this period replication no takes place. This suggested a de novo methylase function for Dnmt1o (Mertineit et al., 1998). In the phase MII on oocyte, characterized with distribution of the proteins of oocyte to two- and four-cell stage embryo, included it inherits a vast store of Dnmt1o

protein. However, there is very limited detection of the Dnmt1o transcript after fertilization. Itis very specific that the Dnmt1 transcript can be detected throughout this period, with the exception of the fertilized oocyte (Fig. 2), (Ratnam et al., 2002; Ko et al., 2005). Analysis of the Dnmt3 family of proteins and activities has not been completed; however, expression of the de novo methylase Dnmt3a is detected throughout preimplantation. Although Dnmt1s is also present throughout this period, the absence of maintenance methylation and the observation that imprinted methylation remains intact, suggests that Dnmt3a, in its targeting role, acts as a maintenance methylase but only on imprinted target sequences.



Dnmt1s	+	+	-	+	+	+	+	+
Dnmt1o	+	+	+	-	-	-	-	-
Dnmt1p	-	-	-	-	-	-	-	-
Dnmt3a	-	+	+	+	+	+	+	+
Dnmt3b	-	+	-	-	-	-	-	+

Figure 2. Dynamics of DNA methyltransferase localization during reproductive development: from germ cell to embryo

The epigenome plays a critical role in embryogenesis. The genome undergoes a two-stage reprogramming process during embryogenesis; first - during the formation of primary germ cells. During this early phase of differentiation, PGCs appear to be methylated as measured by indirect immunofluorescence using a highly specific antibody against 5 methyl cytidine (Seki et al., 2005); after that, both male and female gametes are arrested, the female inmeiotic prophase and the male in mitosis. The significance of this process is to protect the cell lines of the future embryo from mutations and second, methyltransferases to new methylation patterns de novo (Gopinathan and Diekwisch, 2022).

The genome demethylation occurs during early mammalian embryonic development, afew hours after the formation of the zygote as full complect of appropriately imprinted genes are remodeled to restore a uniform diploid nucleus to the newly formed zygote (Li et al., 2008). At the morula stage, gamete methylation patterns are completely obliterated (Bernstein, 2020). Remethylation occurs at the blastocyst stage when methyltransferases (3a and 3b) create de novo methylation patterns. With embryo implantation, the genome is methylated anew, forming a characteristic pattern, which is then maintained of its cells (Gopinathan and Diekwisch, 2022). Using an antibody approach, Dean et al. (2001) found that active demethylation was conserved among widely divergent mammalian species. Investigations in the sheep suggest that there is a partial reduction of DNA methylation in the zygote. A similar analysis in humans, shown that male pronucleus undergoes demethylation in the zygote (Beaujean et al., 2004).

Experimental manipulation suggest that the partial demethylation of conspecific sperm may be related to the absolute amount, and organization, of genomic DNA methylation in the sheep (Jabbari et al., 1997).

The epigenetic load of the oocyte is essential for its developmental potential. Unfortunately, its remodelling often overlaps with potential interfering events such as exposure to assisted reproduction technologies (Hansen et al., 2005) or environmental changes - nutrition, pathologies or accidental exposition to various contaminants (Cortessis et al., 2012). Accurate knowledge of the methylation and hydroxymethylation status of the growing oocyte and on the molecules involved in their remodelling is fundamental to view an overall picture of the early rearrangements that will originate the embryo epigenome.

CONCLUSIONS

DNA methylation plays an important role in the regulation of sheep embryonic development. Methylation levels change several times during embryogenesis. DNA methylation, as a well-established epigenetic marker, has attracted the efforts of many researchers from diverse fields for decades. Combining new knowledge of DNA with histone modification and non-coding RNAs will provide important insights into the mechanisms of chromatin remodeling and regulation of gene transcription, which is instrumental to comprehensive understanding of developmental reprogramming.

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BDELLOVIBRIO BACTERIOVORUS IN BIOFILM AND MICROBIOLOGICALLY INFLUENCED CORROSION INHIBITION

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ABSTRACT

Metal corrosion is one of the major global economic issues. Consisting of destruction of the metal and metal properties, this electro-chemical process is caused by different factors including microorganisms. Even if some microorganisms can influence the metal corrosion without adhering the surface of the metals, biofilm formation on the metal surface is known as one of the influence factors of microbially influenced corrosion (MIC). In this context, inhibiting the MIC can be processed by inhibiting the biofilm formation on the metal surface. Different methods like using antibiotics and biocides are used to fight this community formations. However, resistances to these substances are still developed in the environment and cause high costs in industrial and health fields. By this fact, it is thought that using predator bacteria which can feed themselves on the biofilm forming bacteria or metal corrosion influencing bacteria can be an environmentally friendly method and reduce the cost damage in the industries. In this review, the Gram-negative bacterium *Bdellovibrio bacteriovorus* is the main focused predator bacteria thought to be used to fight against biofilm formation and MIC. This bacterium isolated from different ecosystems (aquatic and soil), has as hosts both gram negative and Gram-positive bacteria. This study helps to understand in general the MIC, biofilm, MIC inhibition (MICI), and the role that can play the *B. bacteriovorus* in the inhibition of both biofilm formation and MIC.

Keywords: *Bdellovibiro bacteriovorus*, Biofim, Corrosion, EPS, Microbially Influenced Corrosion.

INTRODUCTION

Metal corrosion is an electro-chemical mechanism resulting in the destruction of metals and their properties (Wadood et al., 2015; Lou et al., 2021). This phenomenon causes industrial economic loss, since it causes destruction of engineering equipment used in different industries like oil recovery, water cooling towers, and other mechanical systems (Li et al., 2015; Li et al., 2022). Environmental conditions including physico-chemical factors and biological factors, especially microorganisms, are known to impact on the metal corrosion process. Microorganisms can be involved in the initiation, acceleration, or facilitation of the corrosion by directly adhering to the metal surface to form biofilm or changing the environmental physico-chemical conditions of the metal (Li et al., 2022; Lou et al., 2021; Qian et al., 2022).

The abundance of microorganisms in water or liquid ecosystems is found to be reduced at the decrease of nutrient in liquids. However, they can adhere to surface of solid materials like metals or silicon that are in contact with the liquids and form the biofilm. Biofilm consists of pure or different microorganisms that adhere a solid surface and remain motionless in which

they secret organic polymeric matrix known as extracellular polymeric substance (EPS) (AlSalhi et al., 2023; Iroha et al., 2005).

The EPS as well as other chemicals secreted in the biofilm matrix can facilitate the communication between organisms in this microbial community (Quorum Sensing). However, at the same time, this microbial structure can generate a competition between its population which induce physicochemical changes on their microenvironment (Flemming and Wingender, 2010). These changes cause the deterioration of the surface on where the biofilm is formed, thus we talk about corrosion. The output of the corrosion includes organic and inorganic compounds (AlSalhi et al., 2023). These changes don't only impact the metal or the surface from where microorganisms are attached but also cause pollution of the surrounding environments.

The microbial influenced corrosion is estimated at 20% of all corrosion costs (Basera et al., 2019; Kokilaramani et al., 2021). The MIC affects both highly specialised industries as well as our daily living environments like water cooling systems for large air conditioning units, kitchen sink's metal water traps, dental implants, etc… (Kokilaramani et al., 2021; AlSalhi et al., 2023; Fu et al., 2021; Costa et al., 2021). In fact, multiple ways are studied to combat this issue. It consists of chemicals like antibiotics and anti-biofilms developed to inhibit the attachment of the development of cells on the metal surface (Liu et al., 2018; El-Shamy, 2020). However, most of these substances are synthetics and cause significant impacts in the environment or can alter the chemical structure of the metal. In addition, antimicrobial resistance can be developed by the biofilm and the development of new anti-biofilms and antibiotics will still be needed.

In this fact, new strategies to combat the biocorrosion by using microorganisms able to inhibit the biofilm formation are sound to be interesting and efficacies. Some microorganisms are feeding on other microorganisms without causing any infections to higher organisms like plants and animals (Lou et al., 2021). Such microorganisms can be used to control the proliferation of undesired microorganisms in different environments. *Bdellovibrio* Like Organisms are group of Gram-negative bacteria living in different ecosystems like water and soil (Negus et al., 2017). They are prey bacteria that can attack both Gram-negative and Gram-positive bacteria in the environments that are present. Study on biocontrol using these bacteria showed their ability to inhibit the development of *E-coli* in meat like food (Ottaviani et al., 2020). Another study showed the ability of these bacteria in reducing the proliferation of sulfate reducing bacteria (SRB) that are known for their capability to induce metal corrosion in anaerobic conditions (Qui et al., 2016). In this fact, it is thought that these bacteria can be effective in the inhibition of biofilm formation on metals and so the inhibition of microbially induced corrosion. Along this current review, general idea on biofilm, microbially induced corrosion (MIC), microbiologically influenced corrosion inhibition (MICI), and the application of *Bdellovibrio bacteriovorus* against biofilm formation and MIC are developed.

BIOFILM

In the environment, microorganisms can be found as planktonic or sessile cells. Sessile cells are attached to the surface of materials and form through different steps the biofilm structure (Rumbaugh and Sauer, 2020, Rather et al., 2021; Sauer et al., 2022). Biofilm can be defined as a surface attached microbial community embedded in a self-produced extracellular matrix composed of the complex extracellular polymeric substances (EPS) (Sauer et al., 2022; Rather et al., 2021). Microorganisms are directed to form the biofilm structure due to environmental stress including starvation, fighting against chemicals and antimicrobials, unfavourable temperature and pH, and continuous water flow (Castiblanco and Sundin, 2016; Rather et al., 2021). The biofilm formation or development is set in four stages which are detailed in different reviews for the mechanisms of each stage.

The first stage consists of the bacterial adherence on the host material surface: 'Adhesion'. In this stage, bacteria as planktonic cells interact with the host surface and form a firmly monolayer (Tuson and Weibel, 2013; Mahamuni- Badiger et al., 2020). At the second stage, we assist in a local proliferation of microorganisms generating the microcolonies. In this stage named 'biofilm formation or growth', a self-secretion of exopolymeric substances (EPS) is starting and a multilayer of microorganisms is orderly structured (Rabin et al., 2015). The third stage is the 'biofilm maturation' which consists of cell adaptation and development to transform the microcolonies into microcolonies. At this stage, the matrixome components including flagella, pili, amino acids, and exopolysaccharides are under regulation and lead to the development of mature biofilm (Liu et al., 2018). The fourth and last stage is the 'dispersion'. This process is due to environmental conditions such as change in temperature, oxygen, and nutrient composition, or caused by some enzymatic reactions from the EPS components, or physical factors like shearing forces by the liquid flow. These factors induce the detachment of cells from the biofilm structure and open the door for new local infestation, thereby a new cycle of biofilm development (Rather et al., 2021; Mahamuni- Badiger et al., 2020; Percival et al., 2015).

Other than the laboratory manipulated environments, the microbial community in a biofilm structure is far to be composed of a single microorganism. These microorganisms can communicate through the quorum sensing mechanism and form different complex matrix (Rather et al., 2021; Karygianni et al., 2020). Depending on the environmental physico-chemical conditions as well as the microbial community, the EPS is composed of biomolecules such as proteins, polysaccharides, lipids, and nucleic acids (Flemming et al., 2016).

On other hand, EPS is thought to afford drug tolerance in biofilms and develop their antimicrobial resistance. By this fact, the biofilm formation ability of microorganisms is recognized to be among their virulence factors (Wall et al., 2019; Gupta et al., 2016). In this context, the use of antimicrobial compounds against biofilms is more than complicated; because every microorganism in such a micro-community can secret its proper molecules in the biofilm matrix that can have different virulence activities. The EPS form a barrier of ex-situ component like antibiotics by inhibiting them to enter the last layer of biofilms due to the ionic charges of the matrix, by destroying them through exoenzymes secreted by microorganisms in the microcolonies, or by genetic modification of antimicrobial active sites (Wright, 2005; Schroeder et al., 2017). The EPS composition and structure is affected by different factors like the type of microorganisms composed of biofilms, the local stress conditions like shear, and the mono-species or multispecies characteristics of the biofilm (Karygianni et al., 2020).

More of the impacts of the physico-chemical properties of biofilms is the changes of the environmental conditions like pH and oxygen concentration. These changes negatively affect the properties of the materials from which the biofilms are formed. For example, the degradation of microplastics by microorganisms is thought to be more effective with bacteria able to be attached on their surfaces and using these hydrocarbons as carbon sources (Han et al., 2020). In addition, the metal biocorrosion, regardless of its type, ca caused by the cell adherence to the metal surface (biofilm formation) and inducing electrochemical reactions (Yang et al., 2021).

MICROBIALLY INFLUENCED CORROSION (MIC)

MIC consists of deterioration process of metal or other surface caused by the reaction of metabolic reactions on that surface. Biofilm forming on metal induce acceleration of metal corrosion by acting in different ways such as modifying the chemicals transports towards the metal surfaces, removal of the protective films during the biofilm separation stage, causing inequal distribution of oxygen on the metal surface, causing conditions changes on the

oxidation-redaction processes occurred between solution and metal surfaces, and fragilization of the passive layers by changing their inorganic structure and accelerating their decomposition and detachment from the metal surface (Pal and Lavanya, 2022). The type of microorganisms, their metabolic reactions, the environmental conditions including pH, temperature, nutrient availability, metal type, and the addition of new species on the biofilm structure, have important impacts on the metal' MIC (Procópio, 2019; Pal and Lavanya, 2022). Different group of bacteria are registered from previous study for their ability to induce metal corrosion.

Gram negative sulphate reducing bacteria (SRB) are known for their metal corrosive property under anaerobic conditions and are one of the most studied bacterial groups in metal corrosion. In mixed biofilm structure on metal, the SRB are found to inhabit the bottom where the oxygen level is lower due to the colonization of the top layers by aerobic and facultative anaerobic bacteria (Jia et al., 2019). They are thought to act in three different mechanisms during their metal corrosion reaction. The steel corrosion induced by SRB is due to the production of hydrogen sulfide (H2S) in sulfate-containing environments. The sulfide is oxidized on the surface of the steel and produce the thiosulfate which activate the pitting corrosion (Kokilaramani et al., 2021). On the study run by Dou et al. (2018), it is shown that the cause of copper microbially influence corrosion by SRB is their production of sulfide rather than the electron harvesting for their energy production.

As reported by Jia et al. (2019), iron oxidizing bacteria (IOB) are group of bacteria able to oxidize ferrous ions to ferric ions to generate their growth energy and use oxygen as terminal acceptor. This reaction results to the extracellularly iron hydroxides deposit (Jia et al., 2019). IOB are known as corrosion causing agents but together with SRB are known to enhance the microbial corrosion of metals (Liu et al., 2018). It is due to the oxygen-free environment offered by the aerobic and facultative IOB on the biofilm formed on metals (Jia et al., 2019).

Nitrate reducing bacteria (NRB) is another corrosive bacterial group. In the beginning, NRB were used to fight against the development of SRB which are known to have a negative influence on the corrosion of metals. For this purpose, nitrate was injected into the oil and gas industries to promote the multiplication of NRB which use nitrate as a final electron acceptor in a low oxygen environment (Fida et al., 2016; Jia et al., 2019). However, a favourable thermodynamic reaction was reported between the couple formed by this nitrate reduction with iron oxidation. These reactions highlight the role of NRB in the MIC of metals. Other groups like acid producing bacteria, fungi, methanogens are also observed with corrosive role on metals (Kokilaramani et al., 2021; Jia et al., 2019).

MICROBIOLOGICALLY INFLUENCED CORROSION INHIBITION (MICI)

In the previous section, we showed the influence of microorganisms especially bacteria on metal corrosion and destruction. Combatting this industrial issue, traditional methods are applied. Lou et al., (2021) enumerated some protection methods like metal coating with antibacterial materials such as silver, surface treatments, and synthetic corrosion inhibitors. Although these technics are solving most of MIC problems, their negative impacts on the environment and public health, due to the release of toxic materials, limits their uses and incite to an urgent searching for eco-friendly methods to fight against the high loss in industrial and engineering fields (Lou et al., 2021). Previous studies showed some approaches that are thought to be environmentally friends like the use of biocide extracted from the black mustard *Brassia nigra,* using Immunoglobulin A (IgA), and film-forming mixtures of amines, imidazoline and quaternary ammonium compounds (Little et al., 2007; Videla et al., 2004). However, the use of these three approaches is limited on different ways: from the limit to use natural biocide on established biofilm, to the unfavourable use of IgA in medical applications, the toxicity of Alkyl

imidazoline to animals, and the initiation of local corrosion caused by film-forming inhibitors (Little et al., 2007; Videla et al., 2004).

Microorganisms are reported with a dual role in the corrosion process depending to the microorganism types, the environmental conditions, as well as the metal species. When some microorganisms enhance and accelerate the corrosion of metals, others, or the same microorganisms but in different environmental conditions can act oppositely and hindering the MIC (Lou et al., 2021). This process is called microbiologically influenced corrosion inhibition (MICI). Microorganisms act on different mechanisms to inhibit corrosion. Lou et al., (2021) divided the MICI mechanisms into five categories: microbial respiration, competition, secretion corrosion inhibitors, EPS protection, and mineralization. The microbial respiration mechanism consists of both aerobic and facultative anaerobic microorganisms present in metal surface forming biofilm. Facultative anaerobe and aerobic bacteria like *E.coli* and *pseudomonas* have been screened for their ability to inhibit or reduce different metal corrosion processes and the observed results are promised (Jayaraman et al., 1997).

This mechanism is related to the growth conditions of microorganisms, their ability to form biofilm on metal surface, the dissolved oxygen present in the environment, and other environmental conditions like, inorganic nutrient, temperature, and flow velocity (Lou et al., 2021). Wadood et al. (2015) showed the stainless-steel corrosion inhibition role of *Bacillus subtilis* and *Pseudomonas aeruginosa*. This study's results showed through SEM analysis the formation of protective film on the surface of the metal which consist of rod bacterial shape. In addition, authors observed that as long is the incubation time, as high is the decrease of the pH of the medium. This acid production is considered as metabolites production for the bacterial growth. However, an antagonistic reaction has been observed between the two bacteria, since when they are incubated together, the corrosion inhibition is mor important than when they are separately incubated (Wadood et al., 2015).

On other hand, competition between microorganisms can inhibit the microbially induced corrosion of the metals. Some microorganisms can compete other corrosive microorganisms through their metabolic growth systems. They can use the same electron acceptor and reduce the growth of the corrosive microorganisms (Hubert and Voordouw, 2007). Beside this competition, predator microorganisms can use the corrosive microorganisms as host for their growth and inhibit their attachment to the metal surface (biofilm forming inhibition) (Lou et al., 2021). It is the case of *Bdellovibrio* and organisms like (BLO).

APPLICATIONS OF *BDELLOVIBRIO BACTERIOVORUS* FOR BIOFILM INHIBITION AND MICI

Bdellovibrio bacteriovorus is a motile Gram-negative with curved shaped bacterium. This bacterium isolated from different ecosystems such as soil and water, is a predator bacterium known which is feeding from both Gram-negative and Gram-positive bacterial cells (Bratanis et al., 2020; Pantanella et al., 2018). In general, *B. bacteriovorus* attack the prey cells, enter their cytoplasm where they are multiplying. After their multiplication, cells leave the prey cells by causing lysis of their membranes (Bratanis et al., 2020).

Bdellovibrio bacteriovorus and *Bdellovibrio* like organisms are shown in different studies to be with high important uses in the control of pathogen bacteria. In the study conducted by El-Shanshoury et al. (2016), the *Bdellovibrio. sp*, isolated from water shown lytic activity on four antibiotic resistant isolates of *Salmonella sp*. From these results, authors suggest that

the predator bacteria can be used in the treatment of sewage wastewater as antimicrobial agent. In another study, the *Bdellovibrio bacteriovorus* has been revealed with antimicrobial effects against the phytopathogenic strains *Pantoea sp*. and *Xanthomonas campestris* (Odooli et al., 2021). On other hand, experimentations shown that *Bdellovibrio bacteriovorus* could be used during the post-harvest treatment to help the mortality of pathogens like *Esherichia coli* and *Salmonella* which are food borne pathogens (Olanya et al., 2020).

Bdellovibrio species showed effects on both planktonic cells and biofilms. It is reported that the attack to the microorganisms on biofilm structures required high effort than the attack to planktonic cells (Kadouri and O'Toole (2005). However, the reduction of biomass on biofilm formed by different pathogens is observed in previous studies. Kadouri and O'Toole (2005) reported the first anti-biofilm effect of *Bdellovibrio bacteriovorus* on the biofilms of *E. coli* and *Pseudomonas fluorescens* (Kadouri and O'Toole (2005). In addition, *Bdellovibrio bacteriovorus* has significantly reduced the microbial biomass of both pure and multicultures. The mode of action of this predator bacterium on the inhibition or degradation of biofilm formation depends mainly on the prey bacterium. The inhibition of *Staphylococcus aureus*'s biofilm by *Bdellovibrio bacteriovorus* is reported to be the action of extracellular proteases secreted by the predator (Pantanella et al., 2018).

Most of previous studies studying the efficiency of *Bdellovibrio bacteriovorus* and BLO as natural antimicrobial agents, focused on medical importance, biocontrol of plant pathogens and wastewater treatments. Until this date, only one study has investigated the effect of *B. bacteriovorus* on the microbial influenced corrosion caused by SRB. The results of this study show the reduction of corrosion rate of X70 steel from 19.17 to 3.75 mg/dm^2/day after 2 months (Qiu et al., 2016). These results promise potential uses of this predator bacterium to escape from industrial damages which cause important economic cost in different sectors. On other hand, the MIC associated bacteria are of different mode of actions as previously illustrated. Some of them are directly attached to the metal surface (biofilm) some of them secret enzymes or other chemical substances which enhance the corrosion of the metal. Since this predator bacterium can attack both planktonic cells and cells in biofilm structure, it is thought to significantly impacted on the MIC. In addition, previous studies investigating the effects of environmental conditions on *B. bacteriovorus* show high range of pH and temperature resistance. Moreover, any oxygen variation impact on this bacterium was not observed (Williams and Chen, 2020). These data reinforce the idea that the *B. bacteriovorus* is a good natural biocontrol agent against MIC and biofilm formed on metal instruments. However, further studies like effects of this bacterium on different metal surface and on different microorganisms are necessary before applied it on the field.

CONCLUSIONS

Metal corrosion is an electro-chemical process resulting to the destruction of metal and metal properties which cause several industrial issues. It is caused by both abiotic and biotic factors including microorganisms. However, some microorganisms have been shown to play an important role in the inhibition of metal corrosion caused by microbial influence (MIC). *Bdellovibrio bacteriovorus* are a Gram-negative vibrio and Rhod- like shape motile bacteria. They are predators of both Gram-negative and some Gram-positive bacteria. Due to their predation and their high enzymatic activities, these bacteria have been studied for their biotechnological and industrial applications. These bacteria may play a significant role in the inhibition of MIC through their predation activity on the biofilm formed on the metal or on the microorganisms responsible for metal corrosion like SRB. Further studies on their predation

mechanisms should highlight the importance of their use in the fight against this industrial issue (corrosion).

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POTENTIAL APPLICATION OF CANNABIS AND CANNABIS DERIVED COMPOUNDS AGAINST BIOFILM

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ABSTRACT

Many plants including *Cannabis Sativa* are known for their medicinal uses. Cannabis Sativa is an annual plant known for centuries for its different medicinal benefits including antimicrobial effects. Until 2017, this plant has been reported with more than 500 natural constituents in which 120 of them are cannabinoids. Even if the antimicrobial effects of this plant and its extracts have been shown, studying all its natural constituents in the context of antimicrobial agents is still not enough. In addition, antimicrobial resistance constitutes an important public health problem. Microorganisms still develop this mechanism and cause difficulties in treating many infectious diseases. One of the virulence factors in microorganisms is the ability to form biofilm. Biofilm, as a microbial community in which different substances are secreted, is a highly protective form of the microbial community against antimicrobial agents. This highly protective structure should be studied alone for obtaining alternative antimicrobial agents against a such structure. This review resumes information about the response of biofilm structure to antimicrobial agents, and the use of *Cannabis Sativa* as an alternative anti-biofilm agent to fight infectious diseases caused by biofilm-forming bacteria.

Keywords: Antimicrobial resistance, Antimicrobial agent, Biofilm, *Cannabis Sativa*.

INTRODUCTION

Antimicrobial agents are chemicals that destroy microorganisms or prevent their reproduction. The discovery of antibiotics, one of the most important antimicrobial agents, is one of the greatest achievements of the modern medical world. Since the development of antibiotics, millions of lives have been saved. However, to control the action of these antibiotics, microorganisms develop different ways that are often genetically controlled. One of the most important ways is the development of biofilm-mediated antimicrobial resistance.

Bacterial and fungal resistance to antimicrobial therapy is an important threat to human health and a major concern all over the world. Immunocompromised patients, such as transplant recipients and those undergoing chemotherapy treatment, are particularly vulnerable. Their ability to fight infection depends on the effectiveness of their used antibiotics. Therefore, the spread of multidrug-resistant bacteria/fungi threatens the success and development of these therapies (O'Neill, 2016).

Multiple resistance to antibiotics clearly demonstrates the overuse of existing antibiotic drugs and the inability to develop enough new antibiotics on a global scale. Some antibiotics are currently administrated in combination to combat antibiotic resistance. However, predicting that we will soon run out of these options is not difficult. It is estimated that the number of

deaths caused by antibiotic resistance may reach 10 million by 2050 and the cost of this situation is estimated to be 100 trillion USD globally (O'Neill, 2016).

The major problem is the divestment of most pharmaceutical companies in the research and development of new antibiotics. This is due to the uncertainty of successfully developing new antibiotics from compounds identified in the preclinical period. Furthermore, the profit earned on the investment of developing something is known to be less. In these facts, these companies mostly produce and sell existing antibiotics.

The limited number of new antibiotics that have been developed and approved in recent years to reduce antibiotic resistance obscures the prospects for the use of antimicrobial drugs in the future. It also ensures that the issue of antimicrobial resistance continues to be a priority global health issue. The World Health Organization has identified antimicrobial resistance as one of three major threats to health systems globally (https://doi.org/10.1086/652237). Therefore, measures, such as controlling the use of antibiotics, better understanding the genetic mechanisms of drug resistance, and continuing studies to develop new synthetic or natural drugs, should be taken to reduce this problem. The ultimate goal should be to provide the patient with appropriate and effective antimicrobial drugs. Developing new methods to treat antimicrobial resistance is a race against time.

FORMATION OF BIOFILM

Bacteria in the aquatic environment tend to attach to a solid surface and multiply there to form a community known as 'biofilm'. The living conditions in the biofilm are better and safer for microorganisms than their planktonic living environment. While some of the microorganisms leave the surface on which they are attached, others use the nutrients on the surface, multiply and attach irreversibly to the surface by secreting extracellular polymeric substances (EPS). Thus, EPS acts as an adhesive substance and provides the biofilm microorganisms with a "safe life" in which they can grow, reproduce, secrete metabolites, and communicate with other microorganisms. Biofilms protect microorganisms from host immune cells, antibiotics, nutrient shortage, osmolarity, pH, and temperature. Sessile cells can be separated from their biofilms to colonize other regions (Donlan, 2002).

Biofilm formation is an important virulence factor for microorganisms causing chronic infections. Biofilm-forming microorganisms are the main cause of various chronic, hospital-acquired, and medical device-associated infections that are of serious concern or even untreatable nowadays (Wi and Patel, 2018). According to the American National Institutes of Health, approximately 65% of all microbial infections and 80% of all chronic infections are associated with biofilms (Sharma et al., 2019). With the advancement of science, many diseases, disorders, and abnormalities can be effectively managed using various medical devices such as pacemakers, vascular catheters, chronic haemodialysis catheters, prosthetic heart valves and prosthetic joints. However, the effectiveness of these medical devices is severely hampered by the biofilm that forms on them. Due to the complex interactions between microbial cells, host, and biomaterials, treatment of these device-associated infections may be unsuccessful.

INTERACTION BETWEEN BIOFILM AND ANTIMICROBIAL AGENTS

The ability of microorganisms to form biofilms and become resistant to antibiotics causes significant difficulties in disease treatment. In a biofilm under antimicrobial influence, the number of resistant mutants increases, and the resistance genes can spread by horizontal gene transfer to all microorganisms in the biofilm matrix. The main cause of various chronic infections are microorganisms resistant to antibiotics and contagious microorganisms. Most antibacterial agents cannot penetrate biofilms which are the main source of resistant microbial

strains. Consequently, they cannot prevent the proliferation of microorganisms in the biofilm matrix.

The developed antimicrobial agents are mostly tested against free-living planktonic cells. Due to the drug tolerance and the multifactorial nature of biofilm formation, the use of these antimicrobials may be ineffective against pathogenic biofilms. In this fact, combined treatments are needed. The discovery of antibacterial agents that can kill or stop the growth of microorganisms contained in the biofilm and help to prevent infections from the biofilm is urgent.

Plants are one of the most important alternative and effective strategies for reducing resistant microorganisms. Plant-based antibacterial agents have enormous potential to treat a variety of diseases, as they have the power to alleviate infectious diseases and lack adverse side effects, such as hypersensitivity, allergic reaction, and immunosuppression, that are often associated with current antimicrobial agents (Agrawal and Gupta, 2020). Studies have shown that many plant extracts and phytochemicals exhibit antimicrobial properties against pathogens, including clinically resistant bacterial strains (Erdem et al., 2013; Nascimento et al., 2000). Moreover, previous studies confirm the antibacterial activity of plant extracts in animal infection models (Choi et al., 2011; Yunana et al., 2018).

The surfaces of medical devices can be modified with antibiofilm nanoscale biomaterials. Thereby, gold, silver, iron oxide and bimetallic nanoparticles can be made multifunctional either individually or with polymeric substances or drugs. Thus, biofilm formation can be controlled by quorum sensing, cell-to-cell communication, and interruption of multiple drug efflux pumps. The surfaces of medical devices can also be coated with plant extracts. Phytochemicals (including alkaloids, flavonoids), heteroatoms such as N, S, O, and π-electrons in the plant extract contain aromatic rings and are adsorbed on the metal surface through them. The inhibition is mainly attributed to the presence of various organic compounds found in the plant extract (Prabhu and Rao, 2013). *Cannabis sativa L*. is one of these plants which has traditionally been used medicinally for centuries.

CANNABIS PLANT (*Cannabis sativa*)

Cannabis (*Cannabis sativa*), also called Marijuana, is an annual species native to Asia and has been documented as one of the oldest known crops. Cannabis has traditionally been widely cultivated in the world. Apart from its use as a recreational drug, it is used for fiber production, human nutrition, and medicinal purposes (House et al., 2010). Due to its variable low lignin content and bast fibers enrichment, the fibers of the hemp plant are suitable to produce textiles, paper, rope, biofuels, biodegradable plastics, and building materials. They are also suitable for use in the automotive industry, insulation, paint, and animal feed (Singh et al., 2018). Due to the versatility of cannabis, valuable essential oils and resins can be extracted from the flowers and leaves, as well as high-quality cellulose can be extracted from the stems and trunk (Baldini et al., 2018). The cannabis seed, which is called “çedene” or “hemp seed” in Anatolia, has an important place in the nutrition of humans and poultry due to its rich oil content. In addition, cannabis has gained a bad reputation due to its widespread use for pleasure and consequently, its versatile benefits have been ignored for many years.

Nowadays, the production of different products has led to an increase in interest of industrial cannabis. Plants of the genus *Cannabis* produce more than 560 known secondary metabolites with 120 of them are cannabinoid compounds (ElSohly wt al., 2017). Cannabis phytochemicals include primary metabolites such as amino acids, fatty acids, and steroids, or secondary metabolites such as terpenoids, flavonoids, stilbenoids, lignans, and alkaloids (Flores-Sanchez and Verpoorte, 2008).

CANNABINOIDS

C. sativa is increasingly used for its medical treatments against various diseases such as inflammation, cancer, obesity, osteoporosis, multiple sclerosis, vomiting, epilepsy, pain, glaucoma, and anorexia. Besides, it is applied for the treatment of various neurodegenerative disorders including Tourette syndrome, Huntington's disease, Alzheimer's disease, and Parkinson's disease (Grotenhermen and Müller-Vahl, 2012). Apart from these areas of use, *Cannabis sativa* is a plant that offers many interesting features due to its rich metabolic profile such as anti-biofilm and bactericidal effect. Cannabinoids are *C. sativa* metabolites that are effective in the treatment of contagious bacterial infections diseases.

The production of cannabis has been severely restricted and banned all over the world for many years due to its psychoactive properties. However, because of limited studies, their potential for use in both industrial and health fields could not be ignored any longer. In line with the provisions of the regulation published at September 29, 2016 in the Official Journal, number 29842, the production of cannabis, previously prohibited in Turkey, is allowed in the provinces of Amasya, Antalya, Bartin, Burdur, Çorum, İzmir, Karabük, Kastamonu, Kayseri, Kütahya, Malatya, Ordu, Rize, Samsun, Sinop, Tokat, Uşak, Yozgat, and Zonguldak as well as in all districts of these provinces. In the next years, the regulations supporting the establishment of new companies by utilizing cannabis cultivation and the products obtained from it are expected to be made.

ANTIBIOFILM ACTIVITIES OF CANNABIS

Studies have shown the antimicrobial effects of the cannabis plant, its leaf extracts, essential and seed oils, as well as components isolated from it such as cannabinoids, against pathogenic bacteria and fungi (Frassinetti et al., 2020; Feldman et al., 2021). Alkaloids, flavonoids, peptides, tannins, and phenols also found in the *C. sativa* plant are also known for their antimicrobial properties (Chandra et al., 2017). This suggests that many compounds found in cannabis extracts may additionally or synergistically contribute to the antimicrobial activity (Schofs et al., 2021). Although the antibacterial mechanism of cannabinoids effect remains unclear, some modes of action have been recently proposed (Figure 1).

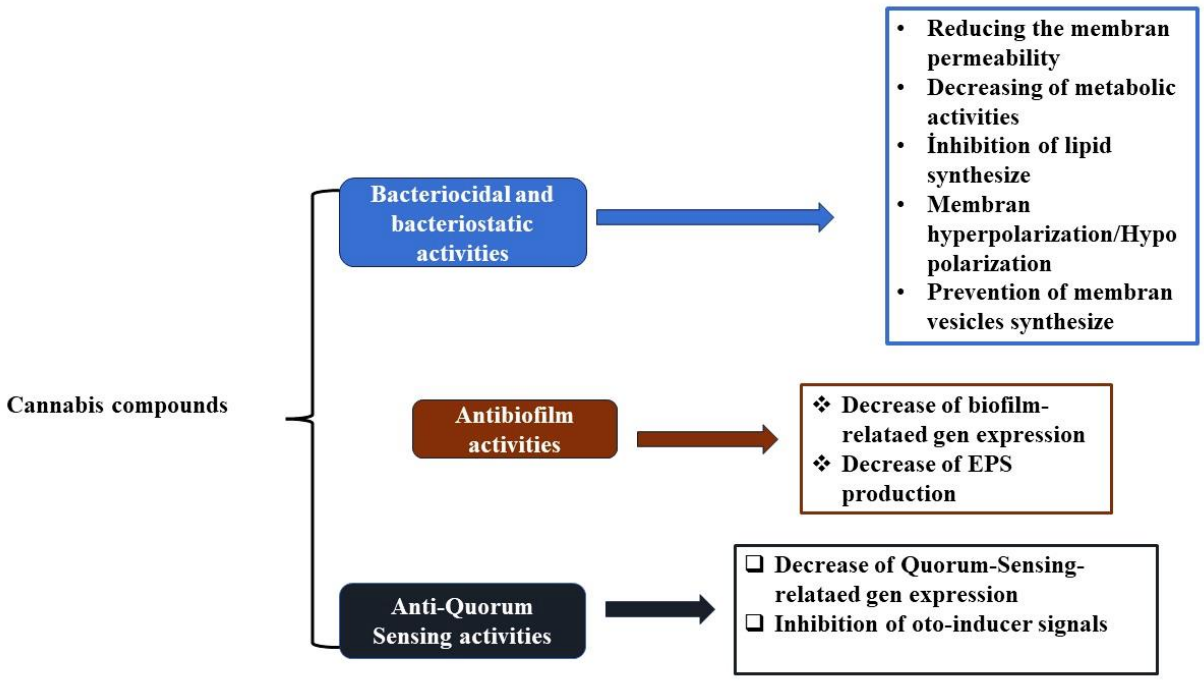

Figure 1: Potential antimicrobial mode of action of cannabis compounds.

One of the modes of action is related to changing the microbial membrane permeability. It was determined that β-caryophyll compounds destroyed the cell integrity and wall structure of *B. cereus* and led to the leakage of intracellular components (Moo et al., 2020).

Another putative mode of action of cannabinoids is the alteration of cell communication through inhibition of membrane vesicles released by bacteria. Cannabidiol that consists of a phyto-cannabinoid obtained from *Cannabis sativa*, was found to be a potent inhibitor of membrane vesicle releasing from *E. coli* VCS257 but the same effect was not detected in *S. aureus* subsp. aureus Rosenbach. In addition, it was determined that cannabidiol used together with selected antibiotics significantly increased the bactericidal effect of several antibiotics in Gram-negative bacteria (Kosgodage et al., 2019). These results suggest that, through different ways including the membrane vesicle inhibition pathway, the cannabidiol can be used with antibiotics selected based on bacterial species to increase antibiotic activity and help to reduce antibiotic resistance.

In one of the limited studies, it was determined that the seeds of *Cannabis sativa L*. showed antimicrobial and antibiofilm activity against *Staphylococcus aureus* (Frassinetti et al., 2020). It was also determined that the biofilm inhibition of *Staphylococcus aureus* by *Cannabis sativa* L. seeds was lower than their minimum inhibition concentrations (MIC) values. Researchers attributed the biofilm inhibition to the high content of phenolic compounds such as caffeoyltyramine and hemp A, B, and C, especially in the cannabis seed extract.

In a study investigating the ability of cannabidiol to inhibit the formation of fungal biofilms, *C. albicans* was exposed to various concentrations of cannabidiol for 24-72 hours and the metabolic activity of biofilms was determined using 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyl-2H-tetrazolium bromide (MTT) analysis method. The study resulted to the observation of fungal biofilm formation inhibition by cannabidiol depending on time and dose (Feldman et al., 2021).

Other study examining the effect of cannabigerol on *S. mutans* biofilm formation and distribution, shown that cannabigerol inhibited biofilm formation with decreased biofilm biomass, decreased biofilm thickness, less EPS production, decreased DNA content and decreased metabolic activity. It was also determined that cannabigerol changed the roughness profile of the biofilm and provided a smoother biofilm surface. However, when researchers examined the effect of cannabigerol on preformed biofilms, they found that cannabigerol reduced the metabolic activity of *S. mutans* with a transient effect on biomass and suggested that cannabigerol could be a potential drug for the preventive treatment of dental caries (Agawi et al., 2021).

Farha et al. (2020) determined that cannabinoids exhibit antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA), inhibit the ability to form biofilms, and destroy preformed biofilms and stationary phase cells that are resistant to antibiotics. They also revealed that the mechanism of action of cannabigerol is to affect the cytoplasmic membrane in Gram-positive bacteria and the inner membrane in Gram-negative bacteria. Researchers have demonstrated that cannabinoids work against multidrug-resistant Gram-negative pathogens (*E. coli, Pseudomonas aeruginosa*) in combination with polymyxin B, demonstrating the broad-spectrum therapeutic potential of cannabinoids (Farha et al., 2020).

In a study investigating the potential role of cannabigerol as an anti-biofilm and anti-quorum sensing agent against *Vibrio harveyi*, it was determined that concentrations of cannabigerol that did not affect planktonic bacterial growth reduced the bioluminescence and biofilm formation of *V. harveyi* regulated by quorum sensing. In addition, it was found in the study that cannabigerol decreased the motility of *V. harveyi* in a dose-dependent manner (Agawi et al., 2020).

CONCLUSIONS

Bacterial or fungal biofilm formation is an important virulence factor that inhibits the control of pathogens and the treatment of infections caused by related microorganisms. Much research has been and is still being done to find alternatives to current antibacterial/antifungal treatments. Studies clearly show that *Cannabis sativa* has antimicrobial activity. However, it is not easy to prevent biofilm-based infections. Recently, although limited, studies in which *Cannabis sativa* is effective on biofilm have also been published.

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TOMATO RESISTANCE GENES MI AGAINST TO THE ROOT KNOT NEMATODE (*MELOIDOGYNE* SPP.) AND MOLECULAR APPROACHES.

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ABSTRACT

Meloidogyne spp. was first detected in the UK but is now a worldwide problem for tomato and other Solanaceae crop production, threatening production both in open fields and greenhouses. If appropriate control measures are not taken 15- 85 % yield losses can take place. Tolerances of the plants themselves, as well as chemical spraying and biological agents, are of great importance for damage prevention. Understanding and engineering these gene mechanisms is of great importance for development of tolerant varieties against *Meloidogyne* spp. Plant resistance(R) proteins recognize pathogen virulence (Avr) determinants and trigger plant defense mechanism. Then the carefully organized dynamic defense regularly emerges as a Hypersensitive Response (HR) and the defense becomes active. As a result of these changes, new studies identified new components of *Mi-1*-mediated resistance to the nematodes. In this study we review the molecular mechanisms of tolerance against *Meloidogyne spp.* in tomato.

Key words: *Meloidogyne* spp., defense mechanisms, host response, *Solanum lycopersicum*, *Mi-1* genes

Introduction

Tomato is one of the most important vegetables grown in the world. They also contain high levels of lycopene, an antioxidant that reduces the risks associated with many cancers and neurological diseases. The homeland of the tomato includes Chile, Peru and Ecuador in western South America. In addition, it was determined that there are 2 endemic wild tomato species in Galapagos Island (Darwin et al. 2003). *Solanum peruvianum* L. is the most common and polymorphic wild tomato species. It has been stated that the possible ancestor of the tomato, which is an annual plant, is the wild cherry tomato (*Solanum lycopersicum* var. cerasiforme) (Jenkins 1948; Akkurt et al. 2013). It has been reported that in ancient Mexico, the tomato was grown for food and was called “tomati” (Boswell 1937). Tomatoes are used fresh or in various forms such as peeled, chopped, frozen, canned, tomato paste, ketchup, pickles in the food industry (Causse et al. 2016).

After it became widespread in the European Mediterranean countries in the 16th century, it is cultivated in many parts of the world in the 20th century (Yazgan and Fidan 1996). 189.1 million tons of tomatoes were produced around the world in 2021. China ranked first in tomato production and harvested area in 2021. Türkiye ranked 3 rd in world tomato production in 2021.

Tomato production; 8.7 tons in 2021/22. According to the 1st Estimation of Crop Production by TURKSTAT for 2023, tomato production is expected to be 13.5 million tons in 2023.Considering these data, the importance of tomato in the country's agriculture is clearly seen.

There are many pests and diseases that cause yield loss in tomatoes. Root-knot nematodes, which are plant parasitic nematodes that feed as endoparasites, also cause serious damage to tomatoes (Bleve-Zacheo et al. 2007). Root-knot nematodes (*Meloidogyne* spp.) are spread all over the world and have a wide host range (Sasser 1980, Karssen and Moens 2006). The four most important pests worldwide are *Meloidogyne javanica* (Treub) *Chitwood*, *Meloidogyne arenaria* (Neal) *Chitwood*, *Meloidogyne incognita* (Kofoid et White) *Chitwood* and *Meloidogyne hapla Chitwood* (Netscher and Sikora 1990). Root-knot nematodes are obligate

parasites that feed only on the cytoplasm of living plant cells (Willamson and Hussey 1996). They become adults after four larval stages (Luc 1990). It is a second instar larva (J2) that penetrates the root and moves to an area near the vascular tissue to create a permanent feeding site (Williamson and Hussey 1996). After J2 enters the root, it moves between the cells in the vascular cylinder and fixes itself when it determines the feeding area (Abad and Williamson 2010). They cause the formation of giant cells in the area where they feed. The swellings that occur on the root surface as a result of growth in giant cells are called galls or galls (Williamson and Hussey 1996). The resulting galls significantly restrict the nutrient and water uptake of the roots from the soil. Then, they cause yellowing, wilting, stagnation in growth, deterioration in fruit quality and decrease in yield. In addition, they increase the formation of diseases by causing soil-borne pathogens to enter through the wounds they open.

It has been reported that *Meloidogyne* spp. causes an annual loss of 157 billion dollars worldwide (Abad et al. 2008). In addition, disease severity increases as a result of co-infection with soil-borne pathogens (Lambert and Bekal 2002). That's why it's so important to management. Cultural measures, physical control, biological control and chemical control methods are used. Chemical control is the most used method for controlling root-knot nematodes (Gowen et al. 2007). Despite this, the use of nematicides is decreasing in some regions of the world (Nyczepir and Thomas 2009). In addition, the prohibition of widely used fumigant such as methyl bromide (methyl bromide) has increased the search for alternatives in chemicals. Studies are carried out on alternative methods of struggle that will not cause the stated results. One of them is the use of biological organisms. The difficulty of adaptation of these organisms to environmental conditions and the cost of the preparations limit their use. Therefore, the use of resistant varieties comes to the fore (Rotino et al. 2002; Toppino et al. 2008). However, host resistance, which is one of the cultural methods, is known as the most effective and environmentally friendly method against root-knot nematodes (Devran and Söğüt 2014).

Resistant varieties provide ease of application and provide an environmentally friendly solution without the need for special tools and equipment (Lopez-Perez et al. 2006; Cortada et al. 2009; Verdejo-Lucas et al. 2009). Resistance prevents the reproduction or development of the nematode through the resistance genes it carries or keeps it at a very low level (Boerma and Hussey 1992, Roberts 2002). Resistant varieties suppress root-knot nematode and reduce the need for chemical control (Williamson 1999).

1. Resistance to Root Knot Nematodes

Resistance to root-knot nematodes was reported for the first time in a wild tomato species, *Solanum peruvianum* (Bailey, 1941). This resistance gene, called the *Mi-1* gene, was transferred to *S. esculentum*, the cultivar of tomato, by embryo rescue technique (Smith, 1944). Today, commercially developed root-knot nematode resistant cultivars carry this gene (Yaghoobi et al., 2005). Many genes (*Mi-2* to *Mi-9*) have been identified against the root-knot nematode, except the *Mi-1* gene (Capnet al., 1993; Yaghoobi et al., 1995; Veremis & Roberts, 1996a; Veremis & Roberts., 1996b; Milligan et al., 1998; Ammiraju et al., 2003). Knowing the characteristics of resistance genes and their responses to nematodes is important for breeding and control.

Plants have developed different defense mechanisms to protect themselves from diseases and pests. Resistance, which is one of these mechanisms, has been defined as the ability of the plant to prevent, eliminate or reduce the attacks of disease agents and pests (Wingard, 1953). For entomologists, the "hardy" plant is less affected by the same population of the pest (Painter,

1951). In general, a nematode-resistant plant is one that can inhibit the growth of the nematode compared to a non-resistant one (Cook & Evans, 1987; Trudgill, 1991; Barker, 1993).

Plants first show a passive response consisting of physical barriers to protect themselves from the pathogen. Thickening of the cell wall as a result of lignin accumulation is one of these barriers (Tör, 1998). Important plant hormones such as salicylic acid, jasmonic acid and ethylene play a role in defense (Kunkel & Brooks, 2002). Another defense mechanism is the hypersensitivity reaction (Hypersensitivity Reaction-HR) created by the resistance genes (Willamson & Hussey, 1996).

The emergence of resistance in plants occurs when the resistance gene (R) in the host and the avirulence gene (avr) products of the pathogen match each other (Flor, 1955). Resistant plants prevent the reproduction or development of the nematode through the genes they carry (Roberts, 2002). These plants protect the plant from nematode damage and reduce the nematode population (Lopez-Pérez, 2006). Tolerant plants, on the other hand, cannot suppress the growth of nematodes, but prevent yield loss (Gonzalez, 2009).

Root-knot nematodes cannot form a feeding zone in a resistant plant (Milligan et al., 1998). In order to create a feeding zone, a hypersensitive reaction occurs immediately in the cell to which it inserts its stylet. In the incompatible interaction of the plant with the nematode, O is produced enzymatically outside the cell and is converted to hydrogen peroxide (H2O2), a compound that can pass through the cell membrane (Bleve-Zacheo et al., 2007). H2O2 begins to accumulate rapidly in the cells, and oxidative combustion occurs along with it. The first symptoms of the hypersensitive reaction resulting from the incompatible relationship appear approximately 12 hours after the nematode inoculation (Dropkin, 1969a; Milligan et al., 1998; Bird & Kaloshian, 2003). As a result, the nematode dies before it can form a feeding place (Verdejo-Lucas et al., 2012). In case of a compatible interaction between the nematode and the plant, H2O2 is produced 12 hours after the nematode enters the plant, but after 48 hours H2O2 cannot be detected. The reason why H2O2 could not be determined is the activity of the genes responsible for the enzymes that prevent oxidative combustion. As a result, structures called giant cells are formed (Apel & Hirt, 2004; BleveZacheo et al., 2007).

Resistance to root-knot nematodes in tomato is provided by the *Mi-1* gene. In tomato, it is a dominant gene called *Mi-1* that provides resistance against *M. incognita*, *M. javanica* and *M. arenaria*. It was named after the nematode species (*M. incognita*) used in tests to determine the resistance status of plants (Gilbert & McGuire, 1956). *Mi* gene was found in *S. peruvianum* (PI128657) and hybrid plant was obtained using embryo rescue technique since it could not be hybridized with culture forms using conventional breeding methods (Smith 1944). The widely used *Mi-1* gene against root-knot nematodes comes from this source (Ammati et al., 1986). Mi-1 gene is 7 homologous genes (*Mi-1.1, Mi-1.2, Mi-1.3* and *Mi-1.4, Mi-1.5, Mi-1.6, Mi-1.7* 2 clusters in the 650 kb region of the short arm of the 6th chromosome of tomato) are available as. Of these homologues, *Mi-1.3* and *Mi-1.5* are pseudogenes. As a result of studies carried out in plants to which homologous genes are transferred, it has been determined that resistance is provided by *Mi-1.2* (Milligan et al., 1998) (Table 1). The cytoplasmic protein encoded by *Mi-1.2* consists of 1257 amino acids. This resistance gene motif is called CC-NBS-LRR. The nucleotide binding site of this structural motif is called NBS (Nucleotide Binding Site), the LRR portion with leucine amino acid-rich repeats (Leucine Rich Repeat) and the helical motif at the amino end of these proteins is called CC (Coiled-coil) (Milligan et al., 1998; Hwang & Williamson, 2003).

Mi-1.2 gene was found to be resistant to *Meloidogyne* species as well as some biotypes of potato aphid [*Macrosiphum euphorbiae* (Thomas)] and cotton whitefly [*Bemisia tabaci* (Gennadius)] B and Q biotypes (Nombela et al. 2003).

Table 1: Characteristics of genes providing resistance to root-knot nematode (*Meloidogyne* spp.) in tomato

Gene	Source	Resistant Species	Temperature	Chromosomal Location	Literature
Mi-1 (Mi)	*S.peruvianum* PI128657	*M. incognita* *M. javanica* *M. arenaria*	<28°C	6	Milligan et al., 1998
Mi-2	*S. peruvianum* PI270435-2R2	*M. incognita*	32°C	-	Cap et al., 1993
Mi-3	*S. peruvianum* PI126443-1MH	*M. incognita*	32°C	12	Yaghoobi et al., 1995
Mi-4	*S. arcanum* LA1708-I	*M. arenaria*	32°C	-	Veremis & Roberts, 1996a
Mi-5	*S. peruvianum* PI126443-1MH	*M. incognita*	32°C	12	Veremis & Roberts, 1996b
Mi-6	*S. peruvianum* P1270435-3MH	*M. incognita*	32°C	6	Veremis & Roberts, 1996b
Mi-7	*S. peruvianum* PI270435-3MH	*M. incognita*	<28°C	6	Veremis & Roberts, 1996b
Mi-8	*S. peruvianum* PI270435-2R2	*M. incognita*	<28°C	6	Veremis ve Roberts, 1996b
Mi-9	*S. arcanum* LA2157	*M. incognita* *M. javanica* *M. arenaria*	32°C	6	Ammiraju et al., 2003

2. Naturally Resistant Resources

Several *Mi*-genes have been detected in some tomato lines, genotypes, and cultivars. These genes confer resistance against root-knot nematodes. Many resources of resistance have been discovered since 1944. Which resistance genes some of these plants contain is still not known. The preferred and safest method for controlling RKNs is in the discovery of new resistant plants. It is important to perform an extensive evaluation of tomato plants whose resistance has not been determined.

2.1. The Mechanism of Natural Resistance

Tomatoes, like all plants, undergo several modes for protection and immunity. The plant has an innate immune system that can recognize pathogen-associated molecular patterns. PAMP-triggered immunity (PTI) is the first defense line of response of the plant to pathogens. The extra cellular receptor proteins, receptor-like kinases (RLK), and receptor-like protein (RLP) are initiation factors and activators of the first defense line. The second defense line is triggered by intracellular proteins that contain a nucleotide-binding site (NBS), a toll-like interleukin receptor (TIR), which is not found in the *Mi-1* gene, and leucine-rich repeats (LRRs). During the second-line defense, there are two modes of pathogen interaction: direct and indirect.

The first pathway depends on a gene-for-gene interaction. In this mode, the receptor protein of tomato directly interacts with the nematode effectors. According to Flor's theory, the inheritance of both resistances in the tomato and the RKN's ability to cause disease are controlled by pairs of matching genes. The first gene, like the *Mi-1* gene, is in the tomato, and the other one is in RKNs and is called a virulence (Avr) gene. One of the responses of this type of defense is localized programmed cell death (PCD), one of the most important responses. This is a type of hypersensitive response (HR) (Figure 3). After the nematode enters the root of the plants; the nematode Avr genes produce effectors that trigger the production and the expression of plant *Mi*-resistant genes in an incompatible interaction. The result, because of this theory, is that no feeding site (giant cell) is formed. The second defense mode is not a direct gene-for-gene interaction, but an alternative mode called the guard hypothesis. The mechanism in this

theory consists of pathogen effectors that trigger the virulence factors/protein of the plant, which finally induces R-gene. In these cases, the virulence factor of nematodes (Avr genes) interacts with tomato accessory protein, resulting in some modification of this accessory protein, which allows for the recognition by plant NBS-LRR proteins that monitor for infection. The last result of this indirect interaction is the prevention of the production and growth of nematodes by the inhibition of the formation of feeding sites.

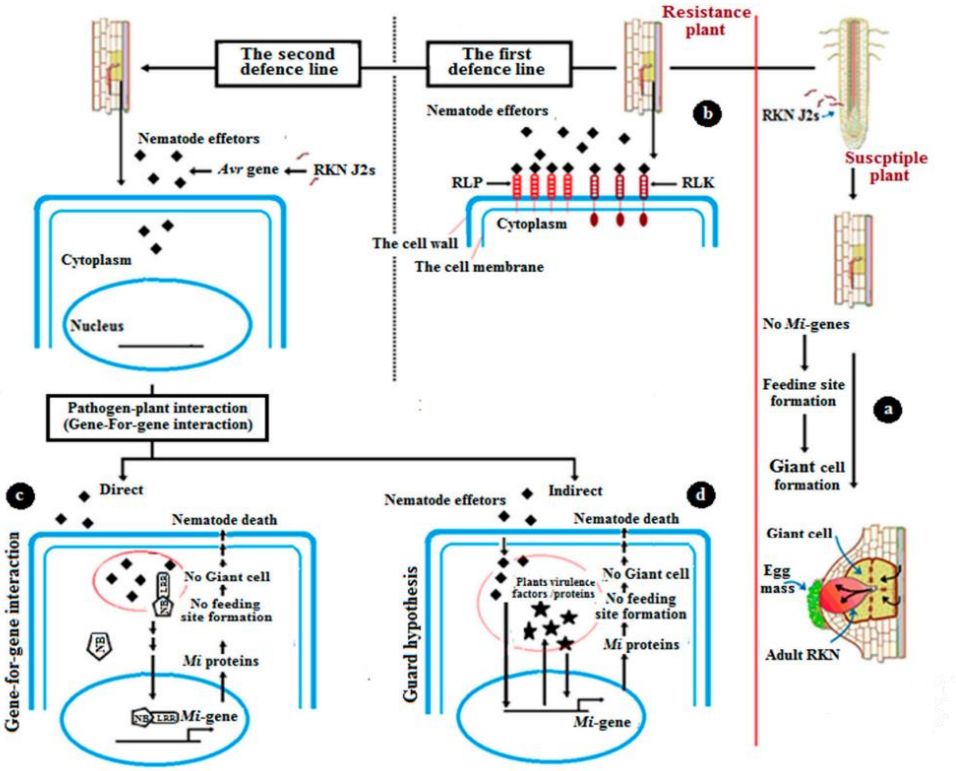

Figure 2. The mechanism of natural resistance against the root-knot nematode (RKN). (a) In susceptible plants, where there are no *Mi*-genes, the nematode completes its life cycle in the root by forming giant feeding cells. (b) In the resistance case, the plant undergoes the first defense line against RKN penetration by the interaction between extracellular receptor proteins, receptor-like kinases (RLK), receptor-like protein (RLP), and nematode effectors. (c) The plant then begins the second defense line, which includes direct gene-for-gene interaction. This theory depends on direct interaction between the receptor protein of tomatoes and nematode effectors, producing *Mi*-proteins, which prevent the nematode from feeding. No giant cell formation is observed. (d) The other second defense line is an indirect pathway, which is referred to as the guard hypothesis. In these cases, the virulence factor of the nematode (Avr genes) interacts with tomato accessory protein.

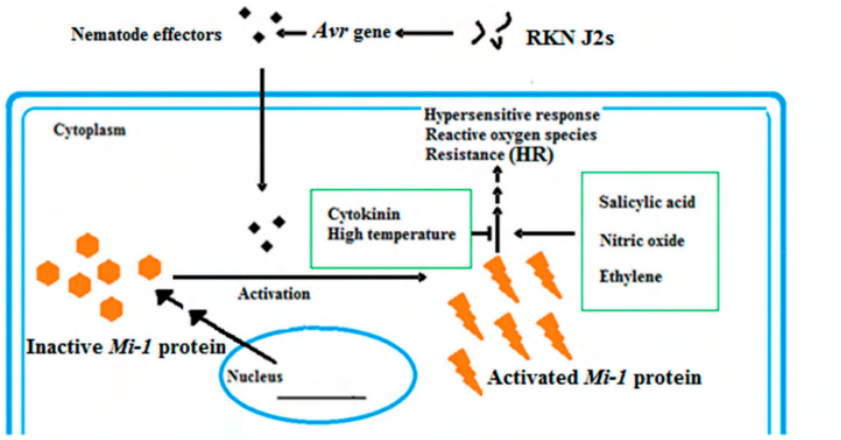

Figure 3. Hypersensitive response of *Mi-1* after nematode infection. The nematode Avr genes trigger the tomato *Mi-1* resistance gene(R-gene) to be active under the salicylic acid pathway with inhibition by both cytokinin and high temperature

3. Different Approaches to Strengthening Natural Resistance

3.1. Marker-Assisted Selectionin Breeding Programs

Marker-assisted selection (MAS) means the use of a binding pattern of linked molecular (DNA) markers for indirect selection in the desired plant phenotype. MAS is based on the concept that the presence of a marker that is tightly linked to the gene of interest indicates the presence of that gene.The improvement of new resistance plants has many benefits. The two most important benefits of using molecular breeding are first that it is less harmful to the environment than pesticides, and second that it is less expensive. Tomatoes are considered one of the most optimal plants for using molecular markers in commercial breeding. Moreover, molecular markers linked to the *Mi*-1 gene have enabled the rapid screening of resistance alleles, without requiring nematode inoculation. The use of molecular marker technologies in sync with new breeding techniques is promising for the a dvancement of tomato breeding.

3.1.1 Genetic Engineering in Controlling RKN

Although molecular breeding is the method that is most applied to achieve resistance against root-knot nematodes in tomato plants, genetic engineering is a future aspiration for further increases in resistance

3.1.2 Transfer Resistance Genes

This strategy is based on two foundations. The first is the transfer of a resistance gene from other plants to tomatoes. The second is the transfer of the *Mi* resistance gene from resistant varieties to susceptible one with high production qualities. Several resistance genes from different plants have been successfully transferred to tomatoes. These tomatoes transformed with new genes reduce diseases in transformed plants. Transgenic tomatoes with these genes would be novel sources for resistance against root-knot nematodes. Moreover, cloned Mi-1 is a good candidate for transfer to susceptible plants. There are more difficulties in understanding the mechanism of R-genes in other plants of the sames pecies or plants of another family. There have been many contradictions in previous studies in the case of other transformed solanaceous

plants with the *Mi-1* gene. Transgenic tomato plants showed reduced chitin content and retardation in embryogenesis in nematode eggs.

3.1.3 Resistance Effectors

Proteinase inhibitors (PIs) are one of the most promising methods for managing nematodes. Proteinase inhibitors are protein molecules secreted by pathogens, which inhibit the function of proteinases.Different types of proteinase have been identified in tomatoes.

Conclusions

Considerable potential has been developed in recent years for improving rootknot nematode resistance in tomato and other crops. The *Mi* gene of tomato has provided effective resistance to three root-knot nematode species for many years. The availability of a clone of *Mi* will allow introduction of this gene into selected varieties and possibly other crops, further expanding its use. However, *Mi* will not solve all root-knot nematode problems; it is not effective against all species or isolates of this nematode. In addition, the failure of *Mi* at high temperature can be a problem in the field. It is possible that in vitro modifications of the cloned gene will improve the range of nematodes controlled by *Mi*. For example, it may be that the partial resistance against *M. hapla* can be improved or the temperature sensitivity can be reduced by modifications in the structure, expression, or signal transduction of *Mi*. Other resistance genes into cultivated tomato using classical or marker-assisted breeding may also broaden the basis of root-knot nematode resistance. As technology advances, cloning of these genes directly from the wild species may be a faster route than conventional breeding for transferring the gene to elite cultivars or other species. However, even now there are virulent root-knot nematode isolates that can infect all currently identified sources of resistance. Continued searches of germplasm are needed to identify new sources of resistance. Artificially engineered resistance based on antisense technology or expression of anti-nematode proteins may be an additional source of resistance. Strategies to best use *Mi* and other genes to maximize their useful lifespans need to be developed. The gene Mi, which confers resistance to several species of root-knot nematode, is present in many modern tomato cultivars. Recent cloning of this gene revealed that it encodes a member of the plant resistance protein family characterized by the presence of a putative nucleotide binding site and a leucine-rich repeat. Although highly effective in many conditions, Mi fails to confer resistance at high soil temperature, and Mi-virulent nematode isolates have been identified in many areas of the world. These findings have stimulated efforts to identify new sources of root-knot nematode resistance. Resistance genes that differ from Mi in properties and genetic position have been identified in Lycopersicon peruvianum. These genes, as well as the cloned Mi gene, provide a resource for broadening the base of root-knot nematode resistance in tomato and other crops.Is pyramiding several resistance sources in selected elite cultivars the best solution or will it promote the spread of supervirulent nematodes? Getting a better understanding of nematode virulence is an important consideration for developing control strategies. As chemical control is reduced, the need for better understanding and implementation of host resistance and pathogen virulence will continue to increase.

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EUNIS HABITATS OF HIGH ANTHROPOGENIC IMPACT IN THE WATERSHED OF THE MIDDLE SECTION OF THE DEVOLL RIVER

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Abstract

The study aims to describe and map the habitat types of the middle section of Devoll River watershed, where human activity is very high and transformative, using EUNIS classification. Results show that 26.5% of the study area is covered by habitats of high human activity. Among them, semi natural habitats created as result of high and persistent human activity occupy 11% and are represented by E5.3(*Pteridium aquilinum fields*), G4 (*Mixed deciduous and coniferous woodland*), G1.7C2 (*Carpinus orientalis woods*). Artificial habitats such as agricultural areas and villages occupy 14% of the study area and are represented by J1 (*Buildings of cities, towns and villages)* and X25 (*Domestic gardens of villages and urban peripheries*). The habitat type C1.33 (*Rooted submerged vegetation of eutrophic water bodies*) represents aquatic habitats with rooted macrophytes of artificial ponds and lakes. Hydropower dams constructed in Moglicë village and Banjë turned a huge area of Devoll River flow into permanent lakes with high fluctuation of water level and with few pioneer and ephemeral vegetation in the lake shore. Habitats C1.2 (*Permanent mesotrophic lakes, ponds and pools*) and C3.5 (*Periodically inundated shores with pioneer and ephemeral vegetation*) are present in these man-made permanent lakes. Although human activity is high, the habitat G4 is very rich in plant species. Among them, many species are of conservation interest such as *Anacamptis pyramidalis, Juniperus oxycedrus, Hypericum perforatum, Orchis sp., Ophrys sp.,* etc.

Keywords: EUNIS habitats, human activity, watershed of Devoll River, plant species of conservation interest

INTRODUCTION

The watershed of the middle section of the Devoll River extends in an area of 1,017 km^2, which makes 3.5% of Albanian territory. It is located in the South-Eastern Albania and it includes territories from Elbasan, Gramsh, Korçë and Skrapar districts (Fig. 1). The area is characterized by a high range of altitudes (from 100 m to 2373 m above the sea level), complex topography, highly variable rock substrates, soil types, and complex hydrological system. The climate conditions are relatively continental and sub-alpine climate prevails but with Mediterranean influences in the lower altitudes (Kabo, 1990; 1991; Norconsult, 2010). These ecological conditions are reflected in a wide floristic diversity, vegetation and habitat types.

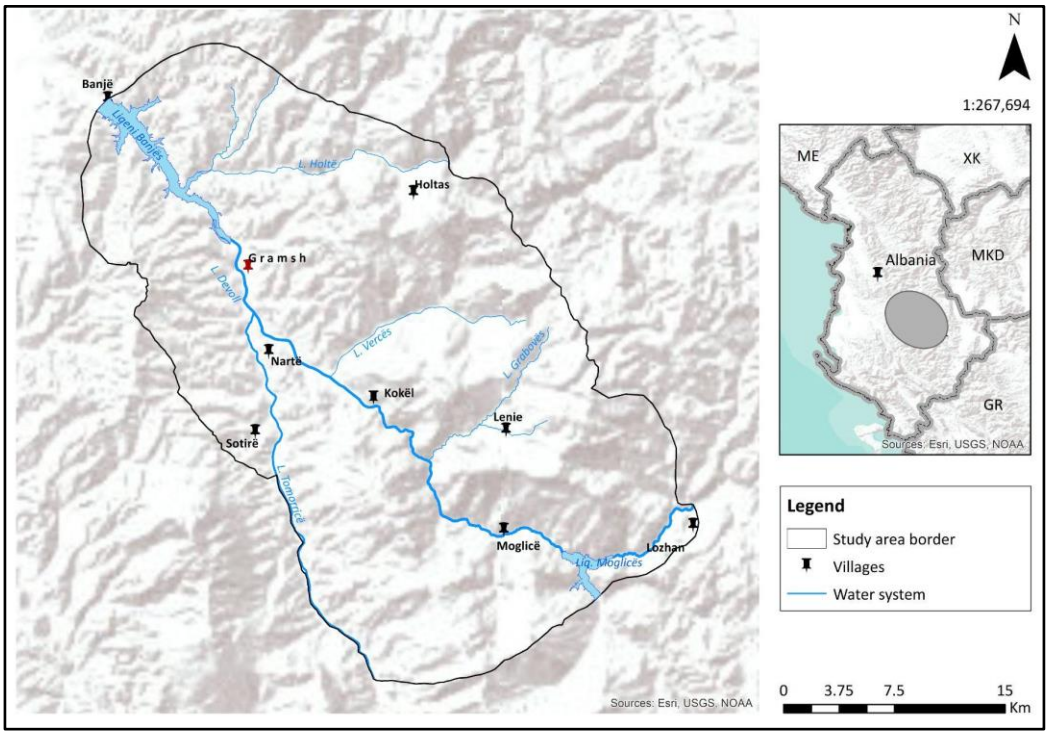

Figure 1. Geographic position of the study area

The first floristic data were published by Markgraf (1927; 1931) and Demiri (1959). Later studies and publications for the area have been mostly sporadic (Paparisto and Qosja, 1976; Gölz and Reinhard, 1984; Vangjeli et al., 2000, a; Barina and Pifkó, 2008; 2011; Shuka and Tan, 2009; Mullaj and Tan, 2010; Meyer, 2011; Mersinllari, 2008; 2014). Studies on the vegetation of this area are even less. Only a few data about the communities of sedge, ash, black pine and alpine pastures in Valamara mountain have been published by Vangjeli (1983), Mersinllari (1988), Hoda (1989), Buzo (1990) and Vangjeli et al. (2000, b). First comprehensive data on habitats according to EUNIS and Annex I of Habitats`Directive classifications were published on period 2016-2019 in the framework of Meço's PhD research thesis (Meço and Mullaj, 2016; Meço et al., 2017, a; et al., 2017, b; et al., 2018, etc).

Flora and vegetation of the area are a precious value for the local community, both as an economic resource and for better life quality. Fire woods, timber, pastures for livestock or medicinal plants are provided by natural resources.

Depending on the type of anthropogenic impact, its intensity and duration, many habitats have lost their naturality completely or partially. Following, new artificial habitats and ecosystems have been created such as agricultural areas, urban areas, industrial areas, infrastructural constructions, artificial lakes etc. Their expansiveness is huge but still they are important for hosting biodiversity values. Therefore, this study is focused on floristic description and mapping of semi-natural and manmade habitats.

MATERIAL AND METHODS

17 field trips were undertaken during 2016 - 2022 as a combination of floristic and phytosociological trips to collect field data. Identification of semi-natural and artificial habitats in the watershed of the middle section of the Devoll River were based on identifying diagnostic species and by comparing the habitat features with habitat description of EUNIS calcification (Davies et. al., 2004). For semi-natural habitats, few phytosociological relevés (Br-Bl, 1964) were also carried out mainly in Carpinus orientalis formations and mixed deciduous forest.

1000 sampling points were recorded as georeferenced data (GPS Garmin) and all of them were accompanied with ecological data and information on floristic composition and vegetation. The plant identification was done according to Flora Europaea (Tutin et al., 1968; et al., 1980; et al., 1993) and Flora of Albania (Paparisto et al., 1988; Qosja et al., 1992; et al.; et al., 1996; Vangjeli, 2015).

Habitat maps were designed in ArcMap 10.1 in WGS 1984. (Environmental Systems Research Institute, New York, NY, USA). Shapefiles were built by interpreting field data and aerial photographs. The main attribute table was created with data such as: coordinates and the main vegetation communities. It was used as layers for geospatial information the orthophotos, which were taken from ASIG (asig.gov.al/english/index.php) and large scale vegetation maps (Corine Land Cover map) (Devillers et al. 1991).

Since the aerial photos are insufficient to separate and delimit all habitat types, repeated field surveys were undertaken, by making new transects. Subsequent field work missions were undertaken as a quality control measure to verify map information.

RESULTS AND DISCUSSION

The geographical position of watershed of the middle section of the Devoll River, the amplitude and the high relief diversity, the rock substrate, the soil types, the climatic and hydrological conditions, have brought in a very rich flora. The species composition depending on the similar requirements to the environmental conditions form a variety of plant associations and habitat types. The presence of the anthropogenic impact is present almost everywhere, and it is very high in a surface of about 35,976 ha or 26.5% of the study area, changing the natural habitats and creating semi-natural or completely artificial habitats such as wood cutting, overgrazed pastures, mixed deciduous forest and pine plantations, agricultural lands, water bodies and lakes, etc.

8 EUNIS habitats of high human impacts are identified and described:

C1.2-*Permanent mesotrophic lakes, ponds and pools*

The Devoll River flow is highly impacted by dam construction in this section. Two large dams, Banja and Moglica, were built, which created two lakes, where large areas were flooded. The most affected are the habitats listed in Annex I of the Habitats Directive, such as: 92D0 (124.91ha); 9340 (107.48 ha); 5110 (83.79 ha); 92A0 (81.5 ha); 5210 (79.86 ha); 9250 (71.82 ha); 3270 (43.69 ha); 9530 (14.47 ha); 9540 (9.45 ha); 6210 (2.74 ha); 9280 (0.39 ha); 92C0 (0.18 ha), reported by Meço and Mullaj (2016) and Meço et al. (2018).

The lakes of Banja and Moglicë have flooded many ~~agricultural~~ lands on both sides of the river, as well. There are 438.04 ha of abandoned arable land and 397.07 of regularly or recently cultivated agricultural, horticultural and domestic habitats, to be flooded.

These two water reservoirs represent permanent lakes with high amplitude fluctuation. Generally, water quality of the basin is good but is polluted by urban wastewaters from Gramsh, Maliq, Korçë and Bilisht (Norconsult, 2010). Due to high water fluctuation and the young age of the basin, the riparian vegetation and rooted macrophytes are not installed. Only, some green algae and pioneer plant species live in the shallow water near the lakeshore and on muddy substrate above the water level in summer.

C1.33-*Rooted submerged vegetation of eutrophic water bodies*

This habitat represents all the artificial water bodies of the study area, with perennial macrophytes rooted in the shallow bottoms of these reservoirs or water basins. The dominant

species belong to the genus *Potamogeton*, alliances Potamion lucentis Vollmar 1947 and Potamion pusilli Wiegleb 1982. Other frequent species are *Myriophyllum spicatum* and *M. verticillatum*. Often, during the summer these aquatic environments dry up completely.

In this habitat type, we have included all the small reservoirs, ponds and artificial fields built by humans, such as Posnovisht Reservoir, Bratila Reservoir and some ponds created to provide water for livestock during the summer period. The total surface of this habitat type in the study area is around 14.7 ha.

C3.5-*Periodically inundated shores with pioneer and ephemeral vegetation*

This habitat type occurs mostly in shallow waters of Banjë artificial lake, close to Trashovica bridge, where the river flow joins the lake. In summer, lake level drops and the alluvial substrate deposited by Devoll River comes to surface. When these alluvions start to dry up, some annual species start growing such are *Bidens tripartita, Persicaria hydropiper, P. lapathifolium, Rumex conglomeratus, Xanthium strumarium, Equisetum telmateia, Cyperus glomeratus, C. fuscus, Echinochloa crus-galli*, etc. The plant composition of this habitat type is very similar to habitat 3270 (*Rivers with muddy banks with Chenopodion rubri pp and Bidention pp vegetation*) of annex 1 of Habitats directive, reported by Meço and Mullaj (2016) on the muddy river bank of Devoll River, partly flooded already by the artificial lakes of hydropowers.

E5.3-*Pteridium aquilinum fields*

The habitat represents the last stage of degradation of forest formations, forming open areas dominated by *Pteridium aquilinum*. The *P. aquilinum* fields appear as a result of massive cutting of trees, followed by burning of the area that clears completely the vegetation from the soil surface and the first heavy rains wash it down from the nutrients. These surfaces, pure in nutrients already, are populated by *P. aquilinum*, which often forms very dense fields, characterized by low floristic diversity. The most common accompanying species are *Fragaria vesca, Brachypodium pinnatum, B. sylvaticum, Viola odorata, Dactylis glomerata, Scilla autumnalis,* etc.

G1.7C2-*Carpinus orientalis woods*

This habitat occurs between 400m and 900m above sea level, on different substrate types, exposition and slopes. *Carpinus orientalis* forests and scrub formations extend widely, but mostly in the northwest part of the study area, on hills along both sides of the old Elbasan-Gramsh road to the Kaçivel village and on hills of Sotirë village. The formations with *C. orientalis* represent a degradation stage of mixed broadleaf oak forest dominated by *Quercus cerris*, often with the presence of *Quercus pubescens* and *Quercus frainetto*. The total surface of this habitat is about 10188.36 ha that makes 10% of the study area or 45.8% of the total oak phytoclimatic layer in that area. As result of intensive cutting, grazing and fires 1685 ha of *C. orientalis* formations are very degraded stage.

The hornbeam formations are dominated by *Carpinus orientalis* cover 70%, but with a high presence of: *Acer platanoides, Colutea arborescens, Cornus mas, Coronilla emerus, Cotinus coggygria, Crataegus monogyna, Fraxinus ornus, Juniperus oxycedrus, Phillyrea angustifolia, Quercus pubescens, Rubus ulmifolius, Acer monspessulanum, Paliurus spina-christi, Cercis siliquastrum, Cornus sanguinea, Hedera helix, Tamus communis, Teucrium polium, Buxus sempervirens*, etc. Among the herbaceous plants there are: *Primula vulgaris, Anemone apennina, Asparagus acutifolius, Brachypodium sylvaticum, Buglossoides purpurocaerulea, Clinopodium vulgare, Cnidium silaifolium, Cynosurus echinatus, Helleborus odorus, Lathyrus niger, Luzula forsteri, Melica ciliata, Potentilla micrantha, Prunella vulgare, Ruscus aculeatus, Satureja montana, Symphytum bulbosum, Thymus longicaulis, Vicia*

grandiflora, etc. In dense formations, the herbaceous layer is often very poor and with a high presence of mosses.

These formations don't have a clear physionomy and are generally characterized by a low floristic diversity, and high presence of species typical for disturbed areas. They are found almost all over the territory of Albania, often forming dense forests with a height of around 3 m and about 20 years old.

G4-*Mixed deciduous and coniferous woodland*

Mixed deciduous forests with coniferous (*Pinus nigra*) are found fragmented at altitudes ranging from 350m to 1200m above sea level, from the hills above Bulçarë village close to Dushku lake. The total surface of this habitat is 771.2 ha or 0.7% of the study area. The most important floristic elements of these forests are *Pinus nigra* mixed with *Quercus cerris, Q. frainetto, Q. pubescens*. Very frequent are also: *Quercus trojana, Carpinus orientalis, Fraxinus ornus, Ostrya carpinifolia, Juniperus oxycedrus, Acer obtusatum, Coronilla emeroides, Cotinus coggygria, Brachypodium pinnatum, B. sylvaticum, Aremone agrimonoides, Digitalis laevigata, Crataegus monogyna, Campanula trachelium, Clematis vitalba, Rubus idaeus, Euphorbia spinosa, Scrophularia canina, Alyssum murale, Dictamnus albus, Inula spiraeifolia, Helleborus odorus, Acer monspessulanum, Pteridium aqualinum, Luzula forsteri, Campanula persicifolia, Myosotis sylvatica, Viola odorata, Dactylis glomerata, Primula veris, Poa bulbosa, P. nemoralis, Veronica chamaedrys, Euphorbia amygdaloides,* etc. Most of these forests have been overexploited as a result of cutting, overgrazing, coppices of new oak branches for livestock, etc.

This habitat type hosts many species with conservation interest. In open woodland or along the forest adge there are found many orchid species such are *Anacamptis pyramidalis, Listera ovata, Neottia nidus-avis, Ophrys apifera, O. scolopax, O. sphegodes, Orchis coriophora, O. mascula, O. morio, O. tridentata,* ect. *Juniperus oxycedrus, J. communis* and *Hypericum perforatum* that have conservation status VU according to Albanian Red List (2013) and LC according to IUCN (2016), are also frequent in this habitat type.

J1-*Buildings of cities, towns and villages*

Rural and urban areas populate the watershed of the middle section of the Devoll River, from the lowest parts along the Devoll River to about 1500m above sea level. The Gramsh town is the largest urban area. The vegetation around these settlements and roads side forms patches dominated by nitrophilous and ruderal species such as: *Urtica dioica, U. urens, Sambucus ebulus, Chenopodium album, Cirsium vulgare. Sonchus oleraceus. Marrubium vulgare, M. peregrinum, Solanum nigrum, Datura stramonium, Portulaca oleracea, Ballota nigra, Parietaria officinalis, Heliotropium europaeum*, etc. Often the vegetation of these disturbed area is dominated by invasive alien species such as: *Robinia pseudacacia, Dittrichia viscosa, Conyza canadensis, Aster squamatus, Ailanthus altissima*, etc. and frequently medicinal plant species are found such as *Tussilago farfara* and *Hypericum perforatum*.

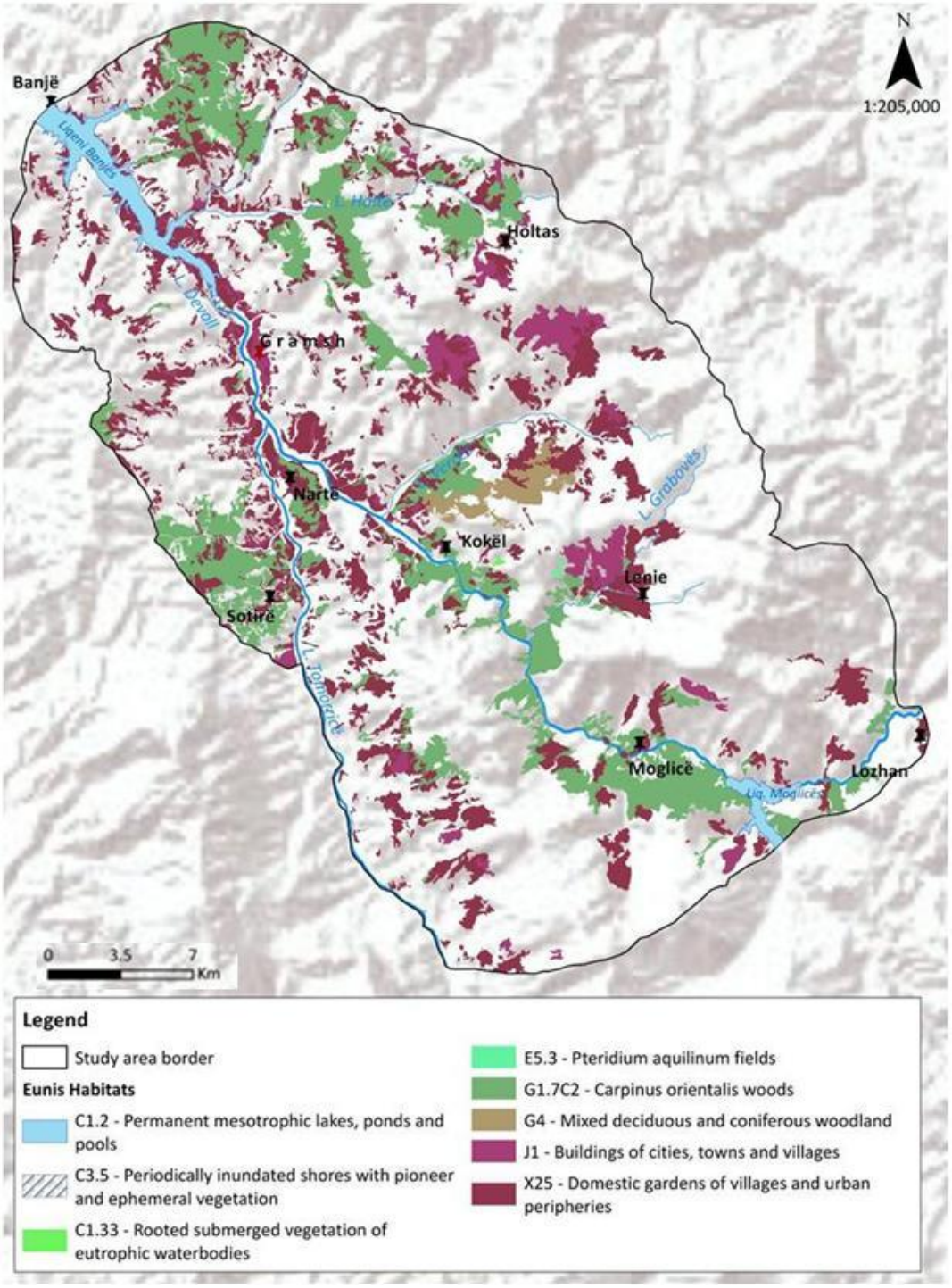

Figure 2. The habitat map of high human impact in the watershed of the middle section of the Devoll River

X25-*Domestic gardens of villages and urban peripheries*

Agricultural gardens of villages and urban peripheries in the study area are widespread in soils that range from shallow and low calcium content (in the upper part of the watershed) to deep soils with higher nutrient content that are formed by alluvial deposits along the river course. The most important agricultural crops of agricultural gardens are: corn, beans, barley, alfalfa, but also by fruit trees (apples, olives, plums, etc.), vineyards and medicinal plants (lavender, sage, cyan, etc.).

The spontaneous flora of these agricultural lands is represented by weeds such as: *Agrostemma githago, Ranunculus arvensis, Papaver rhoeas, Legousia speculum-veneris, Capsella bursa-pastoris,* etc., which are more present in winter crops. Nitrophilous plants such as: *Polygonum aviculare, Amaranthus retroflexus, Chamomilla recutita* etc. are more present in spring crops. A high presence of invasive alien species such as *Dittrichia viscosa, Conyza canadensis* and *Aster squamatus* are often present.

CONCLUSIONS

Due to anthropogenic activities, about 26.5% of the territory of the middle section of Devoll River watershed has lost its naturalness but is still hosting important floristic values in semi-natural and artificial habitats. *Anacamptis pyramidalis, Listera ovata, Neottia nidus-avis, Ophrys apifera, O. scolopax, O. sphegodes, Orchis coriophora, O. mascula, O. morio, O. tridentata, Juniperus oxycedrus, J. communis*, etc., are some of the species of high conservation interest.

Among the described habitat types, semi natural habitats such as E5.3 (*Pteridium aquilinum fields*), G4 (*Mixed deciduous and coniferous woodland*) and G1.7C2 (*Carpinus orientalis woods*) cover 11% of the study area while, the artificial habitats C1.2 (*Permanent mesotrophic lakes, ponds and pools*), J1 (*Buildings of cities, towns and villages*) and X25 (*Domestic gardens of villages and urban peripheries*) cover 15%.

Recent interventions such as the creation of water reservoirs from operation of Devoll hydropower plant inundated 12 habitats of Annex I of the Habitats Directive of the area reported by Meço and Mullaj (2016) and Meço et al. (2018) with a total surface of 620.3 ha.

The major concern for the area, its naturalness, diversity and conservation is the intense and high human activities resulting in a tendency of future man made landscape and complete loss of biodiversity and habitats. For this not to happen, important information should be published and communicated to decision makers and a wide range of stakeholders to strongly and seriously consider conservation measures, nature restoration and management.

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ANTIBIOTIC SUSCEPTIBILITY OF E. COLI AGENT ISOLATED FROM CALF DIARRHEA AND MASTITIS CASES IN SOME REGIONS OF TURKEY

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ABSTRACT

Mastitis affects dairy cattle often and has a significant economic impact. In addition, One of the most prevalent illnesses affecting calves is diarrhea, which costs livestock producers significantly less in terms of economic production. *Escherichia coli* (*E. coli*) is one of the leading infectious agents associated with mastitis and calf diarrhoea. Intimin protein in *E. coli* is encoded by the eae gene and is required to disrupt the host cell's cytoskeleton and facilitate bacterial attachment. For this purpose, the causative agent was confirmed in PCR with the intimine gene region primers. The widespread use of antibiotics on dairy farms is still one of the main causes of the development of bacteria that are resistant to antibiotics.

This study aims to determine the antimicrobial resistance patterns of *Escherichia coli* isolates isolated from bovine mastitis and diarrhea in Aksaray and Van provinces between 2018 and 2022 and to determine the prominent antimicrobial resistance among the isolated strains. For this purpose, antimicrobial susceptibility testing against seven antibiotic groups, including Penicillin, Florfenicol, Azithromycin, Novobiocin, Ciprofloxacin, Amoxicillin/Clavulanic acid and Neomycin, was performed using the disc diffusion method. The study used 178 calf faeces with diarrhea and 135 milk with mastitis, and 32 isolates were obtained. These isolates were subjected to antibiogram testing after confirmation with intimin-K99 primers. While the study's results were resistant to Penicillin, Novobicin and Amoxicillin/Clavulanic acid antibiotics for all isolates, Florfenicol and Neomycin are the most sensitive. While some isolates are susceptible to other antibiotics, some are resistant. As a result, both the detection of virulence factors of the agent and antibiotic resistance should be considered in the fight against mastitis and calf diarrhea, and antibiotics should be administered according to the antibiogram results to prevent the development of resistance.

Keywords: Diarrhea, mastitis, antibiotics, disc diffusion

INTRODUCTION

E. coli, a facultative anaerobic, flagellate, rod-shaped bacterium, belongs to the Enterobacteriaceae family. While the causative agent occurs as flora bacteria in the healthy gastrointestinal tract of humans and ruminants, including milk-producing animals, some can cause disease. One of the most prevalent Gram-negative bacteria associated with diarrhea and mastitis is *E. coli.* (Hasson et al. 2022, My et al. 2023). The inflammation of the breast tissues shapes the case of mastitis. This disease can be seen in many mammalian species, such as domestic dairy cattle (Gomes and Henriques 2016). Mastitis is a disease that affects fertile animals in herds and decreases milk yield. The use of serious antibiotics to treat the disease

causes deaths several times a year and thus causes severe economic losses (Zhao and Lacasse 2008, Stevens et al. 2016, My et al. 2023).

E. coli agents use different virulence factors to infect the host. These virulence factors include colicins, hemolysins, proteases, toxins, fimbria-like adhesion and cell surface (Hasson et al. 2022). There is a need to define virulence characteristics in differentiating normal flora bacteria and pathogenic strains (Güler et al. 2008). Intimin protein in *E. coli* is encoded by the eae gene and is required to disrupt the host cell's cytoskeleton and facilitate bacterial attachment (Suleiman et al., 2020).

As in many bacteria, the multi-drug resistance (MDR) issue in *E. coli* is very current and accepted as a public health threat worldwide. For the factor, livestock such as cattle are included as known reservoirs (Bandyopadhyay et al. 2021). Proper use of antibiotics requires routine antimicrobial susceptibility tests and screening of emerging MRD strains to determine the dose and preferred antibiotic (Hetta et al. 2021, Kareem et al. 2021). There is limited information on antimicrobial resistance profiles for the pathogen in Turkey.

In this study, antimicrobial resistance patterns of Escherichia coli isolates from bovine mastitis and diarrhea in the provinces of Aksaray and Van between 2018 and 2022 were examined, as well as the predominant antimicrobial resistance among the isolated strains.

MATERIAL AND METHOD

Sample collection

Between 2018 and 2022, 178 calf faeces with diarrhea and 135 milk with mastitis were collected from Aksaray and Van provinces, and the samples were transferred to the laboratory at four °C within 3-4 hours.

Bacterial culture and identification

After the milk samples with mastitis and stool samples were delivered to the laboratory, 0.1 mL of the samples were taken for the first isolation and inoculated into 5% sheep blood agar and MacConkey agar. After incubation, the isolates were inoculated into EMB agar as a subculture, and those with a metallic blue-green color were defined as phenotypic. Biochemical tests were performed with Gram stain, catalase, oxidase, and triple sugar iron agar (Momtaz et al. 201A 3). A PCR test was performed to confirm the isolates.

Antimicrobial sensitivity testing

According to previously described techniques, antimicrobial susceptibility testing was carried out using the Kirby Bauer disk diffusion method on Mueller-Hinton agar (Sigma-Aldrich, USA) (Patel et al. 2015). Antimicrobial susceptibility testing against seven antibiotic groups, including tested antimicrobial agents, Penicillin, Florfenicol, Azithromycin, Novobiocin, Ciprofloxacin, Amoxicillin/Clavulanic acid and Neomycin, was performed using the disc diffusion method. After preparation on Mueller-Hilton agar (Sigma-Aldrich, USA) to McFarland 0.5 standard, springs were placed on plates and incubated at 37°C for 24 hours. Sensitivity measurements were determined as sensitive (3), moderate (2), low (1) and resistant (-) according to the diameter of the inhibition zone.

DNA extraction and PCR amplification

Following the manufacturer's recommendations, DNA was extracted from each E. coli isolate using the Qiagen DNeasy Blood and Tissue Kit (Life Technologies, USA) and stored at -20°C until further use. For amplification in each PCR tube content, a total of 25 µl of primers were prepared using a total of 1 µM, four µl of Master Mix, 9.8 µl of sterile ultrapure water and five µl of DNA. This mixture was amplified in a heat cycler with a 35-cycle reaction, each cycle consisting of 1 minute at 94°C, 1 minute at 58°C, and 30 seconds at 72°C after 5 minutes of pre-denaturation at 95°C. As a final extension, after the DNAs were kept at 72 °C for 5 minutes, 1.5% agarose gel containing 5 µg/ml ethidium bromide was prepared and subjected to electrophoresis for imaging. By using 100 bp DNA ladder as marker, bands of approximately 424 to 314 bp in size of intimin primers (F 5- ATA TCC GTT TTA ATG GCT ATC T -3 ve R 5- AAT CTT CTG CGT ACT GTG TTC A -3)- K99 (F 5- TAT TAT CTT AGG TGG TAT GG -3 ve R 5- GGT ATC CTT TAG CAG CAG TAT TTC -3), respectively, were evaluated as positive (Güler and Gündüz 2007).

RESULTS AND DISCUSSION

As a result of the study, Thirty-two isolates were isolated from 313 samples, 178 from calf faeces and 1faecesom milk with mastitis. These isolates were verified by PCR according to the primer sequences of the aeg gene region of the intimin protein, and the results are given in **Figure 1** with gel images.

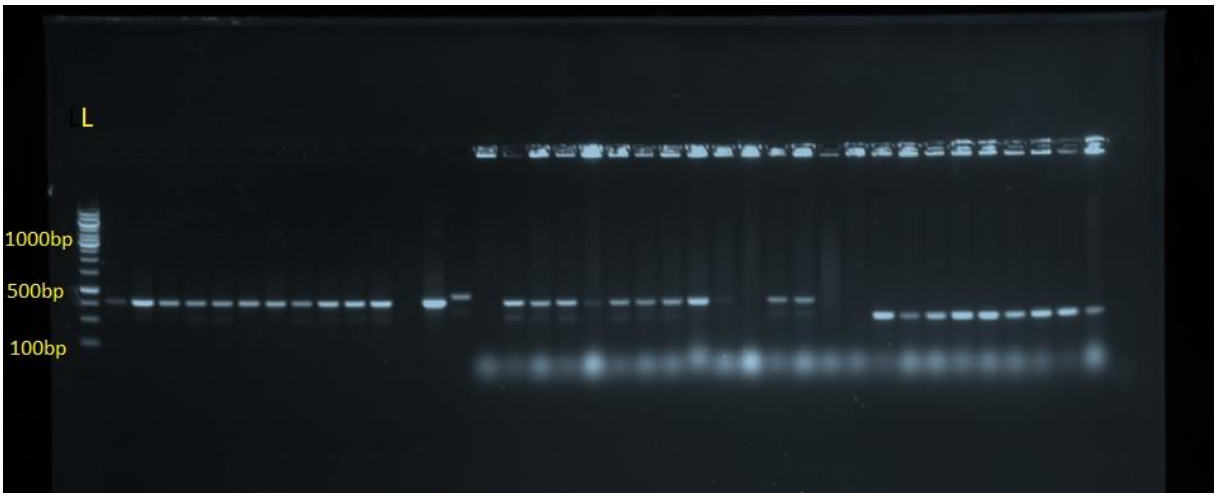

Figure 1. PCR images of 32 E. coli isolates isolated from calf diarrhea and mastitis (L: Leader)

To determine the sensitivity of antibiotics from the isolated *E. coli* agent, Antibiogram Test was performed on 32 isolates obtained in the study, and the results are given in **Table 1**. There are many studies on the virulence characteristics of *E. coli* from calf samples with mastitis and diarrhea (Bag et al. 2021, Dubreuil et al. 2016). It has been reported that K99 fimbria in calves in Turkey was detected in 30.2%, 35% and 16% of calves, respectively, in previous studies (Erganis¸ et al., 1988, Uysal et al., 1992, Güler et al. 2008). This study determined it as positive in 11.23% of calf diarrhea. It has been reported that intiminin, one of the virulence factors, can be isolated from bovine faeces anfaeceslthy calves (Mainil and Daube, 2005).

Table 1. 32 E. coli isolates from calf diarrhea and mastitis were tested for antibiotic susceptibility.

	Isolates	Penicillin	Florfenicol	Amok/clou	Azithromycin	Novobiocin	Ciprofloks	Neomycin
1	***76***	---	3	---	---	---	1	1
2	***80***	---	3	---	---	---	---	1
3	***108***	---	2	---	1	---	3	1
4	***112***	---	3	---	1	---	3	2
5	***114***	---	3	---	---	---	1	2
6	***115***	---	2	---	---	---	1	1
7	***116***	---	3	---	---	---	1	1
8	***117***	---	3	---	---	---	---	2
9	***118***	---	3	---	1	---	3	2
10	***290***	---	3	---	---	---	---	2
11	***314***	---	3	---	---	---	2	1
12	***319***	---	2	---	---	---	---	1
13	***474***	---	3	---	1	---	1	1
14	***475***	---	2	---	---	---	1	2
15	***476***	---	2	---	1	---	2	1
16	***477***	---	3	---	1	---	---	1
17	***478***	---	3	---	1	---	2	2
18	***481***	---	2	---	1	---	1	1
19	***484***	---	3	---	---	---	1	1
20	***485***	---	3	---	1	---	2	2
21	***486***	---	3	---	1	---	1	1
22	***487***	---	3	---	---	---	3	1
23	***490***	---	2	---	---	---	3	1
24	***491***	---	3	---	---	---	3	1
25	***492***	---	3	---	---	---	---	2
26	***493***	---	3	---	---	---	2	1
27	***494***	---	3	---	1	---	---	1
28	***502***	---	2	---	---	---	1	1
29	***503***	---	3	---	1	---	2	2
30	***509***	---	3	---	---	---	3	2
31	***510***	---	3	---	1	---	3	2
32	***511***	---	2	---	---	---	---	1

When the antibiogram results were evaluated, Florfenicol was considered sensitive for all isolates and even the most sensitive for most of them. It can be regarded as sensitive antibiotic preparation, along with neomycin and ciprofloxacin in some isolates. Florfenicol has been used to treat bovine respiratory pathogens in Turkey since 1998. In previous studies, Orden reported the rate of florfenicol resistance in E. coli calves as 1%, Hariharan 11%, Werckenthin 35%, White 92%, Güler 9.3% (Güler et al. 2008). In this study, florfenicol resistance was the highest (100%).

In this study, it was determined that it was resistant to amoxicillin-clavulanic acid antibiotics. Previous studies determined amoxicillin-clavulanic acid resistance in 35%

(Werckenthin et al. 2002) and 12% (Güler et al. 2008), respectively. In addition, all isolates were found to be resistant to Penicillin and Novobiocin antibiotic plaques.

It was found that quinolone sensitivity was higher in strains that produced certain virulence factors, such as K99, eae, and necrotoxin, compared to strains that did not produce these factors (Orden et al. 1999). In another study, multidrug-resistant E. coli isolates were found to be less virulent compared to susceptible ones (Johnson et al. 2004). A definite relationship between antibiotic resistance and virulence has yet to be determined. Many studies also determined multi-resistance in *E. coli* isolated from healthy animals. This shows that microorganisms are resistant to antibiotics in humans with pathogenic strains and that more antibiotics may be exposed (Madoshi et al. 2016). The relationship between antibiotic resistance and virulence may vary depending on antibiotics and virulence factors, and more research is needed accordingly.

CONCLUSIONS

By using primers for the intimin-K99 virulence genes of E. coli isolates recovered from bovine mastitis and calf diarrhea, PCR testing was used to corroborate the study's findings. Later, due to antibiotic susceptibility tests, florfenicol was found to be quite sensitive. The studies examined show multi-antibiotic resistance in E. coli isolates from healthy and infected animals. Regarding public health, it is clear that antibiotic susceptibility tests are essential to reduce unessentialtibiotic use and for accurate diagnosis. In addition, studies between antimicrobial agents and virulence factors need to be developed and done more.

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ANTIMICROBIAL ACTIVITY OF NEW CREAM FORMULATIONS: Hylocereus polyrhizus AQUEOUS EXTRACTS AND *Limosilactobacillus fermentum* MA-7

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ABSTRACT

Knowing the harmful side effects of chemical additives in commercial creams has increased the demand for herbal creams today. Our skin is constantly exposed to physical and chemical threats. *Hylocereus polyrhizus* is known as a tropical fruit with rich bioactive compounds that benefit in many areas. The topical application of probiotics can help protect the skin from a variety of infections. In our study, it was aimed to develop new cream formulations containing *H. polyrhizus* aqueous peel or fruit extracts and probiotic strain *Limosilactobacillus fermentum* MA-7 for topical applications and then to determine the biological activity of these cream formulations. For this purpose, *H. polyrhizus* peel and fruit obtained from Turkey were extracted by sonication method using aqueous solvent. The biological activity of the developed new cream formulations was determined against the test microorganisms by the well diffusion method. The results showed that the cream (control) group did not have a zone of inhibition against all the tested microorganisms. It was determined that the addition of *H. polyrhizus* aqueous extract and *L. fermentum* MA-7 to the cream group increased the antimicrobial activity. The highest inhibition zone diameter of the Cream-Extract-*L. fermentum* MA-7 (CEL) group containing peel extract was determined as 18.68 mm against *S. enteritidis* RSKK 171. The highest inhibition zone diameter of the CEL group containing fruit extract was determined as 17.01 mm against *E. coli* ATCC O157:H7. The results show that the cream formulation containing *H. polyrhizus* aqueous extracts and *L. fermentum* MA-7 can be used as a protective and therapeutic natural agent against some pathogens that cause contamination in our body.

Keywords: Red pitahaya, Cream formulation, Yeast, Bacteria, Pathogens

INTRODUCTION

Antimicrobial substances are synthetic or natural substances that stop the growth or kill unwanted harmful microorganisms (fungus, bacteria, and algae etc.) (Ordu et al., 2018; Ofokansi et al., 2013). Bioactive compounds obtained from plants inhibit the growth of pathogenic microorganisms by mechanisms different from antibiotics and have clinical value in the treatment of diseases resulting from antibiotic-resistant microorganisms (Shankar et al., 2010). Recently, *Hylocereus polyrhizus* is one of the tropical fruit species that has gained popularity and is produced in the Mediterranean Region in Turkey. The popularity of *H. polyrhizus*, which draws attention with its taste and color, is increasing in Turkey (Uğuz and Gezici, 2021; Attar et al., 2022). It is also rich in phyto-albumins, phenolics, betacyanins, and flavonoids, which are extremely valuable for antioxidant potential (Jaafar et al., 2009).

The skin, the largest organ in the human body, acts as a barrier against external factors, including physical, chemical, and bacterial threats (Lolou and Panayiotidis, 2019). Topical

applications of probiotic bacteria have the potential to strengthen the skin's natural defense barriers. There is limited information on the efficacy of topically applied probiotics. The supplements containing probiotics or prebiotics are known to have a positive effect on the skin (Al-Ghazzewi et al., 2014).

The cosmetic products contain organic and inorganic compounds that promote the growth of pathogenic microorganisms that may pose a danger to consumers. Therefore, natural antimicrobial agents are used to increase the durability and safety of cosmetics (Neza and Centini, 2016). Plants and microorganisms provide a wide range of active ingredients acceptable in the cosmetic industry, which can find different applications in the manufacture of cosmetics and personal care products. For example, it can be applied in areas such as the manufacture of creams, protection against UV rays and pollution, production of essence or alleviating the effects of skin aging. (Mahesh et al., 2019; Kentin and Kaarto, 2018; Henkler et al., 2012). The increase in demand for products with natural components in recent years has made consumers aware of the negative effects of using synthetic preservatives (Guzmán and Lucia, 2021).

The purpose of the present study is to investigate alternative uses of cream formulations containing *H. polyrhizus* extract and *Limosilactobacillus fermentum* MA-7 in the pharmaceutical and cosmetic industry.

MATERIAL AND METHOD

Preparation of *Hylocereus Polyrhizus* Peel and Fruit Aqueous Extracts

H. Polyrhizus was purchased in October 2021 from the production greenhouse in Antalya-Turkey. Each fruit supplied weighed ±200-300gr. After the samples were washed, their skins were removed from the fruit and dried at room temperature. The powdered samples were extracted separately with aqueous in 2 repetitions of 10 minutes every day (2 days) using a sonication (Hielscher). *H. Polyrhizus* peel and fruit extracts dissolved with Dimethyl sulfoxide (DMSO) were sterilized with sterile filters (0.22µm).

Microorganisms Suspensions

Candida albicans ATCC 10231 was cultured at 30ºC for 24 hours in Yeast Peptone Dextrose (YPD). *Enterococcus faecalis* ATCC 29212 was cultured at at 37ºC for 24 hours in Tryptic Soy Broth (TSB). *Escherichia coli* O157:H7, and *Salmonella enteritidis* RSKK 171 were grown in Nutrient Broth (NB) for 24 hours.

Preparation of *Limosilactobacillus fermentum* MA-7 for cream formulation

Limosilactobacillus. fermentum MA-7 microorganism was cultured in Man, Rogosa and Sharpe (MRS) medium at 37ºC for 18 hours. The optical density was adjusted to 1.6 at the end of the incubation. Then they were sonication on ice for 15 minutes to use the bacterial lysates.

Antibacterial or Antifungal Activity of Cream Formulation Containing *Hylocereus Polyrhizus* Aqueous Extract and *Limosilactobacillus fermentum* MA-7

The antibacterial or antifungal activity of the cream formulations was determined using the modified method used in our previous study (Asan-Ozusaglam and Celik, 2023). In the prepared antimicrobial cream formulation, a mercantile cream, *H. polyrhizus* peel or fruit aqueous extracts and *L. fermentum* MA-7 isolated from human milk were used. The antibacterial or antifungal activity of the prepared cream groups was determined against the microorganism strains using the well diffusion method. The experiment was performed in triplicate. The culture dishes were incubated under conditions suitable for the test microorganisms.

RESULTS AND DISCUSSION

The biological activity of the cream formulation containing *H. polyrhizus* peel extract is presented in Table 1. The probiotics (Lactic acid bacteria) capable of protecting the skin repair skin damage, UV radiation, prevent age-related skin manifestations, increase skin radiance, and

delay skin aging (Cinque et al., 2011; Htwe et al., 2019). Inhibition zone diameter against test microorganisms was not detected in the control group (C). Inhibition zone diameter was determined against all test microorganisms except *E. faecalis* ATCC 29212 in CE group. The highest Inhibition zone diameter was measured as 18.68 mm in the CEL group against *S. enteritidis* RSKK 171. The inhibition zone diameters of the CEL group against *C. albicans* ATCC 10231 and *E. coli* ATCC O157:H7 were determined as 15.72 mm and 15.66 mm. CEL group (2.17 mm) in *E. faecalis* ATCC 29212 was statistically significant compared to all test groups ($p<0.05$). It was determined that CEL group increased the antimicrobial activity of group C in all microorganisms ($p<0.05$).

Table 1. Antimicrobial activity of *H. polyrhizus* peel aqueous extract cream formulation groups.

Microorganism Strains	**Inhibition zone diameter of peel extract (mm±SD)**				
	Cream Formulation Groups				
	C	**CE**	**CL**	**CEL**	**F(Sig)**
***C. albicans* ATCC 10231**	-^{a}	4.78±0.43^{b}	6.76±0.60^{c}	15.72±0.60^{d}	562.572(0.000)
***E. faecalis* ATCC 29212**	-^{a}	-^{a}	-^{a}	2.17±0.43^{b}	75.001(0.000)
***E. coli* ATCC O157:H7**	-^{a}	3.36±0.45^{b}	6.40±0.24^{c}	15.66±0.42^{d}	1231.351(0.000)
***S. enteritidis* RSKK 171**	-a	1.90±0.46^{b}	1.62±0.46^{b}	18.68±0.17^{c}	2042.613(0.000)

C: Cream, CE: Cream-Extracts, CL: Cream-*L. fermentum* MA-7, CEL: Cream-Extract-*L. fermentum* MA-7

The antimicrobial activity of the cream formulation containing *H. polyrhizus* fruit extract is presented in Table 2. The CE group was determined as 1.38 mm and 2.85 mm in *C. albicans* ATCC 10231 and *E. coli* ATCC O157:H. The highest inhibition zone diameter was detected in the CEL group (17.01 mm) against *E. coli* ATCC O157:H. Addition of fruit extract and *L. fermentum* MA-7 increased the antimicrobial activity of the control group. Except for *C. albicans* ATCC 10231, all CEL groups were statistically significant against other test groups ($p<0.05$).

Table 2. Antimicrobial activity of *H. polyrhizus* fruit aqueous extract cream formulation groups.

Microorganism Strains	**Inhibition zone diameter of fruit extract (mm±SD)**				
	Cream Formulation Groups				
	C	**CE**	**CL**	**CEL**	**F(Sig)**
***C. albicans* ATCC 10231**	-^{a}	1.38±0.44^{b}	6.76±0.60^{c}	6.93±0.08^{c}	271.204(0.000)
***E. faecalis* ATCC 29212**	-^{a}	-^{a}	-^{a}	2.60±0.23^{b}	373.792(0.000)
***E. coli* ATCC O157:H7**	-^{a}	2.85±0.42^{b}	6.40±0.24^{c}	17.01±0.58^{d}	1133.072(0.000)
***S. enteritidis* RSKK 171**	-^{a}	-^{a}	1.62±0.46^{b}	6.98±0.52^{c}	271.844(0.000)

*C: Cream, CE: Cream-Extracts, CL: Cream-*L. fermentum* MA-7, CEL: Cream-Extract-*L. fermentum* MA-7

In our previous study, the biological activity of the cream formulation prepared with *H. undatus* fruit methanol extract and fermentum was determined against *C. albicans* ATCC 10231, *E. faecalis* ATCC 29212, *E. coli* ATCC O157:H7 and *S. enteritidis* RSKK 171 test microorganisms. As a result, it was determined that all CEL groups increased the antimicrobial activity of C groups (Celik and Asan-Ozusaglam, 2023). In a study, cream formulations prepared with *H. undatus* water extracts were used in the treatment of wounds developed in white mice. As a result, the potential of using the prepared cream formulation as an antimicrobial agent in healing wounds was determined (Mahdi et al., 2018). As the studies with *H. polyrhizus* are limited, more literature studies are needed.

CONCLUSIONS

The antibacterial or antifungal activities of the cream formulation developed to evaluate the potential use of *H. polyrhizus* extracts in the pharmaceutical and cosmetic industry were investigated in vitro. The prepared cream formulations showed high antimicrobial activity against the test microorganisms. As a result, the use of *H. polyrhizus* aqueous extracts can be an alternative solution to the prevention of various skin problems by reducing the use of chemical-containing cosmetic products. The results of the study have the potential to contribute to future in vivo studies.

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APPLICATION OF LIPOSOMAL ENCAPSULATED ANTIMICROBIAL BIOACTIVE COMPONENTS IN FOOD PRODUCTS AS NATURAL PRESERVATION

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ABSTRACT

Encapsulation technology is needed to make more durable and effective of alternative natural preservatives and nutritional components. In recent years, liposomal structures have attracted attention and liposomes ensure the preservation of the encapsulated material until the appropriate place and time thanks to controlled or delayed release capability. Liposomal structures prevent the conversion into harmful components during storage and increase the bioavailability. The liposomal encapsulation process provides to be more stable and more durable bioactive compounds in the food and the digestive system. The slowly release of antimicrobial components during storage against microbiological contaminations can be realized without allowing mold contamination and mycelium formation in food products. In addition, which will be carried out on non-chemical "hurdle" technologies in order to control the development of food-borne microorganisms and increase antioxidant activity in order to respond to consumer expectations, aims to produce product formulations suitable for the concept of 'Clean Label'. In addition, in order to respond to consumer expectations, it is possible to control the development of food-borne microorganisms and to produce product formulations in accordance with the concept of 'Clean Label' with liposomal systems suitable for "hurdle" technologies without chemical content.

Keywords: Liposom, natural preservation, bioactive compounds, Clean Label

INTRODUCTION

Bioactive components have important antioxidant and antimicrobial effects and also effective on human health. Encapsulation procedures have implemented to increase the mechanism of bioactive components' action. Liposome encapsulation is an important application thanks to provide the development of controlled release of bioactive components in food and increased stability. One of the biggest advantages of liposomes is to made from natural components. Liposomes can be included in food formulations without the need for any legal regulation due to natural structure. Liposomes are no usage limit compared to chemical origin substances, and so excessive limits lead to no health problems. This feature removes the obstacles to the use of liposome structures in foods.

Liposomes are used to improve the water dispersibility of hydrophobic components, to increase bioavailability and to protect the encapsulated components from adverse conditions such as light, heat, pH, oxidation, hydrolysis or chemical reactions, to enable the delivery of an encapsulated agent to a specific location, to reduce negative effects and particle toxicity. Liposome systems provide to control the circulation in the body by modulation of their size and regulating the release profiles with surface modifications of the bioactive components (Alavi et al., 2017; Lila and Ishida, 2017). Unlike other encapsulation methods, liposomal structures have no negative effect on product rheology properties thanks to very low phosphorylcholine-based lecithin concentration. In addition, the encapsulated components are more resistant to processes

such as cooking and pasteurization with the controlled release of bioactive substances in the liposomal structure.

ENCAPSULATION TECHNIQUES

Encapsulation is an excellent method for the preservation of bioactive, volatile and readily degradable compounds and additives in food applications. The purpose of encapsulation is to protected active ingredient from external factors, to ensure stable in the digestive system and to release slowly, to increase bioavailability, to mask the negative taste and odor, and to prevent the active ingredient from reacting with other ingredients (Delshadi et al., 2020). In the encapsulation process consists of the active components as the core material and the appropriate wall material. The coating agent plays a key role and an ideal coating material should have low hygroscopicity, high solubility, low viscosity, low cost, ability to produce a stable emulsion and provide high protection (Gomez et al., 2018). Lipids, proteins and carbohydrates are widely used as coating material in encapsulation systems. The coating materials are desirable to be inexpensive, plentiful, non-toxic, and compatible with the food matrix (Jafari et al., 2008; Delshadi et al., 2020).

Lipid-based coating agents have excellent functionality in emulsification, film formation and encapsulation of active compounds. These coating materials are less toxic and have many potential uses in industrial applications (Fathi et al., 2012). The lipid-based coating materials are polar lipids (eg monoglycerides, phospholipids) and non-polar lipids (eg triacylglycerol, cholesterol) (Đorđević et al., 2016). Polar lipids such as phospholipids have some properties as biocompatible, suitable for stabilization, preservation and controlled release of active compounds and good surfactants (Đorđević et al., 2016; Shishir et al., 2018). Encapsulation contains microcapsules, submicron capsules, and nanocapsules sizes. Micro and nano encapsulation techniques include high pressure homogenization (HPH), micro fluidization, ultrasonic technique, spray drying, spray cooling/cooling, freeze drying, spray freeze drying, complex coacervation, emulsification (spontaneous, phase inversion, miscellaneous), anti-solvent precipitation, extrusion, electro-spinning and electro-spraying, layer deposition, solid dispersion, fluid bed coating, molecular inclusion in cyclodextrins. Different forms of micro and nano encapsulation systems are reservoir and matrix, emulsions (multilayer emulsions, nano emulsions), lipid nanoparticles (solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid vesicular carriers (liposomes, niosomes, phytosomes, bilosomes), hydrogel particles, molecular inclusion complexes, nanofibers, nanotubes, micelles.

LIPOSOMAL ENCAPSULATION

LIPOSOMES

Liposomes are basically amphipathic vesicles in phospholipid structure, similar in structure to the cell membrane, with polar and nonpolar heads and double lipid layer structure. Liposomes are versatile, biocompatible and biodegradable structures that can be used as carrier systems for unstable components due to their amphipathic properties (Subramani and Ganapathyswamy, 2020). Liposomes were first described in 1965 by Bangham et al. (1965) are small intracellular shaped structures consisting of a closed membrane storing or transporting lipid-based substances. Phospholipids are one of the main groups providing liposome formation. Phospholipids are mainly composed of three-carbon alcohol, glycerol or sphingosine. Common alcohol components of glycerol-derived phospholipids are called phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylinositol (PI). Phospholipids are formed by esterification of the primary hydroxyl group of glycerol with phosphoric acid. The remaining

two hydroxyl groups of the glycerol backbone are esterified to fatty acids (saturated or unsaturated) and form the nonpolar tails of the lipid (Segota and Tezak, 2006). Liposomes consist of two layers of molecules with nonpolar groups. In the liposomal structure, polar head groups are directed outward, while non-polar parts are directed inward. Hydrophobic interactions and Vander Walls bonds that hold long hydrocarbon tails together play an important role in bilayer formation (Bozzuto and Molinari, 2015).

Liposomes are divided into different categories based on their structural properties and composition. Liposomes differ from each other in size and physical morphology, depending on lipid composition and preparation method, and may consist of one or more lipid bilayers. The phospholipid type influences the dimensions and physicochemical properties of the liposomes (Singh et al., 2012). Liposomes according to the composition and mechanism of intracellular delivery as follows: pH sensitive liposomes, conventional liposomes immuno-liposomes, cationic liposomes and long-circulating liposomes (Sharma and Sharma, 1997). Generally, the size change from 20 nm to 5000 nm and consist of one or more lipid bilayers. Liposomes according to lipid composition; preparation method and diameter as follows: multilamellar vesicles-MLV (>500 nm), small unilamellar vesicles-SUV (<50 nm), large unilamellar vesicles-LUV (100-1000 nm), giant unilamellar vesicles-GUV (>1000 nm), Multiple vesicles-MLV (>5000 nm), Oligomellar vesicles-OLV (100–1000 nm) and intermediate unilamellar vesicles-IUV (40-100 nm) (Lasic, 1998; Storm and Crommelin, 1998).

LIPOSOME PRODUCTION METHODS

Bangham method (thin film hydration method); One of the simplest methods for liposome formation in multilamellar vesicles is the thin-film hydration procedure. Thin-film hydration method is the most widely used technique to prepare liposomes (Bangham et al., 1965). The thin-film hydration method consists of sequentially dissolving phospholipids in an organic solvent (mostly chloroform), evaporating the solvent to form a thin film, and then dispersing the dry lipid film in an aqueous phase. In this method, sonication is used to reduce the size of large-sized liposomes (Maja et al., 2020). Apart from this, the methods applied are as follows, solvent (ether or ethanol) injection method, reverse phase evaporation (REV), dialysis, extrusion, spray drying, heating, freeze drying, cross flow injection, microfluidization, membrane contactor, supercritical reverse phase evaporation (SCRPE), improved SCRPE method (ISCRPE), supercritical antisolvent (SAS), depressurization of an expanded liquid organic solution-suspension (DELOS) and ultrasonication method. Each of these methods has different advantages and disadvantages.

LIPOSOMAL ENCAPSULATION METHOD PROPERTIES

Liposomes are preferred in the encapsulation process thanks to biocompatible, biodegradable, no show toxic effects, and high ratio protect of coated material (Laye et al., 2008; Gibis et al., 2012; Chun et al., 2013). One of the most important features of liposomes is that can be obtained from nature components. The natural structure of liposomes enables the usage in food systems without the need for any legal regulation (Taylor et al., 2005). In food science, the liposomal encapsulation method is used to encapsulate antioxidant components, antimicrobial components, enzymes and additives. The liposomal system is used in the encapsulation of many bioactive components, including fatty acids such as gambogenic acid (Tang et al., 2018), resveratrol (Caddeo et al., 2008), tea catechins (Zou et al., 2014) and linolenic acid (Vélez et al., 2019), omega-3 and protein hydrolysates (Li et al., 2015). Liposomal encapsulation offers a versatile approach in terms of preservation and controlled release of sensitive bioactive ingredients, delaying food spoilage, protecting bioactive

ingredients from degradation after consumption, and increasing the bioavailability of ingredients during adsorption (Liu et al., 2020).

Liposome structures improve the solubility of lipophilic compounds in aqueous solutions or hydrophilic compounds in hydrophobic systems. Thanks to high dispersion in water, liposomes can be used to produce low-calorie and fat-reduced products. In addition, liposomes have an important effect in preventing oxidation, removing negative flavors and reducing the energy density of food products (Farrokh et al., 2017). The structural similarity to the cell membrane provides distribution and release some bioactive components to specific areas in the body (Gabizon et al., 2004; Laye et al., 2008). This unique structure allows liposomal nanoparticles to enter the intercellular space in the body. Liposomes have no adverse effects on health and also many health benefits such as liver protection, memory enhancement and inhibition of cholesterol absorption is revealed in studies.

STUDIES ON LIPOSOMAL ENCAPSULATED INGREDIENTS IN FOOD PRODUCTS AS ANTIMICROBIAL AGENTS

The antibacterial activities of clove oil and liposome-encapsulated clove oil were investigated by Cui et al. (2015) and stated that liposome-encapsulated clove oil can be use efficiently as an antimicrobial agent for S. aureus in tofu. In a study by Pinilla and Brandelli (2016) determined the antimicrobial activity efficiency of liposome lysine and garlic extract encapsulated with phosphatidylcholine. Nanoliposome-encapsulated nisin-GE has potential as an antimicrobial formulation for food use. According to results, the use of natural antimicrobial nanoliposomes in dairy products is an important alternative way to improve food quality and shelf life. Lopes et al. (2017) carried out the encapsulation of nisin by nanoliposomes obtained using soybean phosphatidylcholine (PC), pectin or polygalacturonic acid. Antimicrobial activities of liposomes were observed against five different strains of Listeria, and showed the highest activity against L. innocua. In-vitro release studies have indicated that the nisin release rate of PC-pectin and PC-polygalacturonic acid liposomes is lower than that of PC liposomes.

Ghorbanzade et al. (2017) stated that fish oil has important benefits in the daily diet, but applications in food formulations are limited due to strong odor and rapid deterioration. So, fish oil encapsulated with nano-liposomal process and usage in the yogurt formulation. It has been stated that nano-liposome fish oil capsules provide a significant reduction in acidity, syneresis and peroxide values of yogurt. In terms of sensory properties, the addition of nano-encapsulated fish oil in yogurt shows similar properties with the control sample enriched with free fish oil. Pabast et al. (2018) investigated the effects of lamb meat in capsules containing free or chitosan-nano-liposomal encapsulated Satureja khuzestanica essential oil on chemical, microbial and sensory properties of lamb at 4°C for 20 days. As a result of the study, the chitosan-liposom encapsulated essential oil of Satureja khuzestanica could be a promising active packaging material to extend the shelf life of lamb. Lopes et al. (2019), lysozyme and nisin were lipozomal encapsulated with phosphatidylcholine (PC) and pectin or polygalacturonic acid. The co-encapsulation of lysozyme and nisin with liposome has a synergistic antimicrobial effect on L. *monocytogenes* and *S. enteritidis*, but provides greater inhibition against *L. monocytogenes*. The PC-pectin liposomes used in full-fat and skim milk medium reduced the *L. monocytogenes* population by 2 log cfu/ml in whole milk and 5 log cfu/ml in skim milk at 37°C. The L. monocytogenes population remained below the detection limit in milk stored for 25 days under refrigeration temperature. This shows that liposomes can be a promising technology to provide controlled release and stability in complex food systems.

Pinilla et al. (2019) used garlic extract encapsulated with liposome process with phosphatidylcholine and oleic acid as an antifungal agent in bread formulation. They reported that bread samples containing encapsulated garlic extract and free garlic extract (0.65ml/100g

dough) were more microbiologically stable and showed mold inhibition for five days compared to control samples. As a result of the study oleic acid and liposomal garlic extract can be used as natural antifungal agents to improve the microbiological stability of cooked food products due to their thermal properties. In a study made by Lin et al. (2022), a bio-responsive composite liposome with silk fibroin, L-fucose and *Litsea cubeba* essential oil were designed for chicken preservation as antibacterial agent and as results indicated that 20% (v/v) of the composite liposomes could inactivate 99% *Campylobacter jejuni* (C. jejuni).

CONCLUSION

Food safety is an important issue for people in the food production process and consumption. In this respect, food production is faced with many technological challenges due to the increasing demand for naturally additive foods. The natural preservative components are significantly affected by environmental conditions and therefore components must be protected by encapsulation. In the food industry, liposomes have been investigated to deliver proteins, enzymes, vitamins, antioxidants and flavors. Many studies indicate that the efficacy of antimicrobial components is increased with liposome encapsulation. The great advantage of liposomes over other encapsulation technologies is their high stability. As a result, it has been demonstrated that there is a significant potential for use of liposome-encapsulated antimicrobials to improve the quality and healthiness of a wide variety of food products.

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IMPACT OF HESPERIDIN AGAINST GENOTOXIC RISKS CAUSED BY SODIUM FLUORIDE IN MICE LEYDIG

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ABSTRACT

Fluorine is an element with high electronegativity that we encounter in many areas of our daily lives. The element fluorine is found naturally in the air, soil, water, and food. In addition, fluorine is added to toothpastes, mouthwashes, and drinking water to reduce the incidence of dental damage. Although fluorine is among the trace elements necessary for human metabolism, its toxic effects have been determined when exposed to high amounts. The gastrointestinal, endocrine, musculoskeletal, and neurological systems are impacted by excessive fluorine exposure, according to research conducted on both humans and animals. There is also evidence to suggest that fluorine toxicity causes oxidative stress in cells, followed by DNA damage and apoptosis. In recent years, it has been understood that fluorine has toxic effects on the male reproductive system. Fluorine has been found to lower testosterone levels and cause spermatocyte differentiation. For this reason, it is of great importance to evaluate the risk and toxicity of fluorine. The protective effect of hesperidin, a subclass of flavonoid, against the genotoxic potential of sodium fluoride (the most common inorganic form of fluorine) was investigated for the first time in Leydig cells, which are the main cells of the male reproductive system. In this study, sodium fluoride (10 ppm) and hesperidin (20 μM) were applied separately and together for 24 hours to the TM3 Leydig cell line. The genotoxic potential of sodium fluoride was investigated by cell viability, micronucleus, and comet tests in the TM3 Leydig cell. The results indicate that sodium fluoride causes DNA damage by increasing the incidence of micronuclei and comet in Leydig cells. In addition, it has been determined that hesperidin, which has proven natural antioxidant properties, may play a protective role in sodium fluoride-induced genotoxicity.

Keywords: Sodium fluoride, Leydig cells, Hesperidin, Genotoxicity, DNA damage.

INTRODUCTION

Fluorine can be ingested through food, the air, and fluoride-containing dental products, but the main way that fluoride enters the body is through naturally occurring or fluoridated water (WHO, 1994). Fluorine is a highly electronegative element that naturally occurs in salt, which is a compound of sodium, aluminum, and calcium. Fluorine-containing substances are frequently exposed to by both humans and animals. The World Health Organization has determined that the daily exposure dose to fluorine is between 0.7 and 1.2 ppm (Wei et al., 2018). Fresh water sources typically contain 0.01-0.3 ppm fluorine, while sea water includes 1.2–1.5 ppm fluorine. Fluorine can be detected most frequently in black tea (3 ppm), shellfish (2-3 ppm), wine (1-2 ppm), and green tea (2-3 ppm). Fluorine exposure has also increased as a result of the widespread use of fluorine in industry, healthcare, and dentistry (Zhang et al., 2016).

Sodium fluoride (NaF), the most common form of fluorine found in water and food, causes toxic effects when administered in high doses. Studies with different animal models have shown that NaF has harmful effects on the kidneys, lungs, spleen, testis, ovary, brain, and blood (Radovanovic et al., 2021). When studies with most animal models were examined, it was observed that fluoride toxicity also showed toxic effects on the male reproductive system. NaF can inhibit spermatogenesis and cause spermatocyte differentiation since it can pass the blood-

testis barrier (Zhang et al., 2016). NaF is also involved in capacitation, apoptosis, hyperactivation, and chemotaxis in male reproductive cells (Zhang et al., 2017). Additionally, excessive fluorine in the body suppresses zinc levels in the testes and reproductive system, which lowers testosterone levels (Long et al., 2009).

Oxidative stress occurs as a result of an imbalance between free radicals and antioxidants in the cell and causes damage to proteins, lipids, DNA, and other molecules. Living organisms require endogenous antioxidant sources (such as superoxide dismutase, catalase, and glutathione peroxidase) as well as exogenous antioxidants to reduce the effects of oxidative damage (Al-Rikabi et al., 2020). Hesperidin (Hes), known for its exogenous antioxidant effects, is an easily available flavonoid derived from citrus fruits such as oranges, grapefruits, tangerines, limes, and lemons (Pyrzynska, 2022). Hes acts as an antioxidant and has free hydroxyl groups that donate electrons to free radicals. *In vitro* and *in vivo* studies have shown that Hes has antioxidant, anticancer, antimicrobial, anti-inflammatory, and anticarcinogenic activities (Pyrzynska, 2022). In addition, the protective effects of Hes on genotoxicity and cytotoxicity have been confirmed by studies on mice (Shokrzadeh et al., 2015).

Leydig cells were used in this study as a model to clarify the male reproductive system toxicity of NaF, one of the most frequently occurring fluorine compounds in nature. By using comet and micronucleus assays, the genotoxicity of Leydig cells, which are crucial to the male reproductive system and are in charge of testosterone biosynthesis, was examined. Furthermore, the first study in Leydig cells focused on the potential of Hes to protect against NaF-induced genotoxicity.

MATERIALS AND METHODS

Cell culture

The Leydig cells used in the present study were delivered to our laboratory from the Global Bioresource Center of the American Type Culture Collection (ATCC). Cells are cultured in DMEM/F12 culture media with 5% horse serum and 2.5% fetal bovine serum. In our study, NaF and Hes were applied separately and together to the TM3 Leydig cell line. The 10 ppm concentration of NaF, which reduces cell viability to 76%, and the 20 μM concentration of Hes, which shows antioxidant properties in the literature, were chosen for use in the experiments.

Assessment of cell viability

The effects of NaF and/or Hes treatment to TM3 Leydig cells were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-diphenyl tetrazolium bromide (MTT) test to assess changes in cell viability. Cells were seeded at $5x10^3$ cells per 96-well culture dish and incubated for 24 hours in a 37°C CO_2 incubator after being treated with NaF and/or Hes. At the end of the exposure, the kit protocol was applied. The optical densities of the cells were measured with an ELISA device at a wavelength of 540 nm. The viability of the control cells was accepted as 100%, and the viability rates of the experimental cells were calculated relative to the control and expressed as a percentage.

Cytokinesis-Blocked Micronucleus (CBMN) Assay

The method of Fenech (2007) was used in the micronucleus test used to demonstrate genotoxic damage. Micronuclei are formations that occur during the mitosis division of the cell and are not included in the main nucleus, which are indicative of genomic damage. The principle of the test is based on the number of micronuclei in binucleate cells that have completed nuclear division but failed to perform cytoplasmic division by administration of cytochalasin-B. An increased number of micronuclei is associated with genotoxic damage.

TM3 Leydig cells were seeded as $5x10^5$ cells in six-well cell culture dishes for both control and experimental groups, and 150 μM H_2O_2 was used as a positive control. After the 24 h exposure, cytochalasin-B was applied for 20 h. Following the treatment, the cell pellets were incubated in a potassium chloride hypotonic solution for five min. The cell suspensions were

centrifuged after being treated with Cornay fixative (one-unit glacial acetic acid, three-units methanol). The supernatant was removed, the pellet was treated with Cornay fixative again, and the produced cell suspensions were dispersed on the slide. The prepared slides were dyed with a giemsa solution and dried outside. Micronucleus, bud, and cytoplasmic bridge parameters were assessed in at least 1000 cells with mononuclei and binuclei for each group under the microscope. A total of 500 cells with mononuclei, binuclei, trinuclei, and tetranuclei were counted.

Cytokinesis blocked proliferation index (CBPI) was calculated according to the equation given below.

$$CBPI = \frac{(MonoNc)+(2xBiNc)+(3*MultiNc)}{Total\ Number\ of\ cells}$$

"MonoNc" used in the equation represents mononucleated cell, "BiNc" binucleated cell, and "MultiNc" multinucleated (trinucleated and tetranucleated) cell.

Replicative Index (RI), a measure of cell division kinetics, was calculated according to the equation given below by counting at least 500 cells with one, two or more nuclei.

$$RI = \frac{\big((1xBiNc)+(2xMultiNc)\big)+(Total\ No.of\ cells)\big)test}{(((1xBiNc)+(2xMultiNc))+(Total\ No.of\ cells))control} \times 100$$

The percentage of cytostasis (%cytostasis), which provides information about cell kinetics, was calculated according to the equation given below.

$$\%\ \text{cytostasis} = 100 - 100 \times \frac{(CBPItest-1)}{(CBPIcontrol-1)}$$

In addition, the nuclear division index (NDI) was calculated according to the equation given below by counting at least 500 cells.

$$NDI = \frac{(1xMonoNc)+(2xBiNc)+(3xTriNc)+(4xTetraNc)}{(Total\ No.of\ cells)}$$

"TriNc" used in the equation represents the cell with trinuclei and "TetraNc" represents the cell with tetranuclei.

Single-Cell Gel Electrophoresis (Comet) Assay

The comet test was used to detect single-stranded DNA breaks and evaluate genotoxicity more sensitively by modifying the experimental approach and methodologies used by Singh et al. (1988). TM3 Leydig cells were planted in cell culture dishes as control and experimental groups at $1x10^5$ cells per well. Additionally, as a positive control, 150 µM H_2O_2 was applied to the cells. The cell pellet obtained at the end of the experimental period was diluted with PBS. The cell suspension in PBS was mixed with 0.05% low-melting-point agarose to form a homogeneous suspension. The resulting cell suspension was spread on slides pre-coated with agar (1.5% normal melting agarose). These prepared slides were kept in a cold lysis solution for one hour at +4°C. After the lysis process was completed, the slides were incubated in alkaline (pH > 13) electrophoresis buffer for 20 minutes to open the double helix structure of the DNA. After the DNA unwinding phase, the slides were placed in the electrophoresis tank and underwent electrophoresis at 300 mA, 25 V, for 30 minutes. After electrophoresis, the slides were washed with distilled water and treated with a neutralization solution three times at 5-minute intervals. Finally, the slides were stained with 4,6-diamidine-2'-phenylindole DNA dye and left to dry. Each slide was photographed randomly under a fluorescent microscope. Parameters such as comet tail length, tail %DNA content, and Olive tail moment were calculated using the comet analysis program.

Statistical Analysis

The Graphpad Prism 9.0 program was used in the statistical evaluation of the data obtained from the cell viability, micronucleus, and comet analysis program on the concentrations of NaF and Hes used alone and in combination. The One-Way ANOVA method

and Tukey's test were applied for statistical analysis. Results are expressed as mean ± standard error, and $p<0.05$, $p<0.01$, and $p<0.001$ values were considered statistically significant.

RESULTS

The effects of NaF and Hes on Leydig cells viability

The TM3 Leydig cells were tested for viability using concentrations of 10 ppm NaF and 20 mM Hes (Figure 1). When the control and NaF groups were compared in terms of MTT values, a significant decrease was observed in the NaF alone group at the end of 24 hours ($p<0.001$). When only NaF and NaF+Hes groups were compared, a significant increase was observed in the NaF+Hes groups ($p<0.01$). According to the cell viability data we obtained, it was understood that a 10 ppm NaF concentration had a cytotoxic effect on Leydig cells. However, it has been determined that Hes can be used as an effective antioxidant to improve this negative effect of NaF on Leydig cells.

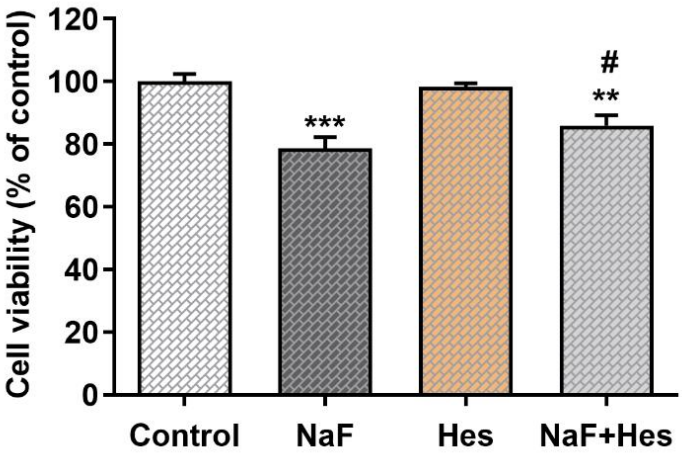

Figure 1. Effects of NaF and Hes on TM3 Leydig cells on cell viability *in vitro*. Each column represents the mean of three independent experiments repeated three times. *in comparison to the control, # in comparison to NaF (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

The effects of NaF and Hes on the micronucleus assay in Leydig cells

Micronucleus, bud formation, and cytoplasmic bridge appearances caused by NaF and Hes in Leydig cells are presented in Figure 2, and the data obtained as a result of evaluating these parameters by comparing them with the control group are presented in Table 1. When the results were examined, there was a statistical increase in the proportions of binucleated cells and mononucleated cells containing micronuclei in the experimental groups where 10 ppm NaF concentration was applied compared to the control ($p<0.05$). Furthermore, a significant decrease in the micronucleus rate was seen in the NaF+Hes group compared to the NaF alone group ($p<0.001$).

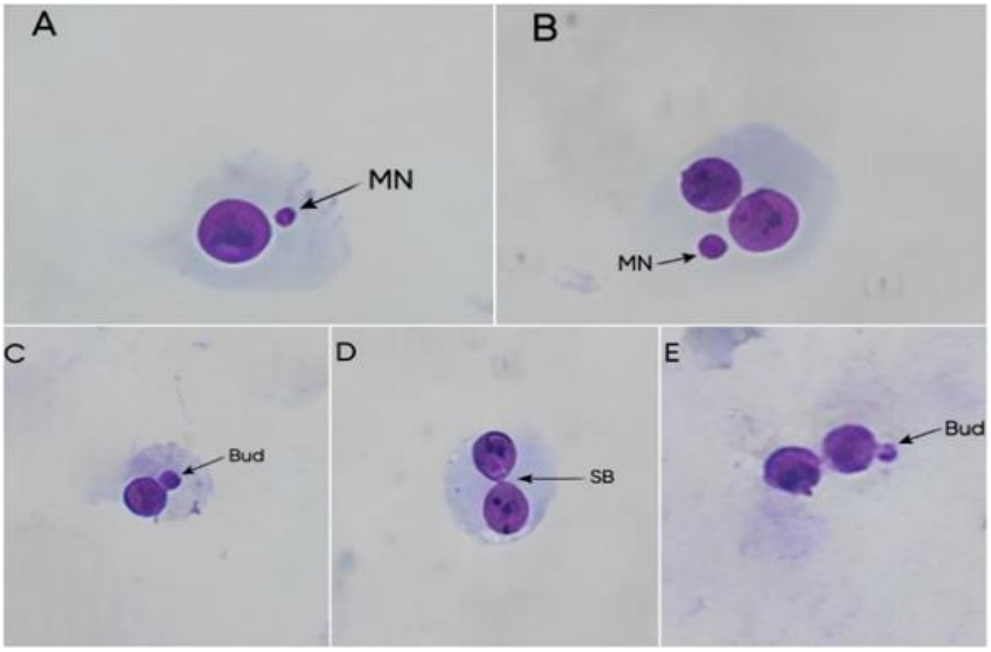

Figure 2. Nuclear abnormalities identified in Leydig cells exposed to NaF and Hes as a consequence of micronucleus testing. A: Micronucleus in a mononucleated cell; B: Micronucleus in a binucleated cell; C: Bud in a mononucleated cell; D: Cytoplasmic bridge in a binucleated cell; E: Bud in a binucleated cell.

Table 1. Micronucleus test results in TM3 Leydig cells treated with NaF and Hes

	Mononucleated cells		**Binucleated cells**		
Groups	MN	Bud	MN	Bud	Cytoplasmic Bridge
Control	1.61±0.3	1.61±0.1	2.28±0.9	0.98±0.1	0.16±0
NaF	8.02±1.2***	5±1.2***	10.54±1.7***	1.61±0.6*	1.24±0.4*
Hes	1.88±0.7	1.29±0.5	1.11±0.1	0.99±0.0	0.0±0.0
NaF+Hes	2.35±0.3$^{\#}$	2.29±0.5$^{\#}$	1.99±0.1$^{\#}$	1.16±0$^{*\#}$	0.25±0.0
PC (H_2O_2)	43,5±2,4	7±1,3***	48.7 ± 1.9***	12.1 ± 1.3***	43.6 ± 2.1***

Each result represents the average of three independent experiments repeated three times. MN: micronuclei; PC: positive control. *in comparison to the control, # in comparison to NaF (*p<0.05, **p<0.01, ***p<0.001).

There was an important increase in the micronucleus, bud, and cytoplasmic bridge rates in Leydig cells after NaF exposure, and our data suggested that Hes reduced NaF-induced genotoxicity. The effects of NaF and/or Hes on CBPI, RI, NDI, and cytostasis values were calculated using nuclear division types observed in the micronucleus test (Figures 3 and 4). The CBPI value, which is a cytotoxicity indicator, revealed a significant decrease in the NaF-treated group compared to the control ($p<0.05$) The RI value, which indicates differences in cellular cytotoxicity, decreased significantly in the NaF group when compared to the control ($p<0.001$). Furthermore, the RI value improved significantly in the NaF+ Hes administered group compared to the NaF alone treated group ($p<0.01$). While the cytostasis value, which shows cell growth and proliferation inhibition, increased in the NaF-treated group, it lowered significantly in the NaF+Hes-treated group ($p<0.001$). According to the data analysis results,

the NDI value declined significantly in the NaF group ($p<0.01$). Additionally, an improvement was observed in the NaF+Hes group compared to the NaF group.

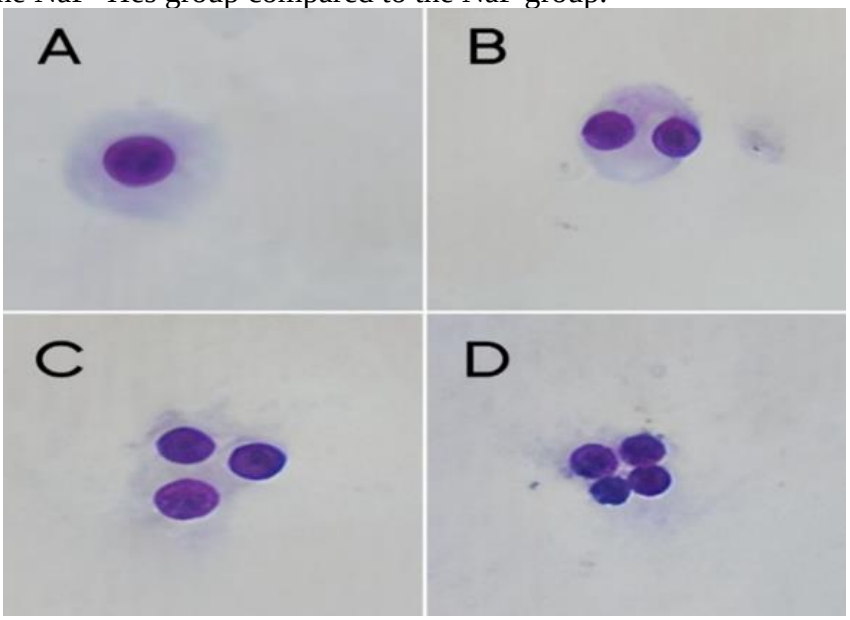

Figure 3. Nuclear division types identified in Leydig cells exposed to NaF and Hes as a result of the micronucleus test. A: mononucleated cell; B: binucleated cell; C: trinucleated cell; D: quadrinucleated cell

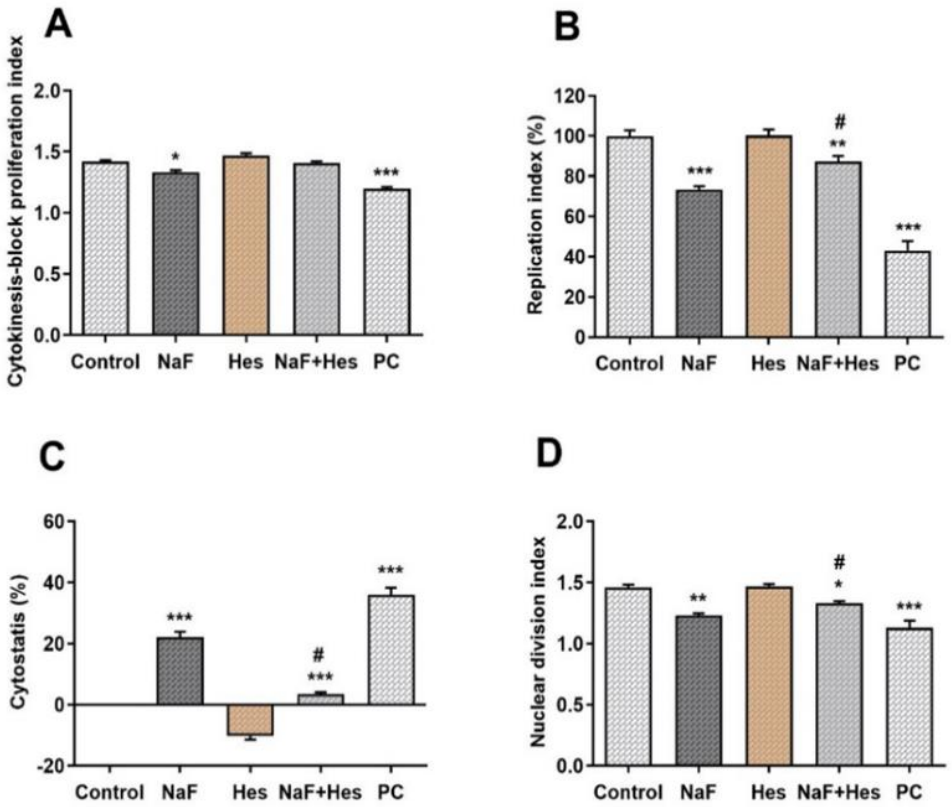

Figure 4. Effects of NaF and/or Hes on (A) cytokinesis-blocked cell proliferation index, (B) replication index, (C) % cytostasis, (D) nuclear division index in TM3 Leydig cells. Each column represents the mean of three independent experiments repeated three times. *in comparison to the control, # in comparison to NaF (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

The effects of NaF and Hes on the comet assay in Leydig cells

DNA damage after exposure of TM3 Leydig cells to NaF and Hes separately and together was evaluated by the comet test (Table 2, Figure 5). Tail length and tail %DNA amount, which are markers of DNA damage, showed a significant increase in the NaF group ($p<0.001$). When NaF+Hes was compared with the NaF-treated group, a significant decrease was observed in tail length, tail %DNA and Olive tail moment parameters ($p<0.05$).

Table 2. Effects of NaF and/or Hes on DNA damage in TM3 Leydig cells.

Groups	Tail Length	Tail % DNA	% Tail Olive Moment
Control	**1.13±0.1**	**8.21±1.4**	**1.07±0.2**
NaF	**21.22±2.4*****	**76.61±11.1*****	**7.12±2.8****
Hes	**0.32±0.1**	**22.36±4.3**	**1.49±0.1**
NaF+Hes	**3.12±0.7**$^{*\#}$	**29.01±3.7**$^{\#}$	**3.15±0.4**$^{*\#}$
Positive control (H_2O_2)	**70.41+- 1.63*****	**97.56+-1.53*****	**10.29+-1.07*****

Each result represents the average of three independent experiments repeated three times. *in comparison to the control, # in comparison to NaF (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

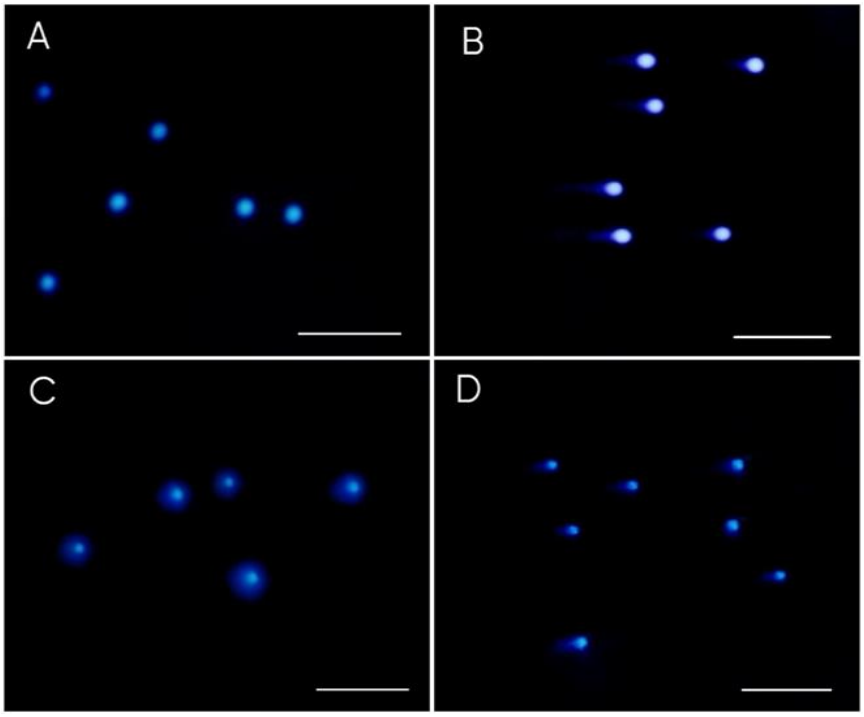

Figure 5. Fluorescence microscope photographs of the alkaline comet assay after applying NaF and Hes to TM3 Leydig cells for 24 h. A: Control; B: NaF; C: Hes; D: NaF+Hes

DISCUSSION

Excess NaF exposure has been proven in studies to have hazardous effects. Studies on the male reproductive system have revealed that NaF has a deleterious impact on reproductive cells, resulting in infertility (Zhang et al., 2006). Understanding and evaluating such effects is critical to defining safe NaF doses and limiting its use. The role of Hes, which has demonstrated antioxidant characteristics, in mitigating the detrimental impact of NaF in Leydig cells was studied for the first time in this study.

Previous research has demonstrated that NaF decreases cell viability. In the osteoblast cell examination by Xu et al. (2008), NaF significantly decreased MTT cell viability at concentrations of 8, 12, and 20 ppm. Another study found that the quantity of NaF affected the viability of MTT cells in mouse Leydig cells at concentrations of 5, 10, and 20 mg/L (Song et al., 2014). Similar to previous research, our *in vitro* study employing Leydig cells showed that

10 ppm NaF decreased cell viability, while Hes had a protective effect against NaF toxicity by raising cell viability.

Genotoxicity occurs when an agent causes damage to the DNA molecule. Currently, many methods are used to measure the occurrence of DNA strand breaks, DNA insertions, and the induction of DNA damage repair. The micronucleus test, one of these methods, is frequently used in scientific studies to determine genotoxic damage. An *in vitro* investigation with osteosarcoma cells revealed that NaF doses of 0, 20, 100, and 200 ppm increased micronuclei, nucleoplasmic bridges, and nuclear buds (Volobaev et al., 2020). Campos-Pereira et al. (2017) showed that NaF caused genotoxic damage in rat bone marrow cells as a result of the micronucleus test. In another study, it was proven that the diazinon substance caused genotoxic damage in human blood cells as a result of the micronucleus test, and they showed that 50 µL of Hes significantly reduced the micronucleus frequency (Shokrzadeh et al., 2015). In parallel with this research, our findings revealed that 10 ppm NaF increased micronucleus frequency, while 20 µL Hes reduced micronucleus damage and therefore potentially improved genotoxic damage.

The comet test is another method for detecting genotoxicity, and it has been demonstrated that NaF induces genotoxicity by causing comet formation in a variety of cells. An *in vivo* study showed that concentrations of 4, 12, and 20 ppm NaF caused an increase in the amount of tail DNA% as a result of the comet test in bone marrow, liver, and kidney cells (Manivannan et al., 2013). Radovanovic et al. (2021) demonstrated that 150 ppm NaF doses caused DNA damage in liver, spleen, and brain cells by increasing the comet DNA tail length and %DNA tail amount. A considerable rise in the percentage of comet tail DNA was discovered in a study using osteosarcoma cells exposed to 20, 100, and 200 ppm NaF (Volobaev et al., 2020). In our study, it was observed that using NaF at lower concentrations increased comet tail length and % tail DNA amount, which is consistent with other findings in the literature. Furthermore, it was discovered that Hes significantly reduced the genotoxicity caused by NaF.

In conclusion, the present study demonstrated that Hes could mitigate Leydig cell damage. Hes considerably increased cell viability and decreased DNA damage. This study revealed that Hes positively modified comet and MN parameters and thereby inhibited cellular damage mediated by NaF.

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PHYTOSANITARY PRACTICES AND OPERATOR EXPOSURE LEVELS

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ABSTRACT

Terrestrial ecosystems are polluted by pesticide residues due to intensive use of phytosanitary products, mainly on cereal crops.

In order to assess the level of exposure of farmers to pesticides and to estimate their potential impact on human health, we carried out a 14-month survey in the wilaya of Khenchela on phytosanitary practices among 368 farmers, including the commune of Remila among 63 farmers (17.11% of farmers). This enabled us to collect data on the phytosanitary practices of farmers in the region through a questionnaire and observations used to estimate risks via the use of mathematical models.

We listed the majority of registered pesticides, and calculated a toxicity risk index for each active ingredient, taking into account both acute and chronic toxicity. Several cases of exceedance of the exposure limits set by legislation reflect the anomalies in the phytosanitary practices of these farmers, most of whom neglect to wear PPE during the preparation of the spray mixture and the application of the treatment.

Key words: Phytosanitary treatment, risk assessment, exposure, active ingredient, Remila.

INTRODUCTION

Cereals are an important part of the food supply for humans and animals **(Karakas ,2011)**. Among these cereals, durum wheat (*Triticum durum* Desf) is one of the oldest species and forms a major part of mankind's diet, hence its economic importance. Wheat provides almost all the nutrition of the world's population in the form of grain foods, 95% of which are produced by the main cereal crops **(Greenway et Munns, 1980 ; Bonjean et Picard, 1990)**.

The consequences of poor phytosanitary application are numerous, and are not limited solely to problems of treatment efficacy, but can also have harmful repercussions on the environment and operators **(Houmy K., 2001)**.

In this context, our study was conducted in the wilaya of Khenchela, among apple growers, to analyze current phytosanitary practices, assess the potential exposure of farmers to pesticides used under usual conditions and perceive their potential impact on human health.

The aim is to demonstrate to farmers the risks associated with the uncontrolled use of pesticides, and the need to respect good phytosanitary practices to protect their health and avoid contamination of the environment (water, soil, air, etc.).

MATERIALS AND METHODS

our study was conducted from 2020 to 2021 (over a period of 14 months) during the main production and processing period for the cereal crop the data collection method is an individual

farmer survey conducted at different sites in the study area, involving 368 producers, including 63 cereal farmers in the commune of Remila Daïra Kais.

The questionnaire included questions on

- ✓ Farm presentation
- ✓ Farmers' knowledge
- ✓ Use of phytosanitary products
- ✓ Storage
- ✓ Personal protective equipment (PPE)

Figure 1 : Realization of the survey with cereal farmers.

The data collected concerns the treatment methods used to control crop diseases and pests, the equipment used for spraying, as well as the various plant protection products used (formulation, dose, frequency, active ingredient, etc.) and protection measures (product-related risk to farmers).

The list of products used was completed by examining empty packaging in the field, agricultural product vendors and packaging stored in the plant.

Observations focused mainly on the preparation of the slurry, since this is a major risk phase for the operator (direct contact with the product).

RESULTS AND DISCUSSIONS

In order to obtain representative results for Khenchela, farms throughout the region were surveyed, including the cereal-growing sectors in Remila.

The study shows that 79% of the 63 farms surveyed have a surface area of [0;10 ha], 5% have a surface area of [10;20 ha] and 11% have a surface area of [20;30 ha], while only 5% have a surface area over [+30 ha].

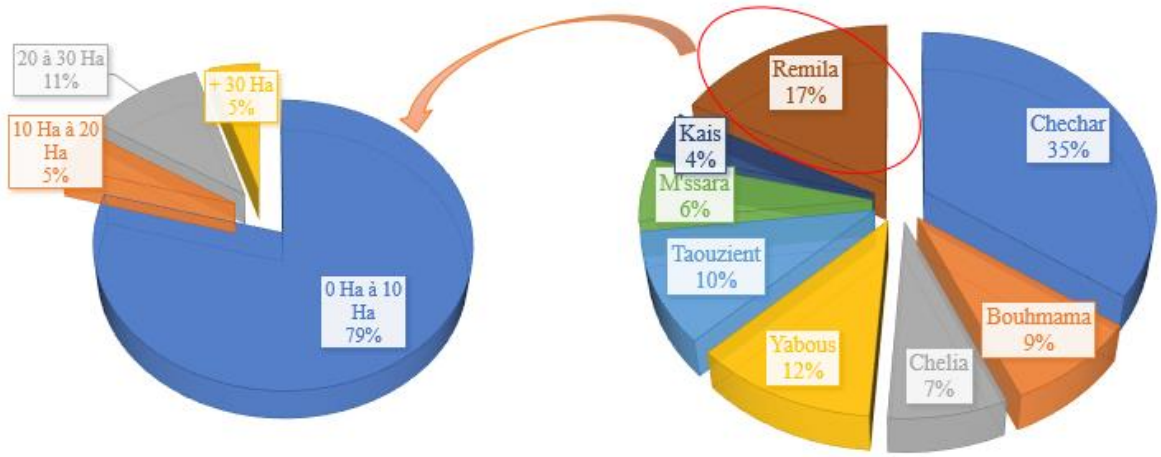

Figure 2 : Surface areas of farms investigated

Application of phytosanitary products

As farm managers are responsible for spraying crop protection products, or for explaining treatment methods (doses to be applied) to applicators, they are the ones most exposed to potential health risks. In order to 62% of farmers wear personal protective equipment (fig. 03).

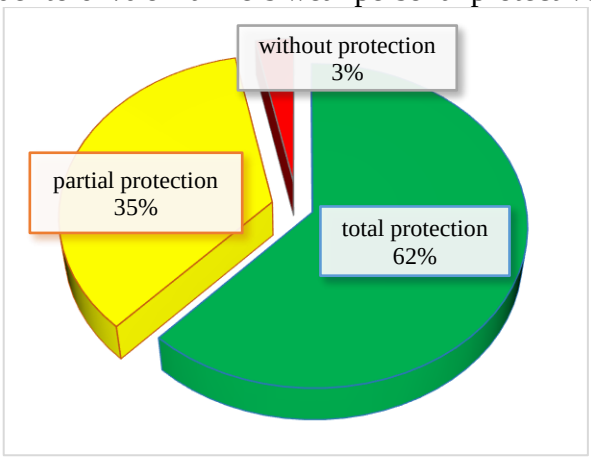

Figure 3 : Personal protection equipment.

The fact that most greenhouse growers in the Ziban region do not use protective equipment is due, on the one hand, to workers' lack of awareness of the real danger posed by pesticides and, on the other, to the lack of such outfits on the market and the unsuitability of those offered by vendors for the working conditions in their greenhouses (high temperatures) **(Schiffers and Mar, 2011).**

Phytosanitary products for cereal treatments

A total of 63 farms surveyed in the Remila region were found to use commercial specialties, all of them synthetic chemicals. Insecticides and herbicides were the most widely used, accounting for 44% and 29% respectively, or 37% of all plant protection products. Followed by fungicide-based products (19%) (fig. 04).

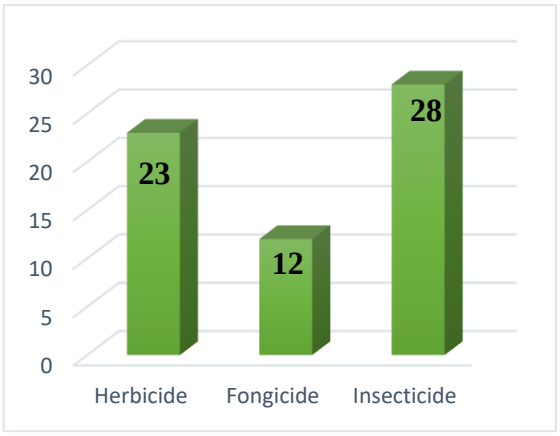

Figure 4 : Categories of crop protection products used on cereals.

The list of products was completed by examining stored packaging and the sellers of phytosanitary products.

The diagram shows that the most useful products in the study area are: Désormone lourd D by 8 farmers (herbicide), ProAct (6 farmers) and Traxos one by 5 farmers (insecticide) and Amistar xtra (6 farmers) and Actara 25 WG by 5 farmers (fungicide) (fig. 05).

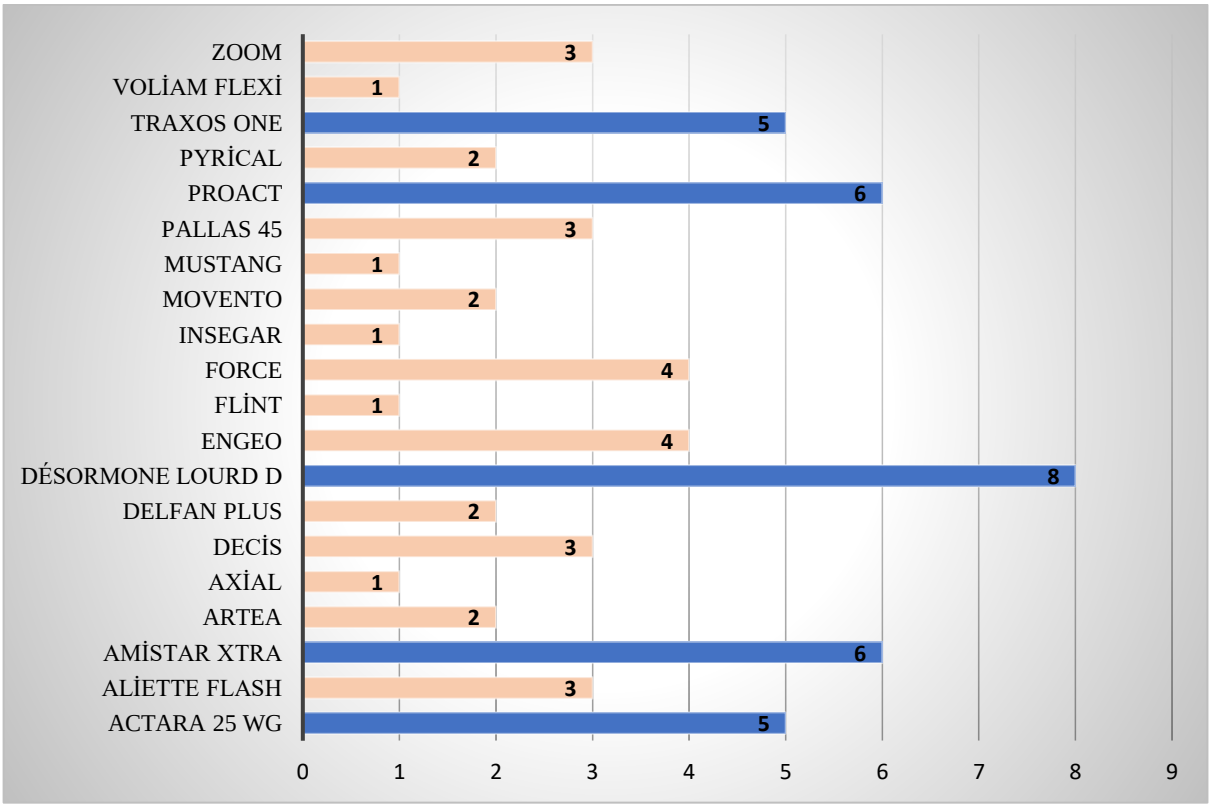

Figure 5 : Phytosanitary products used by farmers of Remila region.

The use of pesticides continues to multiply in many areas, and in large quantities. Approximately 400 phytosanitary products are registered, of which some 40 are widely used by farmers **(Bouziani, 2007).**

Pesticide use is strongly correlated with crop types and local farming practices. In the United States, where field crops (corn, wheat, soybeans) predominate, herbicides are the main category of pesticides used. In France, fungicides account for around half the tonnages sold **(Aubertot et al., 2005).**

21 active ingredients were inventoried, with Titrant 872 g/l (13%), Emamectine Benzoate and 200 g/l Azoxystrobine + 80 g/l Cyproconazole with (10%), Thiamethoxam and 30 g/l Pinoxaden+30 g/l Clodinafop-propargyl+7,5 g/l Florasulm+7,5 g/l Cloquitocet-mexyl with (8%) (fig. 06).

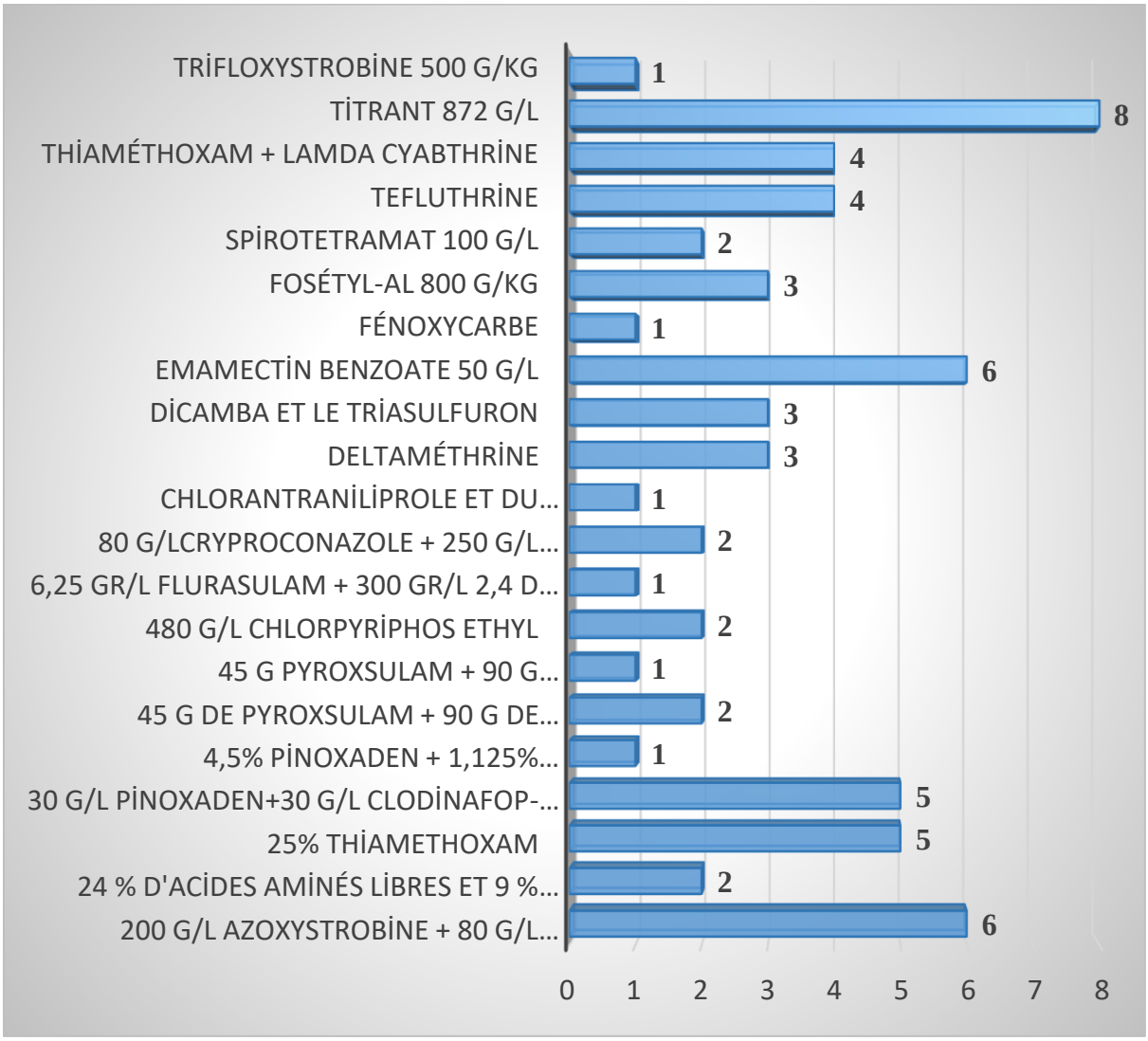

Figure 6 : Distribution of active material handled.

Exposure risk

In the Khenchela region, several noxious or toxic active ingredients have been identified on local markets, on packaging stored at growers' premises or on empty packaging burnt by local farmers after spraying.

Estimation of potential exposure of growers over a working day (mg/kg body weight / day). The following data are integrated: application method, product name, active ingredient, concentration, formulation, PPE, dose and AOEL for each active ingredient. This model enables results to be compared with the AOEL (EU Pesticides database)

1	**THE GERMAN MODEL (GEOMETRIC MEAN VALUES)**					
2						
3	Application method	Tractor-mounted/trailed broadcast air-assisted sprayer				
4	Product	**Bloggo WG**		Active substance	**bloggo**	
5	Formulation type	Liquid		a.s. concentration	100 g/l	
6	Dermal absorption from product	10 %		Dermal absorption from spray	10 %	
7	RPE during mix/loading	None		RPE during application	None	
8	PPE during mix/loading	None				
9	PPE during application: Head	None	Hands	None	Body	None
10	Dose	0,5 l product/ha		Work rate/day	8 ha*	
11	AOEL	0,05 mg/kg bw/dag			*nationellt räknas på 30 ha för boom sprayer	
12						

Figure 7 : The GERMAN MODEL of exposure risk.

Estimated operator exposure without wearing protection, expressed as a percentage of the AOEL, represents 1426% (without protection) and 538% (partial protection) of the AOEL for Chlorpyriphos-ethyl, 990 % (without protection) and 372 % (partial protection) of the AOEL for Emamectine Benzoate, 200% (without protection) of the AOEL for Lambda Cyhalothrin and 181 % (without protection) of the AOEL for 2,4-D-ester S/F de Butylglycol (fig. 08).

Based on these results and the toxicological properties of the preparation, the health risk for operators is considered unacceptable, without wearing protection during all phases of preparation and treatment application. The risk decreases when the operator wears PPE.

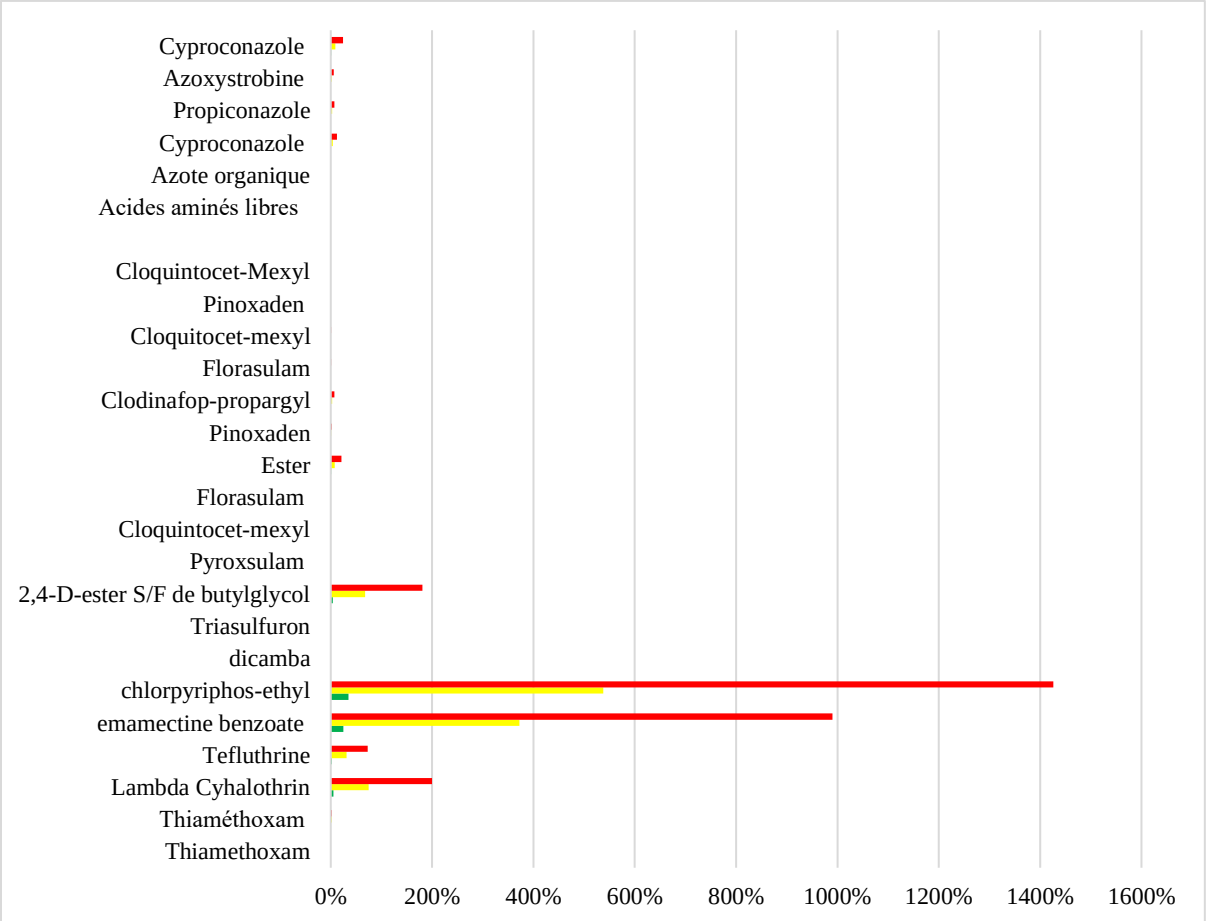

Figure 8 : Operator's exposure level.

CONCLUSION

Cereal growers recognize the risks associated with poor phytosanitary practices: the supply of pesticides from informal channels, the use of toxic phytosanitary products, the total or partial absence of personal protective equipment, and the burning of empty packaging are all practices that expose these operators to danger.

These active ingredients are known to be toxic and could have harmful effects after exposure, especially for farmers who fail to protect themselves when preparing the spray mixture or applying the phytosanitary treatment.

The widespread use of plant protection products, with their adverse effects on human health and the environment, calls for caution and a number of precautions to be taken. To this end, packaging labels include a certain amount of information, including safety information. This information is represented in the form of symbols and colors to better inform users, even the illiterate.

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EFFECTS OF THE PLANT-GROWTH-PROMOTING RHIZOBACTERIA (PGPRS) ON EXPRESSION OF SALT STRESS RELATED GENES IN TOMATO PLANTS UNDER DROUGHT STRESS CONDITIONS

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ABSTRACT

Climate change, hunger, and food insecurity are among the issues that the agriculture sector is dealing with today. During the critical stages of flowering and seed development, tomato plants are vulnerable to drought stress, and elevated carbon levels also result in yield losses. A decline in tomato productivity, an increase in disease, and a fall in fruit quality will all result from the drought. As a result, emerging biotechnological interventions should focus on enhancing plant yield and stress tolerance. The importance of NAC and NHX genes and the benefits of plant growth–promoting rhizobacteria (PGPR) in improving abiotic stress resistance is widely understood. The potential of a group of SINAC and SINHX genes in the control of drought stress tolerance in the presence of a bacterial strain (113-Bacillusis megaterium) in Solanum lycopersicum is the subject of the present study. In this study, the expression level of 4 SINAC genes and 4 SINHX genes was assessed using the real-time PCR technique. In general, in studied genes, in leaf tissues, expression increased at different levels and times of drought stress compared to the control sample. Also, the inoculation of B. megaterium in the leaf tissue has caused an increase in the relative expression of both genes compared to the control samples and also compared to the samples that were only exposed to drought stress. The results indicated that the transcript accumulation of mentioned genes has been regulated under different levels of drought stress. Once naturally tolerant candidate SINAC and SINHX genes have been discovered and the nature of their correlation with drought stress has been known, transgenic technology can be used to build inherent tolerance in future crops.

Keywords: NAC transcription factor, *NHX* family genes, real-time PCR, gene expression, tomato, drought stress

INTRODUCTION:

According to the latest statistics of FAOSTAT (2022), tomato, as one of the most important garden crops, produced over 13 million tons in Turkey in 2020. Also, this product was produced over 251 million tons throughout the world this year. Important nutrients like phenols, flavones, carotenoids, vitamin C, and vitamin A, powerful antioxidants, and minerals like potassium, phosphorus, calcium, iron, and folic acid are present in tomato. Thus, it is frequently consumed both fresh and processed (Tomas et al., 2017). Turkey, for instance, produces tomatoes with a fourth of its total horticultural production (FAOSTAT 2020). The agriculture industry has progressively suffered as a result of recent global climate change. In this regard, it is crucial to focus on thorough research to counter these changes on a global scale (Mahato, 2014). As immobile organisms, plants are subject to a variety of biotic and abiotic stresses that have a detrimental impact on their growth, development, and yield (Lippmann et al., 2019). Plants have created a variety of defense mechanisms to deal with different challenges, including modifications to gene expression and cell metabolism as well as adjustments to plant growth,

development, and performance (Akula Ramakrishna et al., 2011). Drought stress is one of the most significant and prominent abiotic stresses in the world today (Xu and Zhou, 2005). This type of abiotic stress is effective in the plant when soil moisture hits critical levels and atmospheric factors like air heat and solar radiation are the root of ongoing water loss. All plants have efficient defensive mechanisms to endure drought stress, however, these defense mechanisms function differently in various species (Xu and Zhou, 2005). Broadly speaking, plants have been shown to have five defense mechanisms against drought stress: the unfolded protein response (UPR), heat shock response (HSR), epigenetic controls, ROS homeostasis, and the regulations in which hormones are involved (Zhao et al., 2020). Genetic screening of plants to find stress-resistant species and develop them is one of the best approaches to dealing with all sorts of stress (Ermawati et al., 2021).

One of the most significant plant-specific TF families is the NAC (NAM, ATAF, and CUC) domain protein family. No apical meristem (NAM), ATAF1-2, and cup-shaped cotyledon (CUC) are three proteins that share a DNA-binding domain and from which it initially got its name [Aida et al., 1997; Souer et al., 1996]. Typically, NAC proteins have a varied transcription regulatory region at the C-terminus and a conserved NAM domain at the N-terminus (Ooka et al., 2003). Nearly 160 amino acids (aa) residues make up the N-terminal *NAC* domain, which was split into different subdomains (Ooka et al., 2003). Some Subdomains were highly diverse and may give NAC TFs functional variety, whereas some others were often largely conserved (Puranik et al., 2012; Ooka et al., 2003). The C-terminal transcription regulatory regions, in contrast, exhibit high levels of divergence and serve as functional domains by regulating a variety of transcriptional activation activities (Puranik et al., 2012; Ooka et al., 2003). Furthermore, several NAC TFs have transmembrane domains at their C-terminal ends that aid in anchoring to the plasma membrane or endoplasmic reticulum (Seo et al., 2008).

Researchers have identified and classified five subdomains for NAC (A to E). Subdomain A is involved in protein dimerization or heterodimerization. E and B subdomains are responsible for diversity in the function of NAC proteins. The presence of D and C subdomains are necessary for DNA interaction (Puranik et al., 2012). The NAC transcription factor family is one of the efficient genes whose function has been established in the tolerance of diverse biotic and abiotic stressors in plants (Shao et al., 2015). Additionally, studies have shown that this large gene family plays an important role in controlling the synthesis of the secondary cell wall (Zhong et al. 2010), the formation of the stem apical meristem (Aida et al. 1997), embryo development (Duval et al. 2002), and flower growth (Sablowski and Meyerowitz 1998) over the years. The study of this gene family in the past years has attracted the attention of researchers due to the significant role they play in the life of plants and their location so far in many plants such as Arabidopsis (Ooka et al., 2003), rice (Nuruzzaman et al., 2010, pear (Ahmad et al., 2018), tomato Li et al. (2022, etc.) has been identified. Also, the effective role of the large *NAC* family against plants with a variety of biotic and abiotic stresses has been investigated in many research, for example, the role of *NAC* in tomato in drought stress (Jian et al., 2021), aluminum, salinity (Wang et al., 2017) and pathogen attack (Du et al., 2022) have been investigated.

Na+/H+ antiporters, also known as NHXs, which serve as secondary ion transporters for H+ exchange and Na+ or K+ transport across the plant membrane during stressors, are among other genes that have a substantial impact on how the plant reacts to various stresses. Tian). SOS1-like*NHX*, which is found on the cell membrane, and the second category, known as IC-NHE/*NHX*, which contains a multitude of isoforms, are the two primary groups into which NHXs have so far been classified by scientists. According to research by Rodriguez-Rosales et al. (2009) and Leidi et al. (2010), NHXs are involved in the regulation of internal pH and cell development. Different NHX isoforms have so far been shown to have a favorable impact on plants that have experienced a variety of abiotic challenges, such as salinity stress, ionic stress, and nutrient shortage stress (Brini and Masmoudi, 2012).

Beet researchers Kloepper and Schroth discovered in 1981 that rhizobacteria in the soil accelerate beet development by altering the roots and also making the plant more resistant to plant diseases. After further research, these helpful rhizobacteria were termed plant growth-promoting rhizobacteria (PGPR) a few years later, in 1981. Based on where each PGPR acts on the plant cell, Martinez-Viveros proposed classifying PGPRs in 2010. This gives them the names Epgpr and iPGPR, respectively, depending on whether they have an extracellular or intracellular action. Numerous researchers have so far looked into how PGPR affects various plants in various environments. The impact of PGPR, for instance, has been researched so far on tomato production growth, fruit quality, resistance to water stress (Tahiri et al., 2022), salinity stress (Nseri et al., 2022), drought stress (Calvo-Polanco et al., 2016), and Verticillium dahliae stress (Bhattacharyya and Jha., 2012). Cakmakci et al. have also conducted other experiments on the impact of PGPRs on potato, wheat, corn, peas, corn, and cucumber (2006). They can be regarded as biological control agents in biotic and abiotic challenges, effective in enhancing production efficiency, and as biofertilizers in sustainable agriculture due to the great strengths that have been demonstrated in PGPR thus far (Freitas et al. 2007; Yildirim et al. 2011).

This study examines the potential role of a collection of *SINAC* and *SINHX* genes in the regulation of drought stress tolerance when a bacterial strain (113-*Bacillusis megaterium*) is present in *Solanum lycopersicum*.

MATERIALS AND METHODS

Plant Selection and Inoculation: The study used *Solanum lycopersicum* MSC-50 variety. A selected group of these plants were inoculated with *Bacillus megaterium*, a type of PGPR. The objective of this step was to observe how the plant responds to the PGPR treatment.

Induction of Drought Stress: After the inoculation, drought stress conditions were created. This was done by applying three different concentrations of Polyethylene Glycol (PEG), a commonly used substance to mimic drought stress in lab settings. The PEG treatment was administered at two distinct time points: 2 hours and 12 hours after the PGPR inoculation. The doses of PEG and their effects on the plants were detailed in Tables 2 and 3.

Sampling and Tissue Collection: The plants were systematically sampled by collecting both leaves. To maintain the cellular integrity of the samples, they were pulverized using liquid nitrogen. This step was crucial for accurate subsequent analysis.

Sample Preservation: The pulverized samples were stored in Falcon tubes at a temperature of -80 degrees Celsius. This temperature control was essential to ensure the preservation of the biological and biochemical characteristics of the samples.

3.1. Leaf Samples & Treatments

Table 1. Leaf Sample Treatments

Applied Dose of PEG	Samples
(0.25 mM PEG) 31 g of PEG per liter – 1116 g of PEG was used for 36 liters. (36 pots - 1 liter per pot)	**MC:**Control group of MSC-50 tomato variety with no application
	MP1-2h: PEG-treated samples (2 hours)
	MP1-12h: PEG-treated samples (12 hours)
	MBC: Untreated control sample of MSC-50 variety inoculated with 113-*B. megatrium*
	MP1B-2h: PEG-treated samples (2 hours) included with 113-*B. megatrium*
	MP1B-12h: PEG-treated samples (12 hours) included with 113-*B. megatrium*
(0.50mM PEG) 50 g of PEG per liter – 1500 g of PEG was used for 30 liters. (30 pots - 1 liter per pot)	**MP2-2h**: PEG-treated samples (2 hours)
	MP2-12h: PEG-treated samples (12 hours)
	MP2B-2h: PEG-treated samples (2 hours) included with 113-*B. megatrium*
	MP2B-12h: PEG-treated samples (12 hours) included with 113-*B. megatrium*
(0.75mM PEG) 65.5 g of PEG per liter – 1179 g of PEG was used for 18 liters. (24 pots - 750 ml per pot)	**MP3-2h:** PEG-treated samples (2 hours)
	MP3-12h: PEG-treated samples (12 hours)
	MP3B-2h: PEG-treated samples (2 hours) included with 113-*B. megatrium*
	MP3B-12h: PEG-treated samples (12 hours) included with 113-*B. megatrium*

RNA Isolation

RNA isolation was accomplished following a modified version of Bray's (1988) method. About 300 mg of the sample was weighed and placed in Eppendorf tubes, followed by the addition of an extraction solution comprising 50 mM Tris (pH 9), 150 mM LiCl, 5 mM EDTA, and 5% SDS. After vortexing and centrifugation, the upper phase was combined with a phenol chloroform isoamyl alcohol mixture. Subsequent centrifugation separated the supernatant, half of which was treated with 10M LiCl and incubated at +4°C. After centrifugation, the upper phase was discarded, and the remaining supernatant was treated with ethanol, centrifuged, and dried. The pellet was then dissolved in DEPC-treated water.

Purification of RNAs from DNA

RNA purification involved the use of DNase I RNase Free (Thermo) to eliminate genomic DNA from total RNA following the manufacturer's guidelines. The procedure included treating 1 µg

of RNA with DNase I and specific reagents, incubating at 37°C for 30 minutes, and subsequently at 65°C for 10 minutes. The quality of the RNA was assessed through 1% agarose gel electrophoresis. The resulting DNase-treated RNAs were stored at -20 degrees Celsius for subsequent steps.

cDNA Synthesis

The cDNA synthesis process utilized a Thermo Fisher cDNA kit according to the manufacturer's instructions. Sample analysis employed BiO1D software, using 500 ng of RNA based on observed mRNA bands from gel electrophoresis. A 12 µL solution containing 500 ng RNA, 1 µg oligo(dT)18, and dH2O was heated at 65°C for 5 minutes, followed by rapid cooling on ice. In a separate Eppendorf tube, a mixture of 5X reaction buffer, RiboLock RNase Inhibitor, dNTPs, and RevertAid Reverse Transcriptase RNA was prepared. Then, 8 µL of this mixture was added to each sample. Incubation occurred at 42°C for 1 hour, followed by a 5-minute step at 70°C and cooling on ice. The generated cDNAs were partitioned into separate Eppendorf tubes and stored at -20°C to maintain stability.7.

Gene Sequence Identification

The nucleotide sequences of the genes whose expression will be analyzed were obtained via the Solgenomicsdatabase(https://solgenomics.net/organism/Solanum%20lycopersicum/view) and similar genes were searched using the NCBI and its Blast tool. The Gene ID of the genes studied in this research can be found in Appendix 1.

Designing Specific Primers for *NAC* and *NHX* Genes

The sequences of *SINAC* and *SINHX* genes of Arabidopsis was extracted from the TAIR database and in order to find the similar sequences of tomato, using the blast tool on the solgenomics database and the prepared sequences were re-checked for certainty in NCBI and the specific primers were designed in the Eurofins genomics database (https://eurofinsgenomics.eu/en/ecom/tools/pcr-primer-design/). Appendix 2 contains the primer sequences list used in this study. Each primer was evaluated for effectiveness with cDNA produced using standard PCR equipment, and the results were verified on a 1% agarose gel.

Real-Time PCR Test

The Real-Time PCR analysis was performed using a LightCycler 480 II machine from Roche. The RealQ Plus 2x Master Mix Green qPCR Master Kit was utilized with the actin gene as the reference. Peak profiles were established for each gene in the samples, and Ct (Cycle Threshold) values were generated from these profiles. The 2-ΔΔCT method was employed to calculate relative expression values based on Ct values.

RESULTS

Expression Profiles of *SINAC* Genes in Tomato Leaves

The relative expression profile revealed that the *SINAC37* gene was significantly upregulated following PEG treatment across all concentrations tested. Notably, after 12 hours of exposure to MP1, the upregulation was evident in comparison to the control group. The application of PGPR strain 113-Bacillusis megaterium further augmented the expression of *SINAC37*. In

conditions of MP2, the gene exhibited a transient downregulation at the 2-hour mark, followed by an upregulation after 12 hours. Meanwhile, under MP3 conditions, a moderate upregulation was recorded both at 2 and 12 hours post-treatment (Fig. A2).*SINAC40* gene expression saw an upsurge post-PEG treatment, with both 2-hour and 12-hour intervals showing increased transcript abundance relative to the control. Moreover, the presence of PGPR strain 113-Bacillusis megaterium was found to positively regulate *SINAC40* gene expression in tomato leaves (Fig. A3).For both the *SINAC43* and *SINAC45* genes, PEG treatment resulted in a marked increase in transcript abundance at 2 and 12-hour intervals when juxtaposed with the control sample. The introduction of PGPR strain 113-Bacillusis megaterium further modulated the gene expressions, underscoring the combined effects of PEG-induced drought stress and PGPR treatment on the genes' activity in tomato leaves (Fig. A4 & Fig. A5 respectively).

Expression Profiles of *SINHX* Genes in Tomato Leaves

The *SINHX1* gene displayed an upregulation in its transcript levels both at 2 and 12-hour marks, in comparison to the control sample. Furthermore, the presence of PGPR strain 113-Bacillusis megaterium distinctly influenced the *SINHX1* gene's expression patterns (Fig. A6).Similar to *SINHX1*, *SINHX2* gene also manifested an elevated expression profile at both the 2-hour and 12-hour intervals following PEG treatment. The influence of PGPR strain 113-Bacillusis megaterium on the gene was evident, bolstering its expression in the tomato leaves (Fig. A7).The *SINHX3* gene showcased an upregulation in its transcripts at the 2 and 12-hour post-PEG treatment intervals. The inclusion of PGPR strain 113-Bacillusis megaterium further amplified the gene's expression, signifying the synergistic effects of drought stress and PGPR treatment (Fig. A8).Observations for echoed the patterns seen in other *SINHX* genes, with the transcript *SINHX4* abundance escalating at both intervals after PEG treatment. The addition of PGPR strain 113-Bacillusis megaterium further augmented the gene's expression, emphasizing the role of both drought stress and PGPR in modulating its activity (Fig. A9).

DISCUSSION

Tomato cultivars responded to water restriction with a significant proportional fall in yield in semi-arid climate circumstances such as Turkey, also Water stress made plants more vulnerable to pathogenic diseases such as viruses, bacteria, and fungi (Celebi 2014). It is now widely known that several genes, including transcription factors (TFs) that help plants endure adverse conditions, regulate drought tolerance. These genes continue to be prospective genomic candidates for widespread crop breeding (Joshi et al., 2016). Also, globally, drought stress has an impact on plant development and productivity, and *NHX* genes, are well known for increasing drought tolerance in transgenic plants. Several plants have well-defined; nevertheless, nothing is known about *NHX*s in tomato plant (*S. lycopersicum*).

Expression Profile of *SINAC* Genes

In the current study, the expression profile of the tomato NAC gene family was systemically examined. Numerous researche have shown that NAC Transcription factors are present in a wide variety of plant species. and their ability to play a role in controlling plant growth, development, and stress responses (Puranik et al., 2012). Up until this point, this family appeared to be one of the biggest TFs. It was reported that Arabidopsis, rice, grape, apple, maize, chickpea, cassava, sesame, pears, and buckwheat have 117, 151, 79,180, 152, 71, 96, 87, 185, and 80 NAC genes (Ooka et al., 2003; Nuruzzaman et al., 2010; Wang et al., 2013; Shiriga et al., 2014; Ha et al., 2014; Hu et al., 2015; Zhang et al., 2018; Ahmad et al., 2018; Liu et al., 2019).

According to previous studies in the Solanaceae family, a considerable number of NAC genes were overexpressed under drought stress in S. lycopersicum (Al-Abdallat et al., 2015), S. tuberosum (Singh et al., 2013), S. muricatum (Yang et al., 2021), and sweet potato (Yan et al., 2021), and under other abiotic stresses such as S. lycopersicum under Aluminum stress (Jin et al., 2020) or in the development process in S. melongena (Wan et al., 2021). This trend was consistent with the result of the present study where overexpression of a huge number of NAC genes under drought stress and PGPR inoculation has been approved.

Gene expression patterns can typically offer crucial clues for gene activity. Consequently, the expression levels of the 4 SlNAC genes in the leaf of S. lycopersicum were determined using qRT-PCR data. A higher or lower expression level of the studied SlNACs under different conditions (drought stress and PGPR treatment) in the leaf tissue, compared to the control samples was found. These SlNACs demonstrated tissue- and stress-specific expression patterns. These genes may play significant roles in tomato stress tolerance. NAC genes in leaf samples including SlNAC37, SlNAC40, SlNAC43, and SlNAC47 were highly expressed in all doses of drought stress treated samples, indicating that they may be involved in particular drought tolerance system in S. lycopersicum. The specific roles of the tomato SlNAC genes will require further investigation in the future.

4.2. Expression Profile of *SINHX* Genes

For many plants, including A. thaliana (Yokoi et al., 2002), rice (Basu et al., 2014), wheat (Yarra, 2019), sweet beet (Wu et al., 2019), cotton (Ma et al., 2020), and other plants, the importance of NHX gene families under drought and salt stresses have previously been discovered. However, the functionality of NHX genes in S. lycopersicum under drought stress using PGPR has not been studied yet. In this investigation, the genomic expression of four NHX genes in S. lycopersicum was examined. According to the research papers that have been mentioned earlier, the expression level of NHX genes changed significantly in drought and salt-stress-treated samples. Those results are completely consistent with the results of the SINHX gene expression profile in the present study.

Sodium-proton antiporters in tomato plants (S. lycopersicum) facilitate Na+/H+ and K+/H+ exchanges. This contributes to stress tolerance as well as K+ nutrition. NHXs have also been found to increase salinity tolerance in leaves (Zhang and Blumwald, 2001). There was another research which was done by Rodrguez-Rosales et al. (2008) in this regard with the same approach. The SINHXs may also be a part of the responses to drought, according to the expression pattern for different genes and tissues. The tissues' diverse expression patterns suggested that the NHX gene family offers options to breed this plant and overcome the functional restriction imposed by the original gene under drought stress. According to previous studies, it is known that there are many NHX protein isoforms present in tomato plants. Based on a study carried out by Rodríguez-Rosales et al. (2009) the majority of the NHX genes were activated by salt stress in the leaves of tomato (lycopersicon esculentum). It shows that NHX genes play a crucial role and have different functions in the defense system of S. lycopersicum in different tissues.

Regarding the effect of different durations of exposure to drought stress, it is reported that the expression level at different durations of PEG treatment was highly variable in leaf and root tissues of tea (Camellia sinensis) (Paul et al., 2021). This fold change variation was exactly what was observed in this study.

4.3. Plant growth-promoting rhizobacteria

The influence of plant growth-promoting rhizobacteria (PGPR) in bolstering host resilience during abiotic stress periods is well-documented, yet the molecular impact on tomato plants (S.

lycopersicum), which frequently face drought conditions in Turkey, remains largely underexplored. Bacillus megaterium was found to stimulate tomato growth under both normal and salt-stressed environments. Regardless of the conditions, B. megaterium notably boosted the development of tomato plants, leading to more robust roots, shoots, and leaves (Nascimento et al., 2020). This study revealed that the inoculation of tomato seedlings with B. megaterium under normal conditions significantly increased the root and shoot dry weight, resulting in a pronounced augmentation in the overall dry biomass of the tomato plant. Similar observations were recorded under stress conditions, where B. megaterium-inoculated tomato seedlings displayed a substantially higher root and shoot dry weight, leading to an increase in total dry biomass compared to non-inoculated plants. These findings were complemented by the observed elevated NAC and NHX expression levels in PGPR-treated samples exposed to PEG, underpinning the beneficial role of PGPR in supporting tomato plants during drought stress.
While *B. megaterium* boosted expression levels of specific genes involved in the repair of damaged photosynthetic equipment and the preservation of redox equilibrium, it lowered the production of ROS and ethylene. Additionally, *B. megaterium* dramatically changed the metabolic profile to fix salinity-induced physiological disturbances in tomato (*l. esculentum)* (Akram et al., 2019). An observed increase in drought tolerance in this study is completely consistent with the higher level of expression in *NAC* and *NHX* genes in the leaf samples treated with X bacteria in this study.
Yang et al., (2022) also reported that *B. megaterium* could efficiently increase the tolerance of tomato (*S. lycopersicum*) under biotic stresses by affecting a number of functional resistance genes. In addition, Samaras et al. (2021) provided the same result in the transcription pattern of defense-related genes when this genus of rhizobacteria was inoculated into this plant. This rhizobacterium had the same impact as these two previous studies on *NAC* and *NHX* genes in this study.

CONCLUSION

The data obtained from this study will provide essential information for the functional characterization of these genes in tomato under drought stress. In general, we can see that Differential gene expression in NAC and NHX genes were considerable in leaf samples.Also a notable increase in the expression of almost all of investigated SINAC genes has been seen in the leaf specially in 12 hour . This increase in expression at the highest level of PEG has been more considerable than other doses. Also, PGPR inoculation had a positive effect on increasing the expression of the mentioned genes, especially in the second and third doses of PEG. In relation to four SINHX genes, an increase in expression has been seen due to exposure to drought. This increase in expression in samples inoculated with PGPR has increased more in the second and third doses and time has a considerable effect on level of expression .

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Appendix 1:

Table 2. Gene IDs

Gene Name	Ensembl Gene ID
SlNAC37	Solyc04g079940
SlNAC40	Solyc05g009840
SlNAC43	Solyc05g055470
SlNAC45	Solyc06g008360
SlNHX1	Solyc06g008820.2
SlNHX2	Solyc04g056600.2
SlNHX3	Solyc01g067710.2
SlNHX4	Solyc01g098190.2

Appendix 2:

Table 3. Primer Sequences

Primer	Sequence
LeActinF	GCCGGGCGTGATCTTACTGA
LeActinR	AGCTACTCCTGGCGGTCTCC
SlNAC37F	AATGGTGGGACAGCGAGTCA
SlNAC37R	CGGGTCCTAAACGCGCATAA
SlNAC40F	TGTTGGGCGGTATTCCTGCT
SlNAC40R	AACCCGTCCATCCCATTGCT
SlNAC43F	TGTAGCTGCACCTCCTGGTT
SlNAC43R	TGGAGCACTCGCCAATCAGT
SlNAC45F	TGACCCATGGGACCTTCCAG
SlNAC45R	TGTCTTTCCCTGTGGCTTTCCA
SlNHX1F	GCGTCGAGCACCATCTTAGG

SlNHX1R	TCACGGTCAGTAGAGTGCCT
SlNHX2F	CTCCTGCTCCTCGTTCTCCA
SlNHX2R	AAGGACCTGGGTGAAGCTGT
SlNHX3F	GCGAGGGCTGCTAATGTGTT
SlNHX3R	TGACTGCAAAGCAAGGGCAA
SlNHX4F	TGGTGGGCTGGTTTAATGCG
SlNHX4R	TTGGGTGTGGCCAAATCTCG

Appendix 3: Results:
Expression Profile of *SlNAC* Genes in leaves of tomato plants

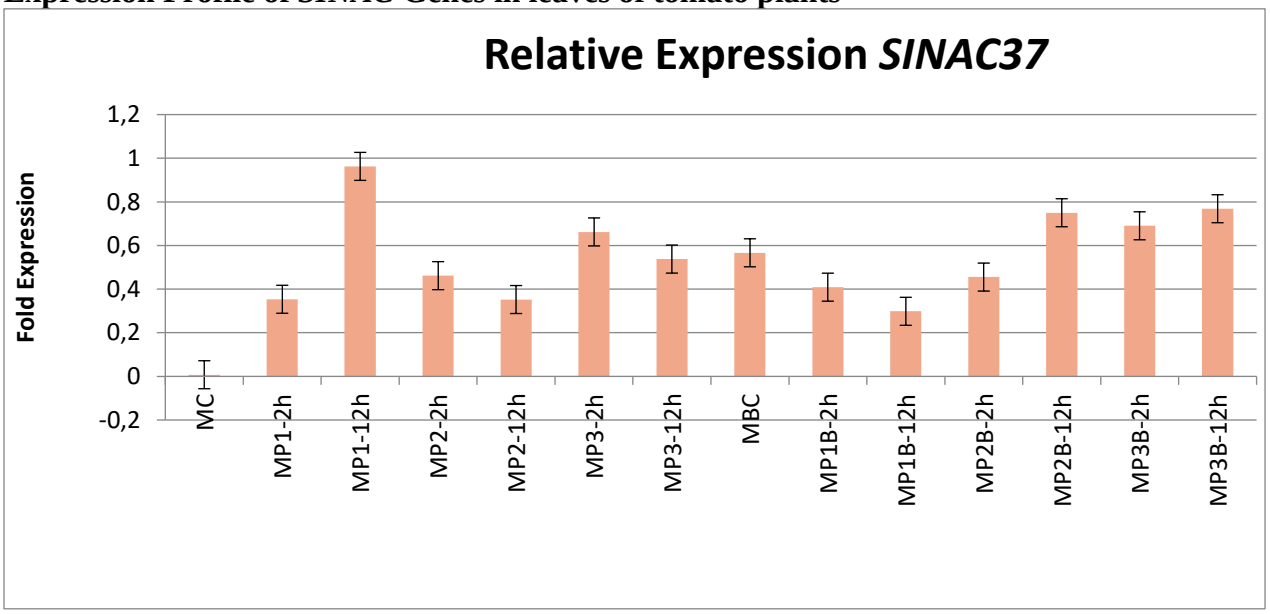

Figure 1. The Relative expression profile of *SlNAC37* in leaves

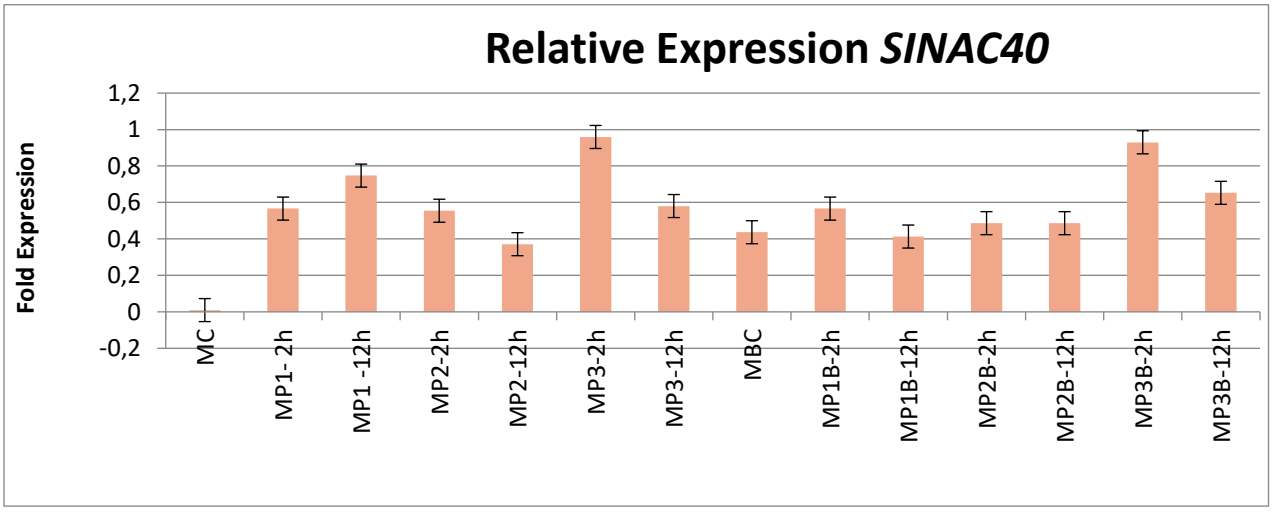

Figure 2. The Relative expression profile of *SlNAC40* in leaves

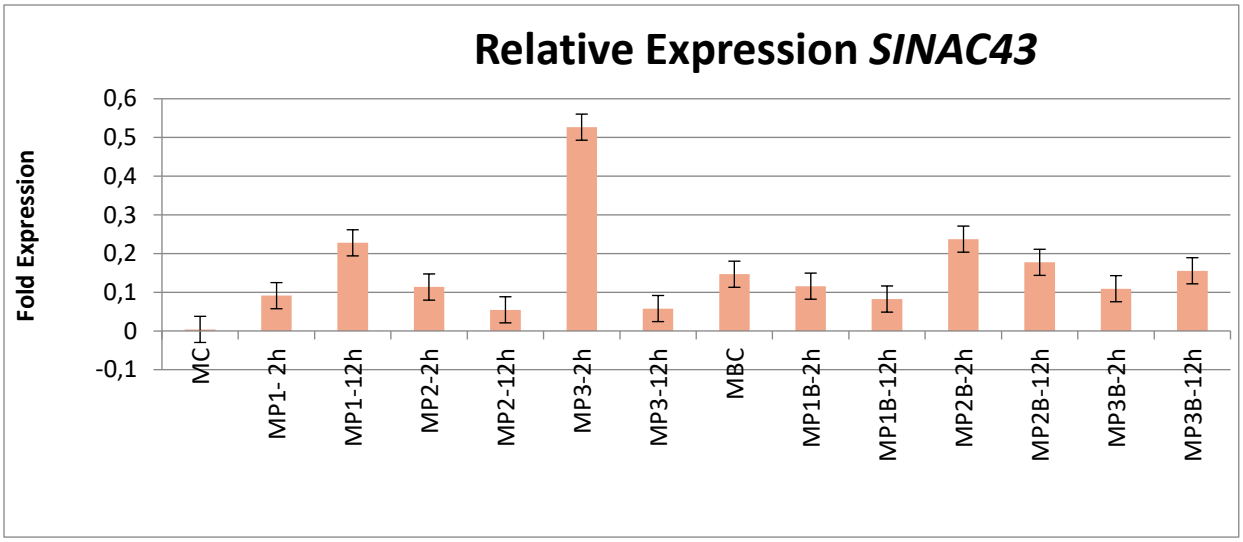

Figure 3. The Relative expression profile of *SINAC43* in leaves

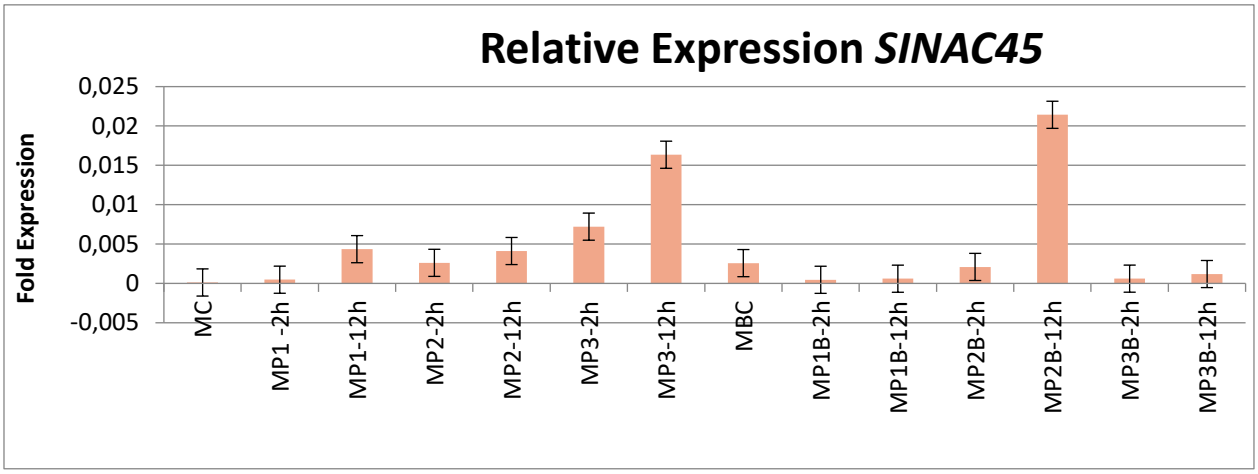

Figure 4. The Relative expression profile of *SINAC45* in leaves

Expression Profile of *SINHX* Genes in leaves of tomato plants

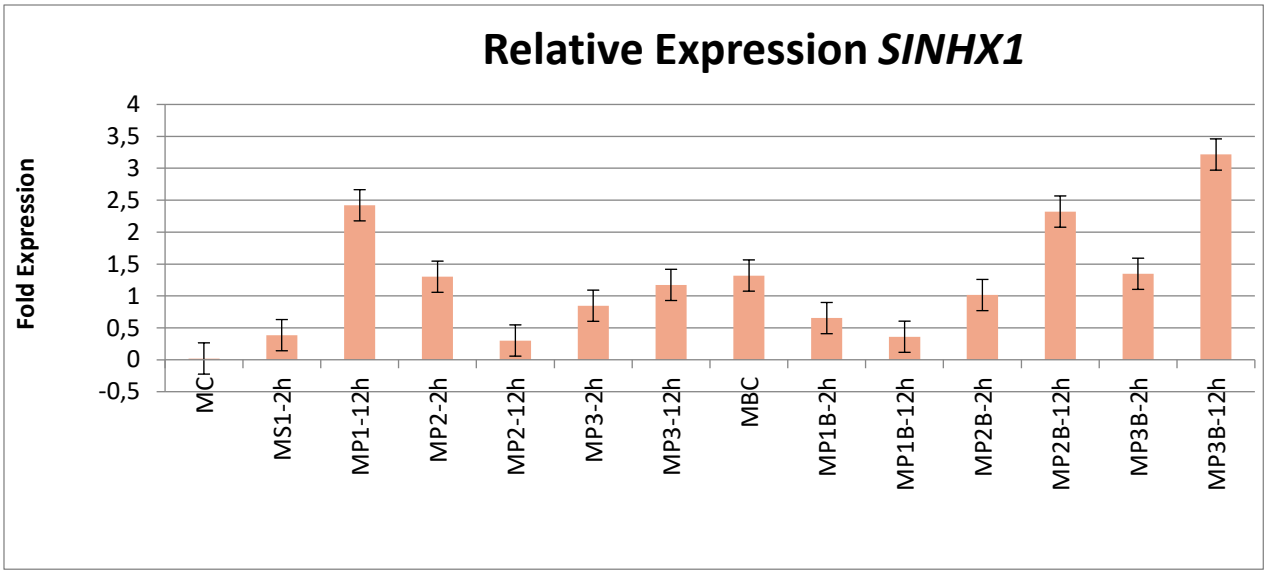

Figure 5. The Relative expression profile of *SINHX1* in leaves

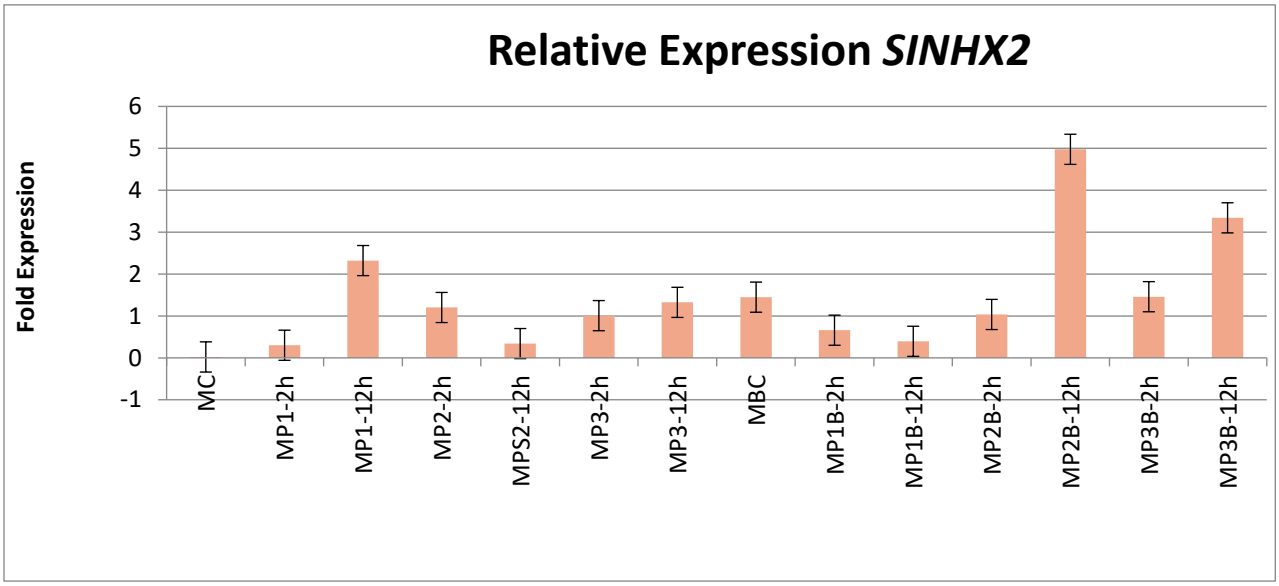

Figure 6. The Relative expression profile of *SINHX2* in leaves

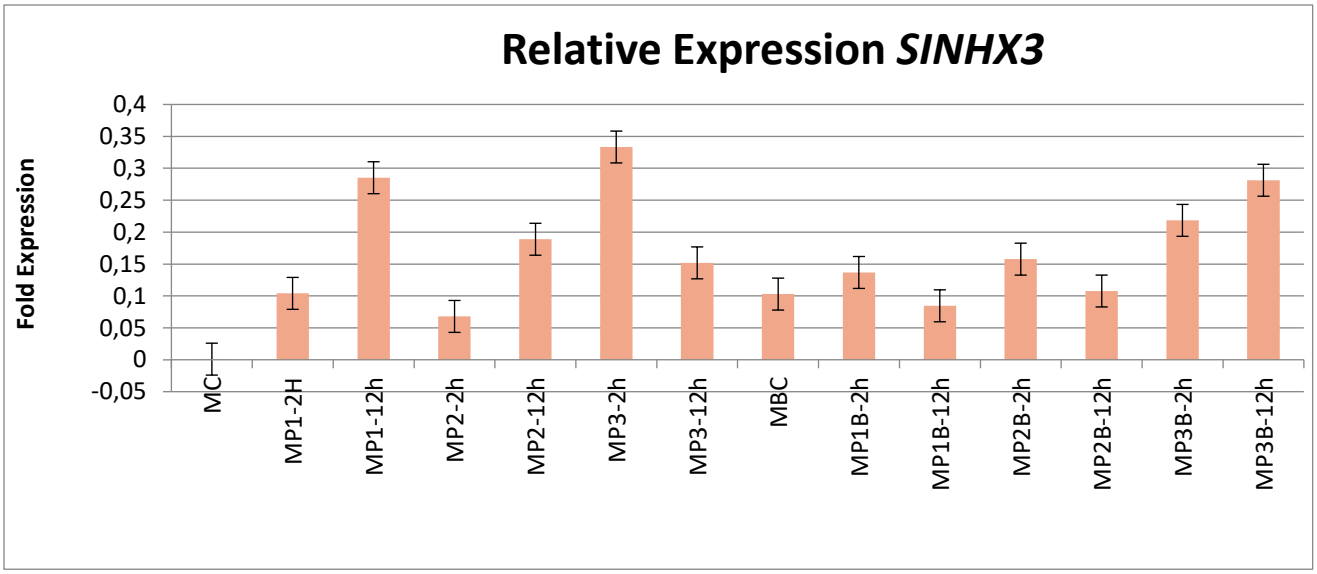

Figure 7. Relative expression profile of *SINHX3* in leaves

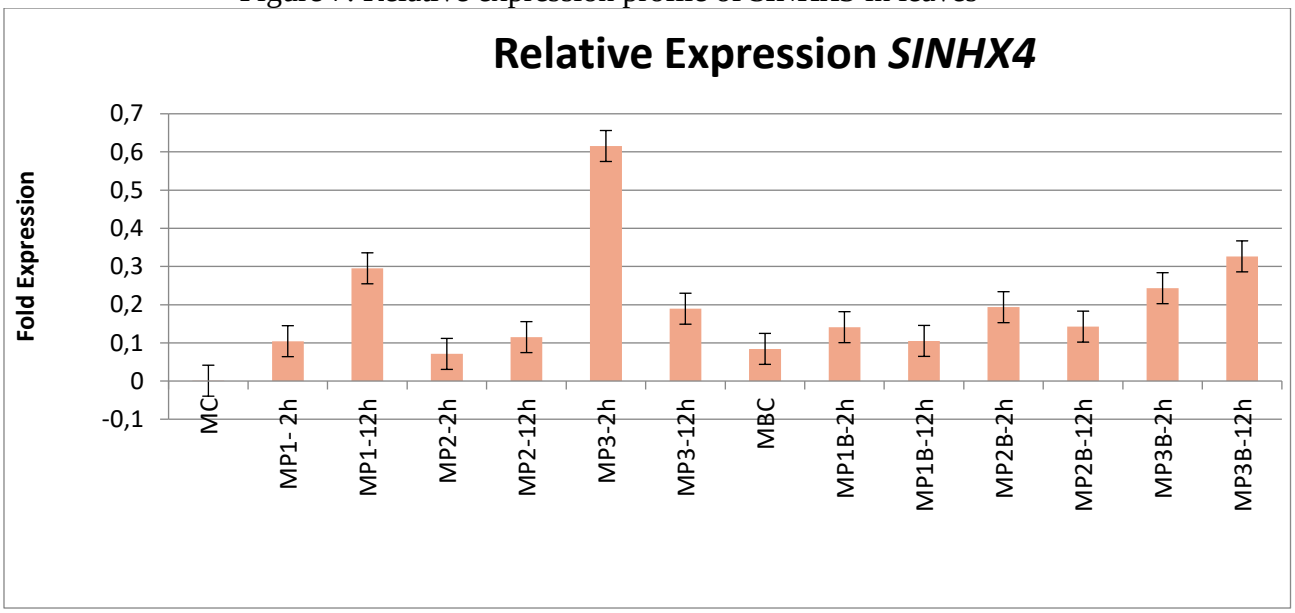

Figure 8. The Relative expression profile of *SINHX4* in leaves

ASSESSING THE IMPACT OF IRRIGATION WATER SALINITY ON MINERAL COMPOSITION IN DIFFERENT PART OF TOMATO

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ABSTRACT

This study aimed to determine the effects of different irrigation water salinity on the mineral contents (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S)) in the leaves, stems, fruits, and roots of tomato plants at the end of the growing period. The research was conducted in two growing seasons between March 2021 - July 2021 and September 2021 - February 2022 under greenhouse conditions. In this study, a randomized block experimental design was used to examine the effects of three different levels of saline irrigation water (EC=0.7-control, 2.5, and 5.0 dS m^{-1}). The result showed that the effects of saline irrigation water differed in both growing seasons. The different salinity levels of irrigation water significantly affected the mineral content of leaves, particularly N ($p<0.01$), K ($p<0.05$), Mg ($p<0.05$), and S ($p<0.05$); the roots, particularly K ($p<0.05$) and Ca ($p<0.05$); and stems, particularly N ($p<0.05$), K ($p<0.01$), and Mg ($p<0.01$), during the fall season. On the other hand, there were no significant differences in the fruit during the fall season. In the spring season, different salinity treatments resulted in significant variations in leaf P ($p<0.05$), K ($p<0.01$), Mg ($p<0.05$), and S ($p<0.001$) contents, fruit N ($p<0.01$), Ca ($p<0.001$), and Mg ($p<0.05$) contents, root Ca ($p<0.001$), and stem S ($p<0.01$) contents. When both growing periods were considered, the differences in mineral content of tomato plants with an increase in salt concentration were in the form of a decrease in N, K, Ca, and P content and an increase in Mg and S content.

Keywords: Abiotic stress, EC_i, greenhouse, salinity stress

Introduction

Irrigation plays an important role for sustainable agricultural production in many parts of the world, particularly in arid and semi-arid regions like the Mediterranean basin (Oron, 2003). However, existing good-quality water resources are insufficient to sustain irrigated agricultural production in arid and semi-arid regions. For the sustainability of water resources, it is critical to enhance water consumption efficiency in irrigated agriculture, which consumes more than 70% of the world's freshwater on a global scale (Singh, 2015). In this scope, it's essential to assess the utilization of low-quality wastewater and saline water resources in agricultural production. However saline water can negatively affect crop quality and yield. Therefore, to

minimize the adverse effects of salinity, it is necessary to understand the salinity tolerance mechanisms of plants. To comprehend these mechanisms, studies have been conducted on the accumulation and transport of ions such as Na, Cl, K, and Mg, and the balance of these ions (e.g., K/Na and Ca/Na) in plant organs when plants are exposed salinity stress (Aziz and Khan, 2001; Parida and Das, 2005; Akay Rastgeldi, 2010; Doğru and Canavar, 2020;). High salt concentrations increase the amounts of Na^+ and Cl^- in the tissues of many plant species, while reducing the levels of K^+, Ca^{+2}, Mg^{+2}, and P (Noshadi et al., 2013; Doğru and Canavar, 2020). Tomato (*Solanum lycopersicum* L.), a rich food source known for its high carotenoid, lycopene, flavonoid, and potassium content (Erba et al., 2013), is one of the most widely cultivated vegetables worldwide, with a production of approximately 189 million tons in 2021, according to FAO (2023) data. The tomato plants, with a salinity threshold value of 2.5 dS/m, is a vegetable that is moderately sensitive to salinity (Maas and Hoffman, 1977). Numerous studies in the literature have extensively investigated the effects of salinity on the growth, yield, and quality parameters of tomato plants (Del Amor et al., 2001; Singh et al., 2012; Tanveer et al., 2020; Karaca et al., 2023;). Nevertheless, it is noteworthy that the effect of salinity varies among different tomato varieties (Alian et al., 2000). Hence, the diverse body of research on this subject is of paramount significance for elucidating the distinct responses of tomato plants to salinity.The objective of this study was to investigate the effects of salinity on the mineral content (N, P, K, Ca, Mg, S) of various organs of tomato plants.

2. Materials and Methods

The research was conducted in two growing seasons between March 2021 - July 2021 and September 2021 - February 2022 under Mediterranean-type greenhouse with a lysimeter system of the Akdeniz University's Agricultural Research Area in Antalya, Turkey (36°53′15″ N, 30°38′53″ E, 31 m altitude above sea level). The greenhouse had dimensions of 9.6 × 25 m and was oriented in a north-south direction, featuring a gothic-style roof. It had natural ventilation through both its sides and roof and was covered with a 200 μm polyethylene film, which incorporated UV, IR, EVA, and AD additives.

The physical properties of the soil are presented in Table 1, and its chemical properties are given in Table 2.

Table 1. The physical properties of soil used in the experiment

Soil particles (%)			Field Capacity	Permanent Wilting Point	Bulk Density
Sand	**Silt**	**Clay**	**(cm^3 cm^{-3})**	**(cm^3 cm^{-3})**	**(gr cm^{-3})**
21	51	28	0.31	0.14	1.38

Table 2. The chemical properties of soil used in the experiment

pH	Lime (%)	Electrical conductivity (EC_e, dS m^{-1})	Organic matter	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
8.1	31.4	0.137	0.9	0.192	3	91	3284	454

Tensiometers were used to monitor soil water content. Irrigation was carried out when the tensiometer readings reached 20 cb, equivalent to approximately 20% of the available water depletion at a depth of 0.6 meters within the profile , achieved by elevating the available water content to the field capacity (Karaca et al., 2023) (Equation 1).

$$I = \frac{P_{V(FC)} - P_{V(AW)}}{100} \times D_s \times A \times P_a \quad (1)$$

where I is the amount of irrigation water (L), $Pv_{(FC)}$ is the field capacity of the soil ($cm^3\ cm^{-3}$), $Pv_{(AW)}$ is the available water content in the soil ($cm^3\ cm^{-3}$), D_s is the soil depth (mm), A is the lysimeter area (m^2), and P_a is the wetted area percentage (%).

In the experiment, the chosen plant material was the OZKAN F1 tomato variety, which is widely cultivated in the Antalya province and is suitable for both spring and autumn planting. The tomato seedlings were transplanted into the plots at intervals of 0.6 × 0.5 meters.

Irrigation was performed using dripper laterals that supplied water at a rate of 2 L h^{-1} under a pressure of 0.1 MPa to the lysimeter plots, with a spacing of 0.5 m between lines and 0.2 m between drippers. Tomato plants were cultivated using a single-stem approach and provided support through strings. Emerging side shoots were pruned at regular intervals. Following the eighth cluster, apical tips were removed from the plant.

A randomized complete block design was used to evaluate the effects of three levels of irrigation water salinity (S) on the N, P, K, Ca, Mg, and S content of different plant parts (leaves, fruits, roots, and stems) of six plants from each treatment group. The electrical conductivity of the irrigation water was S_0 = 0.7 dS m^{-1} (control), S_1 = 2.5 dS m^{-1}, and S_2 = 5.0 dS m^{-1} (Maas and Hoffman, 1977). At the end of each growing period, the leaves, fruits, roots, and stems of the plants were sampled and analyzed for their mineral content. The total N content in the leaves, stems, fruits, and roots of the plant was determined using the Kjeldahl method (Kjeldahl, 1883). The P, K, Ca, Mg, and S contents of plant organs were determined by using an Inductively Coupled Plasma (ICP) spectrometer.

In this study, differences between various treatments were assessed using the ANOVA test conducted with the SPSS Statistics Base v23 (SPSS Inc., Chicago, IL, USA) for overall variations, followed by the LSD test for pairwise mean differences at a significance level of $p<0.05$. In addition, the degree of correlations between the parameters was assessed by considering the r value as suggested by Peck and Devore (2012); strong ($r \geq 0.8$), moderate ($0.5< r < 0.8$) and weak ($r \leq 0.5$).

3. Results and Discussion

Statistical evaluations regarding to the effects of different salinity levels on the mineral content in the organs of tomato plants during both spring and fall periods are presented in Table 3 and 4, respectively.

While the changes in leaf K ($p<0.01$; $p<0.05$), Mg ($p<0.05$; $p<0.05$), and S ($p<0.001$; $p<0.05$) contents due to salinity were statistically significant in the spring and fall growing seasons, the changes in Ca content were not significant. In both growing seasons, leaf K content showed a decrease with increasing salinity (Table 3,4). In the spring growing season, this decrease was 15% and 26% for salinity levels of 2.5 dS m^{-1} and 5 dS m^{-1}, respectively, relative to the control treatment. In the fall growing season, the decrease was 23% and 31%, respectively. In contrast to K, leaf Mg and S contents increased with salinity treatments. Leaf Mg content increased by 121% and 71% in the spring, and by 23% and 31% in the fall, respectively compared to the control. In the spring,leaf S content increased by 35% and 22% in the 2.5 dSm^{-1} and 5.0 dSm^{-1} treatments, respectively, compared to the control. In the fall, there was a 13% increase in leaf sulfur content in the 5.0 dS m^{-1} application, while the 2.5 dS m^{-1} application did not show a statistically significant difference from the control. Under saline conditions, several factors such as reduced root permeability, reduced microbial activity, high chloride levels in the root zone, and low soil nitrogen concentrations result in reduced plant N uptake (Noshadi et al., 2013). Assessing the effect of saline irrigation water applications on leaf N content, it was found that leaf N content was 6% lower in the fall at 5.0 dS m-1 compared to the control treatment.

However, in the spring, the differences between the irrigation water salinity treatments were not statistically significant.

Table 3. Effect of irrigation water salinity on mineral content in the spring growing period

	Salinity (dS m^{-1})	N	P	K	Ca	Mg	S
Leaf	0.7	2.63†	0.31 a	1.84 a	5.44	0.48 b	1.29 c
	2.5	2.60	0.30 a	1.57 b	5.49	1.06 a	1.74 a
	5.0	2.30	0.19b	1.37 b	5.19	0.82 ab	1.58 b
	Significant level	ns	*	**	ns	*	***
Fruit	0.7	2.16 b	0.22	2.63	0.39 a	0.09 a	0.12
	2.5	2.55 a	0.22	3.07	0.32 b	0.10 a	0.16
	5.0	2.07 b	0.21	2.95	0.25 c	0.06 b	0.16
	Significant level	**	ns	ns	***	*	ns
Stem	0.7	1.26	0.14	2.93	2.35	0.28	0.23 b
	2.5	1.37	0.15	4.10	2.08	0.62	0.35 a
	5.0	1.26	0.14	3.73	2.14	0.50	0.33 a
	Significant level	ns	ns	ns	ns	ns	**
Root	0.7	1.23	0.10	1.73	3.58 a	0.14	0.16
	2.5	1.34	0.09	1.16	1.82 b	0.11	0.13
	5.0	1.29	0.08	0.90	1.94 b	0.12	0.13
	Significant level	ns	ns	ns	***	ns	ns

†Each value is the average of measurements from six different plants

*, **, *** and ns, significant at the $p < 0.05$, $p < 0.01$, $p<0.01$ level, and not significant, respectively

Table 4. Effect of irrigation water salinity on mineral content in the fall growing period

	Salinity (dS m^{-1})	N	P	K	Ca	Mg	S
Leaf	0.7	3.26† a	0.26	2.26 a	6.83	0.47 b	2.05 b
	2.5	3.34 a	0.21	1.75 b	9.15	0.68 a	1.98 b
	5.0	3.07 b	0.26	1.57 b	8.05	0.69 a	2.32 a
	Significant level	**	ns	*	ns	*	*
Fruit	0.7	2.06	0.23	3.78	0.37	0.15	0.27
	2.5	1.92	0.26	3.60	0.42	0.17	0.27
	5.0	2.20	0.26	3.78	0.34	0.16	0.24
	Significant level	ns	ns	ns	ns	ns	ns
Stem	0.7	1.47 a	0.12	3.81 b	2.97	0.56 b	0.40
	2.5	1.30 b	0.11	3.28 b	1.97	0.73 a	0.38
	5.0	1.41 a	0.12	4.71 a	2.56	0.50 b	0.46
	Significant level	*	ns	**	ns	**	ns
Root	0.7	1.67	0.10	2.68 a	2.81 a	0.32	0.25
	2.5	1.44	0.11	1.99 b	3.51 a	0.33	0.25
	5.0	1.58	0.11	2.18 b	1.96 b	0.35	0.26
	Significant level	ns	ns	*	*	ns	ns

†Each value is the average of measurements from six different plants

*, **, *** and ns, significant at the $p < 0.05$, $p < 0.01$, $p<0.01$ level, and not significant, respectively

Under saline conditions, many factors such as decreased permeability of plant roots, decreased microbial activity and consequent loss of mineralization of organic compounds in the soil, high chloride concentration in the root zone and decreased soil N concentration are effective in reducing nitrogen uptake by plants (Noshadi et al., 2013). The differences in leaf P content were statistically significant only in spring ($p < 0.05$). At 5.0 dS m^{-1}, leaf P content was 39% lower than in the control.

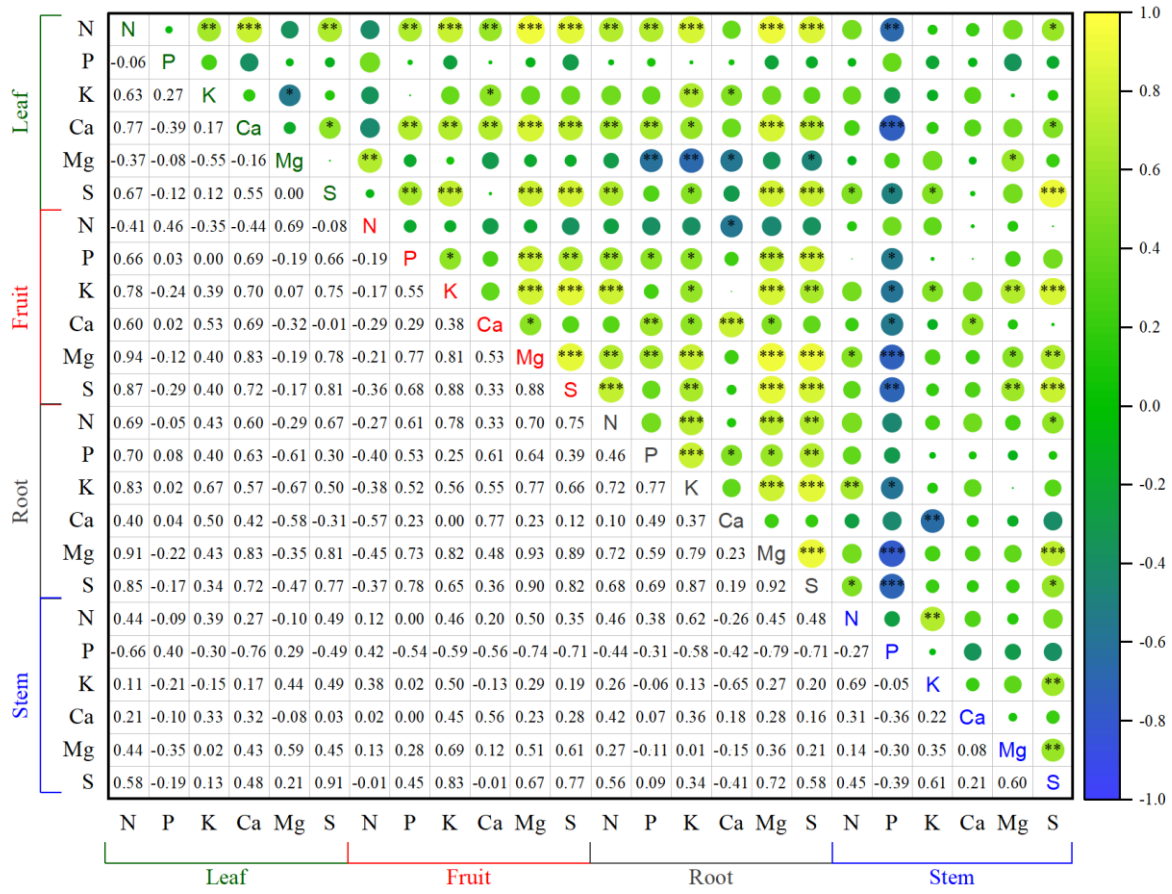
The most significant difference in the mineral content of the fruit, according to the saline irrigation water treatment was the Ca content in the spring growing season. Due to the increase in salinity, there was a decrease of 18% and 36% in the 2.5 and 5.0 dS m^{-1} treatments, respectively, compared to the control. Calcium, which plays a fundamental role in plant growth and development (Hepler, 2005), has a positive effect by regulating Na ions that compete for the same membrane binding sites in the plant, thus protecting the cell membrane from the toxic effects of salt (Tuteja and Mahajan, 2007). In the spring growing season, while the highest fruit N and Mg contents were found in the 2.5 dS m^{-1} treatment, the control treatment was not statistically different from this treatment in Mg content. However, there was 33% decrease in fruit Mg content in the 5.0 dS m^{-1} treatment compared to the control. In the fall period, the differences in the mineral content of the fruit were not statistically significant.

There was a statistical difference in the mineral content of the stem for S ($p<0.01$) in the spring and for N ($p<0.05$), K ($p<0.01$), and Mg ($p<0.01$) in the fall. In the spring, the S content increased in the salinity treatment compared to the control (52 and 43% in 2.5 and 5.0 dS m^{-1}, respectively), as well as in the leaves. During the fall growing season, 5 dS m^{-1} treatment differed from the control only in the potassium content of the stem, which showed a significant increase of 24%. Nitrogen and phosphorus contents of the stem varied according to the control with a salinity of 2.5 dS m^{-1}. In the 5.0 dS m^{-1} treatment, there was a 12% decrease in stem N content and a 30% increase in Mg content compared to the control.

The change in mineral content in the root of the tomato plant in response to salinity stress is particularly noteworthy in the case of Ca during the spring season. It was observed that the Ca content in the roots increased significantly by 49% and 46% in response to treatments of 2.5 and 5 dS m^{-1} salinity treatments, respectively, compared to the control treatment (Table 3). The differences in mineral content in the root zone of the plants in the fall period were in the minerals Ca and K. Both mineral contents decreased at high salinity compared to the control (Table 4).

In this study, we also investigated the correlation between mineral content in different organs of tomato plants under saline conditions (Figure 1). The correlation test shows that various minerals in different plant organs have significant positive and negative correlations under saline conditions. In particular, strong positive correlations were observed, between leaf N content and fruit Mg (r=0.94***) and S (r=0.87***); root K (r=0.83***), Mg (r=0.91***), and S (r=0.85***) contents; fruit Mg and S (r=0.88***), as well as root Mg (r=0.93***) and S (r=0.90) contents; fruit S content and leaf and root S (r=0.81, 0.82 respectively) contents; root S content and fruit K (r=0.83***), root Mg (r=0.92***), and K (r=0.87***) content; and root Mg and leaf Ca (r=0.83***), S (r=0.81***), fruit K (r=0.82***), and S content (r=0.89***). In addition, there was a strong positive correlation observed between leaf S and stem S content (r=0.91***) (Figure 1). On the other hand, weak positive correlations were observed between leaf Ca and stem S contents (r=0.48*); leaf Mg and root S contents (r=0.47*); leaf S and stem N and K contents (r=0.49*). In addition, there was a weak correlation between root Mg, Ca and S contents and fruit Ca (r=0.48*), root P (r=0.49*) and stem N contents (r=0.48*), respectively.

Figure 1. The correlations of mineral substances within different part of tomato plants under saline conditions



4. Conclusions

In this study, we aimed to investigate the physiological changes in tomato plants cultivated with varying concentrations of saline irrigation water. In this contex we examined alterations in mineral content across the leaves, stems, fruits, and roots of the plants. The results revealed that the impact of salinity on mineral content within tomato plant organs varied depending on the growth period. Salinity stress led to decreases in N, P, K, and Ca content, while magnesium Mg and sulfur S levels increased. Furthermore, it was concluded that there were positive and negative correlations between the minerals in the plant organs. The strongest positive correlation was observed between leaf N content and fruit Mg content (r = 0.94***). In contrast, the weakest correlations were identified between stem N and root S content (r = 0.48*), as well as between leaf Ca and stem S content (r = 0.48*). The strongest negative correlation was between root Mg and stem P content (r = -0.79***), while the weakest negative correlation was between leaf Mg content and root S content (r = -0.47*). Salt compounds entering the plant are harmful to the plant when they exceed a certain concentration depending on their type and amount. Salinity is known to be toxic to plants by disrupting nutrition and metabolism (Ekmekçi et al., 2005). Since the differences in the mineral content of tomato plants due to salinity have a direct effect on yield and quality, plant nutrition should be given special attention when growing under saline conditions.

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EFFECTS OF CYCOCEL (CCC) DOSES AND APPLICATION STAGES ON SEED YIELD AND YIELD COMPONENTS OF MUNG BEAN (Vigna radiata (L.) Wilczek)

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ABSTRACT

The aim of this study was to reveal the effects of different cycocel doses and application stages on seed yield and yield components in mung beans. The field experiment was conducted in Adana, Turkey during summer season of 2020 and 2021. Experimental design was split plots based on randomized complete blocks with three replication. The main plots were application stages (seedling and beginning of flowering) and sub-plots were cycocel doses (0, 500, 750, 1000 ppm). In present study, KPS1 excotic genotype was used as a research material. As mean of the years the highest pods per plant, branches per plant, seed weight per plant were observed at 1000 ppm CCC. Cycocel application reduced the plant height and the first podding height. According to combined analysis, greatest seed yield was produced by cycocel application of 1000 ppm with 2530 kg ha^{-1} at seedling stage while minimum seed yield was found in control dose (no cycocel) at seedling stage with 1944 kg ha^{-1}.

Keywords: Application Stages, Cycocel, Mung bean, Seed yield, Yield components

INTRODUCTION

Mung bean (*Vigna radiata* L.) Wilczek) can be grown arid and semi-arid region at the world. Its seeds contain high protein, carbonhydrates and vitamins and its cultivation is widely in Avustralia, Asia, Africa and America (Li et al., 2010; Singh et al., 2013; Dahiya et al., 2015; Abdul Rahman, 2018). Seeds of mung bean can be evaluated as a feed for livestocks and food for human and green manure in the world (Azadi et al., 2013; Nair et al., 2013). Consumption of mungbean is gradually increasing at the world. Mungbean cultivation is not spreading in Turkey, but of landraces of mungbean genotypes are grown in some regions of Turkey (Akdağ, 1995; Dalkılıç, 2010) and it can be succesfully produced for seed in Turkey (Pekşen et al., 2015; Karaman, 2019; Ton, 2021).

Plant growth regulators are natural or synhetic compounds and they are important for increasing seed yield and quality in legumes as in other crops (Kumar, 2021) Cycocel is retarding the plant growth and it is used to prevent lodging in cereals (Kumlay and Eryiğit, 2011). The effect of cycocel application was investigated in some crops, but there are limited studies on the use of growth retards in legumes. It is reported that maximum dry matter in chickpea was obtained from cycocel application of 2000 ppm (Verma et al., 2018). Some studies on various legume crops showed that cycocel application increased some morphological and agronomical traits compared to control in pea (Alan, 1990), in faba bean (Beşer and Adak, 1999), in chickpea (Hajyzadeh, 2008**)** and in mung bean (Kshirsagar et al., 2008). Güler (2010) reported that the greatest seed yield in chickpea was obtained from 1000 ppm cycocel dose applicated in the beginning of flowering. Bora and Sarma (2006) reported that cycocel increased the seed yield and protein content in pea. The studies on mung bean showed that

application of cycocel and some plant growth retards improved seed yield and yield components in comparison to control. (Bhadane et al., 2020). Some plant growth regulators and cycocel inhibites flower shedding, so it can be increased yield in mung bean (Khwairakpam and Kumar, 2019a).

The aim of this study was to reveal the effects of different cycocel doses and applications stages on seed yield and yield components.

MATERIALS AND METHODS

The field experiment was conducted at the research area of Field Crops Department, Faculty of Agriculture, University of Çukurova, Adana, Turkey during summer season of 2020 and 2021. Some meteorological values of Adana for experimental years are present in Table1.

Table 1. Some metorological values in the experimental years

Meterological Parameters	Min Temperature (^{O}C)		Max Temperature (^{O}C)		Mean Temperature (^{O}C)		Relatively Humidity (%)		Total Rainfall (mm)	
Years / Months	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
May	12.9	14.9	40.3	34.0	23.3	23.9	61.0	64.9	66.6	4.1
June	16.4	18.9	39.5	37.7	25.0	25.9	70.9	67.2	38.2	0.4
July	22.9	23.8	37.6	41.0	29.4	30.0	74.3	68.0	0.0	15.8
August	21.7	22.7	41.3	39.8	29.7	30.5	65.9	64.1	1.2	1.2

The texture of experiment soil was siltly-clay loam. The values of pH, salt content, lime and organic matter were 7.25, 0.25 mmhos cm^{-1}, 36.8%, 1.19% respectively. In present study KPS1 exotic genotype provided from Field Crop Department, Faculty of Agriculture, University of Ondokuz Mayıs was used as a research material. This variety showed a good adaptation in the previous experiments conducted in Çukurova conditions. The study was organized in split plots experimental design over randomized complete blocks (RCBD) with three replications. The main plots were application stages (seedling and beginning of flowering) and sub-plots were cycocel (chlormequat chloride 460 g/lt) doses (0, 500, 750, 1000 ppm). The experiment was established on 2th of June in 2020 and 24 th of May in 2021. Each plot was sown in 5 rows of 4m lenght with an inter row spacing of 45 cm and intra row spacing of 5 cm. Fertilizer was applied at a rate of 40 kg N ha^{-1}, 100 kg P_20_5 ha^{-1} before sowing. Rhizobia inoculant was not applied in the experiment. Insufficient nodule formation was observed in the root, hence addition nitrogen fertilizer (Ammonium sulphate, 21% N) was also applied 80 kg N ha^{-1} to plots at seedling stage of plant. The plots was irrigated 4 times during growing period in germination, before flowering and pod stages in both of the years. Harvest was applied in the middle of three rows after eliminating the border rows on September 12, 2020 and August 31, 2021. Net plot area was 3 m x1.35 m=4.05 m^2. Data were recorded from five randomly selected plant in each plot. Plant height (cm), number of main branches per plant, number of pods per plant, number of seeds per plant, 100-seed weight (g), seed weight per plant,(g), seed yield (kg ha^{-1}) were observed.

Data were analyzed according to the split plots experiment design for combined years by using the MSTAT-C a computer software package. Comparisions among the means were made by using LSD (5%).

RESULTS AND DISCUSSION

Plant height

As shown in Table 2, as mean of the years, differences among the application stages were not significant, but plant height was significantly affected by cycocel doses. Plant height varied from 62.4 (1000 ppm) to 74.8 cm (no cycocel). Increasing cycocel doses led to decrease in plant height. Similarly, previous studies recorded that cycocel application reduced plant height in mung bean (Kshirsagar et al., 2008) in chickpea (Güler, 2010). Cycocel application of 500 ppm in faba bean and 300 ppm in chickpea at three leaves stage reduced plant height (Beşer and Adak, 1999; Haiyzadeh, 2008)

Statiscial analysis revealed that year x application stage and year x cycocel dose interactions were no significant effect on plant height. Plant height was also not affected by interaction between cycocel dose and application stage. Plant height was significantly higher in 2020 (73.6 cm) due to greater precipation and relatively humidity and lower temperature in the vegetative stage (June) compared to 2021 (64.1 cm).

Table 2. The effects of cycocel doses and application stages on plant height first podding height and branches per plant in mung bean

Treatments	Plant Height (cm)			First Podding Height (cm)			Main Branches per plant		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Stages (S)									
1	74.9	63.9	69.4	41.6	24.7	33.2	3.2	2.7	2.9
2	72.3	64.3	68.3	35.0	28.6	31.9	3.2	2.7	2.9
LSD 5%	YXS: N.S.		N.S	YXS: N.S.		N.S	YXS: N.S		N.S
ppm	Doses (D)								
0	78.6	71.1	74.8a	42.5	30.0	36.3a	3.1	2.5	2.8b
500	77.0	64.5	70.8ab	38.8	27.7	33.3ab	2.9	2.8	2.9b
750	73.2	61.5	67.3b	36.1	23.2	29.6c	2.9	2.5	2.7b
1000	65.6	59.3	62.4c	35.8	25.8	30.8bc	3.8	3.1	3.4a
Mean	73.6A	64.1B	68.8	38.3A	26.7B	32.5	3.2A	2.7B	2.9
LSD 5%	YXD:N.S.		D:4.85	YxD:N.S.		D:3.29	YXD:N.S.		0.38
CV %			8.3	12			15		

1: Seedling 2: Beginning of flowering

First Podding height

According to mean of the years, application stages didn't affected the first podding height (Table 2). However, effect of cycocel doses was significant on this trait. Increasing cycocel doses reduced the first podding height due to decreasing in plant height. The highest value was obtained from control dose (no cycocel) with 36.3 cm whereas the lowest value was found at cycocel dose of 750 ppm with 29.6 cm These results are in line with report of Beşer

and Adak (1999) who recorded that 500 ppm cycocel dose at pods formation reduced first podding height in faba bean. Statiscial analysis exhibited that there are no significant interactions of year x stages and year x doses. First podding height was higher in the first experimental year (38.3 cm) due to plant height than in the second experimental year (26.7 cm).

Number of main branches per plant

Combined mean showed that branches per plant was not significantly influenced by application stages, but there were significant differences among the cycocel doses in the branches per plant (Table 2). According to mean of the years, branches per plant ranged between 2.8 (0 ppm)-3.4 (1000 ppm) in different cycocel doses. There were no differences among the cycocel doses up to 750 ppm. However, main branches number were improved by application of 1000 ppm cycocel. It is also reported that branches per plant in pea increased with cycocel application (Bora and Sarma, 2006). On the other hand interactions of year x application stages, year x cycocel doses and of application stages x cycocel doses were no-significant for this trait. Main branches per plant in the first year was greater than in interaction was non-significant the second year as in plant height.

Number of pods per plant

As mean of the years, differences among the applications stages were non-significant for pods per plant, but the pods per plant were affected by interaction of year and application stages (Table 3). Pods per plant obtained from cycocel applications at flowering stage were greater in 2020 (34.2) than 2021 (21.8). In mean of years pods per plant was affected by cycocel doses and increase in cycocel doses significantly increased pods per plant and the highest value was achieved by 1000 ppm doses with 33.6 while the lowest value was at control doses with 22.6. Interaction between year and cycocel doses was also significant for pods per plant. The highest value was obtained from 1000 ppm (40.2) in 2020 while the lowest value was found at doses of 0 ppm in 2021 (21.9). Similar to our findings some studies reported that cycocel application increased pods per plant compared to control in mung bean (Bhadane et al., 2020; Khwairakpam and Kumar, 2019b). However, contrary to our findings Güler (2010) reported that the highest pods per plant was obtained from at beginning of flowering in no cycocel application in chickpea. Pods per plant in the first year which is lower temperature and rainfall during the flowering period (July) was greater than in the second year. It was also reported by Warrag and Hall (1984) who more pods per plant was obtained in cowpea at 27/19 ^{0}C than at 33/19 ^{0}C day/ night air temperature.

Number of seeds per pod

Seeds per pod were not affected by application stages in mean of years (Table 3). However year x stage interaction were significant found ort his trait. The highest value was obtained from seedling stage in 2021 while the lowest value was observed in both of applications stages in 2020. Effects of cycocel doses on seeds per pod were significant in mean of the years. Control application (no cycocel) produced minumum seeds per pod (9.1) while maximum value was found in 750 ppm dose (9.8). Nevertheless differences among the other cycocel doses except in control were non-significant for seeds per pod. Interaction of year x cycocel dose was not significant. Seeds per pod in the second year (10.5) were significantly greater compared to the first year (8.6). Increase in pods per plant led to decreasing seeds per pod in the first year. Razzaque et al. (2015) reported that increasing pods per plant decreased the seeds per pod because assimilation used during seed filling is limited.

Seed weight per plant

According to mean of years, significant differences among the stage applications were not observed in seed weight per plant (Table 3). Interaction between year and application stage was significant for this trait. The highest value was achieved by cycocel application at beginning of flowering stage with 14.7 g in 2020. Seed weight per plant was significantly affected by year x dose interaction. Increase in cycocel doses increased the seed weight per plant in 2020 and the highest value was obtained from doses of 1000 ppm. In 2021, the seed weight per plant increased up to 500 ppm and then decreased in higher cycocel doses. As mean of the years, 1000 ppm cycocel dose produced maximum seed weight per plant (15.9 g) while lowest value was obtained from control dose. Similarly, Khwairakpam and Kumar et al. (2019b) recorded that cycocel application improved seed yield per plant in mung bean. Similarly, Bhadane et al. (2020) reported that cycocel application improved seed yield per plant in mung bean. Application stage x dose interaction and differences among the years were not significant.

Table 3.The effects of cycocel doses and application stages on pods per plant, seeds per pod and seed weight per plant in mung bean

Treatments	Pods Per Plant			Seeds Per Pod			Seed Weight Per Plant (g)		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Stages (S)									
1	26.3b	26.6b	26.4	8.5c	10.7a	9.6	12.1b	14.5a	13.3
2	34.2a	21.8b	27.9	8.7c	10.3b	9.5	14.7a	13.7ab	14.2
LSD 5%	YXS: 6.82		N.S.	YXS: 0.29		N.S.	YXS: 1.89		N.S.
(D)ppm	Doses (D)								
0	23.2cd	21.9d	22.6c	8.1	10.1	9.1b	10.6d	13.7bc	12.2b
500	27.1bc	24.1cd	25.6bc	8.6	10.6	9.6a	11.8cd	15.2b	13.5b
750	30.4b	23.8cd	27.1b	8.7	10.7	9.8a	12.7bcd	14.2bc	13.5b
1000	40.2a	26.9bc	33.6a	8.9	10.4	9.7a	18.4a	13.4bcd	15.9a
Mean	30.2A	24.2B	27.2	8.6B	10.5A	9.5	13.3	14.1	13.8
LSD 5%	YXD:4.53		3.21	YXD:N.S.		0.5	YXD:2.93		2.1
CV %	13					6.5			17.8

1: Seedling 2: Beginning of flowering

100-seed weight

Application stages had no influence on 100-seed weight in mean of years. Effect of year x application stage interaction was also not significant (Table 4). The effect of cycocel doses on 100-seed weight was significant. The highest value was observed in 500 ppm (7.22 g) and thereafter decreased in increased dose. However, differences betweeen control and cycocel doses up to 1000 ppm were non-significant. The lowest value was obtained from 1000 ppm of cycocel. Bhadane et al. (2020) reported that cycocel application improved 100 seed weight in mung bean. Combined analysis showed that year x dose and stage x dose interactions were found insignificant. 100-seed weight in the first year (7.35 g) was greater than the second year

(6.48 g). The increase in 100 seed weight in the first year may be due to the decrease of in the seeds per pod.

Seed yield

Application stages and cycocel doses had insignificant effect on seed yield mean of the years (Table 4). Year x stage interaction was also no significant. Mean of the years showed seed yield varied from 2096 (no cycocel) to 2330 kg ha (750 ppm). Seed yield in the first year (1912 kg ha^{-1}) which is higher plant height, lower seeds per pod and later maturation was significantly less compared to second year (2511 kg ha^{-1}).

Table 4. The effects of cycocel doses and application stages on 100-seed weight and seed yield in mung bean

Treatments	100-Seed Weight (g)			Seed Yield (kg ha^{-1})		
	2020	2021	Mean	2020	2021	Mean
Stages (S)						
1	7.45	6.36	6.91	1949	2599	2274
2	7.26	6.59	6.92	1875	2422	2148
LSD 5%	YXS: N.S.		N.S.	YXS: N.S.		N.S.
ppm	Doses (D)					
0	7.29	6.66	6.97ab	1783	2409	2096
500	7.76	6.68	7.22a	1797	2520	2159
750	7.39	6.44	6.91ab	2085	2575	2330
1000	6.99	6.13	6.56b	1983	2538	2261
Mean	7.35A	6.48B	6.92	1912B	2511A	2211
LSD 5%	YXD:0.44		0.43	YxD:N.S.		N.S.
CV %	7.4			10.0		

1: Seedling stage 2: Beginning of flowering

According to combined analysis over the years interaction of application stage x cycocel dose was significant for seed yield (Table 5). The greatest seed yield was produced by cycocel application of 1000 ppm with 2530 kg ha^{-1} at seedling stage application. However, the seed yield obtained from cycocel application of 1000 ppm at seedling stage was similar to cycocel applications of 500 and 750 ppm at seedling stage and dose of 750 ppm at beginning of flowering. The minimum seed yield was found in no cycocel at seedling stage. These results were close agreement with the finding of some studies which cycocel application may improve seed yield in various crops such as mung bean (Bhadane et al., 2020) pea (Bora and Sarma, 2006), chickpea (Güler, 2010) and rape (Pourmohammad et al., 2013).

Table 5. Interaction between application stage and cycocel doses for seed yield in combined mean over the years.

Treatments	Seed Yield (kg ha^{-1})			
Stage/ Doses	0 ppm	500 ppm	750 ppm	1000 ppm
1	1944d	2323ab	2301ab	2530a
2	2248bc	1995cd	2360ab	1992cd
LSD 5%	SXD: 266			

1: Seedling 2:Beginning of flowering

CONCLUSIONS

As mean of the years, the highest pods per plant, branches per plant, seed weight per plant was observed in 1000 ppm CCC. All of the traits were not affected by application stages according to mean of years. Increase in cycocel doses reduced the plant height and the first podding height. Seeds per pod were significant lower in control doses compared to other cycocel applications. Seed yield was affected by cycocel doses x application stages interaction. Greatest seed yield was produced by cycocel application of 1000 ppm at seedling stage. However, the seed yield obtained from cycocel application of 1000 ppm in seedling stage was similar to doses of 500, 750 ppm in seedling stage and doses of 750 ppm in flowering stage. Other traits were not affected application stage x cycocel dose interaction. Present study revealed that cycocel application in mung bean may improve the seed yield.

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INVESTIGATION OF THE EFFECT OF PHAGE APPLICATION ON ANTIBIOTIC RESISTANCE LEVELS OF MULTIDRUG-RESISTANT ESCHERICHIA COLI ISOLATES

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ABSTRACT

Bacteriophages are defined as viruses that infect bacteria. Phages have started to gain importance again because of the increase in antibiotic-resistant bacteria due to the widespread and misuse of antibiotics. These natural killers of bacteria are used in many areas, and the use of phages in combination with antibiotics is an application that has come to the fore in recent years to achieve synergy. This study aimed to investigate the effect of phage application on antibiotic resistance levels of multidrug-resistant *Escherichia coli* isolates. Five antibiotics (ampicillin, fosfomycin, nitrofurantoin, tobramycin, and chloramphenicol) belonging to different antibiotic groups, three lytic phages (M8A, M11A, and M12A) isolated from previous studies, and multi-antibiotic resistant *E. coli* and *E. coli* O157 strains isolated from cattle and chicken were used. Three experimental groups were formed; the *control group*, which was not treated with the phage cocktail; the *phage group*, in which the phage cocktail and bacteria were treated simultaneously; and the *phage mutant group*, which consisted of survivors after treatment with the phage cocktail. Minimal inhibition concentration (MIC) values of the isolates after treatments were determined using the broth microdilution method. According to the results, all *E. coli* O157 isolates were sensitized in the fosfomycin phage group and also in the chloramphenicol phage and phage mutant groups. All strains resistant to nitrofurantoin in the phage group became sensitized in generic *E. coli* isolates. However, MIC values were significantly increased in the phage group in all ampicillin-resistant strains. For tobramycin, MIC values were increased in all isolates in the phage group, while sensitization was detected in the phage mutant group. As a result, it was determined that the combinations of phages and antibiotics caused sensitization in phenotypic antibiotic resistance in multidrug-resistant *E. coli*, but the synergistic effect of phage and antibiotics showed great variability according to the strain and antibiotic. Further molecular studies are needed to elucidate the mechanisms of phage-antibiotic synergism clearly.

Keywords: Bacteriophage, antibiotic resistance, minimal inhibition concentration

INTRODUCTION

Bacteriophages were first discovered by d'Herelle in the early 1900s and successfully used for treatment in a child with dysentery (Roy and Yusuf, 2013; Keen, 2015). With the discovery of antibiotics in the 1930s, the interest in bacteriophages decreased, but the increase in antibiotic-resistant bacteria due to the widespread and misuse of antibiotics caused it to become one of today's biggest problems. The emergence of antibiotic-resistant modified pathogens such as *Mycobacterium tuberculosis, Enterococcus faecalis, Acinetobacter baumannii, Pseudomonas aeruginosa*, colistin-resistant *Escherichia coli*, and methicillin-resistant *S. aureus* creates major problems in the treatment of patients in hospitals (Coelho et al., 2004; Hanlon, 2007; Burrowes et al., 2011). It has become necessary to find alternatives to antibiotics due to several factors. These include the decrease in the effectiveness of antibiotics,

the emergence of resistance mechanisms in bacteria, the limited discovery and production of new antibiotics, and inadequate studies to prevent resistance. In this context, bacteriophages have gained importance again in recent years (Tagliaferri, Jansen and Horz, 2019). These natural killers of bacteria are used in many areas, such as biological control of foodborne pathogens, phage therapy, bio-sanitation (Endersen and Coffey, 2020). The use of phages in combination with antibiotics is an application that has come to the fore in recent years and has been applied in various case studies (Abedon, 2019). Specifically, studies on the sensitization of antibiotic-resistant bacteria by creating a synergistic effect of the combination of antibiotics and bacteriophages are increasing (Abhilash, Vidya, Jagadevi, 2008; Torres-Barceló, 2018). In this study, it was aimed to investigate the effect of phage application on antibiotic resistance levels (ampicillin, fosfomycin, nitrofurantoin, tobramycin, and chloramphenicol) of multidrug-resistant *Escherichia coli* isolates by using the microdilution method.

MATERIAL AND METHOD

Bacterial strains and bacteriophages

In the study, P21x, P67b, P91, and P106 generic *E. coli* isolates isolated from chicken neck skin (Cufaoglu et al., 2022) and 120GA, 180KD, 19RA and 10KoKC *E. coli* O157 isolates isolated from cattle carcass swabs (Ayaz et al., 2015) were used as the test microorganisms. The resistance profiles of the isolates were previously reported by Cufaoglu et al. (2022). In addition, three bacteriophages (M8A, M11A, and M12A) with lytic activity against *E. coli*, previously isolated from slaughterhouse wastewater were used (Gencay et al., 2016).

Determination of phage activities

Before starting the Minimal Inhibition Concentration (MIC) study, the lytic effect of bacteriophages on the test *E. coli* strains selected for use in the study was checked using the spot test method on double-layer LB agar (Figure 1) (Gencay et al., 2016).

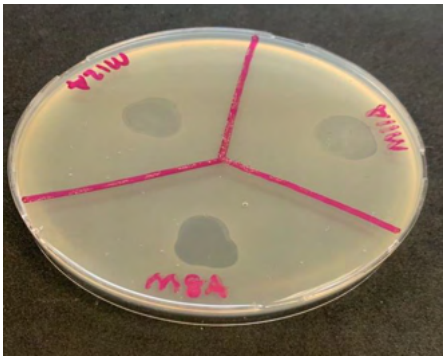

Figure 1. **Plaque image of M8A, M11A, and M12A phages in the *E. coli* ATCC 43895 reference strain.**

Minimal Inhibition Concentration (MIC) Test

Ampicillin, tobramycin, fosfomycin, chloramphenicol, and nitrofurantoin antibiotics, which belonged to five different antibiotic classes, were used. MIC values of antibiotics

according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) (2022) standards are given in Table 1.

Table 1. MIC values (µg/ml) of antibiotics specified for *Enterobacterales* according to EUCAST (2022) standards.

Group	**Antibiotic**	**MIC values**	
		S≤	**R>**
Penicillin	Ampicillin	8	8
Phosphonic	Fosfomycin	8	8
Aminoglycoside	Tobramycin	2	2
Nitrofuran	Nitrofurantoin	64	64
Phenicol	Chloramphenicol	8	8

S: susceptible, R: resistant

In order to determine the effect of the bacteriophage cocktail on the antibiotic resistance of *E. coli* strains, the MIC values of the isolates were determined using the broth microdilution method specified in the international standards of CLSI (Clinical and Laboratory Standards Institute) (2018). In the study, three groups were formed: the *control group*, which was not treated with phage, the *phage group* in which the phage and bacteria were treated simultaneously, and the *phage mutant group*, which was formed by the survivors after treatment with phage. For the phage group, a phage cocktail was obtained by mixing equal volumes of three phages (M8A, M11A, and M12A) (10^{10} pob/ml), and their appropriate dilutions were made. Instead of 100 µl bacteria, 50 µl bacteria (10^5 cfu/ml) and 50 µl phage cocktail (10^7 pob/ml) were added to the wells (MOI 100). In the phage mutant group, phage-resistant bacterial cells that could survive after treatment with phage were used. For this purpose, after the active test bacteria (10^2 cfu/ml) phage cocktail (10^{10} pob/ml) was incubated overnight at 37°C, phage mutant bacteria were separated from the medium by centrifugation. Subsequently, the obtained bacteria were used in the MIC test. The test was performed in three parallel repetitions.

RESULTS AND DISCUSSION

According to the results, it was determined that all *E. coli* O157 isolates were sensitized in the fosfomycin *phage group* and in the *phage mutant group* with chloramphenicol. It was determined that all the strains resistant to nitrofurantoin in the *phage group* became sensitized in generic *E. coli* isolates. However, MIC values were significantly increased in the *phage group* in all ampicillin-resistant strains. For tobramycin, an increase in MIC values was observed in all isolates in the *phage group*, while sensitization was detected in the *phage mutant group*. The MIC values and resistance profiles of the isolates after phage cocktail administration are shown in Table 2.

Table 2. MIC values and resistance profiles of *E. coli* isolates.

	Control Group (µg/ml)					**Phage Group (µg/ml)**					**Phage Mutant Group (µg/ml)**				
	AMP	**FM**	**N**	**TOB**	**CPL**	**AMP**	**FM**	**N**	**TOB**	**CPL**	**AMP**	**FM**	**N**	**TOB**	**CPL**
P21x	2048	32	128	128	512	>8192	16	256	>256	>1024	256	128	64	32	512
P91	4096	16	128	128	256	>8192	32	128	>256	512	512	64	64	32	512
P67b	4096	128	32	256	512	>8192	2	16	256	8	8	64	32	8	8
P106	1024	16	256	2	512	>8192	64	128	32	64	32	32	64	2	128
120GA	8	64	64	1	16	8	2	32	8	1	8	256	64	1	8
19KA	2048	16	64	4	32	>8192	2	64	2	1	128	64	64	4	8
180KD	4096	128	64	256	512	>8192	1	16	256	1	8	128	64	8	8
10Ko	4096	16	64	4	16	>8192	2	64	8	1	256	64	64	2	8
	Control Group					**Phage Group**					**Phage Mutant Group**				
	AMP	**FM**	**N**	**TOB**	**CPL**	**AMP**	**FM**	**N**	**TOB**	**CPL**	**AMP**	**FM**	**N**	**TOB**	**CPL**
P21x	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
P91	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
P67b	R	R	S	R	R	R	S	S	R	S	S	R	S	R	S
P106	R	R	R	S	R	R	R	R	R	R	R	R	S	S	R
120GA	S	R	S	S	R	S	S	S	R	S	S	R	S	S	S
19KA	R	R	S	R	R	R	S	S	S	S	R	R	S	R	S
180KD	R	R	S	R	R	R	S	S	R	S	S	R	S	R	S
10Ko	R	R	S	R	R	R	S	S	R	S	R	R	S	S	S

AMP: ampicillin; FM: fosfomycin; N: nitrofurantoin; TOB: tobramycin; CLP: chloramphenicol

The results of the study revealed that the combinations of phage and antibiotics varied according to the strain and the antibiotic. Other researchers have reported similar results. For example, in an *in-vitro* study conducted by Lin et al. (2018), the interaction of *Pseudomonas aeruginosa* between phage and antibiotics was investigated, and it was determined that the synergy was dependent on both the strain and the antibiotic. In the study, a lytic phage and ciprofloxacin, amikacin, and colistin were used separately and in combination against the clinical strain of *P. aeruginosa* and a synergistic effect was observed. However, complete inhibition of bacterial growth occurred only when the phage was combined with ciprofloxacin. In contrast, in another clinical *P. aeruginosa* strain, no synergistic effect was observed when the same phage was combined with amikacin, aztreonam, ciprofloxacin, colistin, or tobramycin (Lin et al., 2018). In another study, Jansen et al. (2018) reported that a T4-like phage (KARL-1) infecting the multidrug-resistant *Acinetobacter baumannii* strain showed significant synergy with meropenem but more moderate synergy with ciprofloxacin or colistin. In this thesis study, the most significant increase in MIC value was observed in ampicillin and tobramycin in groups where phage and antibiotic were administered simultaneously. The opposite sensitization was observed in the phage mutant groups of these antibiotics. Ampicillin inhibits cell wall synthesis by causing a blockade of the transpeptidase enzyme (Bereda, 2022). Conversely, tobramycin causes inhibition of protein biosynthesis by irreversible binding of aminoglycoside to the 30S subunit of the bacterial ribosome. Obtaining similar results, regardless of strain, against two antibiotics that act in different mechanisms in the study can indicate that phage-antibiotic combinations are antibiotic-dependent.

In this study, it was observed that the effect of phage and antibiotics also varied depending on the strain. For example, it was observed that the MIC values of the generic *E. coli* strains in the phage mutant group in nitrofurantoin decreased, and they went from resistant to sensitized.

It was determined that the MIC values of *E. coli* O157 strains of the same group did not change. Nitrofurantoin acts on bacteria by inhibiting various enzymes, damaging protein synthesis and genetic material (McOsker, 1994). The fact that phage mutant *E. coli* O157 strains are more pathogenic than generic strains and are more capable of surviving phage treatment may influence the profile of resistance to nitrofurantoin. However, simultaneous treatment of phage and antibiotic for fosfomycin caused sensitization in *E. coli* O157 isolates, while an increased MIC value was noted in *E. coli* O157 in the phage mutant group. Considering that fosfomycin inhibits cell wall synthesis by inhibiting peptidoglycan synthesis (Falagas et al., 2016), it may be possible that the simultaneous presence of phage and antibiotic may cause bacterial sensitization due to cell wall synthesis blockade. In phage mutant bacteria, on the other hand, resistance was slightly increased in those that survived due to the selective stress brought by the phage. It was observed that the sensitivity of *E. coli* O157 strains increased, and MIC values decreased in chloramphenicol. However, unlike fosfomycin, sensitization was observed in both phage and phage mutant groups in chloramphenicol. Although the decrease in MIC values in the phage group was higher than in the phage mutant group, the increased sensitivity in both groups suggests that chloramphenicol is related to the inhibition of protein synthesis of the cell by blocking the peptidyl transferase enzyme (Oong et al., 2022).

Many studies have been carried out in recent years to determine the synergistic effect of various phages and antibiotics. These studies show that the use of phages with antibiotics increases the effect of antibiotics and is effective in preventing bacterial resistance. Engeman et al. (2021) used a combination of ceftazidime, ciprofloxacin, gentamicin, and meropenem to determine whether a cocktail consisting of five phages showing lytic effects against *P. aeruginosa* increases antibiotic activity. The investigators found that *in-vitro* combination therapy caused a significant increase in the susceptibility of multi-resistant strains to antibiotics and reported that treatment with ceftazidime, meropenem, gentamicin, or ciprofloxacin in the presence of phages increased the number of *P. aeruginosa* susceptible to these antibiotics in 63%, 56%, 31%, and 81%, respectively. In addition, in a mouse dorsal wound model, seven out of eight mice treated with a combination of ceftazidime and phage cocktail for three days had no detectable bacteria in their wounds at day 4, whereas it was detected that all mice treated with ceftazidime alone or a phage cocktail had ~10^7 cfu bacteria at the wound site. The researchers also tested *P. aeruginosa* bacteria isolated from post-treatment mouse wounds in a waxworm model and observed reduced virulence. Treatment with a phage cocktail in combination with antibiotics has been reported to result in both resensitization of *P. aeruginosa* to antibiotics *in-vitro* and a synergistic reduction in bacterial load *in-vivo*.

There are various mechanisms behind the phage-antibiotic synergy (Segall et al., 2019). In a study by Tkhilaishvili et al. (2018) on the synergy between phage therapy (SB-1) and antibiotics against vegetative and biofilm forms of methicillin-resistant *Staphylococcus aureus* (MRSA), it was reported that antibiotics are effective in killing the vegetative form but ineffective in killing the cells in the biofilm. However, combinations of phages with certain antibiotics markedly reduced bacterial growth within biofilms. The study reported that the simultaneous use of phage SB-1 with rifampin or daptomycin resulted in the synergistic killing of cells within biofilms. It was concluded that pretreatment of planktonic or biofilm-forming cells with phage SB-1 followed by a combination with any of the five antibiotics tested (rifampin, daptomycin, fosfomycin, ciprofloxacin or vancomycin) was more effective than co-treatment in synergistically inhibiting bacterial growth. Researchers have also reported that phage SB-1 not only disrupts the exopolysaccharide component of the MRSA strain biofilm but also reduces the titer of metabolically inactive cells such as stationary phase cells or persisters (Tkhilaishvili et al., 2018). These results are of particular interest, as some phages lyse only actively growing bacterial cells.

CONCLUSIONS

In this study, it was determined that combinations of phages and antibiotics caused sensitization in phenotypic antibiotic resistance in strains with multi-antibiotic resistance, but the synergistic effect of phage and antibiotics showed great variability according to the strain and antibiotic. However, further molecular studies are required to elucidate the mechanisms of phage-antibiotic synergism clearly. Along with antibiotics, phages can potentially prolong the effective life of antibiotics in our 'pharmaceutical arsenal', broaden the spectrum of these drugs, and greatly reduce the burden of last-resort drugs and preserve them for future use. By exploiting the synergistic effect of phages and antibiotics, it is envisaged not only to increase clinical efficacy against multi-antibiotic-resistant bacteria but also to slow or reverse the incidence of antibiotic-resistant bacterial pathogens potentially.

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A PRELIMINARY STUDY ON THE EFFECTS OF MANURE AND ADDITIONAL PHOSPHORUS FERTILIZER IN DIFFERENT RATIO ON FOUR-WINGED SALTBUSH

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ABSTRACT

Pasture improvement is carried out worldwide by using shrub species in regions with marginal climate and soil structure, such as drought and salinity. The four-winged saltbush *(Atriplex canescens* (Pursh) Nutt.) belonging to the *Chenepodiceae* family is one of the shrub species used in the world, especially in the improvement of pastures in arid regions, as it provides quality feed to animals by remaining green during the dry feed period. It is also used to reclamation saline soils by absorbing the salt in the ground. Various researchers have reported that phosphorus deficiency is observed in plants because agricultural soils of Türkiye generally contain high reaction and lime, low organic matter and especially in the pastures of Central Anatolia region because of the arid climate and low temperature, the diffusion coefficient in the soil decreases, and the plants cannot take phosphorus sufficiently. For this reason, in this study, which was established in three replications according to the randomized complete block design, manure and additional phosphorus fertilizer at different rates (Control, Manure (M), M + 40% P, M + 60% P, and M + 100% P) were applied to four-winged salt bushes. The study examined morphological characteristics such as plant height, canopy diameter, new shoot stem diameter, leaf length, and leaf width in the vegetative and generative stages of the four-winged saltbush. In addition, in this preliminary study, the N, P, and K contents of plants in the generative phase and the root contents of these elements at the end of the growing season were examined. In general terms, it can be stated that the morphological characteristics of plants grow more in the generative period than in the vegetative period. In addition, the highest plant height of 115 cm and the tallest plant diameter of 156.25 cm were obtained only from manure (M) applications in the generative period. As a result of the nutrient analysis of the samples taken from the young shoots of the plant during the generative period, the highest protein and N contents were obtained from M+60%P treatment with 18.63% and 2.98%, respectively, and the highest P content was obtained from manure (M) treatment with 0.31%. In the samples taken from the roots at the end of the growing season, the highest N content was obtained from M+60%P treatment with 2.49%, while the highest P content was obtained from manure (M) treatment with 0.357%. This situation can be explained by the fact that plants send the nutrients they produce at the end of the growing season to their reserve nutrient stores in the root zones. As a result of this study, which is a preliminary study for four-winged saltbush fertilization studies, it can be stated that only manure (M) application is sufficient to increase plant growth in phosphorus-deficient soils considering sustainability.

Keywords: Four-winged saltbush, Fertilizer, Manure, Pasture improvement, Phosphorus, Sustainability,

INTRODUCTION

The utilization of fodder plants in rangelands by mowing and grazing causes the nutrients taken by the plants to be depleted in the soil, and the continuation of this situation for many years causes nutrient deficiencies in the soil. This issue is among the factors that reduce pasture productivity (Çınar et al., 2018). For these reasons, Türkiye's pasture yield is approximately 70 kg da^{-1}, one-third of the world's pasture yield (Babalık and Fakir, 2017). In our country, herbaceous plants are primarily used in pasture improvement studies to increase pasture yield. However, the development of herbaceous species takes a long time, and it takes longer and more costly to achieve success in breeding. For this reason, pasture improvement is carried out by using shrub species in regions with marginal climate and soil structure, such as drought and salinity (Acar et al., 2013).

The four-winged saltbush (*Atriplex canescens* (Pursh) Nutt.) belonging to the *Chenopodiceae* family is a C4 forage shrub. It is dioecious, and male and female flowers can be on different plants, or male and female organs can be found in the same flower (Tilley et al., 2012). The plant is a polymorphic species, evergreen in warm climates and deciduous in cold climates (Tan and Temel, 2012). *Atriplex* sp. species provide green fodder to animals during the dry feed period because they are green in the summer months in places where the Mediterranean climate is dominant. *A. canescens*, which grows well in arid and semi-arid areas, grows naturally in the arid regions of North America, from Mexico to Canada. In addition to these regions, it also grows in Australia and areas with a climate similar to the Mediterranean. Saltbush species are also widely cultivated in the Middle East (Erdoğdu et al., 2013). In our country, in recent years, this plant has been included in pasture improvement in the Central Anatolia region, especially in Aksaray and Karaman. In addition, the four-winged saltbush shows salt resistance by accumulating sodium in its specialized salt sacs and thus can grow in saline and sodic soils (Yuan et al., 2016). With this feature, it can also be used in the reclamation of soils in marginal areas' rangelands with saline and boron toxicity.

Manure, which has an essential place in the fertilization of pastures due to its organic origin, is a widely applied pasture improvement method because it increases the organic matter content of the soil and ensures water retention in the soil (Bakır, 1985; Gregorich et al., 1994). In general, manure has high nitrogen content and low phosphorus content. Therefore, manure should be given to pasture areas with phosphorus (Bakır, 1985). Eyüpoğlu (1999) reported that phosphorus deficiency was observed in 58% of the soils of Türkiye. Therefore, it is crucial to supplement phosphorus by fertilization in agricultural production areas in our country. Phosphorus fertilizers increase the roughage rate of plants grown in the pasture (de Groot et al., 2003; Çomaklı et al., 2005). In addition, phosphorus fertilizers also affect the quality of the product and contribute to increasing the tolerance of plants to winter conditions and drought (Kantarcı, 2000; McCauley et al., 2009; Kacar, 2020). When the effect of phosphorus fertilizers on soil was examined, it was found that phosphorus fertilizers that were not taken into the plant structure accumulated in the production areas and plants benefit from this phosphorus accumulated in the soil in the following production period (Moschler et al., 1957). For this reason, as a preliminary study, which is the first of its kind in the region, this study was carried out to determine the effects of manure and different rates of phosphorus fertilizers added to manure on the botanical characteristics and nutrient content of the plant, and the organic matter and available phosphorus in the soil at the end of the growing season on four-winged saltbush grown under Konya conditions.

MATERIAL AND METHOD

Four-winged saltbush seedlings obtained from Konya Forest Nursery Directorate without tubes were grown with tubes in the greenhouse on February 5, 2020 (Figure 1). The plants were planted in S.U. Faculty of Agriculture Prof. Dr. Abdülkadir AKÇİN experimental field

(Selçuklu- Konya) on November 15, 2020, with a row spacing of 3 m and a row spacing of 3 m (Erdoğdu et al. 2013). Parcel spacing was adjusted to be 1 m. Our study was planned as a preliminary study to determine the responses of four-winged saltbush plants to fertilizer, and each parcel contained one plant due to the limited supply of the plant (Karimi et al., 2021). In this experiment, which was established with three replications according to the Randomized Complete Block Design (RCBD), fertilization was carried out on December 29, 2020, during the period of rainfall by following the meteorological data due to changing climatic conditions (Acar et al., 2020). According to the long-term climate average (1990-2019), total precipitation is 330 mm; 290 mm was recorded in the planting year (2020), and 360 mm in 2021. However, the long-term climate mean of monthly average temperatures is at 11,8 ℃, in the planting year (2020) was reported at 13,1 ℃, and 12,5 ℃ is in the harvest year (2021).

Figure 1. Images of plants grown in the greenhouse, planted in the field, and after fertilization.

In the research, a mixture of sheep and cattle manure was used as manure with a moisture content of 5.27%. The chemical content of the barnyard manure used in the research is given in Table 1, and the soil analysis results are shown in Table 2.

Table 1. The result of fertilizer analysis used in the experiment.

Parameters	**Value**	**Parameters**	**Value**
pH	7.09	EC(mS cm^{-1})	7.90
Organic Matter (%)	6.41	Total N (%)	8.53
Water Soluble Ca	1427	Total Ca	76832
Water Soluble K	29413	Total K	30460
Water Soluble Mg	444	Total Mg	6677
Water Soluble P	2127	Total P	5047
Water Soluble B	43	Total B	53
Water Soluble Cu	5	Total Cu	25
Water Soluble Fe	45	Total Fe	4912
Water Soluble Zn	6.00	Total Zn	86

Table 2. Soil analysis results of the trial area

Parameters	Values	Parameters	Values
Texture class	Clay Loam	Available K (mg kg^{-1})	144
pH	7.54	Extractable Na mg kg^{-1}	36
EC (µS cm^{-1})	290	Extractable Mg (mg kg^{-1})	239
Lime (%)	43	Extractable Ca (mg kg^{-1})	4229
Organic Matter (%)	1.43	Available Zn (mg kg^{-1})	1.39
Inorg. N (NH_4 +NO_3-N) (mg kg^{-1})	17.40	Available Fe (mg kg^{-1})	4.39
Available P (mg kg^{-1})	6.30	Available Mn (mg kg^{-1})	15.41
Available B (mg kg^{-1})	0.90	Available Cu (mg kg^{-1})	1.38

The preliminary study applied six treatments (control, manure (M), M + 40%, M + 60% P, M + 80%, and M + 100% P). Since Bakır (1985) recommended 1.0 t da^{-1} of manure for pastures, this amount was applied to the crown projections of four-winged saltbushes according to the application subjects. In addition, 5 kg P_2O_5 da^{-1} phosphorus application for four-winged saltbush was used with TSP (Triple superphosphate) at 100% phosphorus level, and the rates of other levels were adjusted and applied according to this value. In the study, irrigation was done once in July 2021, and hoeing was done twice during spring.

After awakening in spring, some morphological characteristics were examined during the vegetative period (June 2021) and the flowering and transition to the generative period (August 2021). In addition, to determine the nutrient content of the plants during the generative period, plant leaves that had just completed their development during the transition to the generative period just below the main branches or trunk and in full sunlight were taken as representative samples and subjected to certain pre-treatments (washing, drying, and grinding) to be used in the analyzes (Kacar, 2014). In addition, at the end of the growing season in November, root and soil samples were taken from the plant's rhizosphere at a depth of 30 cm to determine the nutrient content (Figure 2).

Morphological properties measurements carried out in the study are given below.

Plant height (PH) (cm): Without disturbing the natural state of the plant, the distance from the soil surface to the very tip of the stems was measured and recorded in cm.

Canopy diameter (CD) (cm): The longest and shortest diameters were measured without disturbing the plant's natural state, and the average value was determined as canopy diameter in cm.

New shoot stem diameter (NSSD) (mm): The stem thickness of the branch of 5 new shoots was measured with calipers and recorded in mm (Aygün and Olgun, 2018).

Leaf length (LL) (mm): The length of 5 leaves of the plant that had completed their development was measured and recorded in mm.

Leaf width (LW) (mm): The thickness of the widest part of the five leaves of the plant that had completed their development was measured with calipers and recorded in mm (Özköse, 2012).

Hay Yield (HY) (kg da^{-1}): Plant samples were taken based on 10-14% benefit of livestock from each plant, and the samples were kept in an oven at 58°C until they reached constant weight and their dry weights were found and calculated in kg da^{-1} (Tamkoç, 1992; Mellado et al., 2012).

Chemical analysis in plants carried out in the study is given below.

Crude Protein Ratio (%): N content was determined in the LECO C/N analyzer according to Dumas Combustion Method given in AACC (2000). The crude protein ratio was recorded as % using the data obtained (N x 6.25) (Merrill and Watt, 1955).

Nutrients [P, K (%)]: 0.2 g of dried sample was weighed, 5 ml of HNO_3 and 2 ml of H_2O_2 were added and dissolved in the microwave. One NIST SRM 1573a leaf sample was added to the

40-cell microwave set as a witness and reference material to ensure the reliability of the analysis. The dissolved samples were made up to 20 ml with distilled water, and the samples were filtered through blue banded filter paper and the total P, K amounts in the filtrate were determined by ICP-OES (Kacar and Inal, 2010).

The soil analysis carried out in the study is given below.

Organic matter (%): After determining the organic carbon by the Walkey-Black method, it was multiplied by a coefficient of 1.724 (Tüzüner, 1990).

Available P (mg kg^{-1}): Determined by Olsen's $NaHCO_3$ method (Bayraklı, 1987).

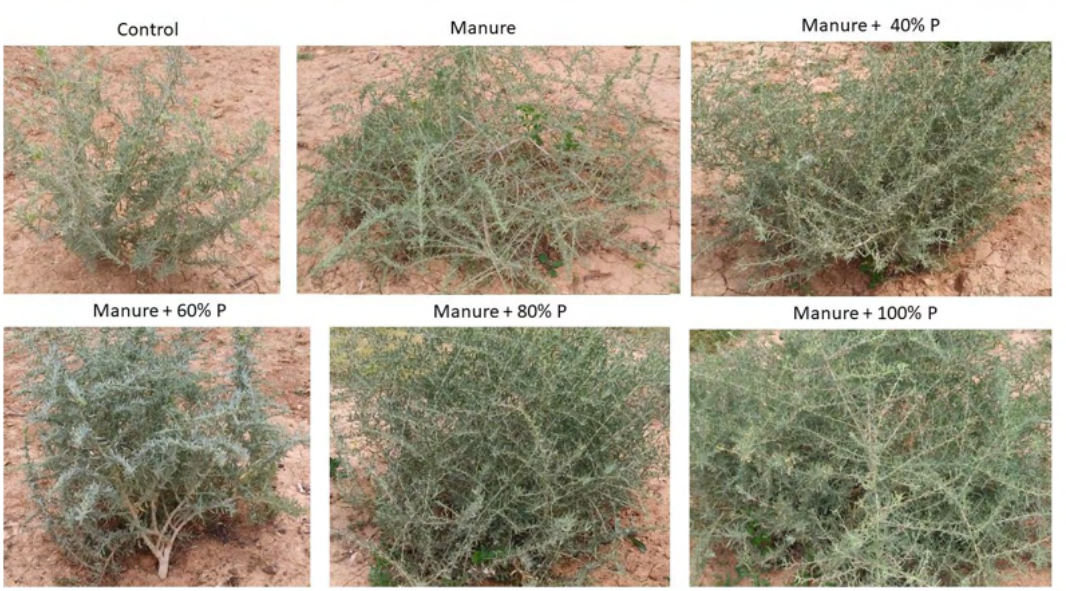

Figure 2. Plant responses to fertilization in September 2021 (30.09.2021)

The data on morphological traits were analyzed in RCBD with two factors (Treatment and Period). The other characteristics were analyzed in RCBD with one factor (Treatment) in the JMP 7 package program, and the Tukey HSD test was performed for each significant trait (Sall et al.,2017).

RESULTS AND DISCUSSION

Results of Morphological Characteristics

The summary of the analysis of variance for the measurements taken within the scope of morphological traits of the research is given in Table 3, and the mean values of the interactions are presented in Figures 3-8. According to the results of the analysis of variance shown in Table 3, it was determined that the period factor was significant at a 1% level in all traits except leaf width. In addition, canopy diameter and leaf length were found to be statistically significant at 5% level, and other characteristics were found to be insignificant in terms of treatment factor. Treatment x period interaction was effective only in leaf length at 5% level, while this value was statistically negligible in other traits.

Table 3. The Summary of variance analysis (F value)***

Source of Variation	DF	PH	CD	NSSD	LL	LW	HY
Total	35						
Replication	2	0.68	0.26	2.38	2.19	0.04	1.31
Treatment (T)	5	2.12	2.98*	2.39	3.49*	2.30	1.99
Period (P)	1	48.75**	78.77**	20.44**	13.18**	0.75	44.91**
T*P	5	0.90	0.99	0.42	2.62*	0.07	0.65
Error	22						
CV (%)		13,43	23.51	18.86	21.59	30.20	31.56

*p<0.05; **p<0.01; ***PH: Plant Height, CD: Canopy Diameter, NSSD: New Shoot Stem Diameter, LH: Leaf Length, LW: Leaf Width, HY: Hay Yield

When the effects of manure and additional phosphorus ratios applied to four-winged saltbush on morphological characteristics were examined, it can be stated in general terms that the treatments increased the growth of plants in the generative period. The most extended plant height of 115.00 cm was obtained from manure (M) application in the generative period (Figure 3). Similarly, in terms of canopy diameter, the largest diameter was obtained from M treatment with 156.25 cm (Figure 4). When the averages of the treatments in terms of canopy diameter were analyzed, it can be stated that M was in group a with 115.63 and control was in group b with 69.38 cm (LSD: 42.81).

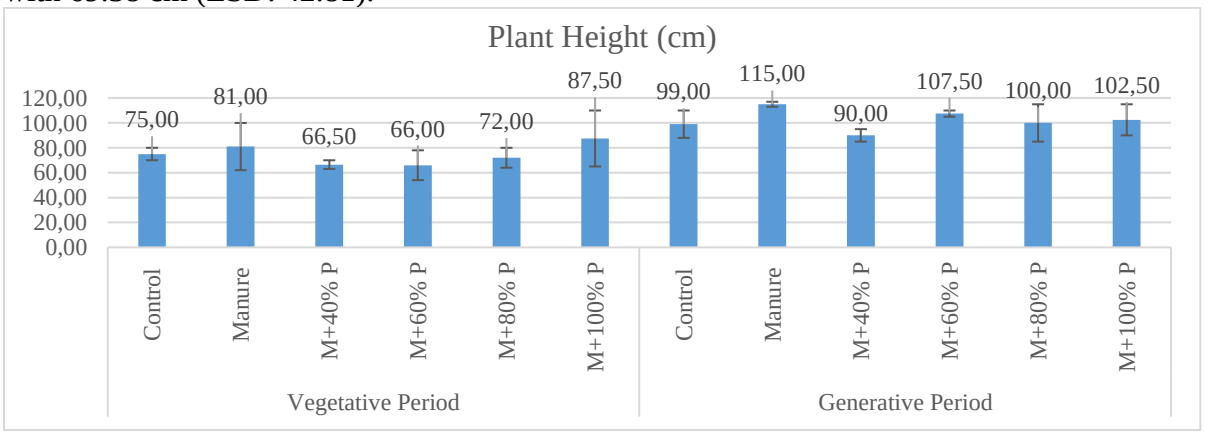

Figure 3. Plant height averages obtained in fertilizer applications to four-winged saltbush.

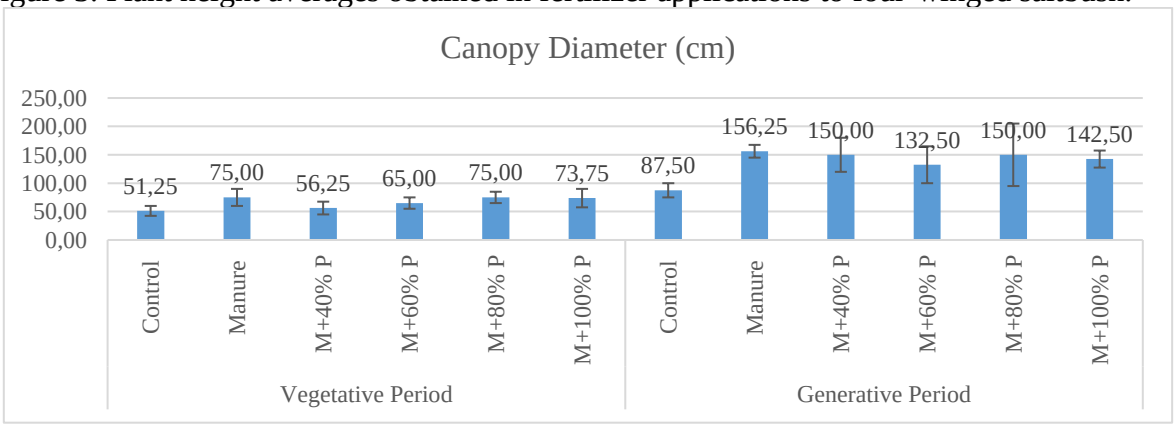

Figure 4. Canopy diameter averages obtained in fertilizer applications to four-winged saltbush

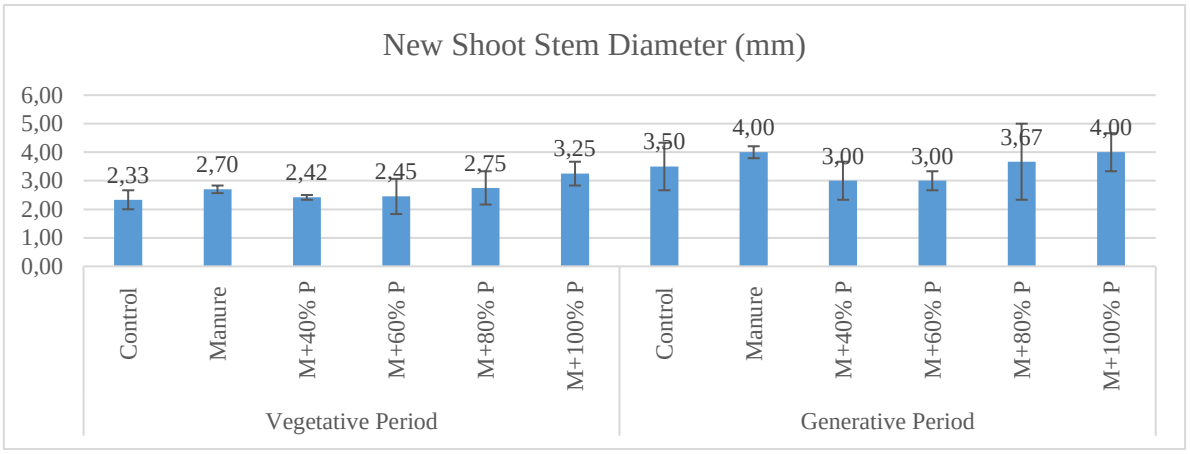

Figure 5. New shoot stem diameter averages obtained in fertilizer applications to four-winged saltbush.

When the effects of manure and additive phosphorus rates on the new shoot stem diameter of the four-winged saltbush were examined, it can be stated that M and M+100%P treatments had the largest diameter width of 4.00 mm in the generative period (Figure 5).

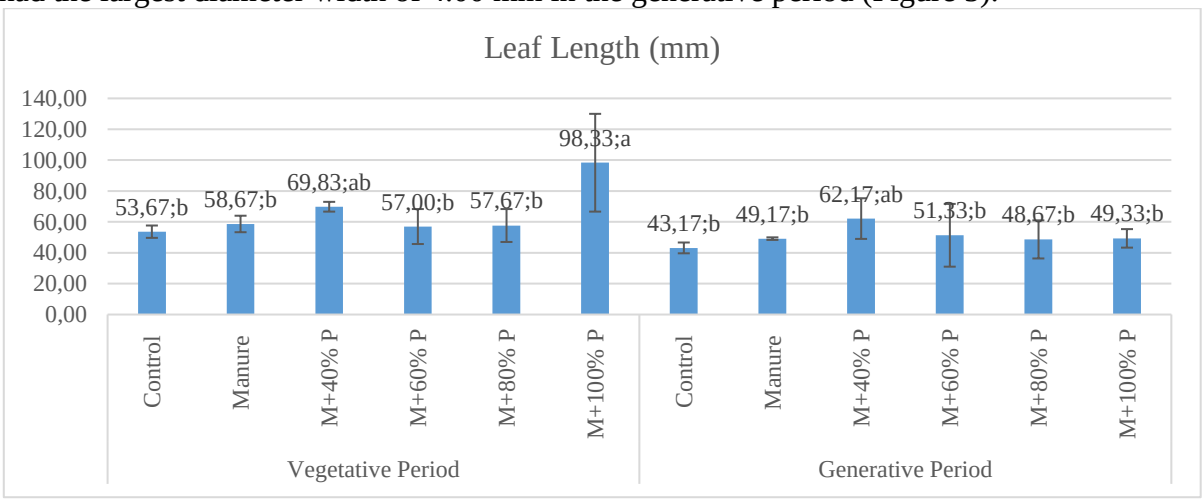

Figure 6. Leaf length averages obtained in fertilizer applications to four-winged saltbush (LDS: 37.36)

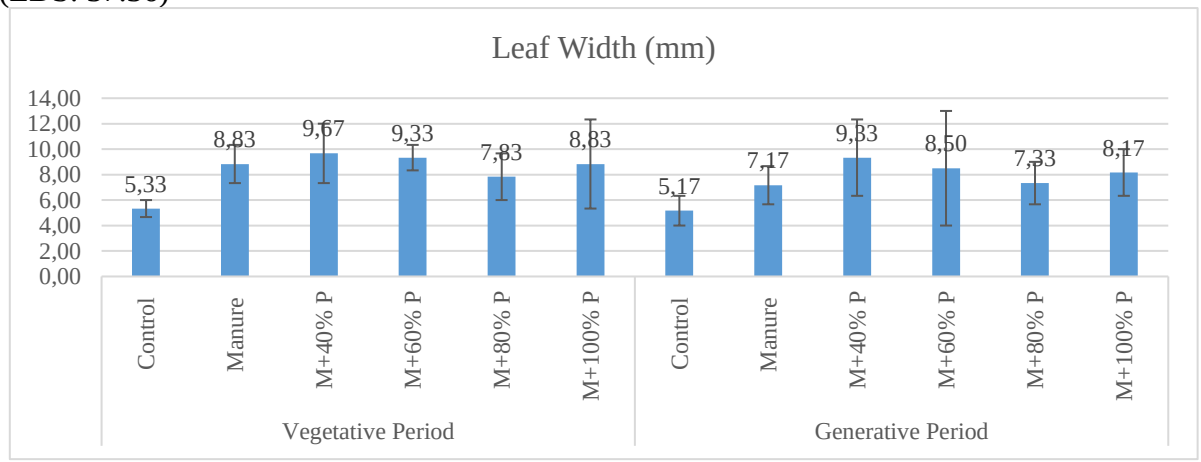

Figure 7. Leaf width averages obtained in fertilizer applications to four-winged saltbush.

According to Figure 6, in contrast to the other traits, the most extended value in leaf length (98.33 mm) was obtained from M+ 100% P treatments during the vegetative period (group a). In addition, the mean values of the treatments were statistically at a 5% significance level divided into two groups, and M+ 100% P application was in group a with 73.83 mm while the

control group (48.42 mm) was in group b (LSD: 22.62). The highest value in leaf width was obtained in the vegetative period, and it was 9.67 mm in the M+40% P treatment (Figure 7).

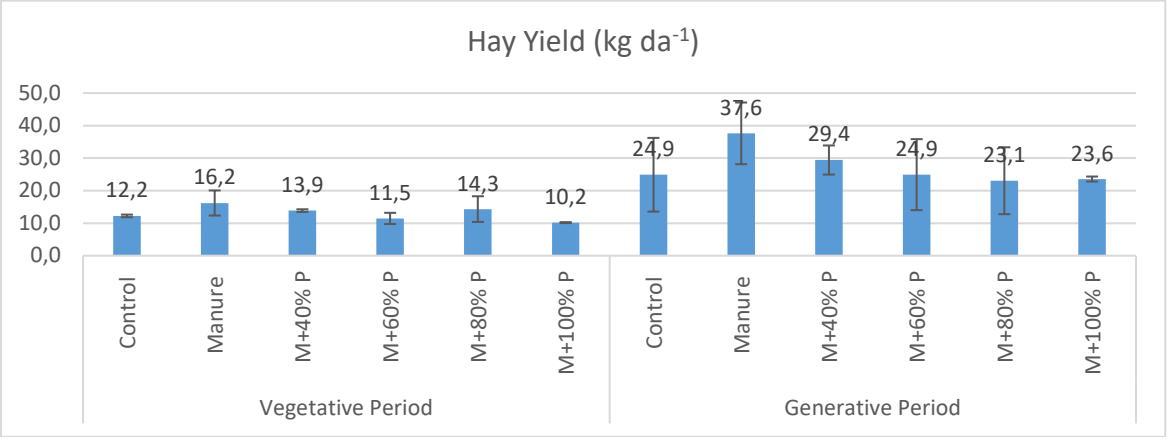

Figure 8. Hay yield averages obtained in fertilizer applications to four-winged saltbush.

The highest value of 37.6 kg da^{-1} was obtained from M treatments in the generative period regarding hay yield. The fact that these yield values were obtained in August is of particular importance due to the limitation of plant species that can remain green and have high yields in that period.

Results of Nutrient Content in the Generative Period

According to the analysis of variance results of crude protein, N, P, and K contents investigated in the generative period in Table 4, except for K content, the other traits were found to be significant at a 1% level. When the mean values and groupings given in Table 5 are analyzed, it can be stated that M, M+60%P, M+80%P, and M+100%P treatments are in group A regarding crude protein and N content. Numerically, the highest value was obtained from M+60%P treatment with 18.63% crude protein and 2.98 mg kg^{-1} N. However, the highest value in terms of phosphorus content was obtained from the M application with 0.31 mg kg^{-1} (group A). The highest value in terms of potassium content was recorded from the M+40%P treatment.

Table 4. Analysis of variance's summary of nutrient element contents analyzed in the generative period (F value)

Source of Variation	DF	Crude Protein	N	P	K
Total	17				
Replication	2	0.60	0.61	2.28	1.78
Treatment	5	17.34**	17.41**	9.99**	2.73
Error	10				
CV (%)		7.12	7.09	9.74	7.84

*:p<0.05; **p<0.01

Table 5. Mean values, standard error (SE), and groupings of nutrient element contents analyzed during the generative period.

	Crude Protein (%)	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})
Control	11.62 ^{B}	1.86 ^{B}	0.18 ^{C}	4.05
M	18.27 ^{A}	2.92 ^{A}	0.31 ^{A}	3.80
M+40% P	14.45 ^{AB}	2.31 ^{AB}	0.22 ^{BC}	4.06

M+60% P	18.63 ^{A}	2.98 ^{A}	0.27 ^{AB}	3.70
M+80% P	17.99 ^{A}	2.88 ^{A}	0.25 $^{A-C}$	3.38
M+100% P	17.82 ^{A}	2.85 ^{A}	0.26 $^{A-C}$	3.50
SE	0.676	0.108	0.014	0.17
LSD	4.35	0.69	0.09	-

Results of Nutrient Content in Root at the End of the Growing Season

When the summary of the analysis of the variance table of N, P, and K contents in roots was analyzed, all traits were found to be statistically significant at a 1% level. At the end of the growing season, the highest value was obtained from M+60%P treatment with 2.49 mg kg^{-1} in terms of nitrogen content in the root, while 0.36 mg kg^{-1} P and 1.64 mg kg^{-1} K content was determined from M treatment in terms of phosphorus and potassium content.

Table 6. Summary of analysis of variance for nutrient content in root at the end of the growing season (F value)

Source of Variation	DF	N	P	K
Total	17			
Replication	2	0.00	0.00	0.38
Treatment	5	2409.55**	137.48**	42.86**
Error	10			
CV (%)		1.68	4.93	14.21

*:p<0.05; **p<0.01

Table 7. Mean values, standard error (SE), and groupings of nutrient contents in root at the end of the growing season

	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})
Control	0.59 ^{F}	0.12 ^{C}	0.66 ^{CD}
M	1.64 ^{D}	0.36 ^{A}	1.64 ^{A}
M+40% P	1.81 ^{C}	0.23 ^{B}	1.36 ^{AB}
M+60% P	2.49 ^{A}	0.23 ^{B}	0.85 ^{C}
M+80% P	2.08 ^{B}	0.20 ^{B}	1.02 ^{BC}
M+100% P	0.78 ^{E}	0.24 ^{B}	0.19 ^{D}
SE	0.015	0.01	0.08
LSD	0.098	0.042	0.503

Soil Analysis Results at the End of the Growing Season

According to the soil analysis results given in Table 8, at the end of the growing season, the figures for soil organic matter and soil available P content were statistically significant at 1% level.

Table 8. Analysis of variance's summary of soil analysis results at the end of the growing season (F value)

Source of Variation	DF	Organic Matter	Available P
Total	17		
Replication	2	0.21	0.58
Treatment	5	43.93**	25.14**
Error	10		
CV (%)		7.02	30.88

*:p<0.05; **p<0.01

When the organic matter remaining in the soil at the end of the growing season was analyzed, it can be interpreted that there may be an increase in the amount of soil organic matter after M + 80%P application with 2.75% (Figure 9).

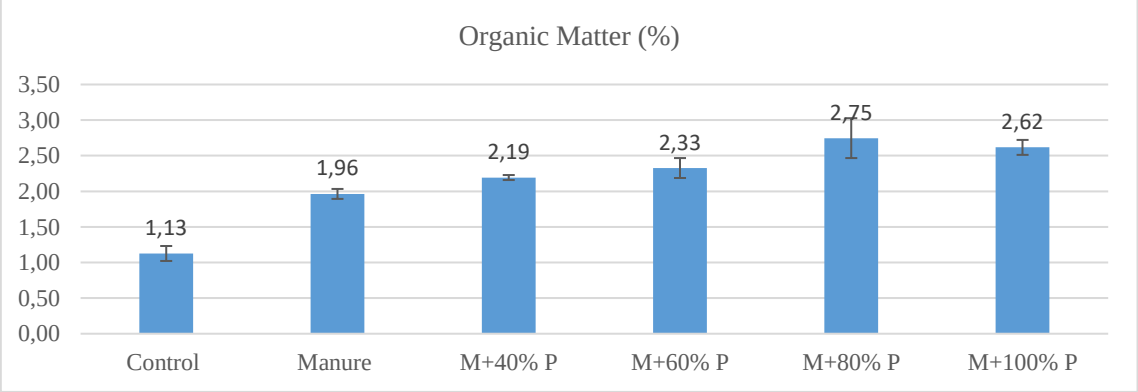

Figure 9. Means of soil organic matter at the end of the growing season (LSD: 0.564)

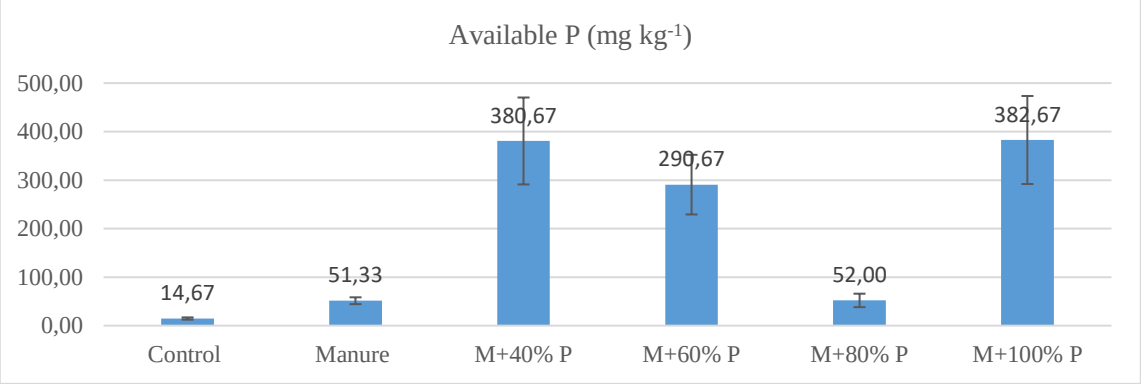

Figure 10. Means of available P in the soil at the end of the growing season (LSD: 223.86)

In terms of soil available P content, M + 100%P application (382.67 mg kg^{-1}) had the highest value, but since the aim of this study was to target the utilization of the additional phosphorus by the plant, M with 51.33 mg kg^{-1} and M + 80%P with 52.00 mg kg^{-1}, which have lower values, stand out (Figure 10).

Discussions

The four-winged saltbush has different levels of ploidy; the tetraploid (2n=36) and hexaploid (2n=54) species are short shrubs with a height between 60 and 150 cm and a leaf

width between 5-10 mm, while the diploid (2n=18) species are between 100 cm and 300 cm tall and have a narrow leaf width (2-5 mm) (Le Houérou, 2000). In addition, as the plant ages, the area covered by the plant on the soil increases. With this feature, it is one of the prominent plant groups in preventing erosion (Tan and Temel, 2012). The plant height ranging from 66.00 cm to 115.00 cm and leaf width ranging from 5.17 mm to 9.67 mm, which we obtained in our study, is close to the short shrub feature.

In northern Mexico, goats were grazed on control pastures and pastures where four-winged saltbush was predominant, and it was reported that there was a change in the diet of goats according to the seasons (spring, summer, fall) in 2007. In the study, in the pasture where saltbush was dominant, goats had an average of 58% four-winged saltbush in their diet, while this rate was 14% in the control parcel (Mellado et al., 2012). For this reason, while calculating the yield of four-winged saltbushes in our study, it was estimated by considering the utilization of the livestock between 10-15%. While 13.04 kg da^{-1} yield was obtained in the vegetative period, this value was recorded as 27.24 kg da^{-1} in the generative period (group A). In Montana, USA 1975, 535 kg ha^{-1} biomass was obtained in the greenhouse with nitrogen (37 kg pure N ha^{-1}), phosphorus (94 kg pure P ha^{-1}) fertilizers, and 67 kg ha^{-1} biomass was obtained with fertilization under field conditions. In the same study, 280 kg ha^{-1} biomass was obtained in the greenhouse, and 56 kg ha^{-1} biomass was obtained under field conditions in plots where fertilizer was not applied (Holechek, 1982). Four-winged saltbushes, which are not native plants of Türkiye, were grown from seeds brought from the USA in 2005 by the Ministry of Food, Agriculture and Livestock. Cuttings from these plants were planted at different planting distances (2 m and 3 m) in Eskişehir (Hamidiye) and Konya (Center and Karapınar) locations in our country in 2011. In the study, a dry leaf weight of 200 g $plant^{-1}$ (22 kg da^{-1}) was obtained at a 3 m planting distance, and it was stated that a 3.0 m planting distance came to the fore in terms of yield. Regarding quality, the highest values were obtained at a 2.0 m planting distance with 18.8% crude protein (Erdoğdu et al., 2013).

Koç et al. (2020) examined the nutrient content of four winged saltbushes planted in 2013 in soil with low organic matter and insufficient P in Konya, Türkiye, 2017, during the dry feed period. The study stated that it contained 10.09% crude protein, 1.76% K, and 0.15% P.

Karimi et al. (2021), who studied the effect of nitrogen and phosphorous fertilizers on four-wing saltbush under saline conditions, stated that applying nitrogen at a rate of 25 and 50 kg ha^{-1} increased shoot fresh weight. However, the phosphorous application had no meaningful impact on the four-wing saltbush performance irrigated with saline waters. Nowadays, microbial fertilizers are gaining importance rather than chemical fertilizers. Noshad et al. (2022), who studied arbuscular mycorrhizal fungi on four-wing saltbush, reported that the application of AMF (*Funneliformis phosphorus, F. mosseae*) increased the plant growth variables such as stem diameter, root length, shoot dry weights, and shoot to root ratio as well as nitrogen and phosphorus uptakes in the root. The application of both AMF types was practical as compared to the control. However, *F. mosseae* indicated better performance, especially regarding the effect on plant growth variables.

In the samples taken from the roots at the end of the growing season, the highest N content was obtained from the M+60%P treatment with 2.49%, while the highest P content was obtained from the M treatment with 0.357%. This situation can be explained by the fact that plants send the nutrients they produce at the end of the growing season to the root zones, their reserve nutrient stores (Altın et al., 2011).

Due to the utilization of additional phosphorus by the plant in soil applications, low amounts of phosphorus were detected in the M application (51.33 mg kg^{-1}) and M+ 80% P application (52.00 mg kg^{-1}). In similar studies (Jat and Ahlawat, 2006; Alam et al., 2007; Makinde et al., 2011), it was stated that the phosphorus uptake from the soil increased with the effect of chemical fertilizers and organic fertilizers. It has been determined that applying

chemical fertilizers and manure or only manure application is more effective because the manure mixed into the soil transforms the soil phosphorus and the phosphorus applied to the soil into a more useful form for the plants (Richardson, 2001). In another study that supports our findings, it was determined that organic fertilizer applications were more effective than chemical fertilizer applications, and it was stated that the presence of organic compounds may be due to the reduction or inhibition of phosphorus binding in the soil (Gatiboni et al., 2003).

CONCLUSIONS

In this study, manure and additional phosphorus fertilizer at different rates were applied to four-winged saltbush; it was determined that M and M+60% P treatments were prominent in terms of morphological characteristics and nutrient content. In contrast, the effect of M+80% P was significant in terms of organic matter in the soil at the end of the growing season. Still, the impacts of M and M+80%P treatments were substantial regarding available phosphorus. As a result of this study, which is a preliminary study for four-winged saltbush fertilization studies, it can be stated that only manure (M) application is sufficient to increase plant growth in phosphorus-deficient soils, considering sustainability. Considering the data obtained in the study, with the increase in organic compounds, they can be used together with chemical fertilizers, or they can be an alternative to chemical fertilizers. At the same time, they can cause a decrease in using chemical fertilizers.

According to the results of this preliminary study, in this and similar studies, because of the different responses of male and female plants to fertilizer, there should be male and female plants in each parcel, and the research should be perennial. In addition, we opinion that for combusting climate change, quality and especially the cold resistance of plants with phosphorus fertilizers is one of the research topics that should be studied in the future regarding sustainability.

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AGRONOMIC PROPERTIES AND L-DOPA CONTENT OF BROAD BEAN (*Vicia faba* L.) GROWN IN DIFFERENT WEED DENSITY

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ABSTRACT

Due to plants not relocating, they adopt allelopathy as a survival strategy when environmental conditions are unfavourable. This situation consists of releasing chemical compounds into the environment that can positively or negatively affect the growth and development of neighbouring plants. One of these chemicals is L-Dopa (L-3,4-dihydroxyphenylalanine), which is not an amino acid and is mostly in velvet bean (*Mucuna pruriens*) and broad bean (*Vicia faba*) plants. Weeds are an important problem in regions like Samsun, where winters and springs are rainy and warm. However, the broad bean has less weed density than other plants. This study was planned to determine the relationship of this situation with the L-Dopa production feature of broad beans. In the experiment, Lara variety, 5 different treatments (weed-free control, weedy control, 1-time hand hoeing, 2-time hoeing, 3-time hoeing) were used in a randomized block design with three replications. Sowing was done on November 3, 2022, with 50 cm row spacing. One week after the last hoeing (April 4, 2023), samples were taken from the roots and stem parts of the plants for L-Dopa analysis. Both fresh pod and dry seed components were made in the plots. In the features, weeds belonging to other families (*Veronica* sp, *Scandix pecten-veneris*, *Lupinus* sp, *Cirsium arvense*, *Fumaria officinalis*, etc.) were more common than wheatgrass. It has been determined that the plant height of the broad bean is 99-131 cm, and it is the longest in weed control. The fresh pod yield was between 5186-7504 kg per decare, and the highest was obtained from the weed-free control; the dry seed yield was between 375.9-481.5 kg, and the highest in hoeing three times. L-Dopa analyses are not yet complete.

Key Word: Broad bean, weed density, L-Dopa

INTRODUCTION

The concept of allelopathy was defined by Hans Molisch (1937) as the mutual suffering of organisms, which was formed by combining the Greek words "Allelo" and "Pathos" (Yılmaz et al., 2021). Conversely, plants use allelopathy as a survival strategy under unfavourable environmental conditions. Allelopathy is the chemicals secreted by plants from different secretory organs, such as roots, leaves, fruits and stems, which positively or negatively affect other plants' germination (İkincikarakaya, 2022). Plants with known allelopathic properties constitute an essential resource for sustainable agriculture. The allelopathic effects of some cultivated plants have an important role in alternation systems and yield increase and in the control of weeds, diseases and pests as a natural method of struggle (Yılmaz et al., 2021).

The high adaptability of the broad beans has allowed it to be cultivated in many countries (Köse et al., 2021). The broad beans have a cultivation area of 2.72 million ha

worldwide. The country with the highest cultivation is China, with 804.3 thousand ha. In Turkey, broad beans have a cultivation area of about 2.6 thousand ha (FAO, 2021). Although it is commonly cultivated in the Aegean, Marmara and Mediterranean regions, it is one of the indispensable products in small-family agriculture, especially in the Black Sea coastal region. The studies we have carried out in Samsun for many years have shown that the broad bean is very suitable for the ecology of the region, that winter plantings are highly yielding, have nitrogen fixation ability and soil healing effects and produce L-Dopa (Bozoğlu and Topal, 2011; Karyel et al., 2016; Topal et al., 2020; Bozoğlu and Bezmen, 2021; Oğuz and Bozoğlu, 2022).

One of the chemicals with an allelopathic effect is L-Dopa (L-3,4-dihydroxyphenylalanine). L-Dopa is a non-protein amino acid secreted within the plant (Soares et al., 2014). It is primarily found in *Mucuna pruriens* (velvet bean) and *Vicia faba* (broad bean) plants and is used in treating Parkinson's disease. The broad bean is one of the few plant species known to produce L-Dopa and has the potential to be developed as a functional food product for Parkinson's disease (Hu et al., 2015). In addition, some studies show that L-Dopa may be effective as a herbicide and insecticide (Soares et al., 2014).

In the study conducted to determine the L-Dopa content of the leaves, flowers and fruits of broad beans genotypes in Samsun, 22 genotypes were used. The difference between genotypes in terms of the amount of L-Dopa in the flowers and fresh fruits of the broad bean was found to be important. This study determined that the amount of L-Dopa in flowers was higher than in leaves and pods (Topal and Bozoğlu, 2016).

In a study to determine the effect of L-Dopa on herbicides, wheat and barley were used as control crops. *Sinapis arvensis*, *Cirsium arvense*, *Papaver rhoeas* and *Lamium amplexicaule* weeds have been found. According to the study results, concentrations of L-Dopa of 1500-3000 mg/L showed a suppressive herbicide effect on the weeds studied without significantly affecting the development of wheat and barley. The most affected species was found to be the *Papaver rhoeas*. In general, it was concluded that the decrease in root elongation was more significant than the trunk elongation (Topal and Kocaçalışkan, 2006).

Etemadia et al. (2018) similarly reported in their study that the highest L-Dopa content was in seedlings, leaves, flowers, young pods, grown pods, roots, and stems, respectively, and increased with drought stress.

In a study examining L-Dopa, vicin and convisin at different stages (seedling, vegetative cycle, flowering and maturity) of broad beans grown under greenhouse conditions, the highest L-Dopa was found in young flower buds. They reported that root and stem are similar and rich in L-Dopa production (Duan et al., 2021).

It is necessary to use environmentally friendly and harmless practices in all cultivation techniques, from seedbed preparation to harvesting and threshing in plant production. Weeds are an important problem in regions such as Samsun, where winters and springs are rainy and hot. Especially when it comes to good agricultural practices and struggle without using herbicides in organic agriculture, activating the cultural struggle and the natural struggle abilities of the grown plants is crucial. Broad beans are a plant of this nature. In our observations, there is less weed density in the broad bean areas than in many other plants in the plant-growing areas. This situation, and very few studies in the literature, have given us the idea of investigating the relationship between the broad bean's L-Dopa-producing property and weed density. This study was planned to determine the effect of different weed densities on the agronomic characteristics of the pod and the affect on L-Dopa production.

MATERIAL AND METHOD

In this study, Lara broad beans variety developed for fresh consumption was used. The experiment was carried out under the conditions of the university's campus on Samsun grounds. The trial soils were clayey, pH neutral, very little lime, and medium in organic matter.

The experiment was set up in a randomized block design with 3 replications. In the experiment, 5 different weed densities (Y_1:weed-free control, Y_2:weedy control, Y_3: 1-time hand hoeing, Y_4:2-time hand hoeing, Y_5:3-time hoeing) were done. The effects of these processes on the agronomic characteristics of the broad beans and the L-Dopa content of the root stems were investigated. Sowing was done by hand on November 3, 2022, in 3 m long rows consisting of 5 rows, 50 cm between rows and 10 cm above rows. Weed removal operations were carried out from mid-December to mid-March. Samples for L-Dopa analyses were received on April 4, 2023, when weed removal was complete. After the dry weights of the samples were determined, they were ground and stored. As of the second half of April, the plants marked on the plots were harvested four times. The ratio of each harvest to the total fresh fruit yield is defined separately. Dry seed harvests were carried out in June, and properties of dry seed were made during this period.

RESULTS AND DISCUSSION

The broad bean is a plant that can be grown in our region for the winter without irrigation. The production of broad beans for both vegetable and dry seeds allows another summer product to develop after harvest (Bozoğlu, 2005). The studies conducted in the region determined that winter sowings were higher yielding than early spring sowings (Bozoğlu and Gülümser, 1994). The growing period of the plant is from November to June. This period is when precipitation and weed density are the highest in Samsun conditions. It is a region where many kinds of weeds are seen every period due to the temperate climate and the high precipitation in autumn and spring. In this study, only species observations were taken from weeds, and their density per unit area was not defined. Weeds started to appear about 1.5 months after the broad beans emerged, and it is time to fight weeds with the high temperatures in January and February. *Veronica sp, Scandix pecten-veneris, Lupinus sp, Cirsium arvense, Fumaria officinalis* and *Lamium sp.* were the most common weeds in our plots.

According to our observations and literature in the region for many years (Frenda et al., 2017), it has a higher competition with broad bean weeds than other winter plants. Because the broad bean is a vigorous growing plant. In addition to the shading effect of the broad beans, the literature suggests that L-Dopa production also suppresses weeds (Soares et al., 2014). In this study, the change in the agronomic characteristics of the broad beans in different weed densities and the L-Dopa content in the root and stem were tried to be determined. In the trial where weedy, weed-free and three different hand-hoeing applications were used, both harvests in the fresh and dry seed periods were done. The results of the variance analysis are given in Table 1, and the means of properties in Table 2.

According to the variance analysis results, it was seen that the block variance was statistically significant in some properties. Using the randomized blocks experiment design shows that these variances correctly minimize the error by subtracting them from the general. Different people sowed each block by hand while the experiment was being set up. This emphasizes that sowing depths are effective in the development of the plant and that the subject should be investigated because we believe that the land soil structure is uniform in the experiment area.

Lara is a variety developed for green purposes. Therefore, fresh harvest observations were taken. The total yield of fresh pods and the ratio of the first harvest to this yield were found to be statistically significant. Yıldız (2018) conducted a study at the same location with 15 genotypes and three control varieties and reported that the fresh harvest was done seven times, and the earliest fresh harvest was in April. The weather was warm during the trial from January to February 2023, and the broad beans bloomed early in February. However, although the flowering was early, the cool weather that came later brought the pod binding period to April again. In this study, only four times fresh harvests were made. It was observed that different weed densities statistically affected the pod yield in the first and fourth harvests (Table 1).

The highest yield was obtained in the application where weeds were taken as soon as they emerged (Y_1: weed-free). Weeds were not intervened at all (Y_2), and two-time hoeing (Y_4) were included in the same statistical group. The exciting thing is that a higher yield is obtained in one hoeing. In our opinion, the reason for this is that the growth period of the plant and the surrounding weeds is more important when hoeing together with the number of hoes made 2 or 1 times.

It was determined that the plant height and the weed density on the number of pods, which are the most important features affecting the yield of the broad beans, have a statistical effect. Plant height was the longest in the control application, where no weed was removed. Kavurmacı et al. (2010). In their study in Hatay, plant height, number of pods per plant, number of seeds per pod and 1000-seed weight were significantly decreased due to weeds. In our study, it was seen that the plant increased in height to suppress them depending on the weed density, and the plant height was the longest in the weeded parcel (Table 2). The number of twigs varied between 3.6 and 4.0 pieces and did not show statistical differences. This indicates that the variety used in the trial is stable in terms of the number of branches. Karayel et al. (2016) reported that the branch number was not different in the frequency trial they conducted under Samsun conditions and that seed yield was positively related to the number of pods, trunk diameter, pod length and hundred seed weights.

Tablo 1. Variance analysis results of some characteristics of broad bean grown at different weed densities.

VS	DF	Total fresh pod weight	First harvest	Second harvest	Third harvest	Dry matter of root	Dry matter of stem	Fourth harvest	Height	Branch	Pod number	Biological yield	Fresh pod yield	Dry seed yield	100 seed weight
Treat.	4	12646.7	67.2 *	137.4	94.3	1.4	30.5	508.3	464.9 **	0.12	55.2 *	615468.6	9089.0	4730.6	273.1
Block	2	35950.0 **	179.8 **	127.1	341.0	6.9	1.3	627.7	956.2 **	1.59	222.3 **	63553.6	25966.1	2010.3	167.1
Error	8	4165.2	16.1	102.1	170.8	2.6	32.4	283.1	50.4	0.48	18.3	382615.2	7075.6	3463.8	202.5

Tablo 2. Mean of some characteristics of broad bean grown at different weed densities.

Treatment	in the fresh pod harvest							in the dry harvest time					
	Fresh pod yield (kg/da)	First harvest (%)	Second harvest	Third harvest (%)	Fourth harvest (%)	Dry matter of root (%)	Dry matter of stem (%)	Height (cm)	Branch	Dod number	Biological yield (kg/da)	Dry seed yield (kg/da)	100 seed weight (g)
Weedy	5186 b	8.1 b	10.9	9.8	73.0 a	15.1	29.9	131.1 a	3.6	23.9 ab	1979.3	399.9	125.4
Weed-free	7470 a	15.1 ab	5.2	22.4	48.7 ab	15.0	23.3	115.3 b	3.7	26.3 a	2844.0	481.5	141.8
1 hoe	5908 ab	18.2 a	22.7	195	37.7 b	16.4	27.0	108.3 bc	3.6	23.0 ab	2193.3	404.2	149.8
2 hoe	3912 b	16.4 a	9.2	23.3	50.3 ab	14.6	26.5	99.7 c	4.0	17.1 b	2218.0	375.9	145.9
3 hoe	5674 ab	20.7 a	7.9	22.5	47.8 ab	15.3	31.5	103.0 bc	3.9	27.7 a	1597.9	410.7	135.9

The number of pods varied between 17.7 and 27.7 pieces. A significant ($P<0.05$) difference was detected between the procedures. It gave the process of hoeing twice the lowest number of pods, which entered the same statistical group as the weedy parcel. In order to eliminate weeds, hoeing was carried out, taking into account the density of weeds. These operations were carried out in December, the last month of 2022, and in the middle of February, depending on the weed density in the hot weather. The broad beans were about 35-40 cm tall during this period but began to bloom with the temperature. Frenda et al. (2017) determined the critical period of weed control as 428 days after emergence for the broad bean. This period is mentioned when the row space is completely closed, and there is flowering. The reason why three hoes were better in our study may have been made during March when the rainfall and the growth of both the broad bean and the weeds accelerated. Removing weeds from 25 to 75 days after crop sowing led to significantly larger yields than on plots that were not weeded. Maximum yield was obtained in both years when weeds were removed thrice at 25, 50 and 75 days after crop sowing (Tawaha and Turk 2001).

Dry seeds yield varied between 375.9 and 481.4 kg per decare, but no statistical difference was determined. The highest yield was obtained in the weed-free parcel, while the application of 2 hoes showed a decrease of about 21% while giving the lowest value. However, this difference from the height of the variance between the parcels was not found to be significant in the experiment. Kavurmacı et al. reported that in 2010, seed yield losses due to uncontrolled weed growth throughout the broad beans crop cycle were 46%.

Frenda et al. (2017) also stated in their study on chickpeas and broad beans that yield losses in broad beans were less than in chickpeas due to weeds. This could be attributable to more vigorous early growth and the plant's greater height, which is related to a more extraordinary shading ability and, consequently, to a better ability to suppress weeds. In addition, we believe it is very important to investigate the L-Dopa content that the broad beans secrete, especially from the roots, due to weed stress.

CONCLUSIONS

Broad beans are not very widely grown in our country. However, its ecological demands and the vital development feature of the plant are remarkable in terms of being an alternative plant. One of the essential ways to ensure the spread of the plant can be realized by accessing new data that will reveal the diversity of use, health and agricultural importance. One of the aims of this study is to determine the change in the agronomic characteristics of the plant by weed pressure in the broad beans under the conditions of our region with high spring rainfall. The broad bean is a plant that shows an absolute need for hoeing because it is a plant capable of nitrogen fixation. However, it also can suppress weeds with its strong development and ability, such as shading.

In the studies we have carried out in recent years, we have shown that broad bean is important for human health due to their L-Dopa content. L-Dopa is a seconder metabolite that is important for the health of the plant and human health. Another goal of this study was to observe whether the broad beans used L-Dopa against weeds. However, this aim could not be achieved because L-Dopa analyses could not be completed until this article was prepared.

The data we obtained showed that although higher data were obtained in weed-free applications in our weed control conditions in terms of the agronomic characteristics of the broad beans, very successful results could not be accepted. The most important situation we have identified is the need to examine precisely in which period the hoe should be made rather

than the number of hoes in the cultural struggle with weeds. In order to obtain healthier data, it will be helpful to repeat the experiments by adding the mentioned situations. We believe that to maintain biological stability and maintain soil and water health, the ability of plants to cope with stress should be better examined, and the broad bean is a plant of this nature.

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BIOLOGICAL AND PHYSICOCHEMICAL INVESTIGATION OF CERTAIN STATIONS OF TUNCA RIVER

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ABSTRACT

This study was carried out in order to determine some water analysis, substrate and environmental data of 3 stations determined in Tunca River in Edirne province in 2012. The first station on the river was determined as Suakacagi Village, which is close to the border where the river enters Turkey, the second station as Degirmenyeni Village, and the third station as Trakya University Tunca Barracks garden. Field studies carried out between June and October 2012 were carried out as monthly periods. During the study, water samples were taken from each station and brought to the biology department laboratory for some analysis (temperature, pH, dissolved oxygen, total hardness, conductivity, suspended solids). In addition, notes were taken by observing the water in the river and the surrounding environments, and as a result, the stations were compared from various perspectives. Months and stations were evaluated physicochemical according to the Bray-Curtis similarity index. The months of September and October and September and August show the most similarity; In terms of stations, the 3rd and 2nd stations were found to be similar at most. In addition, the results of other studies conducted on the river were compared.

Keywords: Tunca River, Physicochemical analysis, Ecology

INTRODUCTION

Freshwater pollution has become a known limiting issue almost all over the world. Lotic ecosystems are among the most vulnerable freshwater bodies to pollution due to their role in municipal wastewater, runoff from agricultural fields, and industrial transport (Kose et al., 2014; Tokatli, 2014; Tokatli, 2015).

It is a necessity to attach great importance to fresh water resources in terms of quality and pollution of surface waters (Cicek et al., 2013; Ustaoglu et al., 2017; Ustaoglu and Tepe, 2019). Monitoring, management and evaluation of water quality in surface waters, especially rivers, is a vital sustainability issue for humans (Boyd, 2015; Wu et al., 2018). Pollution of these unique resources by many anthropogenic, chemical and polluting substances not only degrades ecosystems, but also threatens public health (Tepe and Cebi, 2017; Ustaoglu et al., 2017).

One of the important carbon sources of streams is the leaves that fall from the trees and reach the water. Dried leaves are an indispensable source of carbon, especially for mountain streams. The polymers in the leaf are a source of energy for many benthic organisms. With the deterioration of the leaf, the chemicals it contains, lignin, phenolic compounds, tannin and the nutrient in its carbon affect the nutrient dynamics in the water (Hunter et al., 2003; Welsh, 2007). It has been proven that the activity of certain benthic invertebrates increases with the amount of decaying leaves. Leaf decomposition chemistry and leaf decomposition activities of macroinvertebrates are interdependent. Because the composition of the leaf litter is the most important factor in colony formation of macroinvertebrates. Decomposed plant litter is thought to be important in the distribution of benthic invertebrates (Hunter et al., 2003). In addition, it

can be effective in both leaf rot and colony formation in environmental conditions (Costa and Melo, 2008).

The richness and diversity of the habitat as nutrients and substrate are among the factors that naturally increase biodiversity. However, another factor that should not be forgotten is the flow rate and physicochemical factors. The decay of plants in water is carried out by bacteria and as a result of the intensity of this activity, it is defined by the formation of some toxic gases such as CH_4 and H_2S and the lack of oxygen. A range of environmental factors limit the benthic habitat and change the species composition of the macrozoobenthic invertebrate community (Cupsa et al., 2005).

This study was carried out to evaluate macroinvertebrates from a biological point of view by comparing some water analysis parameters, substrate and environmental data at 3 stations in Tunca River.

MATERIAL AND METHOD

Description of the Work Area

Tunca River originates in the Montenegro Region of Bulgaria and enters Turkey from the Suakacagi location of Lalapasa district of Edirne province. The drainage basin area is 7 884 km^2 and a short distance (12 km) forms the Turkish-Bulgarian border (URL 1) and mixes with the Meric River in Edirne province. It is one of the main tributaries of the Meric River. Its length is 350 km (URL 2). There are two large dams in Bulgaria, Koprinka and Zhrebchevo Dams, on the Tunca River. The average flow value of the Tunca River was determined as an average of 32.09 m^3/sec, according to data from a measuring station in Bulgaria. During the observation period, the highest flow value was 69.36 m^3/sec and the lowest flow value was 18.81 m^3/sec (Orsam, 2011; Tombul, 2014).

Fish such as pike, catfish, mullet, crustacean, barbell, pearl fish, carp, and crucian fish live in the Tunca River. People living in Edirne center and the villages around the river in Turkey use almost every area of the river as fishing and recreation areas. There are many agricultural areas (basic rice crops) around.

Field Study and Sampling

The study was carried out in a 5-month period between June and October 2012. Three stations were identified that characterize the river. The first of the stations is Suakacagi Village, which is close to the border where the river enters Turkey, the second is Degirmenyeni Village, and the third is Trakya University Tunca Barracks (Figure 1). Features of the stations:

– Station 1: Suakacagi Village – This is the area where Tunca enters the Bulgarian border for approximately 300 m. There are many willow and poplar trees around the river. The edges of the water in the river are reeds. There are plant remains as organic material on the ground. The base is sandy and in some areas mud covers the top of the sand. The water level is 50-60 cm around (Figure 2).

– Station 2: Degirmenyeni Village – It is the region that is popularly known as Egribuk. The bottom of the river is muddy and rich in vegetation. The back part of the sampling area consists of a large amount of reeds. It is surrounded by paddy fields and is used as a recreation area. The water level is approximately 75 cm and is the deepest station (Figures 3 and 4).

– Station 3: Edirne Central Tunca Barracks – It is the region where the Tunca River enters the city of Edirne. The water level in the river is approximately 60 cm, the bottom structure consists of mud, but the bottom part is sand. There are small grasses on the shore in its immediate vicinity and it is an open area. Further back, there are trees that are not dense. The opposite shore is wooded (Figure 5).

Figure 1. Tunca River sampling stations: 1. Suakacagi Village (465409.00 D, 4632519.00 K, 47m); 2. Degirmeniyeni Village (461067.00 D, 4623425.00 K, 40 m); 3. Trakya University Tunca Barracks (Edirne) (462965.63 D, 4619414.88 K, 37m)

For water analysis in Tunca River, air and water temperature, pH, dissolved oxygen, total hardness, conductivity, suspended solids parameters were selected for measurement. While the samples were being taken, the air and water temperatures were measured on-site with a thermometer at the stations. Water samples which were taken by a Ruttner water sampler were carried to laboratory. pH, dissolved oxygen, total hardness, conductivity, and suspended solids values were measured in the laboratory with the Hach Lange Brand HQ40D Model Multiparameter device.

Samples taken with a hand mud scoop for macroinvertebrates were diagnosed under the microscope in the laboratory and separated into groups. Different sources were used in the identification process of the groups.

RESULTS

Before the first fieldwork, the weather was quite rainy. In the following week, these precipitations caused the water level to rise by 30-40 cm. However, after 3 weeks, the water level decreased to the normal level due to both the water withdrawal and the very hot weather for the paddy fields, which are abundant in the surrounding area.

In July, when the 2nd fieldwork was carried out, the water levels decreased at all stations, especially at the 2nd station, due to the above-mentioned reasons. This decrease is particularly there were quite a few at the station.

The third fieldwork was carried out in August. The water level decreased considerably due to the hottest months of the year when the 3rd field study was carried out. The water level has receded on the coasts and has decreased considerably, especially at stations 1 and 3. Although the water in the river is less compared to the winter months as usual, there is a current. Islets were formed in the water due to the decrease in the water level from time to time.

Figure 2. Station 1

Figure 3. Station 2

Figure 4. Station 2

Figure 5. Station 3

During the 4th fieldwork in September, the water level continued to decrease, albeit slightly, in the areas outside the second station. The water in the river flows normally at all stations. At station 1, the water colour is yellowish green and has decreased considerably. The ground is sandy and contains invertebrates such as annelids. On the shores, the presence of frogs has been observed. At station 2, the water has a muddy appearance and a cloudy colour. The bottom of the river is mud. In the 3rd station, the soil, sand and plant mixture, the water level decreased slightly compared to the previous month.

The 5th field work was carried out in October and the field work was completed. Since there was no precipitation in this period, the water level did not change much compared to the previous month. The ground and water feature in the river are the same as in September.

Water samples were taken from each station for 5 months and some analysis were done in the biology department laboratory (pH, dissolved oxygen, total hardness, conductivity, suspended solids). In addition, air and water temperatures were measured in situ while taking samples. The results of the physicochemical analysis carried out in the Tunca River was given in Table 1 below.

Table 1. Some physicochemical parameter values in Tunca River

	June			**July**			**August**			**September**			**October**		
Parameters	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Water Temperature (°C)	27	27	28	28	29	29	29	28	28	27	26	26	23	23	22
pH	7,61	7,53	7,85	8,67	8,17	8,17	8,22	8,17	8,27	8,67	8,17	8,17	7,73	7,77	8,08
Electrical conductivity (µS/cm)	583	622	621	616	709	666	596	690	646	626	685	693	677	710	716
Dissolved Oxygen (mg/lt)	11,31	10,15	10,31	4,8	2,5	3,8	3,8	2,9	3,7	4,86	3,97	4,89	7,12	8,01	6,4
Total hardness (FS^{O})	24,4	25	25,4	24.4	25	25,4	24,4	25	25,4	24,4	25	25,4	24,4	25	25,4
Suspended solid (mg/lt)	3,2	3,3	3	269	215	303	75	63	86	29	28	33	13	20	18

(1, 2 and 3 stations)

In Figure 6 and 7, the months and stations were compared according to the Bray-Curtis similarity index according to the results of the river water analysis.

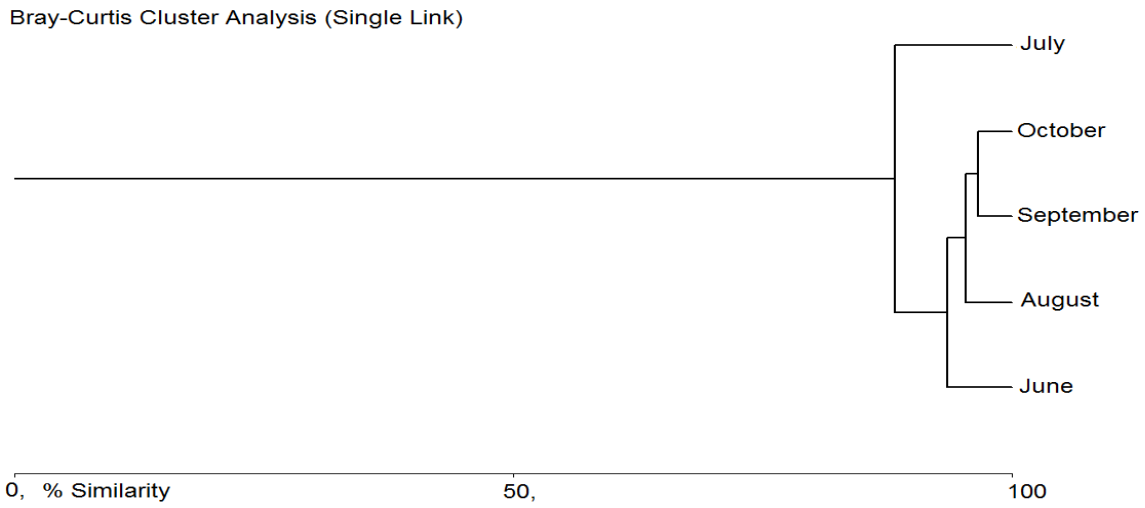

Figure 6. Dendrogram of similarity of months in Tunca River in terms of some physicochemical values (single linkage, Bray-Curtis, log base 10)

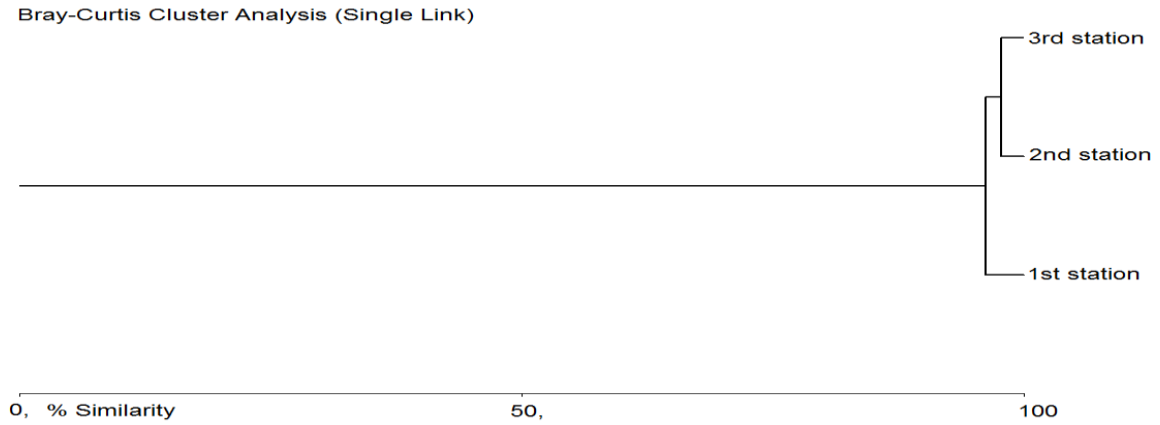

Figure 7. Dendrogram of similarity of stations in Tunca River in terms of some physicochemical values (single linkage, Bray-Curtis, log base 10)

When the Bray-Curtis similarity index is examined; When some physicochemical values of Tunca River are compared as months, September and October (96%), September and August (95%) show the most similarity. The months with the least similarity are June and July (80%) (Figure 6). But the similarity rate is quite high. When the stations are considered in terms of similarity, the most similar stations are 3 and 2, then 1 and 3, and finally 1 and 2 (Figure 7).

Different types of taxa were obtained by sampling soil types with different characteristics with the help of hand scoops at the stations. The individual numbers of these taxa, which are distributed according to stations and months, are presented in Tables 2 and 3.

Table 2. Individual numbers of taxa showing distribution by stations

Taxa	1. station	2. station	3. station
Chironomidae	1549	830	873
Oligochaeta	371	1205	1194
Odonata	27	23	74
Trichoptera	2	185	7
Ephemeroptera	8	96	10
Isopoda	5	24	31
Gastropoda	3	2	54
Other*	19	10	5
Total	1984	2375	2248

Other* = Crustacea, Hirudinae, Plecoptera, Tabanidae, Culicidae

Table 3. The number of individuals in taxa according to months

Taxa	June	July	August	September	October
Chironomidae	558	604	1428	350	499
Oligochaeta	1404	287	211	348	285
Odonata	14	29	45	24	12
Trichoptera	10	7	65	77	35
Ephemeroptera	5	2	35	6	66
Isopoda	4	7	14	19	18
Gastropoda	42	4	10	11	11
Other	5	12	9	12	23
Total	2042	952	1817	847	949

When examined according to the stations, the highest number of individuals is found at the second station. When the distribution by months is analysed, June has the highest number of individuals.

DISCUSSION AND CONCLUSIONS

Monoculture agricultural practices in the region impoverish the soil in terms of many minerals. In order to eliminate this deficiency, inorganic and phosphate fertilizers are used intensively in almost all basin soils. According to the surface water data obtained as a result of the study; It has been determined that many agricultural lands, especially paddy, located around the basin and which are of great importance for our country, create very important pressures on the ecosystem. The data obtained from the statistical analyzes clearly reveal the negative effects on the agricultural system. River waters should be given importance to increase the quality of this most important river basin of the Thrace region, to reduce the stress and pressure on aquatic organisms and to protect the health of the local people.

Since the study generally coincided with the summer period and the temperatures were high due to the dry weather, the dissolved oxygen level was generally low except in June. In July, the suspended solids ratio was high due to the excessive decrease in water. This situation can be explained by the high precipitation and flooding in the spring months in 2012 and the rapid decrease in water in the river due to excessive water withdrawal for agricultural purposes, especially paddy fields, in a short time. These results are consistent with the results of Fritz and Feminelle (2011).

In the study conducted by Camur-Elipek et al. (2006), hydromorphological structures, habitat and physicochemical characteristics of the stations are generally similar. The months that are most similar to each other in terms of seasonal changes are April and May, and the most different from each other are August and January. In the study conducted in Tunca, water temperature, electrical conductivity, chloride, salinity, sulphate, phosphate were found at normal levels. Dissolved oxygen was generally found in abundance and oversaturated due to aquatic vegetation. Total hardness was found at hard water quality level (except winter and October). Nitrate was generally found at the second quality level (only found at the second quality level in September). Nitrite is usually found in the fourth quality level. COD was found at the second quality level (third quality level only in September), while BOD was found at the first or second quality level (third quality level in October only). Regarding the Pearson correlation index, the relationship between the mean number of benthic macroinvertebrates and physicochemical properties and pH ($r = +0.57$, P a 0.05) was directly proportional, while the relationship between the number of macroinvertebrates and Nitrate ($r = –0.99$, Pa 0.05) was inversely proportional. It can be said that these data are consistent with the study in general.

In the research conducted by Tokatli (2015) in the following years, it was observed that the water quality in Tunca downstream was Class III and the river was becoming more and more polluted. According to Cluster analysis results, the river was moderately contaminated. As similar to reported data by DSI, nitrite, ammonia, and phosphate concentrations waters of the Meric, Tuna, and Ergene Rivers were detected at very high levels and they have Class III-IV (highly-very highly contaminated) water quality in terms of these parameters (SKKY, 2004; Uslu and Turkman, 1987). The high nitrite, ammonia and phosphate concentrations in river water are thought to be due to runoff in agricultural areas. According to detected data, the pressure of the Tunca was clearly presented and the pollution levels of the investigated rivers were recorded as Ergene > Tunca > Meric.

Also, in studies on benthos, which were performed in the Bulgarian part of Tunca, Uzunov (1980) recorded 32 and Uzunov and Kapustina (1993) recorded 47 Oligochaeta species in the river. According to Kovachev and Uzunov (1981), the Tundzha River is a relatively polluted river (at the limit between α- and β-mesosaprobity). According to Camur-Elipek et al.

(2006) and Tokatli (2014) also show compatibility with the studies. The chemical fertilizers used in paddy farming areas around the river and the use of all areas by humans have led to the pollution of the river water. It also explains the abundance of Oligochaeta taxon. Kavaz (1997) studied especially benthic macroinvertebrates and physicochemical parameters in her master's thesis, but did not deal with the relationships between them. These results show that the water quality of the river has gradually decreased over time.

Chironomidae and Oligochaeta taxa have a large number of individuals, as was the case in the study conducted by Camur-Elipek et al. (2006) in the Tunca River. In both studies, the total number of individuals was found to be the highest in the second station, which corresponds to approximately the same region, and shows similarity. In addition, Nematoda, Amphipoda and Hemiptera were not found in this study.

According to Fritz and Feminelle (2011), the density and abundance of invertebrates were found to be less in dry months such as June - August than in all rainy months such as September - October. These results are consistent with other taxa except Oligochaeta in the study.

In Tunca River, aquatic fauna is richly found on the bottom of the river. This may be attributed to the fact that the river bottom has different substrate structures, its physicochemical structure, the fact that the river water is in a continuous flow position and the water is rich in vegetation due to its shallowness.

As a result, irrigation, sewage system, variable flow rate, temperature etc. affects the quality of water. The structure of the benthic macrofauna in the Tunca River changes under the influence of environmental variables. In addition, similar studies should be repeated periodically in the Tunca River to determine the future of the river.

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REGRESSION ANALYSIS BETWEEN AGRICULTURAL PRODUCTS AND ATMOSPHERIC CONDITIONS

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ABSTRACT

Number of factors effects agricultural products. Controlling some of them can be relatively easy for instance irrigation is possible if water available or you can improve the quality of soil by supplying appropriate minerals and/or fertilizers. On the other hand, farming threatened by several uncontrollable issues. Atmospheric conditions are one of these tough issues that consists of many parameters such as temperature, wind and air content. In this paper, outcomes of atmospheric issues studied to understand effects to farming. Satellite-based observations utilized in evaluations. Paper presents regression between agriculture and different parameters of atmosphere. Analysis utilizes Copernicus Atmosphere Monitoring Service, Climate Change Service and EUMETSAT.

Keywords: Threatening issues of agriculture, Satellite-based atmospheric analysis, Climate change.

1. INTRODUCTION

Agriculture is an essential part of food supply for mankind. It has evolved as time goes by in the history. Current technology and skills make possible to cultivate wide variety of yields such as wheat, maize and sunflower. Different elements made it simpler to produce different types of agricultural products as well as the amount of harvest. In order to name two types of agricultural facilitators, using different manure strategies or utilizing advanced and/or hybrid seeding, considerable improve farming. On the other hand, life is not always so easy, and humankind may not control every aspect in life and agriculture is not an exception. Atmospheric conditions are one of these threatening factors in farming. Efforts to minimize the influence of atmospheric conditions in agriculture becomes deficient frequently.

Number of previous efforts to analyze atmospheric effects to agriculture includes in situ data collection such as temperature [1], precipitation [2] and wind speed [3]. On the other hand, recent advances made possible to observe them from the space. These modern technologies could be used for diverse elements of atmospheric elements. Moreover, temporal and spatial detection is more flexible based on space-based applications. More than 14.000 satellites have sent, 10.290 currently in orbit and around 7.800 of them are operational as of 01.April.2023, according to [4] (numbers does not include space garbage).

In this study, effects of atmospheric issues analyzed in agriculture. Advanced space technology allows miscellaneous pre and post farming observations and reactions. Paper presents sample space-based agricultural technics. Copernicus Atmosphere Monitoring Service, Climate Change Service and EUMETSAT are example centers to utilize space technologies selected to discuss in this work.

The rest of the paper is organized as follows. In Section 2, atmospheric effects to farming are discussed. In Section 3, various atmospheric related space technologies presented. Section 4 provides samples using these space technologies in agriculture. Section 5 conclude the paper, and it also includes selected future works.

2. ATMOSPHERIC EVENTS AND FARMING

Atmosphere consists of many elements and keeps on fluctuating. This is one of the reasons that it is becoming difficult to plan and react well in advance. Not only the atmospheric seasonal characteristics may repeat inconsistent, but also in and out seasonal variance can be significantly high. To give two examples; summer in Mediterranean coast may be much more rainy than usual. Another example a desert without any snow in fifty or one hundred years may have a huge snow accumulation suddenly. Table 1 shows some atmospheric parameters that can be used in observations and research.

Table 1 Some elements of atmospheric observations

Type	**Description**	**Unit**
Temperature	Quantity of hot or coldness.	Kelvin (K), Celsius (°C), Fahrenheit (°F).
Humidity	Atmospheric moisture.	Units of grams of water vapor per cubic meter air (g.m-3).
Precipitation	Types of water drops (i.e. rain, snow, hail, etc.) on to the ground.	millimeters (mm) or inch of liquid water within the preceding duration.
Air pressure	Barometric pressure applied by air.	millimeters (mm) or inches of mercury (“Hg), pounds per square inch (psi), dynes per square centimeter, millibars (mb), standard atmospheres (atm), kilo Pascals (kPa).
Wind speed	Flow of air speed from high to low pressure.	meters/second (m/s), miles per hour (mph), (knots), feet per second (ft/s), kilometers per hour (km/h).
Wind direction	Direction of air flowing.	Cardinal or compass direction, 0° to 360° can be used.
Gas content	Concentration of atmospheric gases.	Parts per million (ppm).
Air quality Index (AQI)	Ground level ozone	Scale between 0–500

Some of the elements of atmospheric observations listed in Table 1. The relation of farming and these atmospheric elements is given with the following examples. Very high or very low temperatures directly affects farming since it can burn/sear crops. Moreover, appropriate temperature for the seeding, growing and maturity period are important for successful farming. Humidity is another important factor such as suitable growing and adequate amount for the harvesting season. Precipitation sounds one of the crucial factors of farming, however, lots of rain or snow might be harmful. Additionally, if precipitation turns into sleet or hail it can turn into a serious problem. Air pressure is another factor in farming, it can change the quality of products as well as types of yields as pressure varies. Wind speed can be a significant threat to yields as strong winds can easily break or incline plants. Wind direction is important for instance some winds are originating from mild directions whereas others are deriving from stringent regions. For instance, wind from the northern regions for the northern hemisphere may be very tough in winter. Whereas same region has opposite threat from

southern regions in summer. Gas content affects number of farming factors and one of them is the quality and health of the yield.

All the discussed atmospheric elements in this Section can be observed and analyzed by spaced-based systems. The following section discuss some of these space-based observations about atmosphere.

3. ATMOSPHERE AND SPACE TECHNOLOGIES

Although most of the atmospheric events have critical effects to human life in general and farming in particular, limited data and forecast could be utilized until the emergence of space technology. Currently we have many satellites in space, which operate different types of services [4]. At this section, we present some of the important institutions and organizations, which study on atmosphere monitoring using technical advantages of space equipment.

Copernicus Atmosphere Monitoring Service (CAMS) [5] is a service provided by European Centre for Medium-Range Weather Forecasts (ECMWF) wide range of atmospheric data, analysis as well as projects provided through this service.

Climate Change Service (C3S) [6] is one of the services provided by Copernicus Programme of European Union (EU). Service provides climate bulletins, heatwaves, drought reports, etc.

European Organisation for the Exploitation of Meteorological Satellites (EUMETSAT) [7] operates Meteosat GEO satellites over Europe, Africa and Indian Ocean. In addition to these, this organization has different satellite agreements for other geographical regions about meteorologic research.

National Aeronautics and Space Administration (NASA) [8] is one of the oldest and largest organization study in space sector. It has number of projects and diverse instruments. One of the latest space instruments of NASA is TEMPO. It starts operation in the late 2023.

Table 2 shows some of the systems and satellites operated and/or used by these four organizations.

4. ATMOSPHERIC SPACE TECH IN AGRICULTURE

As discussed in the previous section, there are different space-based services available for atmospheric observations and forecasts. Here are some examples with their use. Copernicus Atmosphere Monitoring Service (CAMS) provides number of post and future gas content data. Many of them provided in the printed and some are available in the animated form. For instance, if a certain region is intended to be analyzed for a particular gas content, it is possible to obtain post data. Furthermore, still image maps and even animated maps are available for some cases. For example, ensemble data can be provided for certain area within a period. Thus, it is possible to analyze number of parameters. For instance, it is possible to compare the quality of harvested yields based on these data.

Table 2 Selected space atmospheric observation systems

Owner or partner	**Name of the space system**	**Description**	**Name of sample satellites**
CAMS	JAXA	Methane (CH4) observation with TANSO instrument	GOSAT
CAMS	EUMETSAT	Cloud info. with SEVIRI instrument	MSG
C3S	Copernicus program	Flooding, ice, agriculture, etc.	Sentinel 1, 2, 3 and 6
C3S	EUMETSAT	Gas compositions such as CH4	Sentinel 4 and 5
EUMETSAT	Meteosat GEO	Geostationary satellites (GEO) over Europe, Africa and Indian ocean	Meteosat 9, 10, 11
EUMETSAT	Metop	Low Earth Orbit (LEO) satellites for polar monitoring	Metop B, C
EUMETSAT	Jason 3 and Jason CS/Sentinel 6	Low Earth Orbit (LEO) satellites for sea level monitoring for Europe and US	Jason 3 and Sentinel 6
NASA	TEMPO	Geostationary satellite (GEO) for monitoring air pollution of Northern America	TEMPO

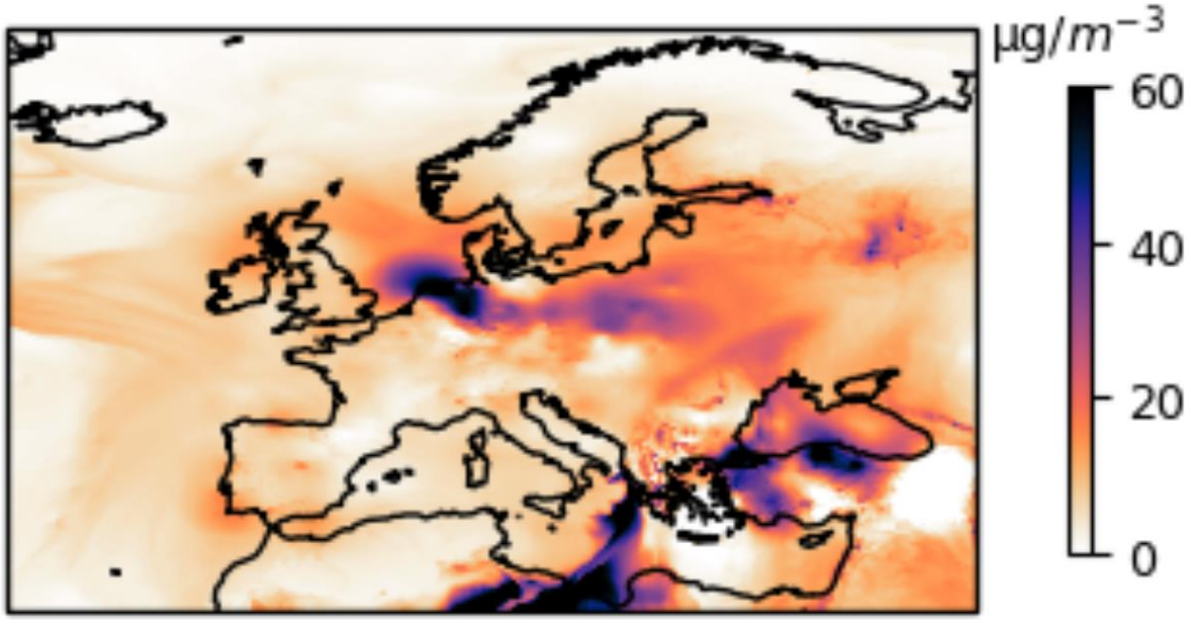

Figure 1 CAMS air quality ensemble of nine air quality data of European region for 01.April.2018.

Figure 1 demonstrates quality of air in European region for 01.April.2018 collected from the datasets of CAMS system. In that specific period, relatively high values of air quality index available in some parts of Turkey including most of the western regions.

Climate Change Service (C3S) works on enormous data sets to provide past, current and future predictions about climate change. It gathers data from different space instruments as well as with in situ equipment. Agricultural predictions, flooding, heavy snow expectations are just to name a few past, current and future forecasts.

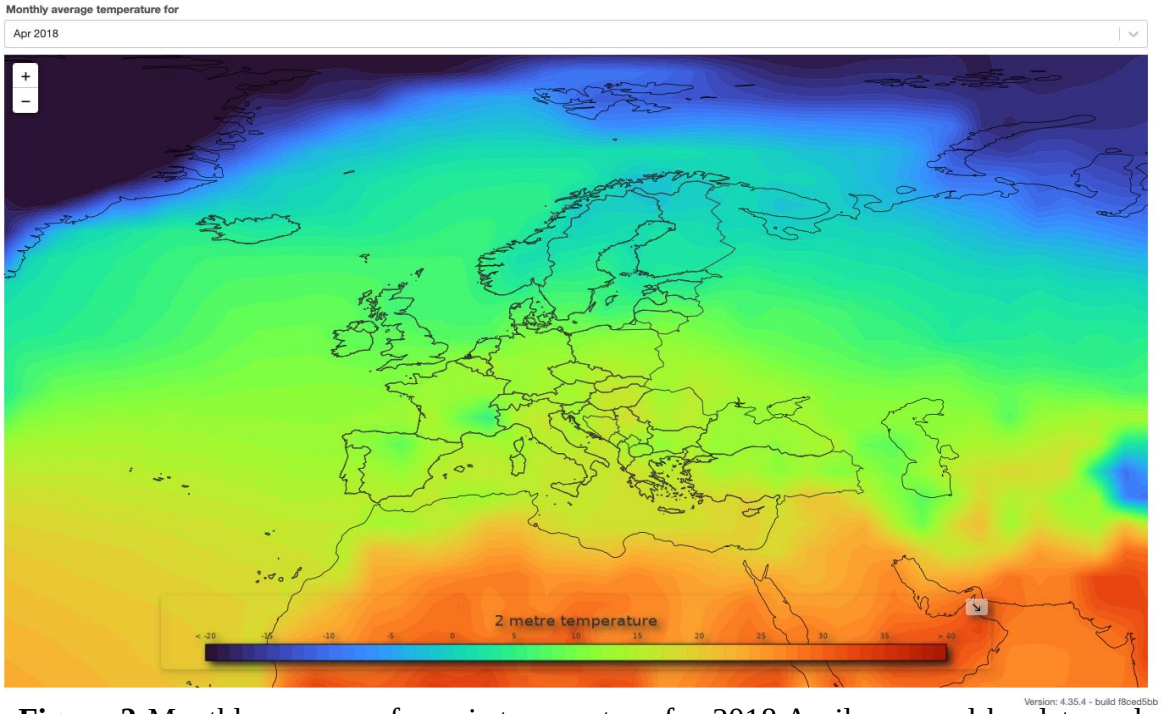

Figure 2 Monthly mean surface air temperature for 2018.April prepared by data and services of Climate Change Service (C3S).

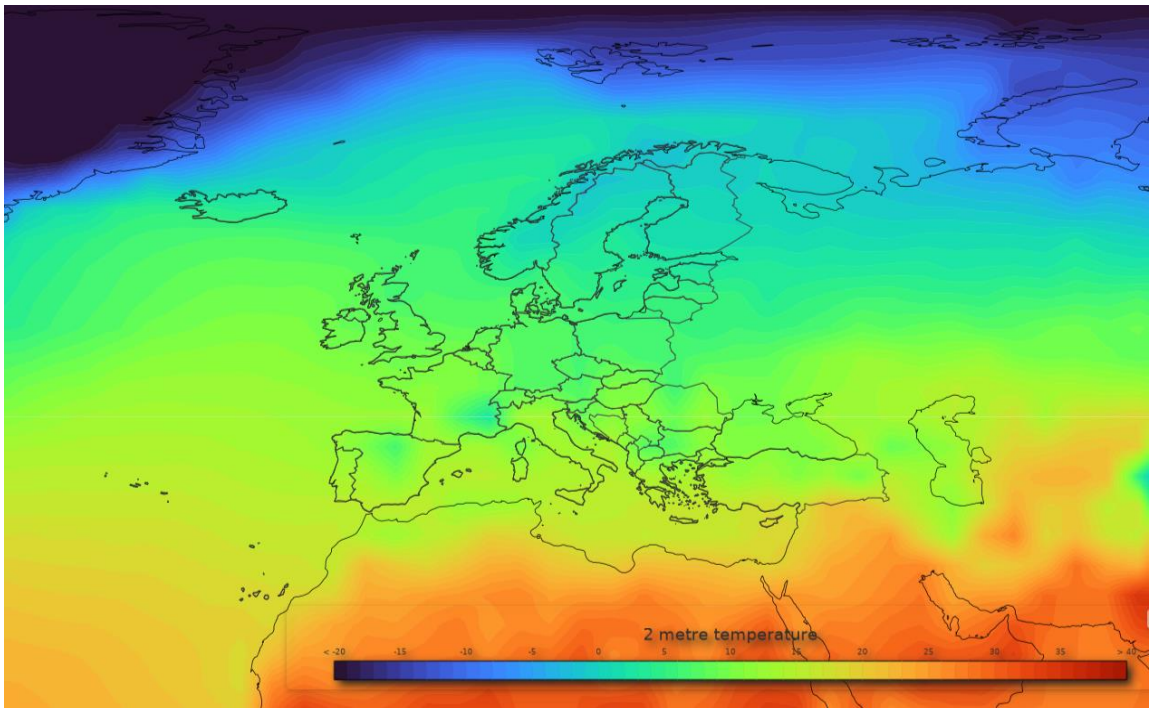

Figure 3 Monthly mean surface air temperature for 2022.April prepared by data and services of Climate Change Service (C3S).

Figure 2 and Figure 3 depict monthly mean surface air temperature for 2018.April and 2022.April respectively. The two figures appear to be identical to each other as an initial

impression, but they are not. One of the reasons for their similarities is that the data comes from monthly average temperature values. Although it is an average value, there is a difference in the figures. While it was slightly colder in April 2022 in regions such as France and Spain, the temperature averages increased in the northern African region. Dissimilarities between two figures are also available in other regions.

Since precipitation is important factor in agriculture, Figure 4 depicts one of the precipitation type, snow, in a particular day as an example. In the selected date 2018.April.01, snow type of precipitation was not occurred, but it was available in some parts of Siberia, Baltic Sea, Northern America, etc. This analysis can be applied to other dates and or to make analysis for the extended period instead of a day.

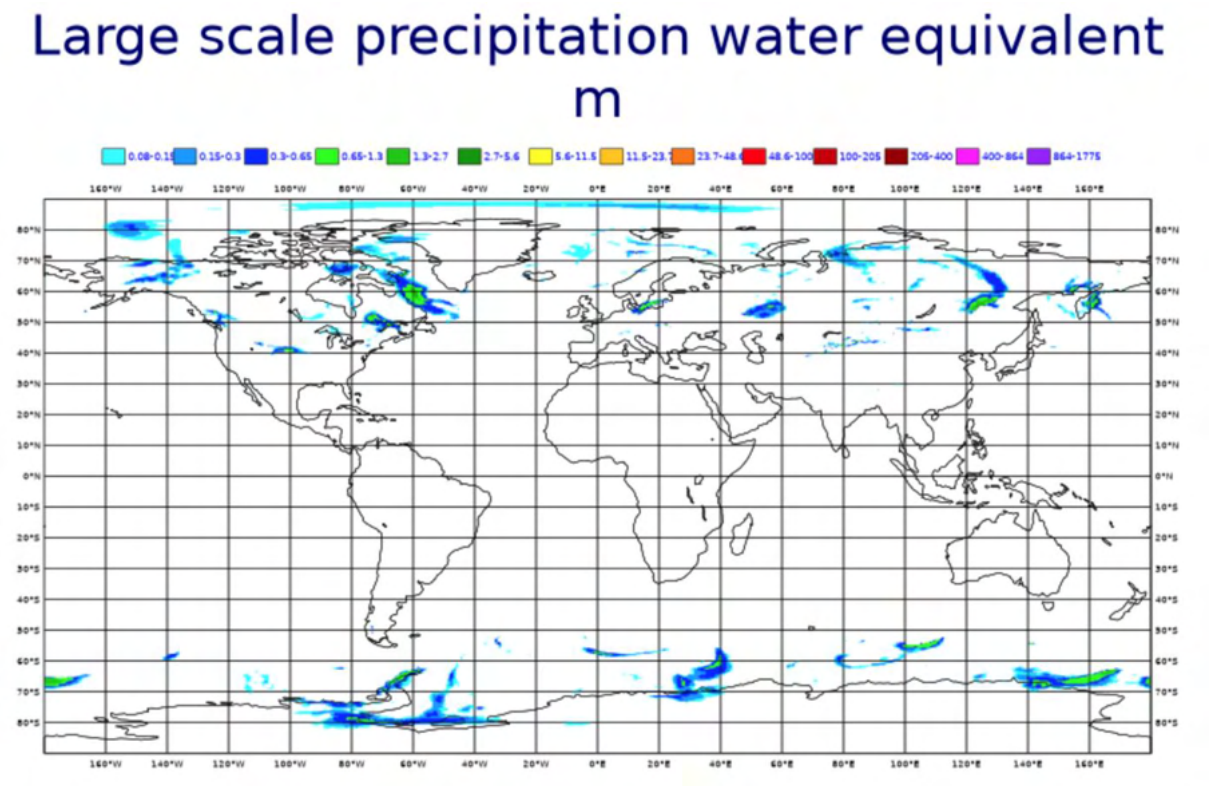

Figure 4 Large scale snow precipitation water equivalent for 2018.April.01 prepared by data and services of Climate Change Service (C3S).

Table 3 shows oil sunflower production in city of Edirne region for 2018 [9] and 2022 [10]. Table 3 indicates area of sunflower production increased from 2018 to 2022 as well as product obtained per unit area.

Figure 1, Figure 2, Figure 3 as well as Figure 4 suitable for agricultural analysis of a region as well as for a specific agricultural product. Moreover, similar observations can be utilized to further extend the research in other fields.

Table 3 Oil sunflower production in Edirne region in 2018 and 2022 years.

Year	Area (Decare)	Quantity (Ton)	Yield (kg/decare)
2018	954.502	237.136	248.4
2022	1.260.318	325.812	258.5

Many implications can be drawn such as; Northern part of Edirne region has better air quality index compared to the rest of the Trakya region. This is because of the fact that less polluter industry available in the region and earth's rotation direction. Considering that air temperatures are increasing, product diversity will probably increase, especially in years with suitable rainfall or in regions with irrigation facilities. On the other hand, air temperatures may also have adverse effect to agriculture that may even burnout the yield. Different sectors can make use of these valuable observations for appropriate predictions. For instance, insurance sector can forecast potential risk of hail for the next season. Since, similar post or future hail map can be generated from these system data sources.

5. CONCLUSION

As discussed in this paper, various satellite equipment and organizations available in the market. Some of them provides free products and services and others requires different amount of money and/or subscriptions. As indicated in this work, their data can be used and/or enhanced for past and future agricultural estimates. These estimates have a positive effect on many areas such as product increase, ideal amount of seed use and appropriate and reasonable fertilizer selection.

As for future works, we are working on these systems for forecasting future scenarios of wheat and sunflower production in Balkan region in general and in Edirne region in particular. Moreover, we conduct our previous work [11] to extend in agriculture field. This prospective study will also an area in the healthy food production and consumption.

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THE EFFECTS OF LUTEOLIN ON ACRYLAMIDE INDUCED OXIDATIVE DAMAGE IN 3T3 EMBRYONIC FIBROBLAST CELLS

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ABSTRACT

Acrylamide, which is used in various fields of industry (paint, textile, cosmetics), is a water-soluble white, odorless and crystal compound. Studies have indicated that high amounts of acrylamide are found in foods such as potatoes, bread, coffee and cereal products. Many studies have been conducted to investigate possible human health risks from acrylamide exposure, along with the determination of daily dietary intake. Previously studies have demonstrated that acrylamide has cytotoxic, neurotoxic, genotoxic and carcinogenic effects. In order to reduce the toxic effects of heat-treated food contaminants, foods with antioxidant properties should be consumed. Luteolin is an antioxidant compound found in significant concentrations in vegetables, fruits, and spices. Luteolin exerts its antioxidant effects by scavenging free radicals responsible for oxidative damage, inhibiting some enzymes that catalyze oxidation, and strengthening endogenous antioxidants. The purpose of this study was to examine the effects of acrylamide on the viability of 3T3 embryonic fibroblast cells, lipid peroxidation, and antioxidant enzyme levels (superoxide dismutase, catalase, and glutathione peroxidase), as well as to show how luteolin protects against acrylamide toxicity. For 24 hours, 2 mM concentrations of acrylamide and/or 10 μM concentrations of luteolin were applied to 3T3 embryonic fibroblast cells. The findings indicate that acrylamide significantly reduces cell viability and antioxidant enzyme activities and increases lipid peroxidation. As a result of the treatment of 3T3 embryonic fibroblast cells exposed to acrylamide with luteolin, it was found that cell viability and enzymatic antioxidant activities increased, and lipid peroxidation significantly decreased. This has led to the discovery that luteolin possesses potent antioxidant properties that protect embryonic fibroblast cells from the cytotoxicity and oxidative damage caused by acrylamide.

Keywords: Acrylamide, Luteolin, embryonic fibroblast cells, oxidative damage, antioxidant system.

INTRODUCTION

Acrylamide is a water-soluble white, odorless and crystalline chemical compound that is widely used in the manufacture of industrial products in the world (Kusnin et al., 2015; Paleologos and Kontominas, 2005; Khezerlou et al., 2018). Acrylamide has been discovered to be produced during the heat processing of food products, in addition to its industrial usage (Lineback et al., 2012). In a 2002 report, the Swedish National Food Administration (SNFA) and Stockholm University first mentioned the occurrence of acrylamide in food products (Keramat et al., 2011). Acrylamide is mainly produced through the Maillard reaction that occurs after cooking foods at temperatures above 120°C. Heat-treated fried potatoes, coffee, bread, breakfast cereals, and biscuits are among the food products containing high amounts of acrylamide (Yaylayan and Stadler, 2005; Michalak et al., 2019; Lineback et al., 2012). Studies indicate that exposure to acrylamide negatively affects human health and causes toxicity (Raffan et al., 2019). The International Agency for Research on Cancer (IARC) has classified acrylamide as a potential human carcinogen as a result of these studies, including it in the

"Group 2A carcinogen class" (IARC, 1994). Although there are various *in vitro* and *in vivo* studies on acrylamide in the literature, there are few studies investigating the toxicity of acrylamide in embryonic fibroblast cells (Hamdy et al., 2022; Hong et al., 2021; Evazalipour et al., 2021; Mahdizade et al., 2021).

Acrylamide exposure occurs by three different routes: oral, inhalation or skin contact (Rifai and Saleh, 2020). Once in the body, acrylamide is rapidly and intensively absorbed from the gastrointestinal tract and enters various organs, including the liver, brain, and kidney (Belhadj Benziane et al., 2019; Yan et al., 2023). Studies have shown that acrylamide increases the production of reactive oxygen species (ROS) and decreases the amount of various antioxidant enzymes involved in their detoxification, leading to disruption of oxidative balance (Zamani et al., 2017; Friedman, 2003). In order to minimize the toxic effects of acrylamide, foods or food supplements with antioxidant properties should be consumed in sufficient amounts in the daily diet (Aslani and Ghobadi, 2016). Flavonoids are a different group of antioxidants that humans receive through food. Flavonoids, commonly referred to as secondary plant metabolites, are compounds with antioxidant characteristics (Zenebe et al., 2001). Luteolin, a flavonoid derivative, is found naturally in the structures of various plant species and is widely included in the daily diet. Luteolin is known to have protective effects against oxidative stress, which causes cell and tissue damage. It acts as an antioxidant by scavenging free radicals responsible for oxidative damage, inhibiting some enzymes that catalyze oxidation, or enhancing endogenous antioxidants (Malacaria et al., 2023; Lin et al., 2008; Mahdiani et al., 2022).

The goal of this study was to determine the potential protective role of luteolin, which has antioxidant capabilities, against the toxicity that acrylamide may induce in 3T3 embryonic fibroblast cells by assessing cell viability, lipid peroxidation, and antioxidant enzyme levels.

MATERIAL AND METHODS

Cell culture and experimental design

The 3T3 embryonic fibroblast cell line used in this study, is a non-tumorigenic cell line derived from 14-17 days pregnant Balb/c mice. This cell line was brought to our laboratory by obtaining from American Type Culture Collection (ATTC): The Global Bioresource Center and is grown by passage regularly once or twice a week under *in vitro* conditions. Cells were maintained in DMEM culture medium with 10% calf serum, 4.5 g/L glucose, L-glutamine, sodium pyruvate, and penicillin-streptomycin solution at 37°C in a humid environment containing 5% CO_2 and 95% air. For acrylamide, the concentration that reduces cell viability to 75% was chosen, while the 10 µM concentration of luteolin used in previous *in vitro* studies was preferred in our study (Li et al., 2019). The experimental groups selected to be applied on the cells were acrylamide, luteolin, and acrylamide + luteolin. 3T3 embryonic fibroblast cells were exposed to the experimental groups for 24 hours.

Cell viability evaluation

The viability of embryonic fibroblast cells after acrylamide and luteolin administration was assessed by measuring formazan development from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Leydig cells were seeded in 96-well plates at $5x10^3$ cells/well in 100 µL experiment medium. The MTT I solution was added to each well after the incubation period, and the plates were then incubated in a CO_2 incubator at 37°C for 4 hours. Each well was then filled with 100 µL of MTT II solution (SDS) and left to incubate in a CO_2 incubator for an additional day. The absorbance was measured at 540 nm using an ELISA reader. The viability ratios of the acrylamide and luteolin-administered groups were represented as percentages based on control, with the viability ratios of the control cells being considered to be 100%.

Biochemical experiments

In this study, 3T3 embryonic fibroblast cells were seeded in six-well culture plates at $5x10^5$ cells per well to perform biochemical experiments. The cells were then treated with acrylamide and/or luteolin concentrations and incubated for 24 hours. After the exposure time was completed, the cells were transferred to ice-cold tris buffer (Tris-HCl, pH:7.2) and the membranes of the cells were disrupted by the ultrasonicator. The cell suspension of lysed cells was centrifuged at in a refrigerated centrifuge. The supernatants were collected and stored at -86°C for analysis of total protein, lipid peroxidation, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity.

Determination of membrane lipid peroxidation

Lipid peroxidation was determined according to the method of Heath and Packer (1968). The principle of the method is based on measuring the amount of the pink-red colored compound formed as a result of the reaction of malondialdehyde (MDA), the product of polyunsaturated fatty acid peroxidation in cells, with thiobarbituric acid (TBA) at pH, in a spectrophotometer at a wavelength of 532 nm.

Measurement of total protein

The smart BCA protein assay kit was used to determine the samples total protein amounts. The principle of this method is that the amount of protein is determined by colorimetrically measuring the purple-colored reaction product formed as a result of the binding of a copper ion to two BCA molecules. The purple-colored reaction product formed within the scope of the method shows linear absorbance with increasing protein amount at 562 nm.

Determination of antioxidant enzyme activities

The Marklund and Marklund (1974) method was used to evaluate the SOD enzyme activity. The principle of the method is based on the inhibition of pyrogallol autoxidation by the SOD enzyme at an alkaline pH. After the experimental steps were performed, the varying absorbance of the test solution was read in the spectrophotometer at 420 nm at 30-second intervals for three minutes.

The Sinha (1972) method was used to evaluate the activity of the catalase enzyme, an important antioxidant. The principle of this method is that the dark blue-black color of chromate acetic acid formed by H_2O_2 turns into light green by heat. The mixtures were then examined in the spectrophotometer at 570 nm against a blank.

The method of Hafeman et al. (1974) was used for the analysis of GPx enzyme activity. In the presence of reduced glutathione, the GPx enzyme catalyzes the breakdown of H_2O_2 to generate oxidized glutathione and water. The principle of the experiment is based on the use of reduced glutathione's 5,5'-Dithio-bis(2-nitrobenzoic acid) reagent to produce a quantified compound at a wavelength of 412 nm.

Statistical Analysis

3T3 embryonic fibroblast cells were assessed by the GraphPad Prism 9.0 program (GraphPad PRISM Software, San Diego, California, USA) to statistically evaluate total protein, malondialdehyde, catalase enzyme, superoxide dismutase, and glutathione peroxide enzymes. The obtained data were statistically analyzed using Tukey's multiple comparison test and a one-way ANOVA test and expressed as mean ± standard deviation. The results were evaluated according to the significance levels of $p<0.001$, $p<0.01$, $p<0.05$.

RESULTS

Cell viability

3T3 embryonic fibroblast cells were administered separately and together with 2 mM acrylamide and 10 μM luteolin concentrations for 24 hours and the resulting viability rates (%) are presented in Figure 1. MTT results revealed a substantial decrease in cell viability in the acrylamide-administered experimental group compared to the control group ($p<0.001$). When

the Acr+Lut group was compared to the acrylamide alone group, there was a significant rise in cell viability ($p<0.01$).

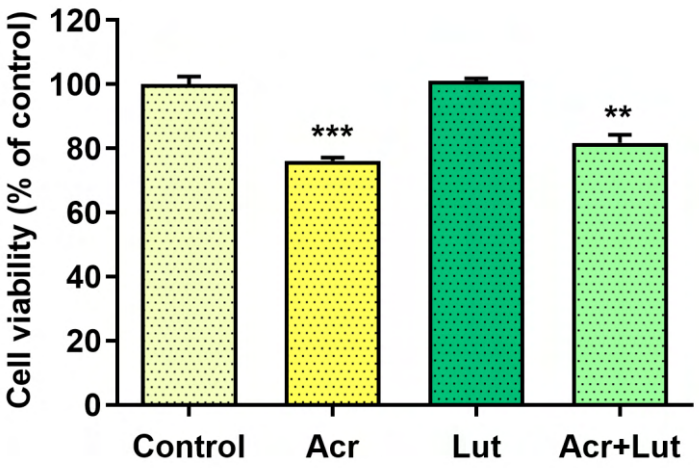

Figure 1. Effects of acrylamide and/or luteolin on cell viability in 3T3 embryonic fibroblast cells The columns indicate the average (±SEM) from three independent experiments. (**p<0,01; ***p<0,001*: compared with control)

Total protein amount

The total amount of protein obtained in 3T3 embryonic fibroblast cells is shown in Figure 2. Acrylamide and luteolin and their combined groups were applied to cells for 24 hours alone and there was no significant difference.

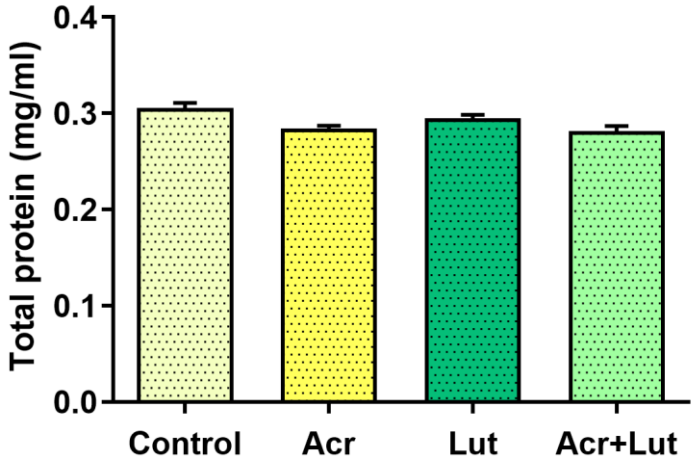

Figure 2. Effects of acrylamide and luteolin on total protein amount on 3T3 embryonic fibroblast cells

Lipid peroxidation

The amount of MDA after 3T3 embryonic fibroblast cells were exposed to acrylamide, luteolin, and their combination for 24 hours is shown in Figure 3. When compared to the control group, the amount of MDA was significantly increased in the group that received acrylamide alone ($p<0.001$). MDA levels significantly decreased (p 0.05) when the group treated with acrylamide+luteolin was compared to the group exposed only to acrylamide ($p<0.05$). According to the lipid peroxidation data, it was determined that the use of luteolin was effective in improving acrylamide-induced lipid peroxidation in 3T3 embryonic fibroblast cells.

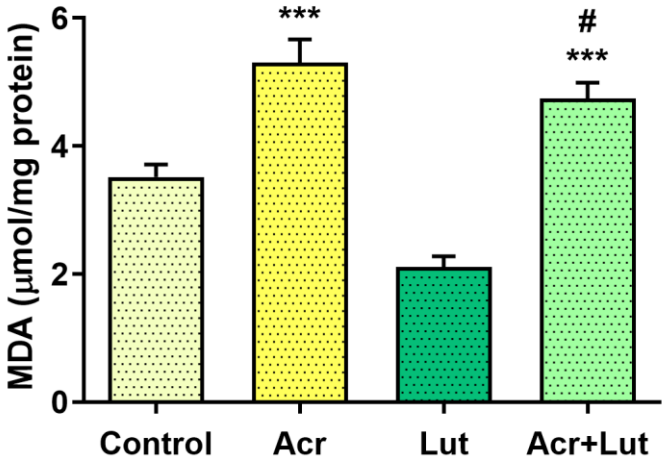

Figure 3. Effects of acrylamide and luteolin on lipid peroxidation in 3T3 embryonic fibroblast cells (*: p<0,05, ***: p<0,001*: compared with control, #: compared with acrylamide)

SOD Activity

The amount of SOD enzyme activity after 3T3 embryonic fibroblast cells were exposed to acrylamide, luteolin, and their combination for 24 hours is shown in Figure 4. The amount of SOD enzyme in the acrylamide-treated group was significantly decreased compared to the control group ($p<0.001$). When the amounts of SOD enzyme in the acrylamide group and the groups treated with luteolin in addition to acrylamide were examined, it showed that the enzyme activity increased significantly ($p<0.05$).

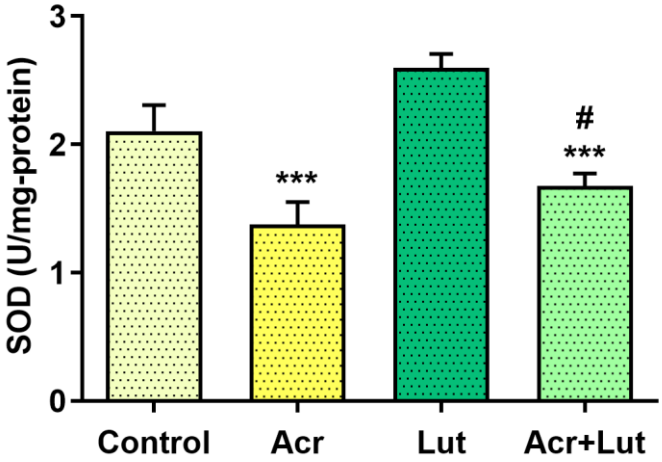

Figure 4. Effects of acrylamide and luteolin on SOD activities in 3T3 embryonic fibroblast cells (*: p<0,05, ***: p<0,001 *: compared with control, #: compared with acrylamide)

CAT activity

Figure 5 displays the findings of CAT enzyme activity after treating 3T3 embryonic fibroblast cells with Acr, Lut, and Acr+Lut for 24 hours. When acrylamide was administered, the CAT enzyme activity considerably decreased compared to the control group ($p<0.01$). The acrylamide + luteolin group revealed a noticeably higher level of CAT enzyme than the acrylamide group alone ($p<0.05$).

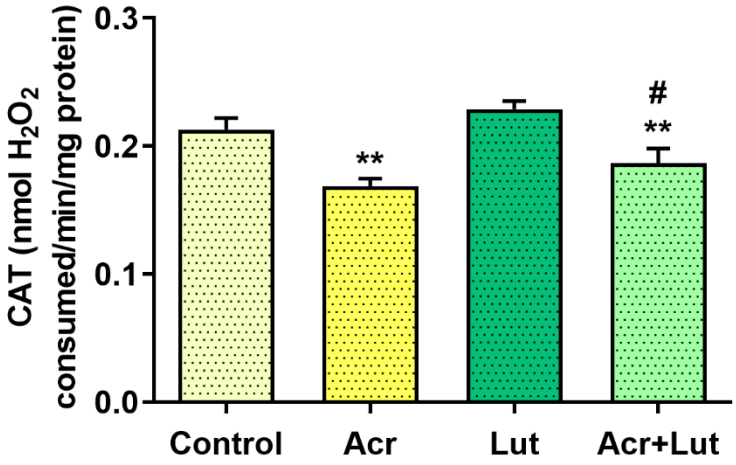

Figure 5. Effects of acrylamide and luteolin on CAT activities in 3T3 embryonic fibroblast cells (*p<0,05; **p<0,01; *: compared with control, #: compared with acrylamide)

GPx activity

Figure 6 indicates the amount of GPx enzymes measured by the quantity of glutathione consumed in 3T3 embryonic fibroblast cells after a 24-hour acrylamide and/or luteolin treatment. According to the results obtained, it was determined that the amount of GPx enzyme decreased only in the acrylamide group compared to the control group ($p<0.001$). In addition, when the groups treated with acrylamide and acrylamide + luteolin were compared in terms of GPx enzyme amount, it was concluded that the enzyme activity increased significantly in the acrylamide + luteolin group ($p<0.05$).

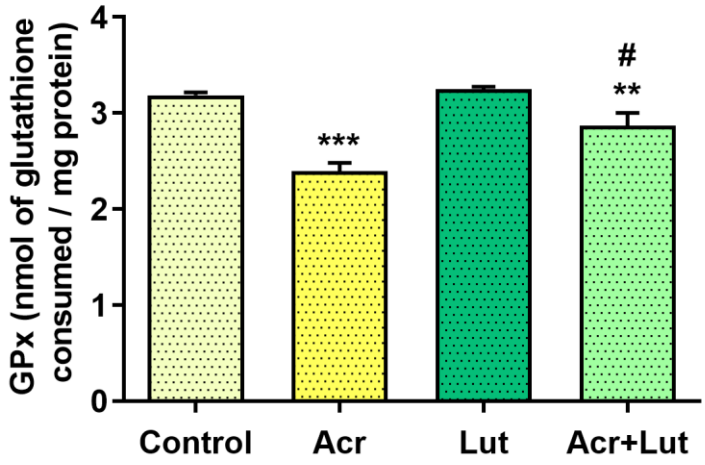

Figure 6. Effects of acrylamide and luteolin on GPx activities in 3T3 embryonic fibroblast cells (*p<0,05; **p<0,01; ***p<0,001; *: compared with control, #: compared with acrylamide)

DISCUSSION

Studies indicate that acrylamide, which has become a considerable problem in terms of food safety, has negative effects on biological systems. Antioxidant compounds have been used in many studies employing different cell types to reduce acrylamide-induced toxicity (Chen et al., 2014; Cao et al., 2008). However, there are limited studies on cell viability in 3T3 embryonic fibroblast cells (Evazalipour et al., 2021; Mahdizade et al., 2021). Cell viability was dramatically decreased in groups exposed to acrylamide at doses of 25, 50, and 100 μM in a

study using human lymphocyte cells. According to this study, supplementing with 10, 25, and 50 μM concentrations of chrysin in addition to 50 μM acrylamide decreased the detrimental effects of acrylamide on cell viability (Salimi et al., 2021). In a different investigation, cultured embryonic fibroblast cells were exposed to acrylamide at concentrations of 1, 2, and 5 mM. It was showed that 5 mM acrylamide significantly reduced cell viability. In the present study, it was discovered that 10 μM luteolin greatly increased cell viability, while 2 mM acrylamide significantly decreased cell viability in 3T3 embryonic fibroblast cells.

An excessive increase in the quantity of ROS produced at particular levels during the regular metabolic processes of cells disrupts the oxidative balance. Biological substances including lipids, proteins, and DNA are damaged by elevated quantities of ROS, resulting in cellular damage (Kohen and Nyska, 2002). Acrylamide can induce oxidative damage by causing excessive production of ROS in cells (Hong et al., 2021; Salimi et al., 2021). According to a study with HepG2 cells, exposure to 10 mmol/L acrylamide significantly increased the MDA level, a lipid peroxidation product. In the same study, anthocyanin extracts (AEP) isolated from blueberries at concentrations of 5, 10, 20 μg/mL were administered to the cells together with acrylamide, and as a result, it was revealed that the amount of MDA decreased (Li et al., 2018). In a 2019 study by Orta-Yilmaz, it was demonstrated that administration to 3T3 embryonic fibroblast cells for 24 hours resulted in significantly higher lipid peroxidation levels at 10, 100, and 1000 mol/L acrylamide concentrations, and that the same study also demonstrated that curcumin treatment combine with acrylamide resulted in significantly lower lipid peroxidation levels (Orta-Yilmaz, 2019). Similar to the above studies, acrylamide has been found to significantly increase lipid peroxidation in 3T3 embryonic fibroblast cells. In groups in which acrylamide and luteolin were co-administered to 3T3 embryonic fibroblast cells, lipid peroxidation levels were significantly reduced. Based on the healing effects of antioxidant compounds such as vitamin C, curcumin and blueberry extract against acrylamide toxicity, the study concluded that luteolin has a healing effect on lipid peroxidation.

Antioxidant enzymes are responsible for the detoxification of ROS, and the changing activities of these enzymes are an important indicator of oxidative damage levels (Adwas et al., 2019). Various chemicals and food pollutants cause the oxidative balance to be disrupted by reducing the levels of antioxidant enzymes in organisms. Earlier studies have shown that acrylamide affects the level of antioxidant enzymes (Orta-Yilmaz 2019; Albalawi et al., 2017). A study with the ARPE-19 cell line found that 0.7 and 1 mM of acrylamide significantly reduced the activity of SOD and CAT enzymes, while 10 μM of carnosic acid significantly increased activity when administrated to cells as a pre-treatment before acrylamide (Albalawi et al., 2017). In a study with 3T3 embryonic fibroblast cells, 100 and 1000 μmol/L acrylamide decreased the activity of the SOD, CAT, and GPx enzymes at the end of the 24 h period, while the enzyme activity of SOD and CAT and GPx increased significantly when supplemented with vitamin C or curcumin (Orta-Yilmaz 2019). In this study, the reduction in enzyme activity resulting from the administration of acrylamide to 3T3 embryonic fibroblast cells was parallel to the above *in vitro* studies. In our study, 2 mM acrylamide decreased the activity of the enzymes SOD, CAT and GPx in fibroblast cells, while 10 μM luteolin significantly increased the activities of these enzymes, playing a protective role in acrylamide toxicity.

CONCLUSIONS

Consequently, our research shown that 2 mM acrylamide decreases cell viability and elevates lipid peroxidation. In 3T3 embryonic fibroblast cells, the study revealed that exposure to acrylamide decreases the activity of the CAT, SOD, and GPx enzymes, which are involved in the intracellular antioxidant defense system. Luteolin has been discovered to be a useful antioxidant molecule against acrylamide toxicity.

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COMPARATIVE METHODS FOR DETECTION OF SUBCLINICAL MASTITIS AT DAIRY COWS IN ORDER TO IMPROVE MILK QUALITY

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ABSTRACT

The control of the health status of the udder is a significant element for obtaining a hygienically and safety milk. The aim of our research was to make a comparative analysis of the methods for determining subclinical mastitis, such as CMT and somatic cell count (SCC/mL) in comparison with electrical conductivity (EC) and lactose as an indirect method for detection of subclinical mastitis. It was determined that by increasing the number of somatic cells in milk (SCC/mL), the percentage of lactose in milk decreases from 4.80% to 4.13%, and the electrical conductivity increases from 4.21 mS/cm to 4.95 mS/cm. The number of somatic cells obtained using the MKC EN ISO 13366-2:2010 method was taken as a standard method for determining the somatic cells in milk, and based on these results, the sensitivity of the other methods was further determined. The results indicate that the California mastitis test (CMT) has 57% sensitivity and 88% specificity, while measuring the electrical conductivity (EC) has a sensitivity of 82% and a specificity of 50%. Whereas the sensitivity of the lactose is 79%, and the specificity is 60%. The sensitivity of the test, the so-called true positive rate, or probability of detection, expresses the percentage of correctly identified infected quarters. According to this with determination of EC and the percentage of lactose, more reliable results are obtained compared to the CMT test. On the other hand the specificity of the test, the ability to detect all negative samples, i.e. healthy cows, better results were obtained with the CMT test.

Keywords: mastitis, California mastitis test (CMT), electrical conductivity (EC), lactose.

INTRODUCTION

Mastitis is still one of the most significant problems in the dairy industry and one of the most expensive diseases affecting dairy cows. The losses that occur are the result of milk reduction, veterinary costs, deterioration of milk quality, and increase in the risk of subsequent mastitis (Lightner, J.K., et al., 1988). These losses are mostly caused by subclinical mastitis, while clinical mastitis can easily be determinate by the farmer (Kaşikçi, G., et al., 2012).

Diagnosing subclinical mastitis can be problematic because the milk still looks normal, but the number of somatic cells is increased (Forsback et al., 2010). These changes can be determined indirectly using several diagnostic methods such as California mastitis test (CMT), pH, chlorides, catalase test, modified White Side test (MWT) (Reddy, B. S. S., et al., 2014) as well as electrical conductivity. These tests are preferred to be used as screening tests for subclinical mastitis and can be easily used and satisfactory and repeatable results can be obtained (Leslie et al., 2002). The diagnosis of mastitis according to the International Dairy Federation (IDF) should be made based on the number of somatic cells (SCC) and the microbiological status of the quarter, i.e. bacteriological cultures of milk samples are the standard method for determining mastitis, which is financially more expensive and therefore not widely used.

For these reasons, the goal was to determine the compliance of several methods with standard protocols for diagnosing subclinical mastitis as somatic cell count. Because, in recent

times, the awareness of consumers who expect quality and safety products obtained from healthy animals is increasing more and more. Precisely because of this, it is necessary to control the quality of milk on the farm itself in order to meet the demands of consumers.

MATERIAL AND METHOD

The milk samples (N=69) were taken from a farm in the Pelagonian region, with a tied cow housing system. First, the milk was milked on a black pad in order to determine if there was clinical mastitis or inflammation of the teat canal, then the milk was milked on California mastitis test (CMT) plates in order to determine if there was subclinical mastitis. Two milk samples per quarter were taken, for determination of somatic cell count (SCC/mL) and for determination of conductivity and physicochemical parameters of the milk. The samples taken were transported to the laboratory at a temperature of 5-8°C in a hand-held refrigerator, and the tests were performed within 24 hours.

The obtained results were grouped into four categories depending on the number of somatic cells. At the same time, the first category referred to normal milk, where the number of somatic cells was ≤ 200,000 cells/ml, while the second, third and fourth categories referred to the number of somatic cells from 200,001 to 400,000 cells/ml; 400,001 to 600,000 cells/ml; and ≥601,000 cells/ml, respectively.

California mastitis test (CMT). The test is based on the action of surfactants (alkylaryl sulfonate) on DNA polymer from leukocytes, during which DNA is separated, and the protein part spontaneously turns into a gel. Interpretation of the results was done as previously described by Galfi A., (2016).

The electrical conductivity (EC) was examined using a HANNA HI 98192 EC/TDS/NaCl/Resistivity conductometer, which has a measurement range of 0-400 mS/cm. The samples were analyzed after milking. During the measurement, the temperature of the samples was 20-25 °C. About 50 ml of milk was taken for analysis.

The number of somatic cells was determined using a fluoro-opto-electronic method, BENTLEY SOMACOUNT CC 150, according to standard MKC EN ISO 13366-2:2010: Milk - Somatic cell counting - Part 2: Instructions for use with fluoro-opto- electronic counter ISO 13366-2:2006. Samples intended for determining the number of somatic cells were previously preserved with bronopol and heated to a temperature of 40 °C in a water bath before analysis in the apparatus.

Physicochemical parameters in milk (fat, protein, lactose, dry matter (SNF), density, casein, pH) were analyzed using LactoScope FTIR Advanced.

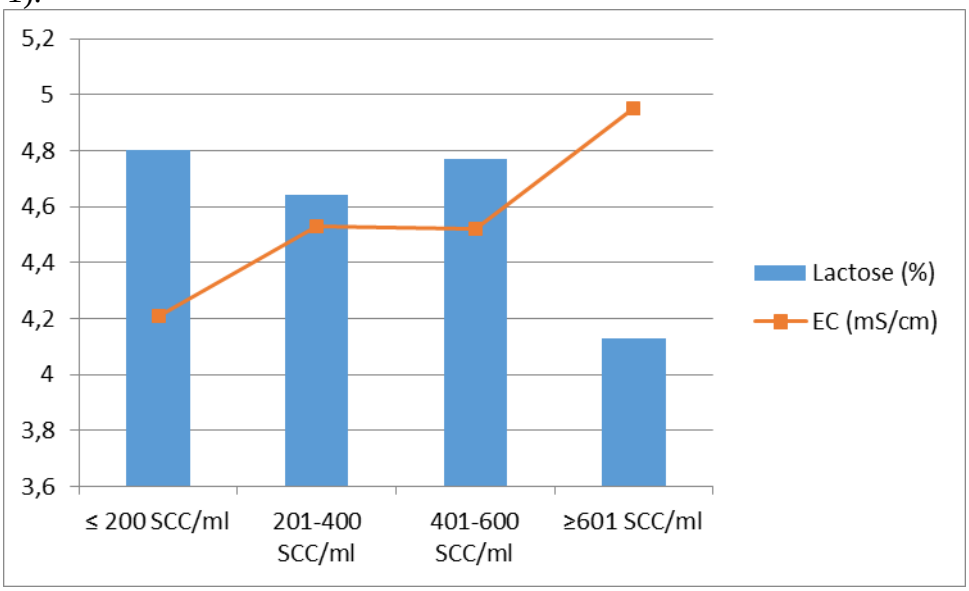
The examination of the sensitivity and specificity of indirect tests was done as previously described by Sharma et al., (2010).

Statistical significance between the studied categories was analyzed at a significance level of 5% ($p<0.05$) and 1% ($p<0.01$) using the Student's t-test. The data are presented in tables and graphs. The results were processed using Microsoft Office Excel and SPSS 20 statistical software.

RESULTS AND DISCUSSION

Monitoring the health status of dairy cows is necessary in order to obtain quality and hygienic milk (Boboš et al., 2012). The somatic cells of the milk are an indicator of the health status of the udder as well as the hygienic quality of the milk. A large number of factors which interact with each other affect the number of somatic cells in milk, such as the lactation period, the number of lactations, i.e. the age of the animals, milk yield, improper milking, stress, chronic diseases as well as mechanical injury to the udder tissue (Laevens et al., 1997; Pyörälä, 2003; Boboš and Vidić, 2005).

The increased number of somatic cells is usually accompanied by changes in the physicochemical composition in raw milk. The results shown in table 1 refer to the changes that occur in the milk composition, as a result of the increased number of somatic cells. Additionally, electrical conductivity in milk gradually increases with the increase in the number of somatic cells (Graph 1).



Graph 1 Changes in EC and lactose depending on the categories according to the number of somatic cells

Significant differences were determined only between the group where the number of somatic cells was over 600,000 cells/ml compared to the rest of the groups ($p<0.05$) (table 1). In addition, although we have an increase in milk conductivity when the number of somatic cells is above 200,000 cells/ml (4.53 mS/cm (201-400 x 10^3 SCC/ml) and 4.52 mS/cm (401-600 x 10^3 SCC/ml), however, no significant differences were observed, which we believe is due to the small number of samples in these two groups (N=8 and N=9, respectively). Additionally EC can have significant variations even in the absence of mastitis which can be due to a number of factors such as stage of lactation, age of cows, milking intervals as well as cow condition (Biggadike et al. 2000). Factors such as milk temperature, pH, and milk fat percentage can have an effect on EC measurement (Qayyum et al. 2016).

Table 1 Changes in the physicochemical composition of milk by category according to the number of somatic cells (N=69)

Categories according to the number of somatic cell count SCC/ml (N=69)	Milk parameters $\bar{x} \pm SD$							
	SCC/ml x 10^3	**Fat (%)**	**Proteins (%)**	**Lactose (%)**	**SNF (%)**	**pH (%)**	**Casein (%)**	**EC (mS/cm)**
≤200 x 10^3 (N=25)	77,92 ± 56,34	2,06 ± 1,78	3,32 ± 0,24	4,80 ± 0,19 [a]	9,07 ± 0,25	6,74 ± 0,06	2,79 ± 0,20	4,21 ± 0,68 [a]
201-400 x 10^3 (N=8)	322,83 ± 231,00	2,23 ± 0,54	3,48 ± 0,49	4,64 ± 0,31 [a]	9,04 ± 0,52	6,77 ± 0,07	2,92 ± 0,38	4,53 ± 2,42 [a]
401-600 x 10^3 (N=9)	466,03 ± 45,46	2,51 ± 0,69	3,65 ± 0,25	4,77 ± 0,20 [a]	8,87 ± 0,31	6,73 ± 0,10	3,13 ± 0,20	4,52 ± 0,64 [a]
≥601 x 10^3 (N=27)	1.415,65± 726,00	2,21 ± 0,64	3,64 ± 0,34	4,13 ± 0,57 [b]	8,61 ± 0,68	6,87 ± 0,11	3,04 ± 0,25	4,95 ± 1,15 [b]

*** Differences in values with different superscripts in the same column are statistically significant at the level: a:b p<0.05**

The CMT test is accepted as a quick, simple and reliable method for identifying cows with altered secretion and subclinical mastitis. At the same time, based on the results of CMT, the number of somatic cells can be indirectly determined in individual milk samples (Galfi A., 2016). Table 2 shows the results obtained using CMT, where the number of somatic cells is taken as a standard. 4% of the examined samples are false positives, while false negatives are 28%. In comparison with the studies of Galfi A., (2016), that value is 13.33% for false positive in the period before the drying of the cows, where bacteriological tests are taken as a standard. Sharma et al., (2010) states that the false positive reaction of CMT is 23.79%, while the false negative is 25.72%. Additionally, according to Varatanović et al., (2010), CMT test was positive at 11 samples, which were determinate previously as bacteriologically negative, on the other hand CMT give a negative reaction in 10 samples, previously determinate as bacteriologically positive.

The sensitivity of CMT in our research was 57%, and in the research of Galfi A., (2016) in dry cows the sensitivity of the test is 75%, while in the early lactation period the sensitivity is 87.5%. Sharma et al., (2010) found a higher sensitivity of the test (86.07%), while Langer et al., (2014) found a lower sensitivity of 60.1% compared to our research. The specificity of the test is 88% in our research, while in the research of Galfi A., (2016) that specificity of the test in the period before the drying of the cows is 86.67%, and in the period of early lactation it is 87.5%. According to the research of Dingwell et al., (2004) the sensitivity of CMT four days after parturition is 82.4%, and the specificity is 80.6%, which indicates that this method can be applied with success in determining udder secretion disorders and subclinical mastitis during the early lactation period. The validity of the test according to the studies of Langer et al., (2014) is 61.56%, Reddy et al., (2014) 73.33%, while according to the results of Sharma et al., (2010) the validity of the CMT is 75.52%.

Table 2. Results obtained with the California mastitis test (CMT) and using SCC/ml as a standard

Test	CMT	
	N	**%**
TP	25	36
FP	3	4
TN	22	32
FN	19	28
Total number of analyzed samples	69	
Sensitivity (%)	57	
Specificity (%)	88	
Validity (%)	68	
PPV (%)	89	
NPV (%)	54	

TP - true positive, FR - false positive, TN - true negative, FN - false negative, PPV - positive predictive value, NPV - negative predictive value

The sensitivity of the test with EC in our research is 82% (table 3). Similar results were obtained by Mansell and Seguya (2003) where they observed a sensitivity of 51%, while Langer et al., (2014) determined significantly low values of sensitivity compared to other authors and it was 12.5%. Galfi A., (2016) states that the sensitivity of the test in the drying period is 74.32%, while during early lactation it was significantly low at 2.86%, and for the specificity of the test, it is 50%. According to research by Mansell and Seguya (2003), the specificity of the test was 71%, while Nielen et al., (1992) observed a high specificity of 94%. In addition, Langer et al., (2014) considers that the possibility of determining the subclinical form of mastitis measured by the Draminski mastitis detector is relatively low 7.6%. While the validity in our research is 55%. According to the obtained results of Galfi A., (2016) when measuring the electrical conductivity with the Draminsky test, it was determined that the validity of the test in the drying period is 52%, while in the early lactation period it is 48.65%. While Langer et al., (2014) determined validity with the Draminski test of 59.05%.

From the results (table 3), it can be noted that the percentage of false positives is high (38%). The validity of manual instruments for measuring electrical conductivity has been investigated by many authors. Musser et al., (1998) indicated that 71% of test positive samples were bacteriologically negative and minor mastitis pathogens were isolated in 11% of negative milk samples. According to Galfi A., (2016) the stage of lactation, type of pathogenic microorganism's plays a significant influence on EC values. Additionally, Seguya and Mansell (2000) observed the lowest electrical conductivity in milk samples infected with major mastitis pathogens. Additionally, during mastitis the electrical conductivity is not always increased (Norberg et al., 2004). Also, Woolford et al., (1998) stated that the difficulties in the interpretation of electrical conductivity measurement results arising from large variations in EC values in uninfected udder quarters between cows, between udder quarters of same cow, as well as between different milking periods in the same udder quarters. Large deviations in the electrical conductivity of milk during the drying period and early lactation are thought to be the result of a physiological increase in chloride concentration in milk (Linzell and Peaker, 1975). Langer et al., (2014) explained that the reduced electrical conductivity of milk in infected udder quarters occurs as a result of increased capillary permeability during intramammary infection

and the transport of sodium, potassium and chlorine ions into the alveolar lumen resulting in to increase their concentration in milk.

IDF experts Hamann J., and Zecconi A., (1998) published a meta-analysis on electrical conductivity (EC) in which they concluded that EC does not provide satisfactory results for the detection of subclinical and clinical mastitis. According to their research the ability of EC to predict clinical mastitis can be considered in two ways. Moreover, if the clinical signs of the animal are taken as a criterion for diagnosis, in that case the sensitivity is 68%, specificity 82%, PPV 58%, NPV 82%. While if the number of somatic cells is taken as a criterion, the sensitivity remains at the same level of 68%, the specificity increases to 88%, the percentage of PPV and NPV is 72% and 85%, respectively. In subclinical mastitis, when intra mammary infection is taken as a criterion, sensitivity is 61%, specificity 66%, PPV 55% and NPV 70%.

Table 3. Results obtained by measuring electrical conductivity (EC) and using SCC/ml as a standard

Test	EC	
	N	**%**
TP	19	33
FP	22	38
TN	13	22
FN	4	7
Total number of analyzed samples	58	
Sensitivity (%)	82	
Specificity (%)	50	
Validity (%)	55	
PPV (%)	46	
NPV (%)	76	

TP - true positive, FR - false positive, TN - true negative, FN - false negative, PPV - positive predictive value, NPV - negative predictive value

As a result of tissue damage during the occurrence of mastitis and the reduction of the synthetic ability of the enzyme system of the secretory cells, there is also a reduction in the biosynthesis of lactose (Pyörälä, S. 2003). According to Pyörälä, S. (2003), lactose can be used as an indicator of mastitis, as it decreases during inflammation. According to the results obtained in our research, the sensitivity is 79%, while the specificity is 60% (table 4). The ability of lactose to determine intramammary infection according to the predicted limits of 4.7% whose value applies when the number of somatic cells is up to 100,000 cells/ml is 60.8% for sensitivity, and 80.6% for specificity (Pyörälä, S. 2003).

Table 4 Results obtained by measuring lactose and using SCC/ml as standard

Test	Lactose	
	N	**%**
TP	19	30
FP	16	25
TN	24	37
FN	5	8
Total number of analyzed samples	64	
Sensitivity (%)	79	
Specificity (%)	60	
Validity (%)	67	
PPV (%)	54	
NPV (%)	83	

TP - true positive, FR - false positive, TN - true negative, FN - false negative, PPV - positive predictive value, NPV - negative predictive value

CONCLUSIONS

Based on our research there is a positive correlation between somatic cells count and electrical conductivity. The highest values were observed in the fourth defined category according to the number of somatic cells (≥600,000 cells/ml) of 4.95 (mS/cm), compared to the normal milk group (≤ 200,000 cells/ml) 4.21 (mS/ cm). Additionally, with the increase in the number of somatic cells in the milk, there is also a decrease in the percentage of lactose. The lowest values were observed in the fourth defined category according to the number of somatic cells (≥600,000 cells/ml) of 4.13%, compared to the normal milk group (≤ 200,000 cells/ml) 4.80%. The best results in terms of the sensitivity of the test were obtained with EC (82%), then with lactose (79%) and finally with CMT (57%), from the total number of analyzed samples. The best results in terms of specificity were obtained using CMT (88%), lactose (60%) and EC (50%), from the total number of analyzed samples. Sensitivity of the test represents the ability of the test to detect all positive, infected individuals, the application of EC and the percentage of lactose gives more reliable results, compared to the CMT test. In terms of the specificity of the test, where its ability to detect all negative, i.e. healthy cows, better results were obtained with the CMT test, which is just another proof that the person performing the test needs training for correct interpretation of the obtained results

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CURRENT BIOTECHNOLOGICAL BREEDING METHODS AND APPLICATIONS IN HEMP (Cannabis sativa L.)

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ABSTRACT

Cannabis (Cannabis sativa) is an industrial plant with a long history and extensive application areas. Thanks to biotechnology, the synthesis and extraction of active chemicals from hemp has been developed, providing a wide variety of treatment opportunities in the field of health. in addition, hemp is utilized in about 60 different industries, including cosmetics, textiles, food, paper, bioenergy and biocomposites. Using these methods, purposes such as micropropagation, optimization, material conservation, production, and breeding are served in hemp. In this study, biotechnological research conducted with hemp in recent years has been examined from a broad perspective.

Keywords: Hemp, Genotype, In vitro culture, Biotechnological methods

INTRODUCTION

Hemp is a versatile industrial plant that has numerous applications. Its secondary metabolites, known as hemp cannabinoids, including THC (Δ9-tetrahydrocannabinol) and CBD (Cannabidiol), are utilized in the medical and pharmaceutical industries. Moreover, hemp is utilized in around 60 different industries, including cosmetics, textiles, food, paper, bioenergy, bio-composites, and biodegradable product manufacturing, as well as the automotive and construction sectors where petroleum and petro-chemistry are used.

Hemp synthesizes chemical compounds in terpenophenolic structures called cannabinoids. When hemp is mentioned, the perception it creates in people is usually related to the narcotic part. THC (Δ9-tetrahydrocannabinol) in the content of hemp is the only psychoactive compound. In this sense, hemp has been used as an arbitrary plant (addictive). For this reason, cultivation of the plant has recently been banned. For many years, hemp cultivation has not been done globally, and the industry, which developed based on hemp, had to change its production direction and techniques. Later, scientists carried out breeding studies and developed hemp varieties with low THC content by using cultural and biotechnological methods. In this way, it has been made possible to make hemp farming worldwide again with permission. Over time, industrial hemp varieties with low THC ratios have begun to be developed in many countries. For Turkey, two hemp varieties were registered by Samsun Ondokuz Mayıs University Faculty of Agriculture and Samsun Karadeniz Agricultural Research Institute in 2021. These varieties are essential for being Turkey's first and local varieties (Aytaç., 2022), and protecting genetically created varieties and using them as breeding material is vital. It may be inevitable that cross-pollinated plants such as hemp show genetic expansion. For this reason, it is crucial to use biotechnological methods to ensure the continuity of the obtained varieties.

Botanical Characteristics of Hemp

hemp (*Cannabis sativa*) is a one-year cultivar with 2n=20 chromosomes belonging to the Cannabinaceae family. The homeland of hemp is known as Asia. Since cannabis is a foreign pollinated plant, it creates a difficult situation for the breeder to collect the desired genes in a plant in breeding studies. For this reason, clonal studies are important especially in dioecious plants such as hemp. In this sense, effective results can be obtained by applying biotechnological methods and principles (Yaman., 2020).

Biotechnological Methods

Tissue culture applications are a method that allows micro-propagation in an aseptic environment and in cultures specially prepared for the plant, and in which plant growth and regulators are used in the prepared environment, the desired material is the same or plants with different gene structures according as the technique used (Kodym et al., 2019). This method is free from diseases, allows rapid reproduction, and is used to protect rootstock plants and in gene transfer. Although it has been reported recently that the success rate of these techniques on hemp is low, significant progress has been made now thanks to optimization studies.

Hemp is a traditionally grown crop and is propagated from its seed. Nevertheless, reproduction is usually done using clonal methods in hemp produced for medical purposes. In this way, the desired product level can be produced without expanding the population. Hemp can be grown under diode-led lamps in clonal propagation, culture vessels, and culture rooms. In this way, many plantlets can be grown in a small area. In this way, in plants grown in these environments, Insect, pathogen, or virus-free plants can be obtained (Monthony et al., 2021).

B5 vitamins and MS salts have been developed to support the culture of hemp, callus induction, and suspension (Mandolino and Ranalli, 1999). Researchers have reported that hemp responds positively to the MS environment and B5 vitamins (Braemer and Paris., 1987).

In a study, the effects of different combinations of plant growth regulators on plant regeneration were investigated. For this purpose, three different monoic hemp cultivars (Bialobrzeskie, Beniko, Silesia) were studied. Cotyledon, stem, and root parts were used as explant sources. Sterilized for 10 seconds in 70% ethanol and 20 minutes in 1% sodium hypochlorite. Prepared explants were kept in the dark for 4 to 7 days at 24°C innocent of plant growth regulators in Knopp medium "(KNO_3 200 mg/L, $Ca(NO_3)$ 2 $_4H_20$ 500 mg/L, $MgSO_4$ $_7H_20$ 200 mg/L)". The cotyledon, stem, and root explants (1 mg/L kinetin and 0.05 Naphthalene Acetic Acid (NAA) mg/l) were transferred to the medium with plant growth regulators. It was incubated in a 24–26°C growth chamber under a 16-hour photoperiod. Three weeks later, the explants were supplemented with plant growth regulators 0.2 mg/L BAP and 0.03 mg/L NAA for callus development. Explants were moved to a medium containing 2.0 mg/L Indole-3-acetic acid (IAA) for root formation. It has been reported that the calluses formed are of the same efficiency in the three cultivars, but there are some characteristic differences. It was reported that differences were observed in terms of water content and callus color, and the best callus induction was obtained from stem explants. Researchers have reported that the root explanatory callus color is white and brown and unsuitable for morphogenesis. In addition, it has been reported that they differ in regeneration. It was reported that the highest regeneration was observed in the cotyledon parts of the Beniko variety (Wielgus., 2008). For this reason, the genotypes studied on success have a direct relationship with the protocol applied.

Anther and pollen culture is an essential protocol for obtaining haploid plants. It is also a vital breeding method applied in breeding studies. In this context, in a study conducted in 2009, anthers collected from seven hemp cultivars (Finola, Jermakowskie, Silistrenskie, W1, Juso11, Bialobrzeskie, Zenit) were cultured for callus induction from anthers grown in vitro to determine the optimal condition in hemp anther culture. Plant regeneration has been studied. It is embedded in the Medium PYL (Medium Pylon Protocol) environment. MS medium modifiers: Plantlet growth was supported with 6-Benzylaminopurine (BAP) (1 mg/L) and NAA (0.5 mg/L), and after culturing, they were placed in a dark environment for two weeks. The cultures were then kept in a photoperiod of 23◦C, 16/8 hours light. While the Jermakowskie cultivar showed the maximum (42%) callus induction rate, it was reported that no callus production was observed in Finola, Juso11, and Silistrenskie cultivars (Luwanska and Wielgus., 2009).

Seed germination, which is the initial physiological stage of plant life, is significant in examining the factors affecting growth conditions and obtaining juvenile tissue as a potency explant for various in vitro procedures. in other words, in vitro seed germination is a variable biological stage that can be affected by environmental and genetic factors (media composition and environmental conditions). In in vitro production, the success rate may decrease due to contaminations. For this purpose, hydrogen peroxide (H_2O_2) applications have been frequently used in tissue environments in recent years. At the same time, since hydrogen peroxide allows the cells in the tissue environment to develop faster, the cells in the medium provide callus formation in a much shorter time. A study conducted in 2022 was carried out to stabilize production, and infrastructure was created with algorithms and artificial neural network technology. The study was designed to explore possible responses to hydrogen peroxide ratios. Five different algorithms were used to predict germination and morphological characteristics of cannabis grown in vitro. These algorithms; Gaussian Process (GP), Extreme Gradient Boosting (XGBoost), Vector Classifier (SVC), Random Forest (RF) models and Multilayer Perceptron (MLP) system. In this study, the Narlısaray hemp variety was studied. The development of seeds in vitro was estimated using five different algorithms. In the study, for the sterilization of plant seeds, they were subjected to surface sterilization with 70% ethanol for 3 minutes, followed by 0.10% HgCl2 (Civachloride) for 10 minutes, then washed three times with distilled water for 5-7 minutes. Seeds were treated with different concentrations of hydrogen peroxide (0.5%, 1.0%, 2.0% and 3.0% v/v) for 24 hours and transferred to MS medium. The medium for in vitro germination was prepared using 0.44% MS, 3.0% sucrose. The medium was gelled with 0.65% agar. The pH of the medium was adjusted to 5.8 with HCl (hydrogen chloride) and NaOH (sodium hydroxide). The medium was autoclaved at 121 °C and a pressure of 1.5 atm for 15 minutes. In addition, 200 mg/L “Sulcid” antibiotic was added to the medium to prevent bacterial formation. All culture media were grown in the growth chamber at 24 °C under white light diode lamps and 16/8 h illumination. Established in eight replicates with ten seeds per replicate. Fresh and dry weight measurements were taken from the plantlets formed after 21 days. Morphological characteristics (seedling fresh weight, seedling dry weight, shoot length and root length) along with germination (%) and plantlet (%) were recorded and the algorithm (RF) giving these values was reported to be the Random Forest model. The most realistic result in estimation. When the seeds were exposed to different concentrations of hydrogen peroxide compared to the control seeds, it was reported that high hydrogen peroxide concentration had positive effects on average germination as well like over germination, shoot length, fresh weight and dry weight (Aasim et al., 2022).

In another study, plantlets were obtained from seed and treated in different concentrations of hydrogen peroxide solutions (0, 1, 3, 5, and 10%) for one day. Surface sterilization was performed with 70% ethanol for 3 minutes and 6% sodium hypochlorite for 5 minutes. The sterilized seeds were then washed with distilled water. For hydrogen peroxide applications, the seeds were directly treated with the indicated concentrations of the solutions. Hydrogen peroxide solution gave the fastest and most successful germination results for hemp seeds, while at higher concentrations, the germination rate decreased, and contamination was observed (Ahsan et al., 2022).

In a study conducted in 2022, a study was conducted on the evaluation of genetic transformation in in vitro reproductions. In this study, cultivars with high cannabinoid (CBD) and cannabigerol (CBG) levels were studied. Simple Sequence Repeat (SSR) technology was used to evaluate genetic stability. Callus was obtained by culturing in MS basal medium with various concentrations of 6-benzyl adenine (BA) or tidiazuron (TDZ) for shoot regeneration. Then, 1-Naphthaleneacetic acid (NAA) was supplemented with 2,4-Dichlorophenoxyacetic acid (2,4-D) or kinetin (KIN), and stem explants were used. For rooting of the formed plantlets, they were transferred to a semi-strong MS medium supplemented with Indole-3-butyric acid (IBA), and rooting was achieved. No somacloning variation was observed in clones propagated by SSR technology. It has been reported that genetic homogeneity is achieved in clones in this culture production. It has been shown that culture protocols developed for in vitro propagation are suitable and applicable for clonal mass dissemination (Ioannidis et al., 2022). The absence of somacloning variation for hemp provides an opportunity for mass production and quickly obtaining a homogeneous population.

An alternative in vitro method has been developed for medicinal hemp production from plant nodes. This method made it possible to obtain new plantlets. The study used ground Stagnum Peat Moss containing sponges in semi-strength MS medium in different hemp varieties. Supplemented with indole-3-butyric acid (IBA) (0, 4.92, 2.46, and 9.84 μm) as growth hormones, Explanations were sterilized with 0.05% Tween-20 and 1% NaOCl, 70% ethanol for 12 minutes. It was then rinsed three times with deionized water. Two culture forms were created with and without aeration. The most effective result was obtained in the IBA (2.46 μm) solution, where the highest rooting was achieved in the environment without aeration, while the maximum growth was obtained in IBA (4.92 μm) in the ventilated containers. It was determined that the presence of IBA and reducing MS to half strength were more effective regarding rooting properties. This result supports the claim of Lata et al., (2010) that it is the most effective hormone in the development of hemp cultures under IBA. In addition, it has been reported by researchers that there is a significant correlation between genotype, culture vessel, and IBA (Ioannidis et al., 2022).

A study in 2021 was conducted on optimizing the in vitro seed germination of cannabis of the Finola cannabis cultivar. To sterilize the hemp seeds, they were treated with 70% ethanol for 60 seconds and rinsed under tap water for 15 minutes and washed with deionized water for 5 minutes in a laminar air flow cabinet. Seeds were sterilized using 12% (v/v) commercial bleach for 12 minutes, then rinsed with deionized water for 3-5 minutes. Then, different concentrations of DKW (Driver and Kuniyuki, 1984) medium (tenth, half and whole), glucose (5% and 2%) were added to the seeds. The pH was adjusted to 5.8 and 30 ml of GA7 (Gibellic Acid) was added for each treatment. As a result of the study, the best root length (8.68 cm) and number of leaves (6.67) were obtained as DKW+5% sucrose, DKW+2% sucrose and 1/2, respectively. Maximum seedling fresh weight (0.37 g) and shoot length (13.99 cm) were observed in semi-strength mMS medium containing 5% glucose. In general, seedling fresh

weight and shoot length decreased in full power environments (DKW and mMS). According to the results of the correlation coefficient, all morphological features were significantly related; The highest correlation was between plant weight and shoot length, and the lowest correlation was between root length and number of leaves (Hesami et al., 2021).

Another study was conducted on developing hemp varieties containing high CBD and CBG in vitro. Plant particles containing axillary buds were taken from the plant and transferred to an MS medium after sterilization. The shoots of the resulting plant were then subcultured in complete and semi-strength MS medium supplemented with various concentrations of 6-benzyl-amino-purine BA (4.0, 8.0 μM) or tidiazuron TDZ (2.0, 4.0 μM). The researchers reported that the highest average shoot number and length were obtained and rooted in adding 4.0 μM BA hormone in complete and half-strength MS mediums. In the same study, after enrichment with different concentrations of IBA (indole-3-butyric acid) 2.0 or 4.0 μM or NAA (α-Naphthalene Acetic Acid) 4.0 μM, it resulted in optimum rooting rates, Average root number and length yield per shoot, 4.0 μM IBA (indole-3-butyric acid) and NAA (Naphthalene Acetic Acid) in the culture medium, which successfully formed approximately 92% of the plant hormone Naphthalene Acetic Acid It has been reported that it is adapted to the conditions (Ioannidis et al., 2020).

In another study on the evaluation of the efficacy of growth regulators, a study was conducted in 2020 to obtain plantlets from hemp shoot tip and node segment tissues under in vitro conditions. This study investigated callus development in environments containing different growth regulators by utilizing cytokinin activities. The cultivar used in the study is the CBD-rich monoecious Eplison68 cultivar. For the sterilization process of the seed, it was kept in 75% ethanol for 1 minute and then in 5% active sodium chloride for 15 minutes. Then it was rinsed five times with deionized water and transferred to an MS medium. It was kept dark (25 ± 1°C) for seven days. Explants were taken from shoot tip and node segment tissues obtained from the seed, while plantlets were in vitro. Explanations were cultured on complete and half-strength MS medium. Daylight was maintained with fluorescents for a photoperiod of 16 hours at 25 ± 1°C under a photosynthetic photon flux density of 120 μmol. It was then transferred to a semi-strength MS medium containing 2% sucrose supplemented with 0.5 mg/L indole-3-acetic acid (IAA). Measurements were taken after 21-28 days. Shoots were examined in 3 different parameters: well-growing, weak-growing, and non-growing. Visual observations determined the shooting status, and IAA and IBA (0.5 mg/L concentration in ½ MS medium) were tested in the rooting status of explants. However, no statistical difference was observed between the two hormones regarding rooting. Different concentrations of tidiazuron (TDZ 0.1–0.5 mg/L), 6-Benzylaminopurine (BAP 0.5–2.0 mg/L), and meta-topolin (mT 0.1–1 mg/L) were used in the MS medium. The explants obtained from plants grown in this medium were compared regarding their ability to form new shoots. The regeneration rate decreased proportionally regarding shoot formation features in subcultured plants. The most effective hormone in MS basal medium for shoot induction has been reported as TDZ (0.5 mg/L). Success has been achieved in obtaining plants from shoots compared to nodal segments (Wróbel et al., 2022).

In a 2021 study to evaluate the regenerative ability on the Yumna7 hemp cultivar, hemp was cultured in MS medium to investigate the effects on embryo, cotyledon hypocotyl and leaf regeneration. Calli formed 10 days, 15 days, 20 days and 25 days after callus formation were collected and transferred to a callus induction medium. During the 4-week incubation, the highest callus yield was observed in explant specimens transplanted after 15 days. Induction frequencies of 5.97% in leaves, 7.65% in cotyledons, and 5.31% in hypocotyls were observed.

The tissues grown here were transferred a regeneration medium at 26°C and uninterrupted light for five weeks. About 6.12% produced shoots, and less than 3% of calli-developed shoots proliferating from the other three explants were reported by investigators. In addition, the study was repeated on 1000 different hemp varieties, and as a result, it was reported that the most effective explant sample was from cotyledons. In addition, it was reported that the success rate of the variety used was statistically significant. The most productive regeneration is reported to be a hemp hybrid, DMG278, with the F2 strain obtained from crossing Red Cherry Berry and Yunma7. This line gave the highest cotyledon regeneration rate at 7.09% (Zhang et al., 2021).

CONCLUSION

Hemp is an industrial plant that can be used in many areas with its history and agriculture dating back to ancient times. On 12/06/1933, hemp farming was banned in Turkey due to its illegal use. It is banned not only in Türkiye but also in many countries. World scientists have reduced the effectiveness of cannabinoids that cause neuropathic effects, such as THC, which is one of the most important reasons for the ban on hemp, allowing its agriculture to continue in a permitted way. In this process, this success has been achieved by using biotechnological methods and principles. It can be difficult to provide genetic stability with cultural methods, especially when working in a plant that is usually dioecious, such as hemp. It may be necessary to use the possibilities of biotechnology in a plant such as hemp. This is important in saving both work and time. In recent years, the scientific world has come a long way in the functionality and usefulness of biotechnological methods in studies with hemp and has achieved successful results. For this purpose, they discovered protocols that serve different purposes by using different media, different plant growth hormones and doses, different sugars and different sterilization methods. As a result of all studies, it was stated that different theories and unique methods should be developed for each genotype, and it was determined that the most important difference in success was due to the genotype.

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DETERMINATION OF ANTIMICROBIAL ACTIVITY OF TOPICAL CREAM DEVELOPED WITH PLANT EXTRACT AND PROBIOTIC

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ABSTRACT

Plants have been used for therapeutic purposes in many diseases from past to present. China rose is known to be used in skin diseases with its antibacterial and antifungal effects. The aim of this study is to evaluate the biological activity of water extract from China rose (*Hibiscus rosa-sinensis*) flowers on some pathogens. Disc diffusion and micro-dilution methods were used to evaluate the biological activity of China rose extract. In addition, an antimicrobial cream was formulated with China rose extract and the probiotic candidate strain *Limosilactobacillus fermentum* MA-7 originated from breast milk. The antibacterial and antigungal activities of the developed topical cream against the test microorganisms were determined by the well diffusion method. In conclusion, the China rose extract showed good biological activity on the test microorganisms. The highest activity was determined against *Staphylococcus aureus* ATCC 25923 with an inhibition zone diameter of 9.36 mm. The MIC and MBC or MFC values of the extract was determined as 12.5-50 µg/µL and 25-50 µg/µL. The developed cream formulations showed variable antimicrobial activity against the test microorganisms. The cream group prepared with Cream-Extract-Probiotic showed the highest inhibition zone (10.46 mm) against *Escherichia coli* O157:H7. The results of the current research show that China rose flower is a suitable candidate for the medical and pharmacological industries. The developed cream formulation containing China rose water extract and probiotic strain may be an alternative in the prevention and treatment of some infections.

Keywords: Cream formulation, *Hibiscus rosa-sinensis*, *Limosilactobacillus fermentum*, Natural additive, Skin

INTRODUCTION

Since medicinal and aromatic plants have the potential to provide benefits in the fields of medicine, cosmetics and pharmacology, the supplement has been attracting attention since ancient times (Abate and Belay, 2022). China rose (*Hibiscus rosa-sinensis*)(Malvaceae), is widely cultivated in subtropics and tropical regions due to its ornamental and medicinal properties (Izquierdo-Vega et al., 2020; Silva et al., 2019). China rose contains bioactive substances with therapeutic benefits such as flavonoids, terpenoids, tannins, alkaloids and saponins (Pawbake et al., 2023). With high medicinal values, China rose has many curative activities, including antibacterial, antifungal and antivital activities (Abate and Belay, 2022; Hema et al., 2022; Sidhu et al., 2023). It is also effective against skin infections, menstrual cramps, hyperlipidemia, hypertension, obesity, anemia and inflammation in folk medicine (Riaz and Chopra, 2018; Shen et al., 2017; Silva et al., 2019).

Skin infections are caused by microbial invasion of the skin layers and can cause life-threatening conditions (Esposito et al., 2016). The skin infections are quite high among hospital infections in recent years (Kaye et al., 2019). Soft tissue and most skin infections can result in surgery, bacteremia and sometimes death (Miller et al., 2015). In recent years, gram negative, gram positive and fungal pathogens have become important causes of acute skin infections (Altuntaş, 2019; Sönmez et al., 2020; Altun et al., 2023).

Live microorganisms that help protect human health when taken in appropriate amounts are called probiotics (Merenstein et al., 2023). Faced with the external environment of the human body, the skin has a large microbiota and functions as a physical barrier to protect the body against pathogens (Habeebuddin et al., 2022). The probiotics have many healing effects on the skin such as moisturizing, whitening, anti-aging, removing body odor and preventing wrinkles (Yu et al., 2022). The probiotics and their lysates can inhibit pathogenic microorganisms that cause skin infections and are used for skin health (Habeebuddin et al., 2022; Joshi et al., 2023).

In the study, the potential uses of cream formulations developed with China rose flower extract and *L. fermentum* MA-7 lysate for pharmaceutical and cosmetic industries were investigated. First, the biological activity of water extract from Chinese rose flower against pathogenic test microorganisms was determined to reveal its potential as a natural antimicrobial agent as an alternative to synthetic antimicrobials. Then, antimicrobial activity of cream formulations containing China rose extract and/or *L. fermentum* MA-7 were determined against clinical test microorganisms.

MATERIAL AND METHOD

Preparation Flower Extract from China Rose

The flower samples of China rose were collected from Alata Horticultural Research Institute (Turkey). After the plant material was washed with distilled water, it was dried in an airy environment. The dried samples were pulverized with a waring blender. The extract was obtained using distilled water in a hot water bath for 2 days (6 hours per day). After extraction, the residue was filtered and the solvent was evaporated. The China rose flower water extract was dissolved in Dimethyl-Sulfoxide (DMSO) and filter sterilized (0.45 µm). The extract was stored at 4°C for the duration of the study.

Test Microorganisms

The antibacterial and antifungal activities of the extract from the China rose flower was tested against four microorganisms. *Escherichia coli* O157:H7 and *Staphylococcus aureus* ATCC 25923 were cultured in Nutrient-Broth (NB) medium at 37°C. *Candida albicans* ATCC 10231 and *Candida glabrata* RSKK 04019 were grown in Yeast-Peptone-Dextrose (YPD) medium at 30°C.

Disc Diffusion Assay

The biological activity of China rose extract against pathogens was determined using the disc diffusion assay. The pathogens prepared at a McFarland concentration of 0.5 were spread on agar medium by dropping (100 µL). 20 µl (2 mg/disc) sample from China rose flower extract was impregnated onto sterile filter discs (Whatman No: 3; Diameter: 6 mm). The discs were placed on agar medium in triplicate. The petri dishes were incubated under conditions suitable for the test microorganisms. At the end of the incubation, the inhibition zone diameter was measured using Vernier calipers. Fluconazole (FCA; 25 µg/disc) and Kanamycin (K; 30 µg/disc) were used as positive controls.

Micro-dilution Method

Minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) or fungicidal (MFC) concentration values of China rose flower extract were determined by the micro-dilution method against test pathogens. The extract was added to each tube containing broth to obtain final concentrations (100-3.12 µg/µL). The microbial suspension at 0.5 McFarland density was added to the tubes. The tubes were incubated under the conditions necessary for the microorganism. At the end of the incubation, the lowest extract concentration that inhibited the growth of the microorganism was determined as MIC. Subsequently, the sample taken from the tubes was inoculated into agar medium and the lowest concentration without growth was determined as MBC or MFC. If the MBC/MIC or MFC/MIC ratio is ≤4, it was determined as

bactericidal, and if the MBC/MIC or MFC/MIC ratio was >4, it was determined as bacteriostatic effect (Al-Shammari et al., 2022; Baj et al., 2020).

Biological Activity of New Cream Formulations

The biological activity of new creams developed China rose flower extract and/or probiotic candidate strain *L. fermentum* MA-7 (Asan-Ozusaglam and Gunyakti, 2019) isolated from breast milk was determined by modifying our previous study (Saglam and Asan-Ozusaglam, 2023). The antimicrobial activity of commercial cream (C) as a control, Cream - Extract (CE) mixture, Cream - Probiotic (CP) mixture and Cream - Extract - Probiotic (CEP) mixture against test microorganisms was determined using the well diffusion method. The pathogens prepared at a McFarland density of 0.5 were dropped (100 µL) onto agar and spread. Cream formulations were added to each well (6 mm) in 3 replicates. After incubation, the diameter of the inhibition zone was measured using Vernier calipers.

RESULTS AND DISCUSSION

Since ancient times, plants have been a source for therapeutic agents. Plants have become leading natural resources in the manufacture of many contemporary medicines and as a result of traditional practices (Alexander et al., 2023). In the present study, the water extract was obtained from China rose flowers to determine its biological activity. The results of the disc diffusion assay of China rose flower extract on the test microorganisms are presented in Table 1. The extract formed zones of inhibition between 6.07 mm and 9.36 mm on the test microorganisms. Among the tested bacteria and yeasts, the most sensitive (9.36 mm) strain to China rose extract was found as *S. aureus* ATCC 25923.

Table 1. Disc Diffusion Test Results of China Rose Extract

Test Microorganisms	**Inhibition Zone Diameter (mm±SD)**		
	China Rose Extract	**Kanamycin**	**Fluconazole**
C. albicans ATCC 10231	6.12±0.12^{a}	16.34±0.84^{a}	14.48 ±0.57^{a}
C. glabrata ATCC 04019	6.58±0.12^{b}	11.68±1.54^{b}	NA^{b}
E. coli O157:H7	6.07±0.06^{a}	17.82±0.42^{a}	NA^{b}
S. aureus ATCC 25923	9.36±0.25^{c}	16.37±1.74^{a}	NA^{b}
F(Sig)	296.742(0.000)	13.558(0.002)	1890.058(0.000)

*No Activity

*Different letters in the column indicate significant difference at $p<0.05$ between samples.

In a study, the antimicrobial activity of water, ethanol and methanol extracts obtained from *H. rosa-sinensis* flowers was determined against test microorganisms (*Listeria monocytogenes* MTCC 657, *Bacillus cereus* MTCC 430, *S. aureus* MTCC 87, *Clostridium perfringens* MTCC 450). The inhibition zone was obtained as 10 mm to 13 mm for water extract, 5 mm to 18 mm for ethanol extract and 5 mm to 15 mm for methanol extract (Karnwal, 2022).

MIC is the minimum concentration of antimicrobial agent that visibly inhibits microorganism growth. MBC or MFC is the concentration at which microorganism growth is completely inhibited (Kowalska and Dudek, 2021). MIC values of China rose extract vary between 12.5 µg/µL and 50 µg/µL. The lowest MIC value was obtained on *C. glabrata* ATCC 04019 (12.5 µg/µL). *C. glabrata* ATCC 04019 has an MFC value of 25 µg/µL as the most sensitive yeast to the extract. Other test microorganisms have an MBC or MFC value of 50 µg/µL. Since MBC/MIC or MFC/MIC values were ≤4, the extract showed a cidal effect on the test microorganisms.

Table 2. MIC and MBC or MFC Values of China Rose Extract

Test Microorganisms	MIC (µg/µL)	MBC or MFC (µg/µL)	MBC/MIC or MFC/MIC
C. albicans ATCC 10231	25	50	2
C. glabrata ATCC 04019	12.5	25	2
E. coli O157:H7	50	50	1
S. aureus ATCC 25923	25	50	2

Ngan et al., (2021) determined the MIC and MBC values of the water extract obtained from *H. rosa-sinensis* flowers on *Helicobacter pylori* ATCC 43504 and ATCC 51932. The results indicated that MIC values were 5 mg/mL and 10 mg/mL and MBC values were 7.5 mg/mL and 12.5 mg/mL, respectively.

The biological activity analyzes of the cream formulations developed with China rose water extract and/or *L. fermentum* MA-7 are presented in Table 3. The control group (C) created a 2.08 mm zone of inhibition on *C. albicans* ATCC 10231, but did not have activity on the other pathogens. While the CE group (2.39 mm) containing the extract did not significantly increase the activity of *C. albicans* ATCC 10231, the CP group with probiotic added significantly increased it with 4.01 mm and the CEP group with 4.60 mm ($p<0.05$). The highest inhibition zone of CE with 6.76 mm was determined on *S. aureus* ATCC 25923. The highest inhibition zone of the CP group was determined with 4.01 mm on *C. albicans* ATCC 10231. Among the cream formulations, the highest zones of inhibition against the test microorganisms was belong to the CEP group. The highest inhibition zone was determined as 10.46 mm against *E. coli* O157:H7 and was significant compared to the other groups ($p<0.05$). The synergistic effect of China rose extract and *L. fermentum* MA-7 inhibited the growth of the test pathogens. This effect will lead to a new alternative for the cosmetic industry.

Table 3. Antimicrobial Activity Results of Cream Formulations

Test Microorganisms	Inhibition Zone Diameter (mm±SD)				
	C	CE	CP	CEP	F(Sig)
C. albicans ATCC 10231	2.08 ± 0.11^{a}	2.39 ± 0.28^{a}	4.01 ± 0.68^{b}	4.60 ± 0.72^{b}	16.529(0.001)
C. glabrata ATCC 04019	$-^{a}$	1.37 ± 0.42^{b}	2.21 ± 0.12^{c}	2.40 ± 0.25^{c}	56.444(0.000)
E. coli O157:H7	$-^{a}$	3.28 ± 0.70^{b}	1.25 ± 0.10^{c}	10.46 ± 0.53^{d}	331.477(0.000)
S. aureus ATCC 25923	$-^{a}$	6.76 ± 0.59^{b}	$-^{a}$	8.53 ± 0.21^{d}	605.635(0.000)

*C: Cream, CE: Cream - Extract, CP: Cream - Probiotic, CEP: Cream – Extract - Probiotic, NA: No Activity

*Different letters in the line indicate significant difference at $p<0.05$ between samples.

In a study, the wound healing potential of a cream prepared with an extract obtained from *H. rosa-sinensis* flower extracted with 95% ethanol was determined. It was determined that the wound formed in rats healed 93.52% faster than the control in 20 days (Mustaffa et al., 2020)

CONCLUSION

In this study, the antibacterial and antifungal activities of water extract obtained from China rose flower on test microorganisms shows that it has a high potential for use as an antimicrobial agent in creams to be obtained for topical uses. In vitro antimicrobial activity of topical cream formulations prepared with China rose extract and probiotic candidate strain *L. fermentum* MA-7 were found to be effective on pathogens.

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DETERMINING THE APPROACH AND EXPECTATIONS ACCORDING TO THE PROFILE OF ENTERPRISERS IN RURAL DEVELOPMENT SUPPORT: THE EXAMPLE OF THE WEST MEDITERRANEAN

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Abstract
The phenomenon of development is the common goal of both developed and developing countries and can be defined as the advancement of human life in the economic and social field and the increase of welfare by changing the economic, social, and political structures of the countries. The phenomenon of development should be achieved through social cohesion. While the development initiatives, which started with industrialization, manifested themselves in urban areas, rural areas were ignored. However, rural areas should not be excluded from the development initiative. This situation, which is seen as social development, has been prevented by industrialization. However, rural development initiatives that started in the 1960s are steps towards the integration of rural areas with urban areas and their inclusion in social development. Rural development initiatives that started in these years found their reflection in Turkey as well as in other countries of the world. The support given to rural areas has been the main basis for this. These supports to the rural areas were primarily applied on a regional basis, but they did not receive sufficient response due to the differences between the regions and the practices were not suitable for the local area. One of the most important supports to rural development initiatives implemented in Turkey is the Rural Development Investments Support Program (RDISP). Within the scope of this program, it is aimed to increase the income level in rural areas, to improve infrastructure, to ensure the integration of agricultural production and agro-industry, to strengthen food security, to create alternative income sources in rural areas, to increase the effectiveness of rural development activities, to increase the level of basic public services, to increase access to services and to create a certain capacity in rural society, taking into account the protection of natural resources. Although these applications are province-based, they were also applied in the provinces of Antalya, Burdur and Isparta, which are the Western Mediterranean Region. In this study, it is aimed to divide the enterprises benefiting from RDISP in the Western Mediterranean Region into groups, to reveal the profile of each group and to examine the benefits of support elements according to the characteristics of these groups.

Keywords: Rural area, Rural Development, Support, Western Mediterranean

INTRODUCTION

Countries attach importance to development to progress in economic and social fields and to ensure social welfare. Development is recognized as a process that involves increasing the level of social welfare and raising living standards. This process includes economic, social, and cultural dimensions. In these dimensions, economic development includes increasing the production power and raising per capita income, social development includes improving education, health and social services, and cultural development includes protecting and developing the society's own cultural values and heritage. However, development is the growth and development of a country by enabling it to become stronger economically, socially, and culturally. For the country to grow and develop, it is desirable for the society to reach the desired

level in economic, social, and political fields. In addition, development is the reduction of human deficiencies emerging in countries by integrating them with ecological balance.

Development is the building of the future of a country. Therefore, in order for development to be balanced, it requires the participation of all dynamics of the country. The human structure, natural resources, economic activities, technological developments, social and cultural structures of the country are these dynamics. Ensuring harmony between these dynamics and developing planning and policies for this is an important factor for development. Countries develop various policies to ensure that there is no discrimination between communities and regions to achieve the desired goals in development. However, the impact of industrialization, the inability to create alternative sources of income in rural areas, the dominance of the agricultural sector in rural areas and the dependence of the sector on nature have caused rural areas to remain in the background. In this framework, rural development policies have started to be seen as a special policy area for individuals living in rural areas to reach humane living conditions, to increase their income levels and to provide them with the opportunities of individuals living in urban areas.

Rural development is defined as "the process of improving the quality of life and economic welfare of people living in rural areas" (Moseley 2003). Rural development is defined differently. According to the Croatian rural development network; "the integral and multi-sectoral and sustainable development of the rural (non-urban) area", according to Atkinson "efforts that are economic and social in nature, aimed at promoting the concepts of retention, growth and expansion in non-urban areas, including improving the quality of life for rural residents" and according to another source; it is explained as "a method of improving the quality of life and financial well-being of individuals living in particularly populated and remote areas" (Anonymous 2023a, Atkinson 2017, Anonymous, 2023b). To define rural development in more detail, it is "the process of increasing people's access to humane living conditions, improving income distribution, increasing income level, ensuring localized developments in social and cultural areas, protecting and utilizing natural resources and reflecting the wealth to the lives of individuals" (SPO 2006).

Rural development policies are policies designed to reveal the efforts made throughout the country to improve the economic, social, social, and cultural opportunities of the communities living in rural areas, to increase the living standards of these communities and to support them to participate in national development (SPO 2000).

Today, both developed and developing countries have increased the importance they attach to rural development. Rural development activities vary from country to country. Even in the USA, there are differences in the rural development program of each state (Gürlük 2001a). In the EU, the basis of rural development activities is to ensure the continuity of production in agriculture, protection of the environment and transparency during the conduct of different economic activities in rural areas (Can 2007). In the Agriculture and Rural Development Report published by the European Union in 2022, it is stated that the European Commission supports rural development through a series of programs and initiatives despite the difficulties encountered, that it is determined to make agriculture and rural development sustainable, and that priorities for the future are set out. The same report calls for continued co-operation between the European Commission, Member States, and stakeholders. In addition, about the rural development activities of the report, it is stated that the Union has helped to improve the quality of life in rural areas and progress has been made in making rural development more sustainable (EC, 2023).

In Turkey, the developments in technology and knowledge level, the increase in the use of machinery in agricultural production have caused the rural labor force to leave agricultural production and employment deficit in rural areas. In addition, the high rate of population growth and limited job opportunities accelerated migration from rural areas to urban areas and Turkey entered a rapid process

of distorted urbanization after the 1950s. Migration and rapid urbanization caused by the development differences between rural and urban areas have created problems both in rural and urban areas. With the planned period, national development plans and programs were envisaged within rural development studies.

In this context, rural development projects have started to be implemented throughout Turkey. Rural development projects cover areas such as development of agriculture and animal husbandry, irrigation, improvement of wetlands, construction of village and forest roads, construction of drinking water ponds, provision of drinking water, increasing agricultural and animal production, afforestation activities.

In addition to general and regional activities for rural development in Turkey, various development-oriented programs are also carried out. One of these is the "Program for Supporting Rural Development Investments (RDISP)"(Taşcıoğlu, 2011).

The program aims to determine the procedures and principles for raising the income level in rural areas, improving infrastructure, ensuring integration of agricultural production and agro-industry, strengthening food security, creating alternative income sources in rural areas, increasing the efficiency of the rural development activities being carried out, increasing the level of basic public services, increasing access to services and creating a certain capacity in rural society, taking into account the protection of natural resources (OJ, 2006).

In this study, it was aimed to divide the enterprises benefiting from the program, which aims to create alternative sources of income in rural areas by evaluating the on-site processing of agricultural products to make rural development activities more effective, to reveal the profile and general structure of each group and to determine the opinions of these enterprises on the program.

MATERIAL AND METHOD

The research is supported by secondary data based on the literature but largely based on original data obtained through a survey based on face-to-face interviews with enterprisers of enterprises benefiting from the Rural Development Investments Support Program in the Western Mediterranean Region. A significant number of these enterprisers (e.g. those who benefit from irrigation investments or process their own produce) are also engaged in agricultural production.

The study was conducted in the Western Mediterranean Region. The Western Mediterranean Region is the region called TR61 in the Classification of Statistical Regional Units (IBBS) Level 2, which covers the provinces of Antalya, Burdur and Isparta in the west of the Mediterranean Region. The region has been home to various civilizations since the early ages due to its geographical location, fertile soils, and rich water resources.

In the study, a survey was conducted with the owner/manager (enterpriser) of 47 enterprises in Antalya province, 26 enterprises in Burdur province and 23 enterprises in Isparta province benefiting from the Rural Development Investments Support Program. Face-to-face interviews were conducted with a total of 96 enterprisers benefiting from the support program in the region in question.

In the analysis of the data, simple descriptive statistics and Cluster Analysis were used to divide the enterprisers into groups according to their level of utilization of the program and to reveal the profile of each subgroup and to develop appropriate policy recommendations for the target groups.

Cluster analysis is one of the multivariate statistical analyses that divides units and objects into classes by arranging them in general. Kaufman and Rousseuw (1990) define cluster analysis as a method that enables to classify the units examined in research by gathering them in certain groups according to their similarities, to reveal the common characteristics of the units and to make general definitions about these classes. Hair and Black (2000), after stating

that the primary reason for using cluster analysis is to find similar (homogeneous) groups of individuals in any data set, define cluster analysis as a collection of objective methods that quantify the structural characteristics of units in observation clusters.

The aim of the analysis is to reveal the similarities of the units according to certain characteristics and to classify the units on the basis of these similarities and to group the units in such a way that they are like each other.

Although cluster analysis is an analysis based on classification theory, it differs in some respects. The most important of these is that the classification technique is used to divide observations into different subgroups, whereas in clustering, sub-clusters are tried to be formed based on p variables (Kendall 1975).

In this study, clustering analysis was conducted to reveal the preferences of enterprisers in determining the type of support in rural development and to classify enterprisers into groups in terms of their characteristics. The main reason for making this distinction is to reveal which support instruments are preferred by the groups to be formed according to the characteristics of the enterprisers. The aim here is to examine the opinions of the groups to be formed among the enterprisers with the same characteristics about the program and to determine the expectations of the enterprises in such supports to be applied in the future. The results obtained from the clustering analysis will be presented in detail in the findings and discussion section.

RESULTS AND DISCUSSION

In the study, the field research was examined in two stages: general descriptive information about the enterprises benefiting from the program and the results of the analysis. Firstly, information about the enterprises benefiting from the program is given in Table 1.

In the Western Mediterranean Region, the highest number of enterprises benefiting from the program is in Antalya province. Although the region is located in the same geography, it shows differences in terms of climate conditions and soil fertility. While Antalya province has the typical climate conditions of the Mediterranean Region, Burdur and Isparta provinces show the common characteristics of the Mediterranean and continental climate zone. This situation also affects the agricultural sector and agriculture-based industry. In addition, due to the entrepreneurial characteristics of Antalya province and the fact that individuals are in closer relations with agricultural organizations, they have more information about such supports.

Table 1. General characteristics of enterprises benefiting from RDISP in the Western Mediterranean Region

		Rate (%)
Distribution of enterprises by province	Antalya	49.0
	Burdur	27.0
	Isparta	24.0
Legal structure of businesses	Company	56.3
	Cooperative, Union	21.9
	VSPU	18.8
	Sole proprietorship	3.1
Status of interviewees in enterprises benefiting from the program	Business manager	52.0
	Business owner	24.0
	Cooperative, Union President	18.0
	Operating partner	6.0
Education level of the interviewees	Primary School	6.0
	Middle School	12.0
	High School	28.0
	Associate degree	20.0
	University (Undergraduate)	32.0
	Postgraduate	2.0

When the enterprises benefiting from the support in the region are classified according to their legal structures, it is seen that the highest number of investments are made by limited, joint stock and collective companies. It is observed that companies utilize more than half of the total economic investment (56%). After the companies, development and irrigation cooperatives and unions (22%) and Village Service Provision Unions (VSPU) operating under district governorships (19%) made the most investments. Individuals or bilateral partnerships were the least beneficiary enterprises (3%). 56% of the enterprises benefiting from the program are in company status and 80.9% of the total beneficiary enterprises in Antalya province and 52.2% of the enterprises benefiting from the support in Isparta province are in company status in terms of their legal structures. On the other hand, in Burdur province, due to the effective work of the provincial governorship and the fact that they see the program as an opportunity, Village Service Provision Unions (approximately 60%) were the enterprises that benefited the most from the program.

Within the scope of the survey, most interviews were conducted with people who were in managerial positions such as business managers, accountants, etc. and who were actively working in the application to the program. At the company level, interviews were mostly conducted with company managers or company owners, chairmen or partners in cooperatives and unions, chairmen or managers in VSPU, and partners in sole proprietorships.

It was observed that the education level of the interviewees was generally high. This feature is directly related to the beneficiary status of the program. Because there is a one-to-one relationship between having information about the program and the education level of the people. This situation emerged from the general structure of the interviewees during the observations made during the survey period.

Within the scope of the program, many projects have been supported throughout Turkey. Information on the provinces and fields of activity of the enterprises receiving grant support in the Western Mediterranean Region is given in Table 2.

Table 2. Fields of activity of enterprises benefiting from RDISP by provinces distribution

Activity	**Antalya**		**Burdur**		**Isparta**		**Total**	
	Quantity	Rate (%)	Quantity	Rate (%)	Quantity	Rate (%)	Quantity	Rate (%)
Processing, packaging, storage	31	66.0	12	46.2	16	69.6	59	61.5
Drip irrigation	9	19.1	11	42.3	4	17.4	24	25.0
Capacity expansion	7	14.9	-	-	3	13.0	10	10.4
Sewerage, road	-	-	3	11.5	-	-	3	3.1
Total	**47**	**100.0**	**26**	**100.0**	**23**	**100.0**	**96**	**100.0**

Within the scope of the program, enterprises in the region that determined their own field of activity benefited from grant support for processing, packaging, and storage (61.5%). This situation is valid for all three provinces in the region. Agricultural production is intensive in the region due to the fact that the climate and soil fertility is suitable for fruit and vegetable production compared to other regions of Turkey in general. Intensive agricultural production increases the desire to meet post-production services from within the region. In this context, the program has been an opportunity for the enterprises in the region, and the need for processing and packaging of the products produced has been met to a certain extent. In addition, the need for storage of agricultural products produced in the region has been met through the support program. In this respect, it has been observed that the support program has benefited the rural areas of the Western Mediterranean Region.

The analyses of the enterprises benefiting from RDISP supports were summarized and divided into two clusters according to the non-hierarchical K-means clustering method. Individuals in cluster 1 constitute 54% of the total population and those in cluster 2 constitute 43% of the total population. Information about the clusters obtained is given in Table 3.

Table 3. Group and characteristics of enterprises benefiting from RDISP according to cluster analysis.

Criteria	**1st group**	**2nd group**
Field of activity of enterprises	Activity related to processing, packaging, and storage	Irrigation activity
Project subject of the enterprises	Animal and herbal products	Irrigation activity
Legal structure of businesses	Company	Cooperative and Village Service Union
Interviewees	company owners/managers	president
Education level of interviewees	Elementary and high school	Associate degree and undergraduate
Other sources of income the business	Have additional income	No additional income

Table 3. continued

Criteria	**1st group**	**2nd group**
Membership status of the operator to the agricultural producer organization	no membership	has a membership
How the courses to be organized in the region should be	Practical courses with a subject expert	Meeting, seminar etc.
Reason for businesses to do the project	Being an enterprise that the region needs, Support is an opportunity to establish an economic enterprise, Processing its own product,	Protecting water resources Saving natural resources and energy
Type of investment of enterprises in the project	establishing new businesses and upgrading technology	Increase capacity
Those working on project implementation and reporting	Business managers and private consultant	Personnel working in the company
Businesses' source of information about the program	Meetings organized by the provincial directorates of the Ministry and friends	Provincial Chamber of Commerce and Industry, governorship units
Whether the enterprises have other applications for support	Businesses with more than one application	Businesses with a single applicant
Difficulties encountered by enterprises during project application	Businesses facing difficulties in applying	
Businesses' intention to make other investments in the region in the future	They will make other investments	They will not make any other investments
Investments that enterprises intend to make in the future	Cotton processing, animal husbandry, milking unit, greenhouse cultivation, cold storage, and packaging facility	
Benefits of the program to the region	Collective decision-making and social solidarity	
Problems with the program according to enterprises	Excessive demand for equity capital, long investment period and the necessity of private consultancy	The evaluation period is long, the number of documents required is high, the support rules are strict, and the Ministry staff do not have sufficient knowledge
The idea of businesses to develop environmentally friendly projects and ensure environmental protection	Businesses that do not harm the environment and pay special attention to environmental protection	
Increased job opportunities in rural areas		Businesses arguing that their investments in rural areas will increase job opportunities for people living in rural areas
The situation of using the knowledge and experience of the company owners/managers	Businesses that think that their own knowledge and experience are important in business management	
Experts make business decisions		Businesses that advocate that the final decision on management and other issues in businesses should be taken by specialists
Adequacy of the amount of support		Businesses that agree that the amount of support is sufficient

Table 3. continued

Criteria	**1st group**	**2nd group**
Reasons for businesses to choose project topics	Thinking it will bring good income.	Reasons for businesses to choose project topics
Goals that businesses want to achieve with the project	Evaluating products To evaluate the existing resources of the region Increase capacity. The need of the business Contributing to the development of the region Technology innovation Exporting	To utilize the resources of the region The need of the region Protecting water resources Contributing to the development of the region Increasing efficiency Meeting the needs of the producer
Businesses' expectations from the government regarding the overall program	Increasing the monetary amount of support Expansion of area of activity Providing support separately according to sectors Giving to people who will produce. Separate grant for building construction	Expanding the scope of support Benefiting from the same support subject for a second time
Reasons for the continuation of the program according to the company owners/managers	Investment opportunity for businesses Providing financial contributions to businesses Increasing employment	Contribution to the region Making investments that cannot be made in the region. Protection of natural resources Ensuring effective use of resources Saving time and labor
Problems with the program according to the company owners/managers	Taxes within the support amount Lack of financial support Too many documents requested. Long evaluation period after application	No problem
Aspects of PSRDI that need improvement according to company owners/managers	The amount of money in support should be increased. Support should be tax-free. The number of required documents should be reduced. Should be given differently according to the sector. Support should be given to projects to encourage production. Facilitate the application process	No need to improve. Support for cooperatives should be diversified and prioritized

CONCLUSIONS

Development is the reflection of the changes to be made in social, economic, and cultural structure on human life, reaching the desired living conditions of people, increasing their income levels economically. In order to realize this, it is necessary to use the natural and human resources and technological structure of the country. For this purpose, the creation of policies should be done in a planned manner.

To realize the development initiative, it should be carried out without regional distinctions. Since the focus of development is seen as urban areas, more importance should be given to rural areas where agriculture and food products are produced, nutrition needs are met, and alternative income opportunities are limited. For this purpose, rural development policies have been implemented.

Rural development activities vary from country to country. Along with the developments in rural development in countries, various policies are developed and implemented for the development of rural areas in the world.

In Turkey, rural development activities and policies have been increasing in parallel with development initiatives in recent years. The "Rural Development Investments Support Program", which was established according to the principles specified in the National Rural Development Strategy (NRDS) issued for this purpose, is one of the most important ones.

The main purpose of this study is to categorize the enterprises benefiting from the RDISP in the Western Mediterranean Region into groups and to reveal the profile of each group. According to the results obtained in line with this main objective, it is examined how the supports for rural development should be on the basis of enterprises, and what kind of priorities and expectations the enterprises prefer in future such programs and/or supports.

According to the clustering analysis applied in the study, enterprises are divided into two groups.

The first group of enterprises benefiting from the support element are enterprises with company status that apply to the program for the processing of agricultural products, which is the next stage after the production of both plant and animal products, or for technology renewal in the existing enterprise. The second group of enterprises are agricultural producer organizations such as cooperatives and unions that support the producers to carry out the irrigation activities necessary for agricultural production.

The first group of enterprises benefited from the program to purchase equipment for processing.

While interviews were conducted with managers and company owners in the first group enterprises, interviews were conducted with the heads of agricultural producer organizations in the second group enterprises.

While the education level of the interviewees in the first group enterprises was primary school and high school, the education level of the interviewees in the second group enterprises was associate degree and bachelor's degree.

In the first group of enterprises, it is seen that the enterprises have different income-generating sources other than the program application, while in the second group, there are no income sources because they are agricultural producer organizations, and they are non-profit organizations in line with the objectives and principles of producer organizations.

It was observed that the first group enterprises were not members of agricultural producer organizations because they were engaged in commercial activities.

While the first group of enterprises wanted the courses for rural areas to be in the form of applications, the second group of enterprises preferred more general applications such as meetings and seminars.

While the first group of enterprises think that the main reasons for doing the project are that there is an enterprise that the region needs, the grant support received is an opportunity to establish an enterprise in the economic sense, and to process their own products, the second group of enterprises are to protect water and natural resources and to save energy.

The first group of enterprises benefited from the support program to establish new enterprises and renew their technologies. The second group of enterprises applied to the program to make use of the water resources of the region, to make the existing irrigation systems work more efficiently and to open more areas for irrigation.

While the first group of enterprises worked with enterprise managers and private consultants in the preparation and reporting of the project in the application to the support program, the personnel of the producer organizations worked in the second group of enterprises. The first group of enterprises encountered various difficulties during the application due to the fact that they made the preparation and reporting of the project through managers, private consultants, and technical staff.

The information sources of the first group enterprises about the support program are the meetings held by the Ministry of Agriculture and Forestry. The second group of enterprises

received information about the program from both public institutions and chambers of commerce and industry.

The first group of enterprises applied to benefit from the support program by making more than one application. The second group of enterprises did not have a second application.

The opinions of the two groups of enterprises about the functioning of the support program are as follows.

The first group of enterprises think that they will establish facilities for processing, packaging, and storing agricultural products in the future, thus more people and enterprises will benefit from the support program and alternative income and employment opportunities will be provided to the rural areas. This situation, which is important for rural development studies, is thought that the incomes of individuals living in rural areas will increase and rural development studies and supports will provide the desired effect in rural areas. In addition, this group of enterprises stated that with the increase in the support program, they will transfer other economic investments to the region in the coming years. On the other hand, since the second group of enterprises received support from the program only for irrigation-based projects, they do not have any thoughts about investing in the region in the future.

The first group enterprises add that with such support elements, besides the economic benefit that the enterprises will provide to the region, there will be elements that support social solidarity and collective decision-making.

When the problems encountered within the scope of the support program are examined, it is seen that the most important problems are the high demand for the amount of equity of individuals or companies, the long investment period and the necessity of a special consultancy system, while the second group of enterprises consider the long evaluation period, the excessive amount of required documents and the strictness of the support rules as the most important problems.

The opinions on the development of environmentally friendly projects are considered important for the first group enterprises in terms of developing projects that do not harm the environment and thus creating projects that respect the environment and protect nature in rural development studies.

Within the scope of the opinion on increasing job opportunities in rural areas, especially the enterprises with agricultural producer organizations, which are the second group of enterprises, are in the position of enterprises that advocate that investments to be made in rural areas will provide new job opportunities for the rural community.

When the reasons for the enterprises to choose the project subjects within the scope of the support program are analyzed, the first group of enterprises are of the opinion that such supports will bring alternative and good income to the region and the rural community, the products produced in the region will be evaluated with the establishment of an enterprise that the region needs, the needs of the enterprise will be met with financial support for new technology and modernization, the support will be seen as an opportunity for those who want to establish an enterprise in the region, the enterprises producing in the region will process their own products and increase regional and local production. In the second group of enterprises, the determination of the project subjects came to the forefront due to the fact that an enterprise that is needed in the region will be established by providing services to the rural area, the sustainability of natural resources will be ensured by protecting water resources, water saving will be ensured with irrigation systems and there is a support element within the field of activity of the enterprise.

The objectives that the enterprises want to realize with the project are to evaluate the products of the first group enterprises, to evaluate the existing resources of the region, to improve the business capacity of the region, to reach the elements needed by the enterprise, to contribute to the development of the region, to renew the technology and to export, which will

contribute to the development of production and the region in general. In the second group of enterprises, the evaluation of the existing resources of the region, especially the evaluation of water resources, meeting the irrigation needs of the region, protecting water resources and transferring them to future generations, contributing to the development of the region, increasing the yield of agricultural products with irrigation and meeting an important need of the enterpriser has been determined as the target to be achieved in the project.

Enterprises have various expectations in the support program. The first group of enterprises expect that the monetary amount of the support should be increased, the fields of activity should be extended to the whole rural area, the support should be given in different qualities and quantities according to the sectors, the support should be given to the people who will produce, and a separate grant support should be established for the construction of buildings. The second group of enterprises demanded that the scope of the grant capacity should be expanded and that they should be able to benefit from the existing support program for the second time.

When the reasons for the continuation of the program according to the enterprises are examined, the first group of enterprises are the enterprises that argue that the program is an investment opportunity for the enterprises, provides financial contribution to the enterprises and increases the employment opportunities of the region. The second group of enterprises, on the other hand, want the program to continue for reasons such as the protection of natural resources related to the natural structure, efficient use of resources, saving time and labor, contributing to the rural development activities of the region, and making investments that cannot be made in the region.

As for the problems related to the program, the first group enterprises consider the reduction in the amount of support due to the fact that the value added tax rate is included in the grant program, the fact that the amount of support is not sufficient financially for enterprise establishment, capacity increase and modernization, the excessive amount of documents required before the application and at the time of implementation of the project, and the long evaluation process of the application as the most important problems of the program. The second group enterprises argue that there are no problems with the program.

In this study, the expectations of groups with similar characteristics from rural development policies were investigated. Rural development studies have differences from other policy implementations. In rural development policies, the characteristics of the rural area, its potential, the social, economic, and social structure of the rural community, etc. need to be analyzed. It is expected that the analysis of the rural area and the society and the support elements to be made according to the social structure of the social structure, the groups showing similar characteristics in the face of situations and events are classified and the implementation of rural development policies for these groups can increase the effectiveness of existing policies.

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DETERMINATION OF SUPPORT PREFERENCES OF ENTREPRENEURS UTILIZING SUPPORTS POLICIES FOR RURAL DEVELOPMENT BY CONJOINT ANALYSIS

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ABSTRACT

Countries attach importance to development for the continuity and sustainability of societies. Economic, social, political, etc. of development It is expected that it will change in areas, and this will be reflected in society. Development starts with economic indicators and can be achieved with improvement in social indicators. Changes in economic indicators around the world have gained rapid momentum mainly in industry and service sectors. The change in the industrial sector, which started especially in the urban area, caused the rural area to remain in the background. Elimination of the separation of urban and rural areas, which is necessary for social development, has begun to be achieved by supporting rural development studies. Different models have also been used in rural development studies, and they have changed due to the general characteristics of the rural area. Various studies have been made and are being carried out for the development of rural areas in Turkey. In recent years, various programs have been implemented to support rural development studies. The main purpose of these programs is to increase the income level in rural areas, to improve the infrastructure, to ensure the integration of agricultural production and agro-industry, to strengthen food security, to create alternative income sources in rural areas, and to increase the effectiveness of the rural development studies, considering the protection of natural resources. The most important of these programs is the Rural Development Investment Support Program (RDISP). In the program, which includes the support given on a provincial basis, it is aimed to process and evaluate agricultural products, and to ensure the integration of agriculture and industry in rural areas. However, within the scope of the support, the preferences of the business owners/ manager (enterprisers) in rural development supports were not considered. Support preferences of entrepreneurs are important in terms of entrepreneurial activity. Support elements and types are a situation that encourages entrepreneurs to start businesses.

This study, it is aimed to determine the support preferences of the enterprisers benefiting from Rural Development Investment Support Program in the Western Mediterranean Region, which includes the provinces of Antalya, Burdur, and Isparta, by using conjoint analysis. Thus, it is aimed to determine the degree of influence of the policy set that maximizes the utility of the business owners/ manager and the characteristics of the manufacturer in this policy preference.

Keyword: Rural development, Support policies, Conjoint analysis

INTRODUCTION

Development is the common goal of developed and developing countries. Development can be defined as the change in the economic, social, and political structures of countries and the progress and welfare of human life in the economic and social fields. Harris (1992) defines development as the growth of the economy, change in its structure, improvement of income distribution and improvements in the political and cultural spheres. In the Special Specialization Commission (SPC) Report of the State Planning Organization (SPO) for the 9th Development

Plan, development is defined as "the process of increasing people's access to decent living conditions, improving income distribution, raising income levels, ensuring locally appropriate developments in social and cultural areas, protecting and using natural resources, and reflecting wealth on the lives of individuals" (SPO, 2006a). Within the framework of the human dimension, development is the mobilization of the existing power to reduce the human deficiencies that arise in countries to a great extent and to increase the welfare of people in material terms. In the light of comprehensive definitions, development is not only the increase in the income of individuals in economic terms, but also social and cultural developments and the phenomenon of living in greater social welfare.

For development to be balanced, it is necessary to ensure harmony between elements such as population dynamics, natural resources, economic activities, technology level, social and cultural structures of the country. As a result of the analysis of these factors, planning and policy formulation and development processes have an important role in the success of development. To achieve the expected goals in development, improving the qualifications of the society in terms of education, health, and manpower, raising the standard of living, eliminating the differences between regions and settlements should be one of the most important goals (Anonymous, 2002).

The economic dimension of development started especially with the industrial revolution. This change in the industrial sector has led to the development of the economic structure and its reflection on human life and the concentration of the population in urban areas where the industrial sector is intense. The inability to create alternative sources of income other than the agricultural sector in rural areas has caused this change to bring urban areas to the forefront and rural areas to be ignored.

The first foundations of rural development were started to be established with the traditional rural development approach, which started in the 1960s, with the increase in ideas about the lack of distinction between urban and rural areas for social development. Today, different models and methods of rural development approaches have been applied, rural development policies have been harmonized with sustainable development policies and changes in rural structure have been tried to be achieved.

The change in the industrial sector in the world has also been experienced in our country, especially with migration and rapid urbanization, which has led to the emergence of various problems in both rural and urban areas and the development at the national scale has not reached the desired dimensions.

With the Planned Period, various strategies were developed and put into practice in order to increase infrastructure and public services for rural areas and to accelerate rural development (SPO, 2006b). In addition to the basic legal regulations on rural areas, issues such as rural development, village problems and village development have been mentioned in all national development plans, and the priority targets for rural areas have been determined in these plans and programs.

The main purpose of rural development policies is to improve the economic, social, and cultural opportunities of the communities living in rural areas, to bring these communities to the national level of living, and to ensure their full participation in national development (SPO, 2000). A significant portion of the world's population lives in rural areas, and these communities provide their development in economic and socio-cultural areas, especially in the agricultural sector, with their own means or through external support (Taşcıoğlu, 2011).

With the planned period in Turkey, approaches to rural areas and rural development were generally different from the previous period. In addition to the basic laws enacted in the previous period, industrialization, modernization in agriculture and urbanization were considered in rural development and it was emphasized that rural development was a part of national development and should be handled together (Çağlar, 1986). In addition, regional development projects were implemented during this period and continue to be implemented.

In addition to general and regional activities for rural development in Turkey, various development-oriented programs are also carried out. One of these is the "Program for Supporting Rural Development Investments (RDISP)", which was established according to the principles set out in the National Rural Development Strategy (NRDS) published in 2003 and put into practice after being published in the Official Gazette dated 06.04.2006 and numbered 26131. The Program aims to determine the procedures and principles for raising the income level in rural areas, improving infrastructure, integrating agricultural production and agro-industry, strengthening food security, creating alternative sources of income in rural areas, increasing the efficiency of ongoing rural development activities, increasing the level of basic public services, increasing access to services and creating a certain capacity in rural society, taking into account the protection of natural resources (OJ, 2006).

The scope of the program is determined as the issues related to what needs to be done in order to encourage and support the economic activity investments of real and legal persons for the processing, evaluation, and marketing of agricultural products and the investments of organizations for the rehabilitation of existing infrastructure facilities in order to ensure economic and social development in rural areas within the provinces determined for the projected investments based on equity capital to be made individually and/or collectively by agricultural enterprisers (business owners/managers) in rural areas within the framework of development plans and programs and the National Agricultural Strategy.

Within the scope of RDISP, projects for village-based Irrigation facilities that ensure participation with a bottom-up approach, develop local capacity and organization, have the potential to create employment, increase and diversify entrepreneur incomes, encourage the increase in the level of education and entrepreneurship of the female population, and are based on the development and expansion of small and medium-sized industries based on agriculture are supported.

This study, it is aimed to determine the support preferences of the enterprisers benefiting from Rural Development Investment Support Program in the Western Mediterranean Region, which includes the provinces of Antalya, Burdur, and Isparta, by using conjoint analysis. Thus, it is aimed to determine the degree of influence of the policy set that maximizes the utility of the enterprisers and the characteristics of the entrepreneurs in this policy preference.

MATERIAL AND METHOD

The research was carried out with original data, supported by secondary data based on the literature, but largely obtained through a survey based on face-to-face interviews with enterprises benefiting from the Rural Development Investments Support Program in the Western Mediterranean Region.

The study was conducted in the Western Mediterranean Region. The Western Mediterranean Region is the region called TR61 in the Classification of Statistical Regional Units (IBBS) Level 2, which covers the provinces of Antalya, Burdur and Isparta in the west of the Mediterranean Region, with Muğla and Denizli in the west, Afyon and Konya in the north, Karaman and Mersin in the east, and the Mediterranean Sea in the south. The region has been home to various civilizations since ancient times due to its geographical location, fertile soils, and rich water resources.

A "field survey" covering the enterprises benefiting from the Rural Development Investments Support Program was conducted in the area called TR61 according to the Statistical Regional Units Classification (IBBS) Level 2, which includes the provinces in the Western Mediterranean Region (Antalya, Budur and Isparta). Information on these enterprises was obtained from the Support Branches of the Provincial Directorates of Agriculture.

In the study, 47 enterprises in Antalya province, 26 enterprises in Burdur province and 23 enterprises in Isparta province benefiting from the Rural Development Investments Support

Program were surveyed. Face-to-face interviews were conducted with a total of 96 enterprises benefiting from the support program in the region in question.

Conjoint Analysis methods, one of the multivariate analysis techniques, were used to analyze the data. Conjoint, as a word, means collective participation. The word Conjoint was formed by combining the words consider and joint (Churchill and Lacoubicci, 2002). If a Turkish equivalent is desired, it can be called "Analysis of Relationships", "Association Analysis" or "Composite Analysis" (Yiğit, 2008). With conjoint analysis, it is possible to define the service as combinations of quality levels and to determine the quality levels and the detailed judgments of individuals towards that service (Gill and Sanchez, 1997). Conjoint analysis is a multivariate analysis technique used to analyze individuals' preferences for different combinations of measured and unmeasured attributes. According to another definition, Conjoint analysis is defined as a method of systematically evaluating and estimating a decision maker's choice of a limited number of alternatives (Joel, 2002). This analysis is a method that tries to determine which features a newly developed or already existing product or service should have, to reveal the preference behavior of individuals who benefit from this service and to determine the most desirable features of the service.

In this analysis, it is assumed that the value people place on a service corresponds to the sum of the benefits they derive from all its identified attributes, and that they will then use that service in proportion to the benefits they derive from it. Utility is a highly subjective phenomenon that varies from person to person. It would therefore be difficult to know without the help of Conjoint analysis. The analysis is widely used in a wide range of fields and can be used in new service planning to determine the impact of innovations and in efforts to improve existing achievements.

The starting point of Conjoint analysis is based on "Total Benefit Theory". In the partial benefit contribution model, the partial benefits of each attribute level of the product are independent of each other and the sum of the partial benefits of these attribute levels constitutes the total benefit.

In Conjoint analysis, two different calculation methods are used to determine the importance levels of policy-related features. The first one is to determine the difference between the partial utility values of each attribute. The other way is to calculate the relative importance levels of the combinations. The difference between the partial utility values of the attributes is the difference between the two attribute levels with the highest and the lowest partial utility value. This value shows the relative importance of each level of each combination in the combination. In measuring the relative importance between combinations, the partial benefit change values calculated for each combination are proportioned to the total partial benefit change value.

When applying Conjoint analysis, it is important to determine the variables and measurement methods at the beginning. The stages start with defining the problem and determining the research purpose, and end with determining the variables and levels and collecting and evaluating the data accordingly.

The purpose of Conjoint analysis is to determine the priorities and options that affect the outcome in the decision phase (Schweikl, 1985). The first step in the analysis is the selection of the preference function that will determine the effect of the factor characteristics that have an impact on the preferences of the people participating in the analysis on the decision. This function is the basis for determining the partial values of the factor attributes that affect the preferences of the participants in the analysis (Gutsche, 1995; Green and Srinivasan, 1978). The most used models are the ideal vector model, the ideal point model and the partial benefit model (Gustafsson, 2003).

As in all statistical studies, the first step in conjoint analysis is to determine the decision mechanism and objectives of the research problem. The point to be considered at this stage is

that the research problem can be solved by defining preferences between variables and variable levels.

Within the scope of the Conjoint study, the selection of the factors and their levels to be included in the cards to be shown to the interviewee is a critical step. For this reason, the researcher should pay attention to the following points while determining the characteristics and levels of the product or service:

- Factors should be determinative in a way that they could influence individuals' choice. Any factor that is not related to choose should not be included in the study. However, the inclusion of factors that are important but do not create differences between preferences will make it difficult for the respondent to decide (Hair et al. 1995).
- Factors should provide complete and meaningful information about the service and be realistic.
- Factors should be practical and represent a single concept. The use of factors that include more than one dimension such as quality should be avoided.
- Factors should be easily communicated by the interviewee to enable a realistic assessment.
- The number of factors included in the analysis directly affects the reliability and statistical validity of the results. In addition, when the number of factors and factor levels is increased, the increased number of parameters will either lead to the presentation of more cards or to a decrease in the validity of the parameters.

In addition, many factors may cause respondents to be reluctant to participate in the research, as it would take too much time.

In this study, conjoint analysis was used to determine the support preferences of the enterprisers benefiting from RDISP in agricultural policy and rural development policy. Thus, the policy set that maximizes the benefit of the enterprisers and the degree of influence of the characteristics on this policy preference of the enterprisers were determined. At this stage, first of all, 5 factors required for the policy were determined and these are support type, support amount, support area, investment period and tax exemption. While determining the factors and factor levels, the factors and factor levels previously given in the supports for this field and given within the scope of this program were used. Factor levels according to these factors are given in Table 1.

Table 1. Factors and Factor Levels Used in Conjoint Analysis

Factors	**Factor Levels**			
	1	**2**	**3**	**4**
Support area	Animal husbandry	Greenhouse cultivation	Irrigation	Manufacturing industry
Type of support	Cash payment	Building construction	Machinery purchase	-
Support amount (rate)	25%	50%	75%	-
Investment period (months)	9	12	15	-
Tax exemption	None	2 years	3 years	-

The combinations to be used in the analysis according to factors and factor levels were determined as 16 in the SPSS package program. Accordingly, the combinations were formed as shown in Table 2.

Table 2. Conjoint Analysis Combinations

Card No	Type of support	Support amount (rate)	Support area	Investment period (months)	Tax exemption
1	Machinery purchase	50%	Greenhouse cultivation	12	None
2	Machinery purchase	50%	Animal husbandry	9	3 years
3	Cash payment	50%	Animal husbandry	9	2 years
4	Building construction	75%	Animal husbandry	15	None
5	Cash payment	25%	Irrigation	9	None
6	Cash payment	25%	Greenhouse cultivation	15	None
7	Building construction	25%	Greenhouse cultivation	9	3 years
8	Cash payment	75%	Manufacturing industry	12	3 years
9	Cash payment	75%	Greenhouse cultivation	9	2 years
10	Machinery purchase	75%	Irrigation	9	None
11	Building construction	25%	Irrigation	15	None
12	Machinery purchase	25%	Manufacturing industry	15	2 years
13	Cash payment	25%	Animal husbandry	12	None
14	Building construction	50%	Manufacturing industry	9	None
15	Cash payment	25%	Manufacturing industry	9	None
16	Cash payment	50%	Irrigation	15	3 years

RESULTS AND DISCUSSION

The study was examined in two stages: general descriptive information about the enterprises benefiting from the program and the results of the analysis. First, information about the enterprises benefiting from the program is given below.

In the Western Mediterranean Region, the highest number of enterprises benefiting from the program was in Antalya province. In Burdur and Isparta provinces, the need for agriculture-based industry differs from Antalya due to the nature of the investments made, and this is also reflected in the program.

When the enterprises benefiting from the support in the region are classified according to their legal structures, it is seen that most investments are made by limited, joint stock and collective companies. More than half of the support program (56%) was used by companies. After companies, development and Irrigation cooperatives and unions (22%) and Village Service Provision Unions (VSPU) operating under district governorships (19%) made the most investments. Individuals or bilateral partnerships benefited the least from the program (3%) (Table 3). In Antalya and Isparta provinces, companies benefited the most from the program, while in Burdur province, Village Service Provision Unions (approximately 60%) benefited the most from the program due to the effective work of the provincial governorship and the fact that they saw the program as an opportunity (Table 3).

Table 3. Distribution of legal structure of program enterprises by province

Legal Structure of Support Beneficiaries	Antalya		Burdur		Isparta		Total	
	Quantity (person)	Rate (%)	Quantity (person)	Rate (%)	Quantity (person)	Rate (%)	Quantity (person)	Rate (%)
Company	38	80.9	4	15.4	12	52.2	54	56.3
Cooperative, Union	7	14.9	5	19.2	9	39.1	21	21.9
VSPU	1	2.1	15	57.7	2	8.7	18	18.8
Sole proprietorships	1	2.1	2	7.7	-	-	3	3.1
Total	**47**	**100.0**	**26**	**100.0**	**23**	**100.0**	**96**	**100.0**

The survey was conducted with the people who are in the managerial positions such as business manager, accountant, etc. in the enterprises benefiting from the program and who are actively working in the application to the program. This also shows that the results are directly proportional to the legal structure of the enterprises. At the company level, interviews were

mostly conducted with company managers or company owners, chairmen or partners in cooperatives and unions, chairmen or managers in VSPU, and partners in sole proprietorships.

The education level of the interviewees was generally high. This feature is directly related to the beneficiary status of the program. Because there is a one-to-one relationship between having information about the program and the education level of the people. This situation emerged from the general structure of the people interviewed during the observations made during the survey period.

Within the scope of the program, enterprises that determined their own field of activity in the region benefited from grant support for processing, packaging, and storage (61.5%). Agricultural production is intensively carried out in the region since the climate and soil fertility is suitable for fruit and vegetable production compared to other regions of Turkey. Intensive agricultural production increases the desire to meet post-production services from within the region. In this context, the program has been an opportunity for the enterprises in the region, and the need for processing and packaging of the products produced has been met to a certain extent. In addition, the need for storage of agricultural products produced in the region has been met through the support program. In this respect, it has been observed that the support program has benefited the rural areas of the Western Mediterranean Region.

Within the scope of the program, other supports other than processing, packaging and storage grants were also benefited from. While 25% of the enterprises benefited from the support for drip Irrigation, 10.4% received grants for capacity increase and 3.1% received grants for infrastructure works such as sewerage and road construction.

Accordingly, the evaluations of the surveyed individuals about each alternative were taken and the evaluation of the individuals on the subject was made on a 10-point scale. In the scoring system, 1 point was accepted as the highest score for the alternative preferred by the individuals.

Individuals who benefited from the support were asked to rank the cards obtained as a result of the orthogonal design according to their preferences. Everyone’s ranking was subjected to the Bretton-Clark Conjoint Designer process and the partial utility coefficient, the degree of importance calculated for each factor and the preference ranking of everyone were calculated.

When the results of the analysis are evaluated, it is revealed that the most important factor in the support preference of individuals is the "support area". The degree of influence of the support area on the decision of individuals to benefit from support was calculated as 38.23%. After the support area, the second most important factor in the decision of individuals to benefit from support is "investment period". The degree of influence of the investment period on the decision of individuals to benefit from support was calculated as 16.25%. The third most important factor in the decision of individuals to benefit from support is "type of support". The degree of influence of the type of support on the decision of individuals to benefit from support was calculated as 15.57%. "Tax exemption" is the fourth most important factor in individuals' decisions to benefit from support. The degree of influence of tax exemption on individuals' decision to benefit from support was calculated as 15.42%. Finally, the fifth and last factor in individuals' decisions to benefit from support is the "amount of support". The degree of influence of the investment period on the decision of individuals to benefit from support was calculated as 14.53% (Table 4).

Table 4. Results of Conjoint Analysis

Factors	**Factors levels**	**Partial utility (Part worth value)**	**Significance levels (%)**
Support area (SA)	Animal husbandry (SA1)	**0.925**	**38.232**
	Greenhouse cultivation (SA2)	0.917	
	Manufacturing industry (SA3)	-0.291	
	Irrigation (SA4)	-1.551	
Investment period (months) (IP)	9 (IP1)	**0.331**	**16.246**
	12 (IP2)	-0.081	
	15 (IP3)	-0.250	
Type of support (TS)	Machinery purchase (TS1)	**0.666**	**15.572**
	Cash payment (TS2)	-0.265	
	Building construction (TS3)	-0.401	
Tax exemption (TE)	3 years (TE1)	**0.102**	**15.418**
	2 years (TE2)	0.086	
	None (TE3)	-0.188	
Support amount (rate)(SAR)	50% (SAR1)	**0.419**	**14.532**
	75% (SAR2)	0.023	
	25% (SAR3)	-0.442	
Total			**100.000**
Pearson's R Value = 0.983	Significance = 0.0000		
Kendall's tau Value = 0.833	Significance = 0.0000		

Within the framework of the findings obtained in the research region, it can be said that the most important feature in the optimum policy choice that gives the highest total benefit in the support decision of the enterprisers benefiting from RDISP in the Western Mediterranean Region is the "area of support" to be provided to the region. It is seen that enterprisers and administrators primarily pay attention to the area of support in the investments to be made in their regions. This situation shows that the bottom-up implementation in the EU in recent years, especially in rural development studies, is also suitable for the region in question. As a matter of fact, in relation to the investments to be made in a region, cooperation with local stakeholders or non-governmental organizations of that region is requested first. This is based on the fact that local stakeholders have knowledge about the shortcomings and potential of the region. It is seen that the individuals who will benefit from the support first pay attention to the area to be supported and prefer to benefit from the support accordingly.

The partial utility values of each factor level show the effect of those levels on individuals' preferences. The factor level with the highest partial utility value is the most preferred option by individuals. Accordingly, the factor level with the highest partial utility score in the support area factor is "animal husbandry" with 0.925. Animal husbandry is followed by "greenhouse farming" with a benefit score of 0.917, "manufacturing industry" with a factor score of -0.291 and finally "Irrigation" with a benefit score of -1.551. These data show that in the selection of the support area, the livestock breeding activity of the enterprisers benefiting from the program in the region is the factor level with the highest partial benefit for the region.

In the investment duration factor, the factor level with the highest partial benefit score is "9 months" with 0.331. This factor level is followed by "12 months" with a benefit score of -0.081 and "15 months" with -0.250. In the selection of the investment period given in the supports, enterprisers who benefit from RDISP in the region prefer a period of 9 months.

Table 5. Total Utility Values of combinations in Conjoint Analysis

No	TS	Partial utility	SAR	Partial utility	SA	Partial utility	IP	Partial utility	TE	Partial utility	Total utility
2	TS1	0.666	SAR1	0.419	SA1	0.925	IP1	0.331	TE1	0.102	2.443
1	TS1	0.666	SAR1	0.419	SA2	0.917	IP2	-0.081	TE3	-0.188	1.733
3	TS2	-0.265	SAR1	0.419	SA1	0.925	IP1	0.331	TE2	0.086	1.496
9	TS2	-0.265	SAR2	0.023	SA2	0.917	IP1	0.331	TE2	0.086	1.092
7	TS3	-0.401	SAR3	-0.442	SA2	0.917	IP1	0.331	TE1	0.102	0.507
4	TS3	-0.401	SAR2	0.023	SA1	0.925	IP3	-0.250	TE3	-0.188	0.109
13	TS2	-0.265	SAR3	-0.442	SA1	0.925	IP2	-0.081	TE3	-0.188	-0.051
14	TS3	-0.401	SAR1	0.419	SA3	-0.291	IP1	0.331	TE3	-0.188	-0.130
6	TS2	-0.265	SAR3	-0.442	SA2	0.917	IP3	-0.250	TE3	-0.188	-0.228
12	TS1	0.666	SAR3	-0.442	SA3	-0.291	IP3	-0.250	TE2	0.086	-0.231
8	TS2	-0.265	SAR2	0.023	SA3	-0.291	IP2	-0.081	TE1	0.102	-0.512
10	TS1	0.666	SAR2	0.023	SA4	-1.551	IP1	0.331	TE3	-0.188	-0.719
15	TS2	-0.265	SAR3	-0.442	SA3	-0.291	IP1	0.331	TE3	-0.188	-0.855
16	TS2	-0.265	SAR1	0.419	SA4	-1.551	IP3	-0.250	TE1	0.102	-1.545
5	TS2	-0.265	SAR3	-0.442	SA4	-1.551	IP1	0.331	TE3	-0.188	-2.115
11	TS3	-0.401	SAR3	-0.442	SA4	-1.551	IP3	-0.250	TE3	-0.188	-2.832

In the form of support factor, the factor level with the highest partial benefit score is "machinery purchase" with 0.666. Machinery purchase is followed by "cash" with a benefit score of -0.265 and "building" with -0.401. In the choice of the type of support, enterprisers who benefit from RDISP in the region prefer to receive machinery directly.

In the tax exemption factor, the factor level with the highest partial benefit score is "3 years" with 0.102. This factor level is followed by "2 years" with a benefit score of 0.086 and "none" with -0.188. In the choice of taxation in the supports provided, enterprisers who benefit from RDISP in the region prefer that the business they will establish be exempt from tax for 3 years.

Finally, the factor level with the highest partial utility score in the support amount factor is "50% grant" with 0.419. This factor level is followed by "75% grant" with a benefit score of 0.023 and "25% grant" with -0.442. According to the enterprisers benefiting from the program in the region, the factor level with the highest partial benefit when choosing the support amount is the support amount with "50% grant" rate (Table 5).

The average and total utility values of the combinations (question cards) presented to the enterprisers within the scope of Conjoint analysis and the priority order of individuals in policy choice are given in Table 5. The total utility value is the sum of the factor level scores and the combination with the highest total utility value is defined as the policy set that provides optimum utility for individuals. The combination with the lowest total utility value provides minimum benefit to the enterprisers.

According to the enterprisers, the optimum policy pattern that provides the maximum utility is card or combination number 2 with a total utility value of 2.443. The second most preferred combination by the enterprisers is card number 1. As can be seen from the above, machinery and cash grants are the most preferred forms of support for the owner, manager, shareholders, or heads of cooperatives/unions. As for support, 50% and 75% grants are preferred by the enterprisers. Animal husbandry and greenhouse cultivation are the most preferred sectors in the region. However, keeping the investment period short is seen as a preferred practice by individuals. The policy support set that provides the minimum (least)

benefit to individuals is determined as combination number 11 with a total benefit score of -2.832. This result shows that individuals do not prefer building construction, 25% support rate and irrigation investments (Table 5).

CONCLUSIONS

Development is the process of increasing people's access to humane living conditions, improving income distribution, increasing the level of income, ensuring localized developments in social and cultural areas, protecting, and utilizing natural resources and reflecting the wealth to the lives of individuals. Development is a target that countries want to reach and a series of developing movements.

Developments and development initiatives in countries have been to the detriment of rural areas and in favor of urban areas. For centuries, it has been accepted that urban areas are the focal points of development and progress. However, in recent years, this idea has started to change in many countries, especially with the demonstration that no distinction can be made between urban and rural areas for social development.

Policies have been established to make rural development efforts more efficient. These policies are the policies that reveal the efforts made on a national basis to improve the economic, social, and cultural opportunities of the communities living in rural areas, to increase the living standards of these communities, and to support them to participate in national development. However, these efforts have ceased to be the domestic policy of countries and have become an international issue in the world. Rural development activities vary from country to country. With the developments in rural development in countries, various policies are developed and implemented for the development of rural areas in the world. In Turkey, rural development activities started in the early years of the Republic and rural development policies were implemented with various regulations in the following years. One of these practices is the "Rural Development Investments Support Program". The program aims to create a certain capacity in rural society.

In this study, a "field research" covering the enterprises benefiting from the Rural Development Investments Support Program in the provinces in the Western Mediterranean Region was conducted. A survey based on face-to-face interviews was conducted with a total of 96 enterprises benefiting from the support program in the said region. According to this

When the results of the Conjoint analysis were evaluated, it was revealed that the most important factor in the support preference of individuals was the "support area". The degree of influence of the support area on the decision of individuals to benefit from support was calculated as 38.23%. After the support area, the second most important factor in the decision of individuals to benefit from support is "investment period". The degree of influence of the investment period on the decision of individuals to benefit from support was calculated as 16.25%. The third most important factor in the decision of individuals to benefit from support is "type of support". The degree of influence of the type of support on the decision of individuals to benefit from support was calculated as 15.57%. "Tax exemption" is the fourth most important factor in individuals' decisions to benefit from support. The degree of influence of tax exemption on individuals' decision to benefit from support was calculated as 15.42%. Finally, the fifth and the most important factor in the decision of individuals to benefit from support is the "amount of support". The degree of influence of investment duration on the decision of individuals to benefit from support is calculated as 14.53%. According to these results, the enterprises benefiting from the support in the region preferred the support combination of machinery in the form of support, 50% in the support amount (rate), livestock breeding in the support area, 9 months in the investment period and 3 years in tax exemption. In addition, according to the enterprisers, this combination is accepted as the policy pattern that maximizes their total benefits.

Conjoint analysis reveals the results obtained in the field of agricultural policy and the opinions of the people who benefit from the support. The study has created a entrepreneur-oriented approach that reveals the thoughts of the enterprisers on how the support for rural areas should be and can answer the question of how the support should be. As a result of this approach, the study is an important resource for policy makers.

In line with the results obtained from the study, the objectives of the support program, scope, support area, investment period, form of support, amount of support, what kind of support they benefit from the support program due to what kind of features of the support, according to which features the enterprises benefit from the support to operate in rural areas and which support combination the enterprises prefer were carried out through the example of enterprises benefiting from RDISP in the Western Mediterranean Region. With the findings obtained, the creation of support units according to the wishes, expectations, and potential of local knowledge in support policies for rural development will ensure that rural development efforts will achieve the desired success.

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ASSESSMENT OF PHENOLIC CONTENTS IN BASIL GROWN INDOORS AND OUTDOOR CONDITIONS

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ABSTRACT

The accumulation of plant phenolic compounds varies according to the conditions of the environment in which plants are grown. The lighting conditions the plant is exposed to are among the most important factors for plant production and metabolite content. This study aimed to assess the quantity of phenolic substances with HPLC equipment in basil (*Ocimum basilicum*) grown in two different conditions. In each light condition, the phenolic contents of basil plants significantly changed depending on the natural daylight and grow cabinet lighting conditions. The results showed that the quantity of rosmarinic acid, rutin, eugenol, chicoric acid, benzoic acid, methyl chavicol, chlorogenic acid, vanillic acid, caffeic acid, and TPC significantly increased under natural daylight. However, the level of cinnamic acid, quercetin, and TFC did not alter under both conditions. Overall, natural daylight condition is the most suitable lighting strategy to increase the phenolic content of sweet basil.

Keywords: *Ocimum basilicum*, Phenolic compound, Rosmarinic acid, Light exposure

INTRODUCTION

Environmental factors such as light spectrum, watering, temperature, and nutrients in agriculture are important ingredients for plant development and growth (Sutulienė et al., 2022). Light is one of the major elements that regulate plant behavior depending on light quality, quantity, duration, and direction (Dou et al., 2018). Photosynthetic characteristics are physiological traits indicating plant responses to environmental stress. Synthesis of primary molecules, sugars, is directly related to photosynthesis, and the accumulation of secondary metabolites through various biosynthetic pathways via intermediate molecules is also affected by environmental factors (Chutimanukul et al., 2022a).

Plant growth conditions play a crucial role in the yield and secondary substance accumulation in herbs. Among abiotic factors, light is a key abiotic factor that drives photosynthetic production and regulates physiological responses in plants. The primary and secondary responses of plants are triggered by changes in photoperiodic and photosynthetic light conditions (Chutimanukul et al., 2022b). Light quality has a profound effect, influencing complicated responses in plant morphology, physiology, biochemistry, and gene expression to regulate plant growth, morphogenesis, chloroplast, and secondary metabolite accumulations (Xu et al., 2020). Light situations influence the synthesis of phenolic compounds as a secondary metabolite in the growing conditions of the plants. Accumulation of the metabolites is also varied according to plant tissues at different developmental stages of herbs (Maurya and Sangwan, 2020; Chutimanukul et al., 2022b).

Basil is raised as a traditional and medicinal herb in Türkiye, and the phytochemical content of the plant varies depending on the growth and development conditions. The study was aimed to compare the effect of indoor and outdoor plant growth conditions on phenolic compound content in green sweet basil (*Ocimum basillicum* L.)

MATERIAL AND METHOD

Basil growth conditions

The sterilized basil seeds were seeded in a seedling tray containing garden soil, perlite, and vermiculite (2:1:1). After the approximately four weeks, the seedlings having four true leaves were transferred to plastic pots, then plant growth conditions were carried out in two different environments. For the indoor conditions, a controlled growth chamber was used to continue the experiment at 25 °C under 500 μmolm^{-2} s^{-1} white fluorescence 14:10 h light/dark cycles. For the outdoor conditions in the periods of April to August, the plants were grown in pots under open air conditions covered with a transparent plastic sheet to protect them from rain. The basil plants were watered with tap water, and sweet basil leaves were sampled in liquid nitrogen from approximately 4-month-old plants. For phenolic compound analysis with HPLC, the samples were kept at − 80 °C.

Analysis of phenolic compounds with HPLC

The samples were powdered in liquid nitrogen and extraction was done in methanol-chloroform solution (4:1). The mixture was sonicated for 45 min at 37 °C and then centrifuged at ambient temperature. The upper solvent phase was filtered to analyze total phenolic, flavonoid, and individual components.

The quantitative analysis of basil extract was performed HPLC (Shimadzu, Japan) by transferring to vials with an automatic injection of the samples (20 μL). Methanol and acidified water (2% acetic acid, v/v) was used as mobile phases by executing in reverse-phase C18 analytical column (250 mm × 4.6 mm, 5 μm, GL Sciences) at 25 °C. The flow rate was 1 mL min^{-1} and elution gradient was as follows: 0–2 min, 13% methanol; 2–7 min, 22% methanol; 7–30 min, 40% methanol; 30–50 min, 75% methanol and 50–59 min, 90% methanol; 59–67 min 95% methanol. The detection of basil phenolic compounds was determined by comparing the standard compound's chromatographic profiles (Elmastaş et al., 2017).

Determination of total phenolic and flavonoid contents

The contents of the total phenolic and flavonoid of basil extract were briefly analyzed according to earlier methods (Pękal and Pyrzynska, 2014; Genç et al., 2019). For TPC, folin-ciocalteu reagent, basil extract, and 2% $NaHCO_3$ were mixed, and after two hours of incubation, the absorbance was read at 765 nm. For TFC analysis, basil extract, CH_3COONa, and $AlCI_3$ were pipetted to tubes, and following 30 min incubation, the absorbance was measured at 425 nm.

RESULTS AND DISCUSSION

Based on the phenolic compound study results, rosmarinic acid, rutin and eugenol were major metabolites both for basil leaves grown indoors and outdoors conditions. On the other hand, it was determined that the content of all three metabolites in plants grown under external conditions was approximately 17, 4.7, and 4.4 times higher, respectively (Fig. 1). Based on study results at the pot study; the level of methyl chavicol, chlorogenic acid, vanillic acid, and caffeic acid were significantly higher in basil cultivated outdoor environment. However, cinnamic acid and quercetin contents did not significantly change in both conditions (Fig. 2). Also, it was calculated that chicoric acid and benzoic acid had 5-fold more content in basil grown in outdoor conditions. Total phenolic content of basil leaves was changed from 9.12 to 42.47 mg g^{-1} in the plants cultivated in indoors and outdoors environment, respectively. However, total flavonoid content did not show any significant change according to the growing conditions of the plants (Fig. 3).

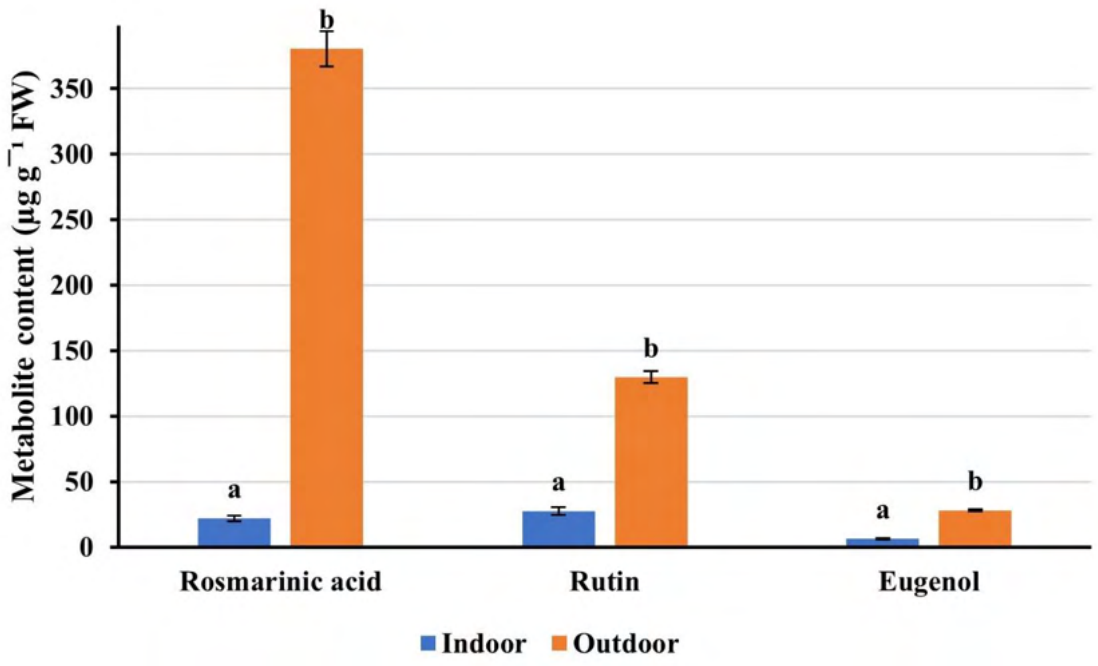

Figure 1. The major metabolites of basil leaves at the pot study

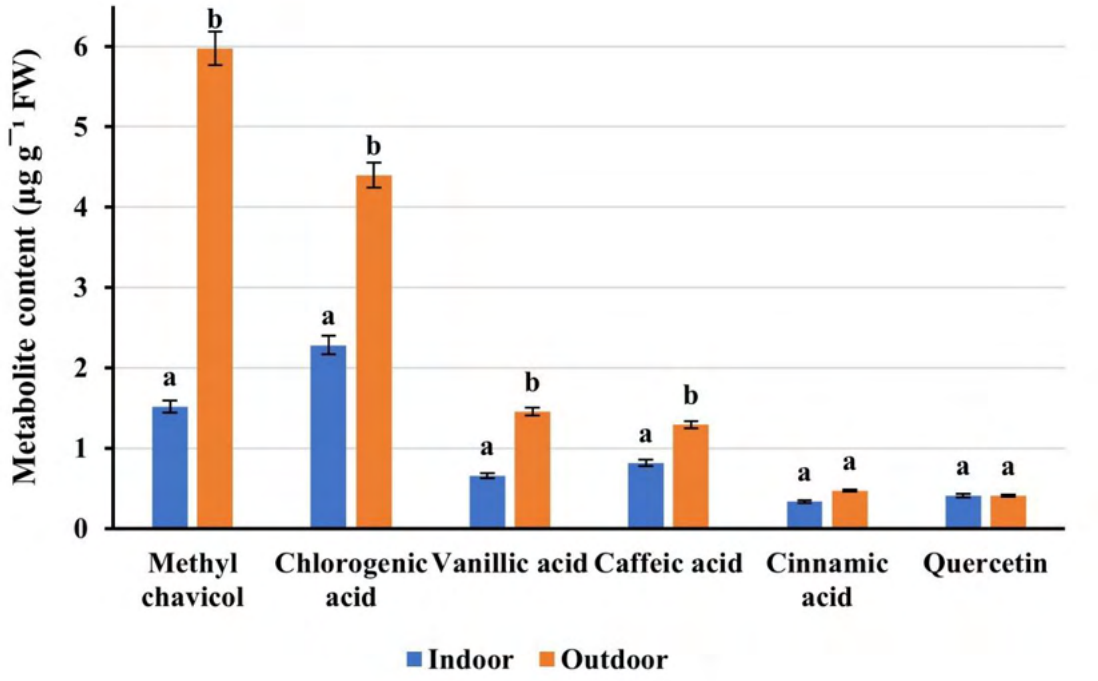

Figure 2. Six metabolites detected in basil leaves at the pot study

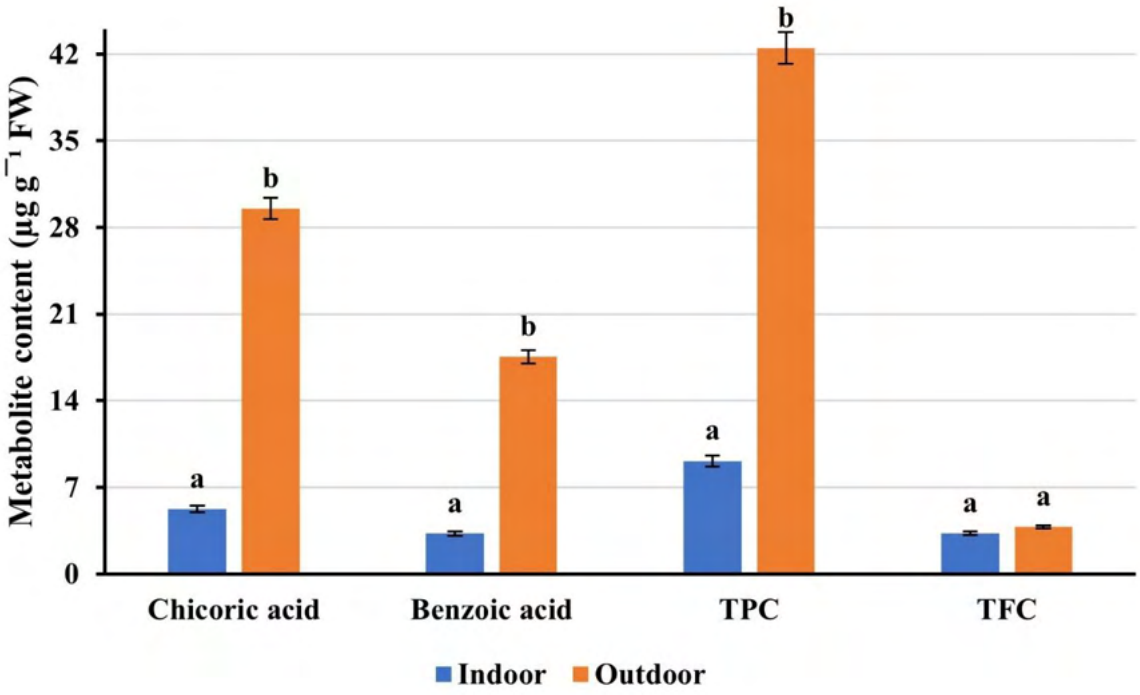

Figure 3. Chicoric acid, benzoic acid, TPC and TFC contents in basil leaves at the pot study (Concentration for TPC and TFC is mg g^{-1}).

The results of the study showed that the phenolic content of the basil mainly increased in the outdoor condition compared to the plant growth chamber conditions. The previous study on the effect of different shading treatments on the phenolic profile of basil declared that the highest phenolic contents for chlorogenic acid, caffeic acid, and rosmarinic acid were detected in unshaded plants compared to the various shading treatments (Castronuovo et al., 2019). Chutimanukul et al (2022) reported that TPC content in basil cultivars notable highest in the plants under the lights treatments treated with % 25 red and % 75 blue spectrum (Chutimanukul et al., 2022b). It may be said that the current study results are consistent with the different light application results mentioned.

CONCLUSIONS

Based on the study, the phenolic profiles of the basil exhibited different accumulations under cultivated in indoor and outdoor conditions. The increase of some phenolic substances in plants grown outdoors may be associated with different wavelengths of light to which plants in the open environment are exposed, while indoor plants are exposed only to white-fluorescent light. However, in the future, more detailed studies can be carried out on the change of plant phenolic profile by growing plants grown outdoors under more controlled conditions.

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EVALUATING THE IMPACT OF STRAW MULCH ON LOW-INPUT ULTIVATION OF TWO PARSLEY VARIETIES (PETROSELINUM CRISPUM (Mill.) Fuss)

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ABSTRACT

Leafy parsley (*Petroselinum crispum* (Mill.) Fuss) is an aromatic herb from the Apiaceae family, widely used in culinary, cosmetic, and pharmaceutical applications. It is distributed across all continents and is one of the oldest herbs used as a spice in food, with its leaves commonly utilized in fresh, frozen, or dried forms. Organic mulch is applied in the ecological cultivation of parsley to protect, maintain, and improve soil quality, as well as to reduce weed growth and erosion. The study was conducted on two parsley varieties, 'Domaći lišćar' as flat-leaf parsley and 'Moos curled', as curly parsley. The field experiment was carried out in the region of Pamići in Istria, on red soil with shallow to moderately deep depth and low to moderate rockiness. Organic mulch, in the form of straw, was applied in a 10 cm thick layer between the rows of parsley plants. The research investigated the effects of straw mulching on yield components and leaf quality, including vitamin C, chlorophyll, carotenoids, and the content of essential oil in the leaves of both parsley varieties. Initially, straw mulching slowed down the growth of parsley, but ultimately resulted in increased fresh herb yield. It was observed that straw mulching affected leaf quality, as the parsley leaves contained more water compared to those from the non-mulched control plots. There was a tendency of reduced vitamin C and pigment content, while the content of essential oil remained unchanged.

Keywords: leafy parsley, ecological cultivation, strew mulch, yield, quality

INTRODUCTION

Petroselinum crispum (Mill.) Fuss., better known as leafy parsley, is an aromatic plant from the Apiaceae family (Umbelliferae). The name of the genus *Petroselinum* was derived from the Greek word "petra" (Πέτρα) for rock or stone and the Latin word "selīnum" for plants growing on rocky and stony soil. The name of the species "crispum" comes from the Latin word "crispus," meaning curled (Mathe, 2020).

Parsley is primarily used in the kitchen as a condiment, for garnishing, or for flavoring food (Chenard et al., 2005). The advantage of this vegetable and spice is that it is used in various forms, such as fresh, frozen, or dried. It also finds applications in cosmetics for perfume, soap, and cream production, as well as in pharmaceuticals (Sidra et al., 2014).

Leafy parsley is recognized as a rich source of vitamin C, carotenoids, flavonoids, and essential oils (Buchter-Wisbrodt, 2005). The vitamin C content in parsley leaves ranges from 150 to 180 mg per 100g of fresh leaves, and the carotenoid content is on average up to 5 mg per 100g of fresh leaves (Kišgeci and Adamović, 1994). It is also a good source of mineral salts, primarily calcium, potassium, magnesium, and phosphorus (Pokluda, 2003), as well as iron, vitamins A, B, C, and bioactive compounds from the group of carotenoids, particularly lutein-zeaxanthin and beta-carotene (Chenard et al., 2005).

As an aromatic plant, parsly contains from 3% to 7% of essential oil in mature fruits, the root contains about 0,1%, while in dried leaves essential oil content vary between 0,8% and 1,0% (Kišgeci and Adamović, 1994). The fresh leaves contain from 0,041 to 0,121 ml essential oil per 100 g of fresh weight (Gruszecki and Walasek-Janusz, 2022). Essential oil of parsley is yellow to yellow-green in color, with a pleasant aromatic smell and taste. The dominant components of the essential oil are apiol and myristicin, which vary depending on the chemotype and variety. The aroma of parsley is determined by four components: 1,3,8-p-menthatriene (Chenard et al., 2005), apiol, β-phellandrene, and myristicin (Petropoulos et al., 2010). The characteristic taste of parsley comes from 1,3,8-p-menthatriene (Chenard et al., 2005).

Due to its favorable phytonutrient content, parsley leaves are used for medicinal purposes in the treatment of skin diseases, hypertension, hyperlipidemia, urinary tract infections, and as a diuretic (Chauhan and Aishwarya, 2018). Parsley leaves also contain furanocoumarins, averaging up to 0,2% (Mathe, 2020), which are phototoxic substances that, in combination with UV radiation, can cause a phototoxic reaction on the skin, resulting in phototoxic dermatitis (Agyare et al., 2017), an acute skin inflammation characterized by redness and blisters. The use of parsley as a natural deodorant is linked to its high chlorophyll content (Agyare, 2017).

Parsley's origin is in the Mediterranean region, where it has been cultivated since ancient times, and throughout history, parsley's popularity led to its spread to various parts of the world, to all continents. It is believed that the first wild parsley populations from Sardinia were transferred to England, where it was first cultivated in 1548 (Agyare et al., 2017). Leafy parsley is cultivated in most Mediterranean countries, Europe, America, and also in tropical regions, including East and West Africa. In tropical regions, such as Southeast Asia, leafy parsley is grown on a smaller scale (Agyare et al., 2017). In Turkey, parsley is widely distributed and grown in gardens and fields (Yanardağ et al., 2003), with an annual production of approximately 108.604 tons (Coskun et al., 2023 according to TUIK, 2021).

Within the European Union, the cultivation of leafy parsley is estimated at an average of 5.000 hectar. Significant areas of leafy parsley production are in Germany, France, the United Kingdom, Poland, the Netherlands, and Belgium. Apart from open fields, parsley is cultivated as well as fresh potted herbs. The production of potted parsley is intensive in the Netherlands, Germany, Belgium, France, Great Britain, and Poland (Mathe, 2020). According to the same author, basil (*Ocimum basilicum* L.) dominates the production of fresh potted herbs in Germany, making up 47% of the total, followed by parsley ranking second with a share of 19%.

The European Union's common variety list includes 215 varieties of leafy parsley. Notably, the list includes local races and land races, which are of special interest (European Commission, Plant Variety Database, 2023, available at https://ec.europa.eu/food/plant-variety-portal/index.xhtml, accessed on July 31, 2023). In the Republic of Croatia's variety list, there is a single registered variety of leafy parsley, "Domaći lišćar." This variety is preserved, and its seeds are recognized as "standard seeds." The preservation and maintenance of this variety are carried out by Podravka d.d. from Koprivnica (Republic of Croatia Variety List, 2023, available at https://www.hapih.hr/wp-content/uploads/2023/08/Sortna-lista-Republike-Hrvatske-01082023.pdf, accessed on July 31, 2023).

In cultivation, parsley is usually grown as a biennial species, but it is often cultivated as an annual crop. It is recommended to plant parsley as the second crop in a crop rotation, after crops fertilized with fresh manure. Fertilization and supplementation should be done using permitted organic fertilizers, fully mineralized organic fertilizers, or mineral fertilizers. Monoculture cultivation of parsley is not recomended, and a rotation period of 5 to 7 years on the same plot is advisable (Mathe, 2020).

Parsley is cultivated by direct sowing in rows. The germination process is slow at soil temperatures of 3-4°C and can take two to three weeks or even up to a month, depending on soil moisture availability. The first harvest of parsley leaves occurs when the plants develop 10-15 leaves, approximately 10 to 12 weeks after sowing. Harvesting is done by cutting the leaves at least 2 cm above the vegetative apex to promote regeneration. Throughout the year, multiple harvests can be done every 21 to 30 or more days, depending on the growing conditions.

During the prolonged germination period and after seed germination, weeds thrive vigorously, presenting a significant challenge in organic farming. The limited opportunity for inter-row cultivation before row formation and visibility intensifies the issue.

Organic mulching is used to slow down, hinder, and prevent weed growth. However, mulching also brings several additional benefits to cultivation, such as retaining soil moisture, protecting the soil surface from crust formation, and shielding it from overheating. As a result, mulching positively influences the plant's growth. The observed climate changes significantly modify production conditions and risks in agriculture. Mulching, as a soil protection measure and weed growth reducer, can contribute to mitigating the negative effects of climate change. This research was conducted to investigate the impact of straw mulching on morphometric characteristics and the quality of leafy parsley.

MATERIAL AND METHOD

The field experiment was conducted at an altitude of 340 m in Pamići, Istria, with geographical coordinates 45°09'36"N 13°52'41"E. Two standard varieties of leafy parsley were tested in the experiment: the mid-early variety "Domaći lišćar" (Bio Valentin) and the early variety "Moos curled" (Marcon). Parsley was cultivated with and without the use of mulch. (Figure 1). The "Domaći lišćar" variety has a declared vegetation period of 80 days, while the "Moos curled" variety ranges from 70 to 85 days.

Parsley was sown in the 16th week, on April 20, with an average monthly air temperature of 12°C. The average soil temperature at a depth of 10 cm was 8,8°C, and the total rainfall in April amounted to 37,4 mm. The annual precipitation sum was 1.309,8 mm (Croatian Meteorological and Hydrological Service, https://meteo.hr/klima.php?section=klima_podaci&param=k2_1 accessed on July 29, 2023).

The parsley was sown densely in rows with a row spacing of 25 cm. The cultivation of parsley was done on a red soil, shallow to moderately deep, low to moderately rocky, in cartographic unit 12/13 of the Istrian pedological map (Figure 2).

The soil was poor in physiologically active phosphorus, with 1,35 mg100 g^{-1}, well supplied with physiologically active potassium, with 15,6 mg100g^{-1}, and had a heavier mechanical composition with an average clay content of 30%. The soil pH value was 6,55. Before sowing, organic fertilization was carried out with 0,35 kgm^{-2} of fully mineralized manure, which was incorporated to a depth of 15-20 cm. Parsley was grown passively, under stress conditions, without irrigation and additional fertilization, initially with the addition of mineralized organic fertilizer and after germination with the addition of organic mulch.

The field experiment had a randomized design with four replications, covering a total area of 80 m^2, with each individual plot measuring 5 m^2 (Figure 3). The germination period was 17 days. After germination, a 10 cm thick layer of straw mulch was applied. In this experiment, various parameters were monitored, including plant height, leaf number, fresh and dry parsley leaf yield. Additionally, qualitative parameters such as vitamin C content, pigments, and essential oil were analyzed in fresh leaf samples.

Figure 1. Leafy parsley, varieties “Domaći lišćar” and “Moos curled” with straw and in control variant

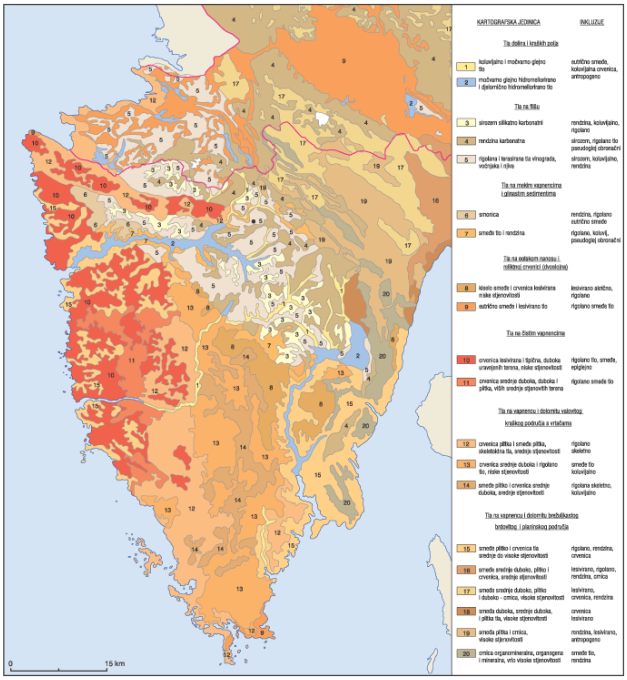


Figure 2. Pedological map of Istria (Bertoša i Matijašić ed., 2005)

2-4	4-4	1-4	3-4
4-3	3-3	2-3	1-3
3-2	1-2	4-2	1-2
1-1	2-1	3-1	4-1

Figure 3. Field experiment with parsley

The vitamin C content (mg100 g^{-1} FW) was determined by titration with 2,6-dichlorindophenol according to the AOAC standard method (2002). Fresh parsley leaves (10 g

±0,01) were homogenized with 100 ml of 2% oxalic acid and filtered. Then, 10 ml of the filtrate was titrated with 2,6-dichlorindophenol until a pink color appeared in the solution.

Pigment content, chlorophyll a, chlorophyll b, and total carotenoids were determined in a 96% ethanol extract of 0,5 g of fresh parsley leaves. The plant material was homogenized with quartz sand and $MgCO_3$, and then 10 ml of ethyl alcohol was added. Filtration was carried out using a water pump. The filtrate was quantitatively transferred to a 25 ml measuring flask and topped up with ethyl alcohol. Pigment concentration was determined spectrophotometrically, according to the method described in Pompelli et al. (2012).

The essential oil was obtained by hydrodistillation using an adapted Neo Clavenger apparatus according to ISO standard 6571-2008, in dry material, with a duration of 3 hours.

Statistical analysis was performed using IBM SPSS Statistics, version 23, applying Two-way ANOVA and Post-hoc Tukey's test with $p \leq 0,05$ and 0,01. In case of a significant interaction between the tested factors, One-way ANOVA and post-hoc Tukey's test were conducted with $p \leq 0,05$ and 0,01.

RESULTS AND DISCUSSION

The aim of the research was to determine the impact of mulching on quantitative and qualitative parameters in the cultivation of two varieties of flat-leaf parsley. The harvests were conducted thre times, at 84, 135, and 190 days after sowing. The observed quantitative and qualitative parameters were influenced by different factors with varying intensity: variety, mulching, harvest time, and their interactions. The results of the statistical analysis on the influence of each individual factor and their interactions are presented in Table 1.

Table 1. Effects of main factors and thair interactions on morphometric parameters and quality in leafy persley (Two way ANOVA, $p \leq 0,01$)

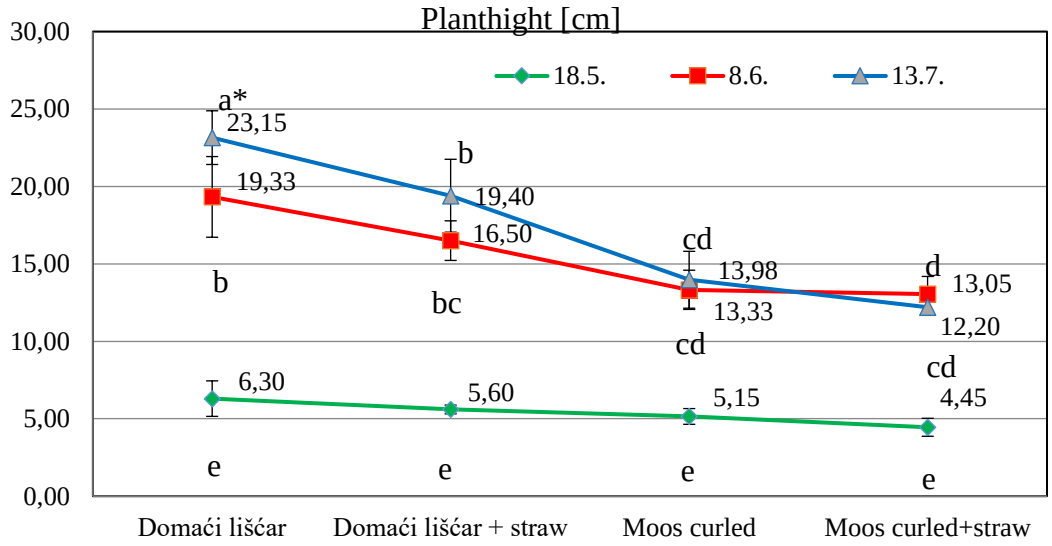
Parameter / *Factor*	Plant hight	Leaf number	Yield fresh leaves	Yield dried leaves	Vit C content	Essential oil content	Chl. A	Chl. B	Chl. AB	Carote-noids content
A -variety	+	-	+	+	+	+	-	-	-	-
B- mulching	+	+	+	-	+	-	-	-	-	-
C- harvest	+	+	+	+	+	+	+	+	+	+
AxB	-	-	-	-	-	-	-	-	-	-
AxC	+	-	+	+	+	+	-	-	-	-
BxC	-	+	+	-	+	-	-	-	-	-
AxBxC	-	-	-	-	+	+	-	-	-	-

Plant height, leaf number, fresh leaf yield, and vitamin C content were affected by the variety, mulching, harvest time, and interactions between these factors. Dry leaf yield and essential oil content depended on the characteristics of the variety and harvest time, as well as the interaction between these factors. Regarding pigment content, only influence of the harvest time on pigment concentration was statistically confirmed. A detailed overview of the impact of these factors is presented below in graphs 5 to 10 and in Table 2.

The height of leafy parsley just before harvest ranged from 13,5 to 23,5 cm, with variations depending on variety and treatment. According to Mathe (2020), parsley's plant height at the time of harvesting typically falls between 8,0 and 25,0 cm, and curly-leaf parsley

generally has a noticeably lower plant height compared to flat-leaf parsley. For commercial harvesting of flat-leaf parsley, the recommended plant height is usually in the range of 20 to 25 cm (Prez et al., 2021).

Mulching with straw had a significant impact on the plant height of the "Domaći lišćar" variety during the third measurement, as shown in Figure 5.



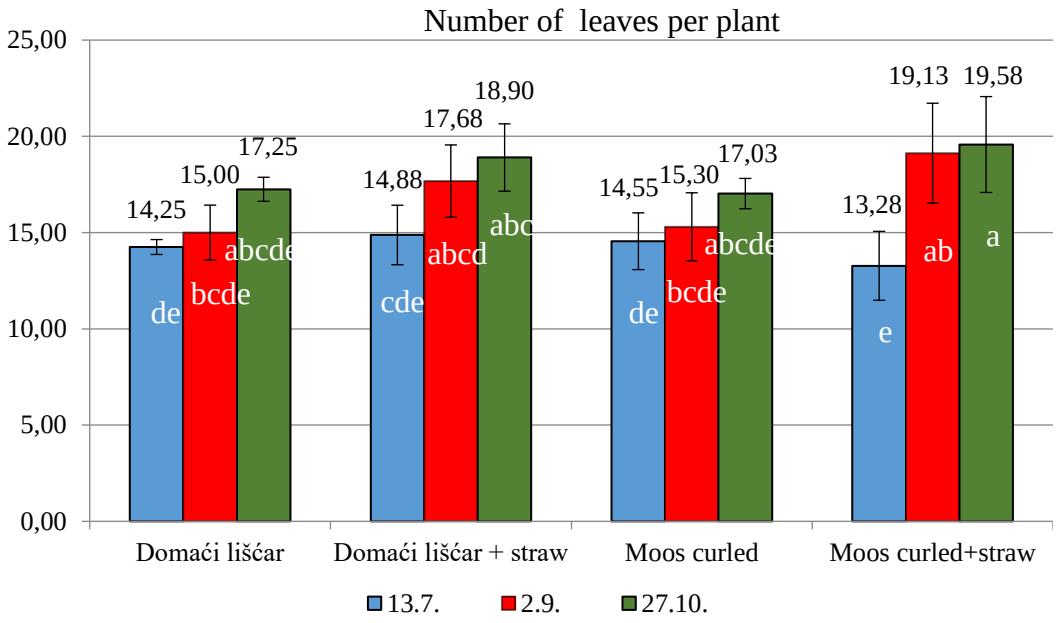
*different letters indicate significant differences

Figure 5. Hight of the parsley plants [cm] 28, 49 and 84 days after sowing

In the mulched variants of the "Domaći lišćar" variety, the plants were noticeably shorter compared to those grown without mulch. The differences in height just before harvest were statistically confirmed, as well as the differences between the varieties (in non-mulched variants). The dry straw layer in mulched variants between the parsley rows led to the absorption of moisture from the soil, resulting in reduced moisture availability for the plants during their initial development compared to the control plants. Additionally, organic material undergoes microbial degradation, and it is expected that a portion of the available nitrogen for the plants was also utilized by microorganisms. The results of the study by Crossman et al. (1997) support this finding; mulching with silver and white plastic film significantly contributed to reducing the height of parsley. However, in the same study, straw mulch did not have a significant impact on plant height compared to the control.

Before the parsley was collected for harvesting, careful attention was given to observing and counting the number of leaves within its rosette. The average number of leaves before the first harvest ranged from 13 to 15, in the second harvest from 15 to 20, and in the third harvest from 17 to 20 (Figure 6).

Based on the statistical analysis, there is no statistically significant difference in the number of leaves among different varieties of parsley. However, the number of leaves is significantly affected by the harvest. The number of leaves per plant increased from one harvest to another. A significant increase in the number of leaves was found in the "Domaći lišćar" variety in the mulched variant between the first and third harvests, and in the "Moos curled" variety in the mulched variant of the first harvest to second and third harvest terms (Figure 6).



*different letters indicate significant differences

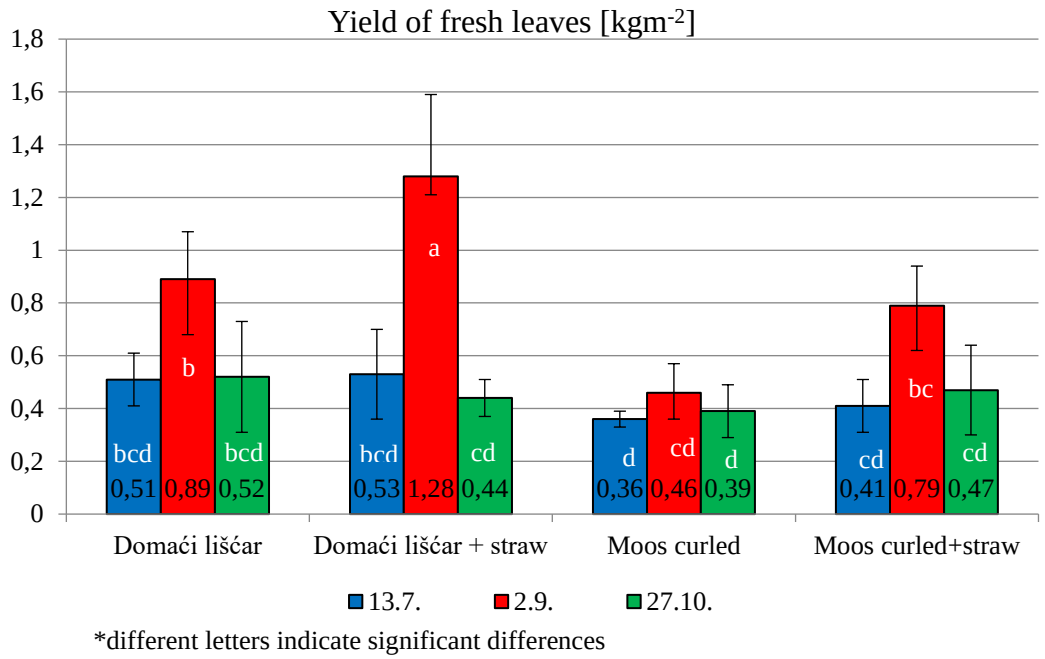
Figure 6. Number of leaves per plant 84, 135 and 190 days after sowing

The yield of fresh parsley leaves varied between harvests and treatments significantly, the highest yield of fresh parsley leaves (Figure 7) was determined in the "Domaći lišćar" variety with straw mulch in the second harvest, and the yields of the first and third harvest did not differ significantly.

Hoda et al. (2022) confirmed significant differences in parsley leaf yield between the first, second, and third harvests in the same growing season, with the highest yield achieved in the second harvest. The total yields of fresh parsley leaves were 2.275 kgha^{-1} in the first, 2.356 kgha^{-1} in the second, and 1.835 kgha^{-1} in the third harvest. The notable reduction in yields can be attributed to the chosen planting density and the quantity of plants within each designated area. In the context of this study, parsley was sown using a notably broader row spacing of 60 cm, accompanied by a 5 cm interval between individual plants within the row. Similar findings were reported in a study by Osińska et al. (2012), where the yield of parsley leaves per 1 m^2 varied between 0,55 and 3,57 kg. The highest yield was observed during the second out of three harvests for all the investigated varieties.

In the study conducted by Kołota and Adamczewska-Sowińska (2012) on parsley cultivation using black mulch film, fleece, and plastic tunnel cultivation, significantly higher yields were observed on fleece with 17,6 tha^{-1} and black film with 15,9 tha^{-1}, compared to the control variant. However, the yield of parsley grown in the plastic tunnel with 15,0 tha^{-1} did not show a significant difference from the control variant, which yielded 13,7 tha^{-1}.

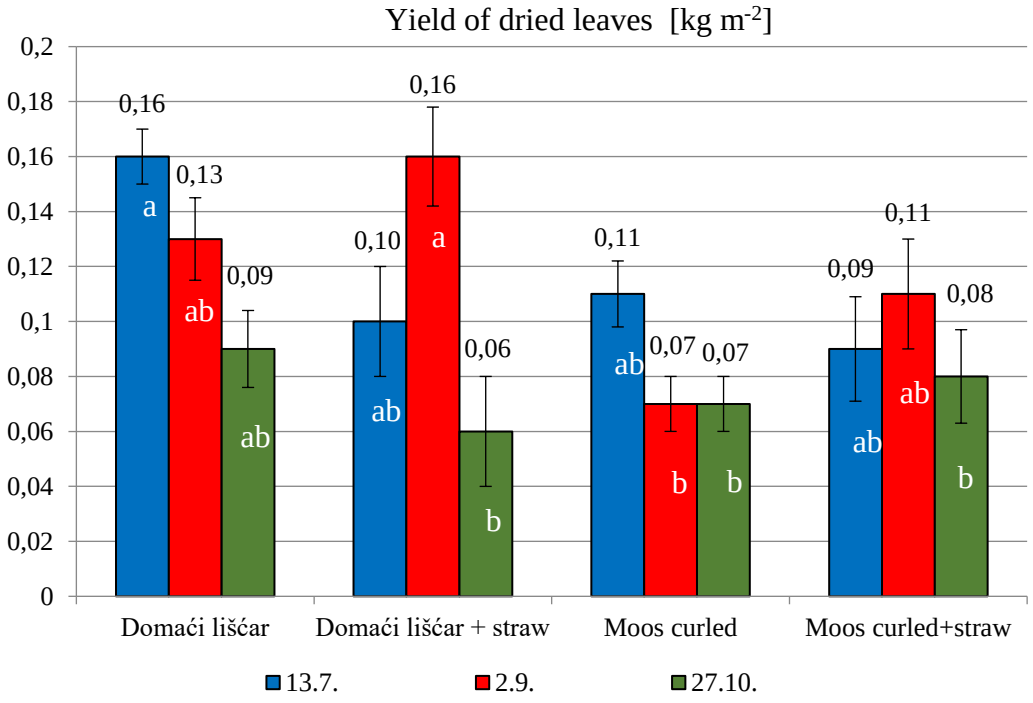
Fresh harvested parsley leaves were dried at room temperature in a well-ventilated room. The average mass loss during drying ranged between 74,3% and 85,5%. The moisture content after drying varied between 9% and 11%. The total mass loss of fresh parsley leaves from the first harvest was on average 74,3%, 88,5% in the second harvest, and 85,5% in the third harvest.



*different letters indicate significant differences

Figure 7. Yield of fresh leaves [kg m^{-2}] 84, 135 and 190 days after sowing

The average mass loss over the three harvests for the "Domaći lišćar" variety without mulching was 78,9%, and with mulching was 84,9%. For the "Moos curled" variety, the mass loss during drying was 82,23% and 83,36%. Fresh parsley leaves from the mulched variants contained more water than leaves from the non-mulched variants, as evident from the yield of dried leaves (Figure 8).



*different letters indicate significant differences

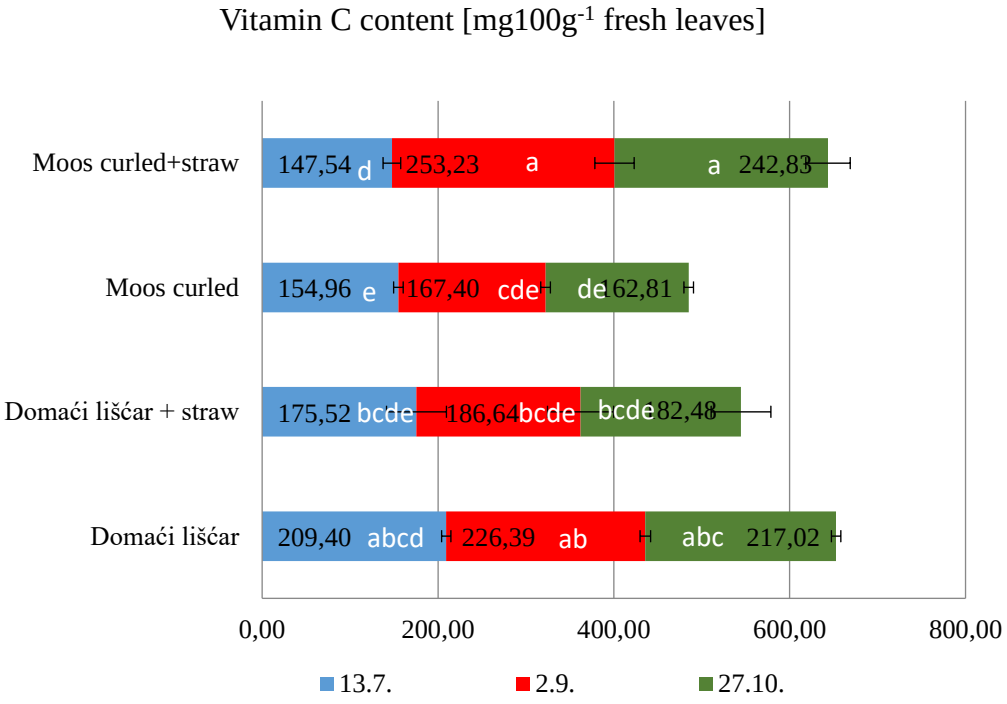
Figure 8. Yield of dried leaves [kg m^{-2}] 84, 135 and 190 days after sowing

The study confirmed significant differences in the yield of dried parsley leaves of „Domaći lišćar“ in mulched variant between second and third harvest term.

In the study conducted by Carrillo-Lopez et al. (2010), it was determined that fresh parsley leaves, on average, contain 87,42% water. Mathe (2020) reported that hermetically packaged dried spices, including parsley leaves, typically retain residual moisture ranging between 4% and 6%. As a result, the ratio of fresh to dry parsley leaves varies between 10:1 and 7:1, depending on the final moisture content achieved during the drying process.

The findings from the research conducted by Crossman et al. (1997) suggest that although mulching with straw and white and silver films may result in higher yields of fresh parsley leaves, it does not necessarily lead to higher yields of dried parsley leaves. In other words, the increase in fresh leaf yield due to mulching does not directly lead to a proportional increase in the yield of dried parsley leaves.

The vitamin C content in fresh parsley leaves ranged between 147,54 and 253,23 $mg100g^{-1}$ (Figure 9). These results align with generally reported range of 150 to 180 mg vitamin $C100g^{-1}$ by Kišgeci and Adamović (1994). The results are consistent as well with the findings from Osińska et al. (2012), with 98,88 to 312,7 $mg100g^{-1}$ vitamin C in fresh leaves, where the vitamin C content varied depending on the specific parsley variety.



*different letters indicate significant differences

Figure 9. Vitamin C content [$mg100g^{-1}$ fresh leaves] 84, 135 and 190 days after sowing

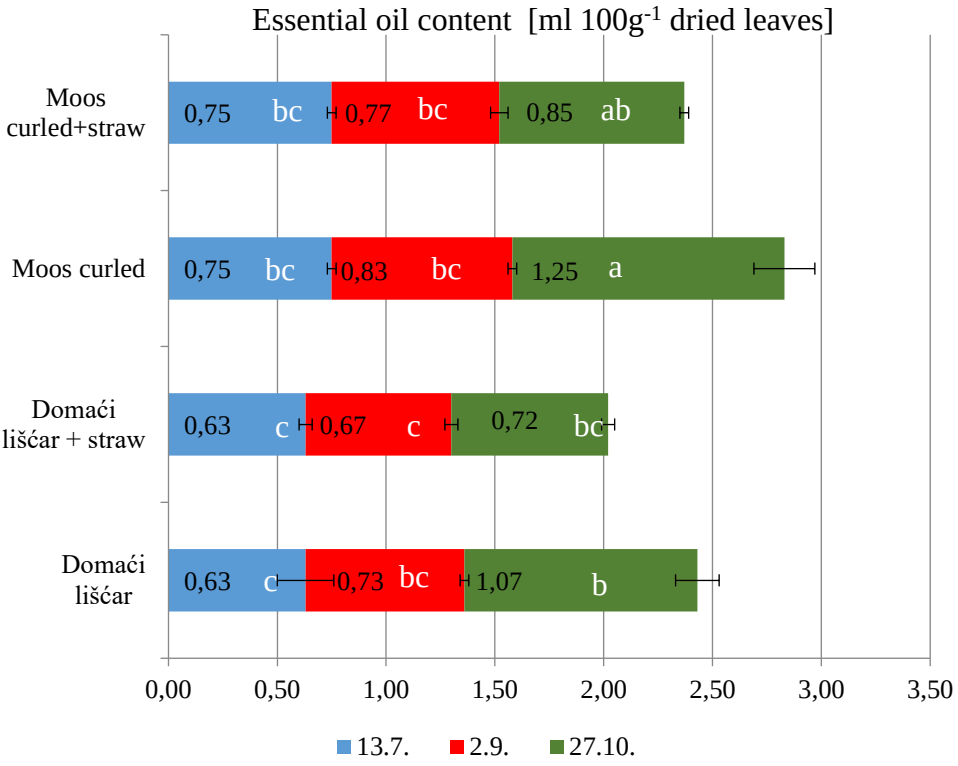
Furthermore, the study revealed significant variations in vitamin C content among different parsley varieties and also differences between terms of harvest. Mulching with straw had no impact on vitamin C content in the "Domaći lišćar" variety, while in the "Moos curled" variety in mulched variant showed a significantly higher vitamin C content. In the second harvest parsley leaves contained the highest vitamin C level, while the leaves from the first harvest had the lowest content. These findings are consistent with Osińska et al.'s (2012) research, which compared three parsley varieties across three terms of the harvest.

The research conducted by Kołota and Adamczewska-Sowińska (2012) on six distinct parsley varieties, using synthetic mulches, highlighted notable differences between different

harvest periods. This indicates that the timing of the harvest can have a substantial influence on the quality of parsley. Moreover, the type of mulching material used also played a role, with fleece being the most beneficial mulching material. However, mulching with black plastic film did not affect the vitamin C content in fresh parsley leaves, indicating that its impact on parsley quality might be limited.

As shown in the research conducted by Prez et al. (2021), the vitamin C content in fresh parsley leaves significantly varies depending on the growing conditions. Parsley cultivated in open fields exhibited 2,6 times higher vitamin C content compared to parsley grown under controlled conditions, such as in a chamber. Furthermore, the difference was even more pronounced, with 5,4 times higher vitamin C content, for parsley grown in a greenhouse, for instance, potted parsley.

The analysis of essential oil content in parsley leaves revealed a significant variation, ranging from 0,63 to 1,25 ml100g^{-1} of dry weight (Figure 10). These range in essential oil content generally aligns with the results of a study examining six different parsley varieties, which reported essential oil content ranging from 0,38 ml100g^{-1} in the "Hamburger Schnitt" variety to the highest content of 1,18 ml100g^{-1} of dry weight in the "Mooskrause" variety (Franz and Glasl, 1976).



*different letters indicate significant differences

Figure 10. Essential oil content [ml 100g^{-1} dried leaves] 84, 135 and 190 days after sowing

The literature on parsley and other herbs has consistently emphasized the significant influence of various factors on the content of essential oil, including the specific variety, growth period, and cultivation conditions (Proz et al., 2021; Dudaš et al., 2016; Petropoulos et al., 2009; Gruszecki et al., 2009; Petropoulos et al., 2004).

The current study's results are in line with the existing literature, as it also demonstrated significant variations in the essential oil content among different parsley varieties. Additionally, the influence of harvest timing on essential oil content was statistically confirmed for both

varieties in the non-mulched variant, particularly between the first and third harvests. These findings further underscore the importance of considering multiple factors when assessing and optimizing the essential oil content in parsley.

Earlier studies on various herbs, such as basil (*Ocimum basilicum* L.), have statisticaly confirmed effects of mulching on essential oil content. Specifically, the utilization of white plastic mulch resulted in significantly increased essential oil content across two distinct basil varieties. This increase was as well influenced by both the variety of basil and the timing of harvest, as demonstrated by research conducted by Dudaš et al. (2009). Similar findings were also observed by Gruszecki and Walasek-Janusz (2022) in their study on root parsley, where the essential oil content was analyzed in both the root and leaves. In the leaves, the yield of essential oil varied significantly among the tested varieties. However, the oil yield per growth season differed between the root and leaves. The content of essential oil in the root did not vary significantly depending on the growth season, while in the leaves, it exhibited greater variability and was higher during the second growth season, characterized by higher temperatures and dry periods.

The content of *chlorophyll a* in parsley leaves varied between 1,08 to 1,23 mg100g^{-1} of fresh weight (Table 2). For *chlorophyll b*, the measured values ranged from 0,16 to 0,95 mg100g^{-1} and finally, total *chlorophyll a+b* ranged from 1,21 to 2,18 mgg^{-1} of fresh weight. The determined chlorophyll content are within the range reported by Papista et al. (2002) and Chenard et al. (2005), who stated chlorophyll a content as 75,89 to 159,19 mg100g^{-1}and chlorophyll b as 16,37 to 36,14 mg100g^{-1}in fresh parsley leaves.

Variations in chlorophyll content in parsley leaves, according to Chenard et al. (2005), are influenced by fertilization rates. Abd El-Hameed et al. (2018), in their research on the influence of harvest terms on four parsley varieties (Local, Peione, Gigante, Bravour) in Egypt, found that the average total chlorophyll content (mean of four varieties) decreased in the third harvest to 2,14 mgg^{-1} compared to the content in the first (2,52 mgg^{-1} of fresh weight) and second harvests (2,67 mgg^{-1}).

Total chlorophyll content in *Trigonella foenum graecum* L. leaves fell within a comparable range, with significant differences based on the harvest term. In the non-mulched variant, the content of total chlorophyll was 23,55 mg100g^{-1} of fresh weight. However, in the mulched variant with straw, the content increased to 32,89 mg100g^{-1} of fresh weight after 60 days of cultivation and further to 35,32 mg100g^{-1} of fresh weight after 90 days (Nabil et al., 2019).

The content of total carotenoids in "Domaći lišćar" parsley leaves varied between 0,03 and 0,05 mgg^{-1}(Table 2). The carotenoid content in parsley leaves of both "Domaći lišćar" and "Moos curled" varieties is comparable to the values reported in the literature, with 5 and 8 mg100g^{-1}in fresh parsley leaves (Lešić et al., 2004; Kišgeci and Adamović, 1994).

As stated by Kamel (2013), the carotenoid content within fresh parsley leaves varies between 29,02 and 40,0 mgkg^{-1}. There is an observed trend of decreasing carotenoid content as a result of microwave drying, with the decline being influenced by the duration of the drying process.

Higher values of total carotenoid content were determined in the study by Dobričević et al. (2019) for different parsley varieties from a partially newer assortment. The determined carotenoid concentration averaged 0,8 mgg^{-1} for the "Petra" variety and 0,16 mgg^{-1} in the fresh leaves of the "Mooskrause" variety.

Table 2. Chlorophyll and total carotenoids content in leafy parsley [mgg^{-1} fresh leaves]*

Harvest**	I		II		III	
Chlorophyll a content ± *Standard deviation (SD)*						
Domaći lišćar	1,21ab	*0,04*	1,12abc	*0,08*	1,17abc	*0,02*
Domaći lišćar + straw	1,19abc	*0,06*	1,10abc	*0,07*	1,23a	*0,01*
Moos curled	1,23a	*0,02*	1,08bc	*0,07*	1,22ab	*0,03*
Moos curled+straw	1,23a	*0,01*	1,06c	*0,06*	1,22a	*0,02*
Chlorophyll b content ± *Standard deviation (SD)*						
Domaći lišćar	0,66a	*0,03*	0,23b	*0,01*	0,17b	*0,01*
Domaći lišćar + straw	0,81a	*0,02*	0,22b	*0,03*	0,16b	*0,05*
Moos curled	0,95a	*0,07*	0,24b	*0,02*	0,17b	*0,01*
Moos curled+straw	0,84a	*0,05*	0,22b	*0,03*	0,16b	*0,01*
Total Chlorophyll content (a+b) ± *Standard deviation (SD)*						
Domaći lišćar	1,87a	*0,26*	1,34b	*0,07*	1,34b	*0,04*
Domaći lišćar + straw	2,00a	*0,29*	1,31b	*0,05*	1,38b	*0,03*
Moos curled	2,18a	*0,11*	1,31b	*0,06*	1,37b	*0,09*
Moos curled+straw	2,06a	*0,16*	1,21b	*0,07*	1,38b	*0,03*
Totall carotenoids ± *Standard deviation (SD)*						
Domaći lišćar	0,038bc	*0,004*	0,054a	*0,001*	0,040ab	*0,002*
Domaći lišćar + straw	0,032bc	*0,001*	0,049a	*0,002*	0,042ab	*0,001*
Moos curled	0,036bc	*0,002*	0,049a	*0,003*	0,040abc	*0,002*
Moos curled+straw	0,036bc	*0,003*	0,048a	*0,001*	0,041abc	*0,002*

* siginificant differences in content of pigments only between harvesting period, ANOVA, Tukey's post-hoc test, $p \leq 0,01$
**first harvest 84 days (13.7.), second 135 days (2.9.) and third 190 days after sowing (27.10.)

Lower carotenoid content values in parsley leaves were found in the study by Proz et al. (2021), with 112,91 $\mu g g^{-1}$ of fresh weight for parsley grown in open-field conditions and 22 to

30% higher values for parsley grown in controlled conditions with additional white LED lighting (460-560 nm), providing a greater supply of green and blue light.

In summary, the main finding of this study is that straw mulching does not have a significant effect on the carotenoid content in parsley leaves. Additionally, there were no significant differences in pigment content between the "Domaći lišćar" and "Moos curled" varieties. The study did reveal significant variations in pigment content depending on the different harvest terms for multiple parsley harvests. These results emphasize the importance of considering the harvest timing when assessing the pigment content in parsley leaves.

The study on garlic demonstrated that mulching with straw and plastic mulch film had no significant effect on the carotenoid content. The carotenoid quantities in garlic leaf samples from both the straw-mulched variant (3,98 $mg100g^{-1}$) and the plastic film variant (3,91 $mg100g^{-1}$) were not significantly different from the control (3,98 $mg100g^{-1}$) (Anwar et al., 2020). However, the study was unable to determine a significant impact of harvest terms within the same year due to the single garlic harvest, and differences in carotenoid content between two consecutive garlic growing seasons with mulch were not determined.

The study conducted by Osińska et al. (2012) revealed significant variations in the total carotenoid content of parsley leaves across different harvest periods, involving multiple harvests and various parsley varieties. The carotenoid content in the 'Amphia' variety was 3,57 $mg100g^{-1}$ in the first harvest, 1,64 $mg100g^{-1}$ in the second harvest, and 2,61 $mg100g^{-1}$ in the third harvest. For the 'Festival' variety, the respective values were 1,57 $mg100g^{-1}$, 1,66 $mg100g^{-1}$, and 1,61 $mg100g^{-1}$, while for the 'Verta' variety, they were 1,65 $mg100g^{-1}$, 1,43 $mg100g^{-1}$, and 1,54 $mg100g^{-1}$ of fresh parsley leaves in the third harvest. These findings underscore the impact of harvest timing on the carotenoid content in parsley leaves among different varieties.

CONCLUSIONS

Based on the research results, we conclude that mulching significantly affects plant height, number of leaves, fresh mass yield, and vitamin C content in parsley leaves. However, mulching did not have significant impact on the yield of dried leaves, the content of essential oil, and pigment content. The yield of dried leaves and the content of essential oil varied depending on the variety and harvest time, while the pigment content was statistically influenced only by the harvest time.

Further research on a larger number of varieties and other mulching materials, as well as expanding the analysis to include mineral content, nitrate accumulation, and specific components of essential oil, could provide additional and more precise information on the impact of these factors on quantitative and qualitative parameters in parsley cultivation.

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PHENOTYPIC AND GENOTYPIC RESISTANCE OF MERCURY AMONG *Escherichia coli* ISOLATES FROM VEGETABLES

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ABSTRACT

Vegetables are an important part of a healthy and balanced diet and are commonly consumed raw or undercooked by humans. Vegetables have been implicated in many foodborne outbreaks of *Escherichia coli* infections. The presence of *E. coli* in vegetables can indicate that fecal contamination has occurred. Heavy metals from polluted soils and environmental wastes enter via the roots of plants and accumulate in variable concentrations in the roots, leaves, and fruits of vegetables. The existence of heavy metal-resistant pathogens in vegetables contaminated with highly toxic heavy metals such as mercury poses a serious risk to human and environmental health. This study aimed to determine the resistance to mercury (Hg) phenotypically by the broth microdilution method and genotypically by PCR for the presence of the mercury reductase encoded by the *merA* gene. Out of the *E. coli* isolates from vegetables, five isolates (45.5%) were resistant to Hg. Furthermore, only one of these Hg-resistant isolates carried the *merA* gene, which is associated with mercury resistance. Consequently, it may be critical to evaluate the presence of mercury-resistant pathogens found in vegetables because they pose a human health risk.

Keywords: *Escherichia coli*, mercury resistance, *merA* gene, vegetables, broth microdilution method, PCR

INTRODUCTION

Fresh fruits and vegetables are an excellent source of nutrients, minerals, and vitamins for humans, as well as an important basic raw material for the food industry (Carlin, 2007). Vegetables as an essential component of a healthy and balanced diet are commonly consumed raw or undercooked by people. They are extensively exposed to microbial contamination through contact with contaminated water used to irrigate, animal or human feces used as fertilizer, contaminated seeds, untreated manure, food handlers, production facilities, insect vectors, and slicing tool (Bhunia, 2008; Carlin, 2007). As a result, these conditions may lead to spoilage and a loss of quality. Microbial contamination of vegetables is a significant human health concern. In addition to bacteria, molds and yeasts that cause spoilage in vegetables, pathogenic microorganisms also cause various foodborne disease outbreaks associated with the consumption of raw fruits and vegetables (Carlin, 2007; Luna-Guevara et al., 2019; Pintor-Cora et al., 2021).

Escherichia coli, an *Enterobacteriaceae* family member, is prevalent in humans and animal intestinal microflora. They are usually released into the environment, where they may contaminate water and soil, and hence fruits and vegetables, particularly if untreated manures are used as fertilizers (Bhunia, 2008). Vegetables as a common source of *E. coli* contamination have been documented in many studies (Holvoet et al., 2013; Freitag et al., 2018; Luna-Guevara et al., 2019). Pathogenic *E. coli* strains can cause a variety of illnesses including septicemia, neonatal meningitis, pneumonia, gastroenteritis, hemolytic uremic syndrome, dysentery, and urinary tract infection (Bhunia, 2008). In recent years, it has been a major concern that an

increasing number of *E. coli* outbreaks have been associated with the consumption of contaminated vegetables, including sprouts, spinach, lettuce, coleslaw, and salad, in developing countries (WHO, 2018).

Heavy metals are widespread in the environment. Heavy metals from polluted soils and environmental wastes enter plants through the roots and accumulate in variable concentrations in the roots, leaves, and fruits of vegetables (Bhunia, 2008). Therefore, in their natural environments, bacteria are continuously exposed to various metals, which leads to the survival of metal-tolerant cells due to mutations. Extrinsic resistance determinants in pathogens have been to the development of heavy metal resistance (Vats et al., 2022). Heavy metal resistance in various pathogenic bacteria has been shown in previous studies (Wickramanayake et al., 2020; Cufaoglu et al., 2022; Ejaz et al., 2022; Dahanayake et al., 2019). In addition to antimicrobial agents, heavy metals are frequently used in animal husbandry, aquaculture, and human and animal health, which can promote the dissemination of antimicrobial resistance through co-selection (Seiler and Berendonk, 2012). Some researchers found that heavy metals enhance resistance to antimicrobials in bacterial isolates (Yazdankhah et al., 2014; Ejaz et al., 2022; Vats et al., 2022; Anedda et al., 2023). Due to its toxicity, persistence in the environment, and bioaccumulative nature, heavy metal contamination is a major hazard to human health and ecological environment safety (Seiler and Berendonk, 2012).

The presence of heavy metal-resistant pathogens in vegetables contaminated with highly toxic heavy metals such as mercury, which is an extremely hazardous heavy metal, poses a significant risk to both human health and the global environment. Therefore, this study aimed to determine the resistance to mercury (Hg) phenotypically by the broth microdilution method and genotypically by PCR for the presence of the mercury reductase encoded by the *merA* gene.

MATERIALS AND METHODS

Bacterial isolates

In the present study, a total of 11 *E. coli* isolates obtained from various vegetable samples, including 3 spinach, 3 lettuce, 2 arugula, 2 black cabbage, and 1 lamb's lettuce, were examined. Fresh vegetables, not precooked or frozen, were collected from various supermarkets and public bazaars. The *E. coli* isolates were previously identified using traditional biochemical tests and a PCR for the *uspA* gene (*E. coli*-specific universal stress protein A) (Chen and Griffiths, 1998; Scheutz and Strockbine, 2005). All *E. coli* isolates were cultured overnight at 37 ºC in Brain Heart Infusion broth (BHI) (Merck, Germany). Isolation of genomic DNA from the *E. coli* isolates was performed using the cetyl trimethyl ammonium bromide (CTAB) method for PCR detection of the heavy metal mercury resistance gene, according to Ausubel et al. (1991). The DNA was stored at -20°C after being dissolved in Tris-EDTA (TE) buffer.

Detection of MIC of heavy metal mercury

The heavy metal mercury ($HgCl_2$) used in this study was obtained from Sigma-Aldrich (Sinopharm Chemical Reagent Co., Shanghai, China). The broth microdilution method was used to quantitatively determine the minimum inhibitory concentrations (MICs) of heavy metal mercury (CLSI, 2012; He et al., 2016; Dahanayake et al., 2019). Mercury (Hg) concentration ranged from 400 to 0.78 µg/mL. For MIC determinations, a 96-well U-bottom sterile polystyrene microplate (LP Italiana) was used. MICs were defined as the lowest heavy metal concentration that completely inhibited organism development after 18-20 hours of incubation at 37 ºC. The tests were carried out in triplicates. *Escherichia coli* K-12 strain was used as a quality control in the heavy metal resistance test (Dahanayake et al., 2019).

Detection of heavy metal mercury resistance gene by PCR

Detection of mercury resistance gene

Resistance to Hg was also evaluated genotypically using PCR for the presence of the mercury reductase encoded by the *merA* gene. The *merA* primers were merA-F: 5'-GAGATCTAAAGCACGCTAAGGC-3' and merA-R: 5'-GGAATCTTGACTGTGATCGGG-3', which were predicted to yield a 1011 bp product (Misra et al., 1984). All PCR experiments were performed in a DNA thermal cycler (Bio-Rad T100). The PCR reaction mix (50 µL) contained 5 µL of 10X PCR buffer (100 mM Tris-HCl pH 8.8, 500 mM KCl, 0.8% [v/v] Nonidet P40) (Thermo Fisher Scientific), 2 mM $MgCl_2$ (Thermo Fisher Scientific), 200 µM dNTP mix (Thermo Fisher Scientific), 0.4 µM primer (Oligomer Biotechnology), 1.5 U Taq DNA polymerase (Thermo Fisher Scientific), 4 µL (50 ng) isolated DNA and 31.7 µL molecular grade water (AppliChem). The PCR cycling conditions were carried out with the following setup: 94°C for 3 min and 30 cycles of denaturation (30 sec, 94°C), annealing (30 sec, 57°C), extension (1 min, 72 °C), and final extension (5 min, 72°C). The amplified *merA* products were analyzed by electrophoresis (BioRad) on a 1% agarose gel containing ethidium bromide in 1X Tris borate EDTA (TBE) buffer with a 100 bp Plus DNA ladder (Vivantis, Malaysia). The gels were visualized using UV transillumination (DNR Minilumi Bioimaging Systems, Israel).

RESULTS AND DISCUSSION

This study investigated the resistance of *E. coli* isolates obtained from various vegetables to heavy metal mercury phenotypically and genotypically. Table 1 shows the phenotypic and genotypic results for mercury (Hg) resistance using the broth microdilution method and PCR for the presence of the mercury reductase encoded by the *merA* gene. Five *E. coli* isolates (45.5%) exhibited resistance to Hg at a MIC value of 12.5 µg/mL. Furthermore, only one of these Hg-resistant isolates carried the *merA* gene, which is associated with mercury resistance.

Table 1. Phenotypic and genotypic resistance to mercury (Hg) heavy metal among the *E. coli* isolates from vegetables

No	Isolate	Origin	Phenotypic resistance to mercury heavy metal MIC (µg/mL)	Genotypic resistance to the mercury resistance gene *merA*
1	V13	Spinach	12.5	-
2	V14	Spinach	12.5	-
3	V15	Spinach	6.25	-
4	V17	Lettuce	12.5	+
5	V22	Lettuce	6.25	-
6	V23	Lettuce	6.25	-
7	V30	Arugula	6.25	-
8	V35	Arugula	12.5	-
9	V38	Black cabbage	12.5	-
10	V39	Black cabbage	1.56	-
11	V40	Lamb’s lettuce	6.25	-
E. coli K-12 strain			6.25	-

According to phenotypic heavy metal mercury (Hg) results, the MICs were found to range between 1.56-12.5 µg/mL (Table 1). Yang et al. (2020) reported that the MICs of Hg for *E. coli* and *Salmonella* strains from chicken farms and retail meat ranged from 12.5 to 25 mg/mL. In contrast to our results, the higher MIC values of Hg for *E. coli* isolates from chicken, cattle, and sheep carcasses, slaughterhouse wastewater ranged from 3.12 to 50 µg/mL, as previously documented by Cufaoglu et al. (2022). The MIC concentration for Hg in *E. coli* isolates from Mediterranean mussels and sea snails in the Southeastern Black Sea varied from 100 to 400 µg/mL (Terzi and Civelek, 2021). Contrary to these results, Sipahi et al. (2019) found that all *E. coli* isolates from cattle stool samples were phenotypically sensitive to Hg.

Several studies have demonstrated that various bacterial species other than *E. coli* develop resistance to Hg and other heavy metals (Seiler and Berendonk, 2012; Yazdankhah et al., 2014). A study published in China by He et al. (2016) found Hg MIC values for *Vibrio parahaemolyticus* isolated from fresh shrimp in Shanghai fish markets ranged from 50 to 6.25 µg/mL. In Korea, the mercury-resistant phenotype was not found in any of the *Aeromonas* spp. isolates from Pacific abalone obtained from retail and wholesale markets (Wickramanayake et al., 2020). In a reported study on Gram-positive bacteria, all *Bacillus* isolates exhibited high resistance to Hg, with MICs ranging from 125 to 180 µg/mL (Singh et al., 2013).

The presence of the *merA* resistance gene was identified in the *E. coli* isolates examined, and the products of PCR were visualized using agarose gel electrophoresis, as demonstrated in Figure 1.

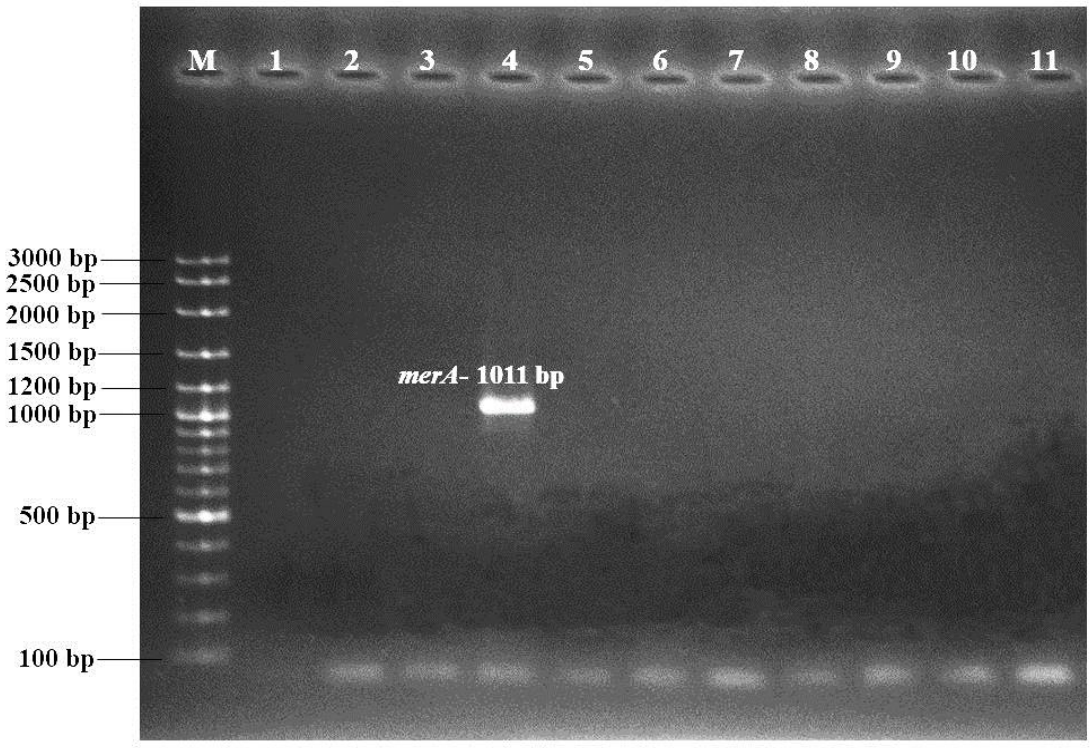

Figure 1. Agarose gel electrophoresis of the PCR product of the *merA* (1011 bp) gene in *E. coli* isolates from vegetables. Lane M: 100 bp DNA ladder (Vivantis, Malaysia). Lane 4: The *merA* positive *E. coli* isolate recovered from lettuce in this study. Lanes 1, 2, 3, 5, 6, 7, 8, 9,10, and 11: Negative results for the *merA* gene in the *E. coli* isolates.

Based on the results of genotypic mercury (Hg) resistance encoded by the *merA* gene, a low frequency of the *merA* gene was observed in *E. coli* isolates (2.5%) and *Salmonella* isolates (11.4%) from chicken broiler farms and retail meat (Yang et al., 2020), similar to our results (9.1%). Similarly, resistance to Hg, indicated by *merA*, was less prevalent (11%) in European honey bees (Apis mellifera) (Fry et al., 2023). On the other hand, Cufaoglu et al. (2022) revealed that the *merA* gene was present at a greater frequency in the chicken, cattle, and sheep origin *E. coli* isolates (50%). Resistance to the heavy metal mercury is common in *E. coli* strains of veterinary significance. The mercury resistance gene, *merA*, was found in 79% of the clinical avian *E. coli* isolates (Bass et al., 1999). In a study reported by Çelik et al. (2023), the presence of the *merA* gene was detected in 11 *E. coli* isolates, of which 5 (27.8%) were mussels and 6 (37.5%) were shrimp. Dahanayake et al. (2019) also observed the *merA* gene in 17 (47%) *Aeromonas* spp. strains from the Manila Clam (*Ruditapes philippinarum*) in Korea. Similarly, Wickramanayake et al. (2020) found that the *merA* gene was positive at a rate of 41% in *Aeromonas* spp. isolates from Pacific abalone.

CONCLUSIONS

This study demonstrated the phenotypic and genotypic resistance profiles of *E. coli* from vegetables against the heavy metal mercury. Consequently, it may be critical to evaluate the presence of mercury-resistant pathogens found in vegetables because they pose a human health risk.

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EFFECT OF USING PROBIOTICS SUPPLEMENTED WHEAT INSTEAD OF CORN IN THE DIET ON PERFORMANCE AND SLAUGHTERING CHARACTERISTICS OF BROILERS

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ABSTRACT

The aim of this study was to determine the effects of diets using 1 g/kg probiotics supplemented 50 and 100% wheat instead of corn on the performance, carcass characteristics and visceral weight of broilers. In the study, a total of 120 male Ross 308 broiler chicks at the day-old were randomly allocated to 3 treatment groups with 4 replicates of 10 chicks each. Treatment groups were formed from diets using corn as a grain source (Wheat0), wheat with 1 g/kg probiotics added at the rate of 50% of maize (Wheat50), and wheat with 1 g/kg probiotics added at 100% of maize (Wheat100). Performance parameters were determined on the 10th, 25th and 42nd days, and carcass and visceral weights were determined at the end of the study (42nd day).

Effect of using probiotics supplemented wheat in the diet on body weight, body weight gain, and feed intake of broilers was statistically insignificant ($P>0.05$). Compared the control group (Wheat0), 11-25th days for Wheat50 and Wheat100 groups, as cumulative for Wheat100 feed efficiency improved ($P<0.05$). Relative carcass decreased in Wheat50 group and relative abdominal fat decreased in Wheat100 ($P<0.05$). According to the results of this study, it was determined that the addition of probiotics and the use of wheat instead of whole corn (100%) in male broiler diets improved feed efficiency and reduced fattening.

Keywords: wheat, probiotics, broiler, performance, carcass

INTRODUCTION

Breeding studies in poultry and the high level of applicability of hybrid production have maximized the productivity of these animals for today. However, to obtain maximum production from animals, improvement of genetic structure and environmental demands is not sufficient alone, and they should be fed with diets based on products such as corn and soybean meal that are highly digestible and do not contain anti-nutritional factors. This situation creates concerns in terms of sustainability for our country and some other countries where corn and soybean cultivation is not sufficient. In these countries, corn and soybean are supplied by importation and increase foreign dependency with high foreign exchange loss. The use of cereals, such as wheat, which is more suitable for the ecological structure of our country and has more production, instead of corn in the nutrition of broilers, maintains its currency. However, the anti-nutritional factors and biased directions in these products have limited the use of these products.

Wheat is the grain with the most energy after corn due to low cellulose and high starch content. However, the non-starch polysaccharides (arabinoxylan, beta-glucan) it contains cause problems such as doughing and wet litter, and therefore yield loss. The energy content of wheat is approximately 90% of corn. In addition, the protein content is almost twice that of corn. While wheat can be used up to 25% in broiler diets, this amount can be up to 50% with the addition of enzymes. This amount means that it can be used instead of almost all of the corn.

In many studies conducted since the 1970s with the aim of using probiotics as growth factors in poultry, positive or negative results have been obtained regarding animal performance (Jernigan et al., 1985). In laying hens given Lactobacillus cultures, egg production increased by 3.03%, feed efficiency by 7.41%, while fertility and hatching rates were not affected (Krueger et al., 1977). At the end of the 21-day trial in the first of two separate studies conducted by adding L. acidophilus to chick diets, Watkins et al. (1983) stated that the body weight gain and feed efficiency were negatively affected by 0.4% and 3.3%, respectively. In the second, they reported that despite the increase in body weight gain by 2.31% as a result of the 49-day trial, feed efficiency was not affected. In contrast, Alp et al. (1993) noted that the supplementation of Lactiferm-L5 (Streptococcus faecium) alone and together with some antibiotics to broiler diets did not have a significant effect on performance, abdominal fat weight, and serum cholesterol. In a different study conducted by the same researchers, the effect of probiotics added to oxidized broiler diets on fattening performance, ascites formation, blood oxidation and antioxidant status was investigated, but no statistically positive results were found (Alp et al. 1999).

The aim of this study was to determine the effect of using wheat with probiotics supplement instead of 50% and 100% corn on performance and slaughtering characteristics in broilers.

MATERIAL AND METHODS

The experiment was carried out to randomized arrangement design with three dietary treatments. A total of 120 1-day-old Ross 308 male broiler chicks were randomly distributed among three trial groups. In each experimental group, there were four subgroups, each with 10 chicks. The animal and feed raw materials were obtained from commercial companies and the diets were prepared in the Feed Unit in the Selcuk University Faculty of Agriculture Prof. Dr. Orhan Düzgüneş Animal Husbandry Research and Application Facility. In the study, diets containing wheat with probiotics supplement were used instead of 0% (control, Wheat0), 50% (Wheat50), and 100% (Wheat100) corn (Table 1). In the study, the probiotics strain *Bacillus velezensis* (10^{11} CFU/g) obtained from a commercial company was used. The birds were raised in environmentally controlled house and pens were 150 × 150 cm. During the trial, ahemeral lighting (23 hours/day) was applied, water and feed were given ad-libitum.

During the experiment, body weight and feed intake were determined as g/chick by group weighings at the hatching, 10^{th} day, 24^{th} day, and final (42^{th} day) of the trial. Body weight gain was also found from these measurements. Feed conversion ratio was calculated as g feed/g gain with *feed intake / body weight gain* formula.

Table 1. Treatment diets using different levels of wheat instead of corn and nutrient contents of diets

Ingredients	Treatment Diets								
	Wheat0			Wheat50			Wheat100		
	Starter (0-10. days)	Grower (11-25. days)	Finisher (26-42. days)	Starter (0-10. days)	Grower (11-25. days)	Finisher (26-42. days)	Starter (0-10. days)	Grower (11-25. days)	Finisher (26-42. days)
Corn	48.14	51.28	56.40	26.69	26.65	28.90	---	---	---
Wheat	---	---	--	25.00	26.10	28.90	53.00	54.04	59.38
Soybean meal	42.70	39.00	33.80	35.40	37.00	31.80	33.44	35.00	29.50
Corn gluten	---	---	---	4.00	---	---	4.00	---	---
Soybean oil	5.40	6.30	6.80	5.00	6.80	7.40	5.65	7.50	8.10
Limestone	0.70	0.60	0.60	0.85	0.65	0.60	0.85	0.73	0.65
Dicalcium phosphate	2.20	2.00	1.75	2.08	1.95	1.75	2.05	1.85	1.68
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine	0.17	0.26	0.12	0.30	0.28	0.12	0.33	0.31	0.16
DL-methionine	0.34	0.21	0.18	0.33	0.22	0.18	0.33	0.22	0.18
Calculated nutrient contents									
Metabolizable energy, kcal/kg	3003	3105	3203	3007	3101	3200	3008	3105	3203
Crude protein, %	23.012	21.530	19.496	23.052	21.496	19.541	23.082	21.504	19.508
Calcium, %	0.973	0.872	0.794	0.976	0.873	0.789	0.964	0.873	0.785
Available phosphorus, %	0.489	0.445	0.396	0.480	0.448	0.409	0.486	0.441	0.409
Lysine, %	1.288	1.291	1.063	1.288	1.283	1.042	1.291	1.284	1.046
Methionine, %	0.675	0.521	0.467	0.679	0.528	0.466	0.678	0.526	0.463
Methionine+cystine	0.974	0.899	0.815	1.057	0.915	0.823	1.065	0.922	0.829

[1]Premix provided the following (per kg of diet): manganese 80 mg; iron 60 mg; copper 5 mg; iodine 1 mg; selenium 0.15 mg; vitamin A 8800 IU; vitamin D_3 2200 IU; vitamin E 11 mg; nicotinic acid 44 mg; Cal-D-Pan 8.8 mg; vitamin B_2 4.4 mg; vitamin B_1 2.5 mg; vitamin B_{12} 6.6 mg; folic acid 1 mg; biotin 0.11 mg; choline 220 mg.

*__Wheat0__: Group using 100% corn as grain source, __Wheat50__: Group in which 1 g/kg probiotics added wheat is used instead of 50% of the corn, __Wheat100__: The group in which 1 g/kg probiotics added wheat is used instead of 100% of the corn.

Determination of relative carcass and visceral organ weights

At the end of the experiment, two broilers at six weeks of age from each subgroup were euthanized by cervical dislocation. Carcass, thigh+drumstick, breast, abdominal fat, liver, gizzard, pancreas, and were weighed with a 0.01 g precision scale, and then their relative weights were determined. Relative weights of carcasses and some organs were calculated as percentage of body weight. On the other hand, relative weights of thigh+drumstick and breast were determined as a percentage of the carcass.

Data were analysed in the SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA) with a model of one-way ANOVA, using the group mean as an experimental unit. Differences among the group means were determined by Duncan's range tests. A probability value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The effect of using at the levels of 50% and 100% wheat with probiotics supplement instead of corn in broiler diets on performance is demonstrated in Table 2.

Table 2. The effect of using probiotics (1 g/kg) supplemented wheat instead of corn (50 or 100%) in the diet on the performance of broilers

Parameters	Treatments*			Standard error	*P* value
	Wheat0	Wheat50	Wheat100		
Body weight, g/broiler					
Hatching	41.38	42.07	40.63	0.267	0.076
10. days	247.0	251.3	257.8	2.23	0.136
25. days	1175.0	1198.3	1218.5	14.13	0.497
42. days	3213.8	3260.0	3306.1	36.33	0.629
Body weight gain, g/broiler					
0-10. days	205.6	209.3	217.2	2.33	0.110
11-25. days	928.0	947.0	960.7	13.22	0.642
26-42. days	2038.8	2061.7	2087.6	27.06	0.796
0-42. days	3172.4	3217.9	3265.5	36.29	0.623
Feed intake, g/broiler					
0-10. days	270.9	275.5	273.4	1.19	0.322
11-25. days	1258.5	1249.9	1218.0	12.88	0.441
26-42. days	3385.2	3359.5	3311.3	27.89	0.593
0-42. days	4914.7	4884.8	4802.8	39.96	0.542
Feed conversion ratio					
0-10. days	1.318	1.316	1.262	0.0131	0.133
11-25. days	1.356[a]	1.322[b]	1.268[b]	0.0140	0.013
26-42. days	1.662	1.632	1.587	0.0136	0.057
0-42. days	1.550[a]	1.519[a]	1.471[b]	0.0115	0.003

***Wheat0**: Group using 100% corn as grain source, **Wheat50**: Group in which 1 g/kg probiotics added wheat is used instead of 50% of the corn, **Wheat100**: The group in which 1 g/kg probiotics added wheat is used instead of 100% of the corn.

[a,b:]Within a row, values not sharing a common superscript are statistically different; $P \leq 0.05$.

The use of 50% and 100% wheat with 1 g/kg probiotics added instead of corn in the diet did not affect the body weight of broilers statistically (P>0.05). The body weights of the broilers were 40.63-42.07 g in the hatching, 247.0-257.8 g on the 10. day, 1175.0-1218.5 g on the 25. day and 3213.8-3306.1 g on the 42. day. In the study, the body weight gain was 205.6-217.2 g on the 0-10. days, 11-25. days 928.0-960.7 g, 26-42. days 2038.8-2087.6 g and 0-42. days 3172.4-3265.5 g, but the body weight gain was not affected by the treatments considerably (P>0.05). Feed intake was not affected by the use of probiotics supplemented wheat instead of corn in the diet (P>0.05). Feed intake according to periods as follows: 0-10. days 270.9-275.5 g, 11-25. days 1218.0-1258.5 g, 26-42. days 3311.3-3385.2 g, and 0-42. days was 4802.8-4914.7 g. The use of 50% and 100% wheat with 1 g/kg probiotics supplement instead of corn in the diet of broilers at 0-10. days (1.262-1.318) and 26-42. days (1.587-1.662) did not affect the feed efficiency statistically (P>0.05). In the second period (11-25. days) of the study, feed efficiency in Wheat50 (1.322) and Wheat100 (1.268) groups was significantly improved (P<0.05), compared to the control group (Wheat0) (1.356). In the 0-42. days, feed efficiency of Wheat100 group (1.471) improved considerably compared to Wheat0 (1.550) and Wheat50 (1.519) groups (P<0.05). In most cases, improved growth has been associated with increased feed intake in broilers fed a diet containing probiotics (Abdel-Raheem et al., 2012; Landy and Kavyani, 2013; Lei et al., 2015). Kırkpınar et al. (2018) reported that the addition of probiotics to wheat-based broiler diets improved body weight but did not affect other performance parameters. Afsharmanesh and Sadaghi (2014), Mookiah et al. (2014), Zhang and Kim (2014), Lei et al. (2015) stated that dietary supplementation of probiotics improved broiler growth rates. However, these results are not compatible with the current study. Mountzouris et al. (2010),

Hung et al. (2012), Fajardo et al. (2012), Shim et al. (2012), and Zhang and Kim (2014) demonstrated that the use of probiotics in broiler diets improved the feed efficiency. However, feed intake and feed conversion ratio were not affected by the treatments in current study. These outcomes are similar to the feed efficiency results in the current research. Differences between studies may be due to differences in the dosage and composition of the administered probiotics, diet components, and variations in the physiological status of animals.

The effect of using at the levels of 50% and 100% wheat with probiotics supplement instead of corn in broiler diets on slaughtering characteristics is given in Table 3.

Table 3. The effect of using probiotics (1 g/kg) supplemented wheat instead of corn (50 or 100%) in the diet on the slaughtering parameters of broilers

Parameters	**Treatments***			**Standard error**	***P* value**
	Wheat0	**Wheat50**	**Wheat100**		
Carcass[1]	76.17[a]	74.09[b]	76.31[a]	0.428	0.041
Thigh+drumstick[2]	27.23	27.74	26.85	0.289	0.497
Breast[2]	36.31	36.50	39.33	0.662	0.103
Abdominal fat[1]	1.13[a]	0.80[ab]	0.59[b]	0.0899	0.026

***Wheat0**: Group using 100% corn as grain source, **Wheat50**: Group in which 1 g/kg probiotics added wheat is used instead of 50% of the corn, **Wheat100**: The group in which 1 g/kg probiotics added wheat is used instead of 100% of the corn.

[1]% of body weight, [2]% of carcass.

[a,b:]Within a row, values not sharing a common superscript are statistically different; $P \leq 0.05$.

The use of 50% and 100% wheat with probiotics added instead of corn in the diet did not statistically affect the relative thigh+drumstick (26.85%-27.74%) and breast (36.31%-39.33%) weights of broilers ($P>0.05$). However, the relative carcass and abdominal fat were affected by treatments ($P<0.05$), and the carcass ratio in the Wheat50 (74.09%) group was considerably lower than the Wheat0 (76.17%) and Wheat100 (76.31%) groups. In the research, the relative abdominal fat weight was statistically decrease in the Wheat100 group (0.59%) compared to the control (Wheat0) group (1.13%).

The effect of using at the levels of 50% and 100% wheat with probiotics supplement instead of corn in broiler diets on performance is shown in Table 4.

Table 4. The effect of using probiotics (1 g/kg) supplemented wheat instead of corn (50 or 100%) in the diet on the visceral weights of broilers

Parameters[1]	**Treatments***			**Standard error**	***P* value**
	Wheat0	**Wheat50**	**Wheat100**		
Liver	1.57	1.90	1.68	0.069	0.123
Gizzard	1.34	1.48	1.29	0.050	0.287
Pancreas	0.217	0.205	0.193	0.0104	0.680

***Wheat0**: Group using 100% corn as grain source, **Wheat50**: Group in which 1 g/kg probiotics added wheat is used instead of 50% of the corn, **Wheat100**: The group in which 1 g/kg probiotics enzyme added wheat is used instead of 100% of the corn.

[1]% of body weight

[a,b:]Within a row, values not sharing a common superscript are statistically different; $P \leq 0.05$.

The use of 50% and 100% wheat with probiotics added in the diet instead of corn did not statistically affect the relative liver (1.57-1.90%), gizzard (1.29-1.48%), and pancreas (0.193-0.217) weights of broilers ($P>0.05$). Kırkpınar et al. (2018) reported that the addition of probiotics to wheat-based broiler diets had no effect on slaughtering characteristics. Similar results were found by Mountzouris et al. (2010), Hung et al. (2012), Fajardo et al. (2012), Shim et al. (2012), and Zhang and Kim (2014), these results are partially similar to the present study.

In the current study, body weight, feed intake, carcass yield, and slaughtering characteristics were not affected by the treatment diets, except for abdominal fat. In addition,

while the feed efficiency improved in the Wheat100 group, the carcass yield decreased in the Wheat50 group. According to these results, it was observed that feed efficiency improved, and fat accumulation decreased with the use of probiotics supplemented wheat instead of whole corn in the feeding of broilers.

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EFFECTS OF AQUAFABA AS ALTERNATIVE PLANT ADDITIVE ON PHYSICAL, TEXTURAL AND SENSORY CHARACTERISTICS OF EGGLESS TURKISH PASTA (ERIŞTE)

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ABSTRACT

Consumers experience health problems with egg consumption and also preference of vegan and vegetarian nutrition leads to the search for alternative egg substitutes in new product development. This research determined the quality and acceptability of Turkish noodle (Erişte) substituted with 25%, 50%, 75% and 100% chickpea aquafaba instead of egg. Erişte were analyzed for physical, textural, and sensory properties compared with sample containing egg. An increase in substitution led to a rise of 23.81% in water uptake, 23.74% in volume increase and 40.19% in the cooking loss. The addition of up to 75% aquafaba increased the firmness of erişte significantly compared to the control sample. Erişte sample containing 100% aquafaba showed significantly $p < 0.05$ higher values in L* (75.11) and hue angle (94.85), while lower values in a* (-2.13), b* (24.30) and saturation index (24.39). The odor (7.00), taste (7.00), appearance (7.00), chewiness (7.00) and overall acceptability (6.88) of samples containing aquafaba were found more acceptable than control sample (4.00, 5.50, 5.90, 5.95 and 5.47, respectively). Based on our results, possible to produce erişte with acceptable sensory properties, and good physical quality product by adding up to 50% aquafaba.

Keywords: Aquafaba, egg-less noodle, physical properties, textural properties

INTRODUCTION

Turkish pasta (Erişte) is one of the traditional Turkish foods was generally produced from wheat flour, salt and egg. Milk, whey powder and some other additives can also be added in different regions of Turkey (Özkaya et al., 2004). The high rate of egg component used in pasta products can reduce production and consumption due to both the cost of the product and various reasons. So, many issues such as changing consumer preferences, increasing allergens, improving food safety, improving nutritional balance, reducing price variability and supporting environmental sustainability have increased the interest in researching egg substitutes and alternatives (Grizio and Specht, 2018).

Plant-based proteins are one of the most important food components to use as egg substitution in product development. Recently, the viscous liquid obtained from cooked chickpeas or other legumes and pulses called ‘aquafaba’ according to the Latin origin of water (aqua) and beans (faba) has been recently used as eggs replacement in many foods (Erem et al., 2021). Some studies in the literature revealed that aquafaba has many functional properties such as water and oil holding capacity, emulsion stabilizer, foaming, gelling and thickening in various formulations (Mustafa and Reaney 2020) and can be used as an egg substitute in vegan products (Raikos et al., 2020).

Aquafaba has used in meringue (Stantiall et al. 2018), sponge cake (Mustafa et al. 2018) and vegan mayonnaise (Raikos et al., 2020) as an emulsifier instead of egg. To our knowledge, no studies have been conducted on the inclusion of aquafaba in the formulation of egg-less Turkish pasta (Erişte). The objective of this study was to investigate egg substitutes for Turkish pasta using aquafaba. The effects of aquafaba on physical, textural and sensory properties of eggless pasta were evaluated.

MATERIAL AND METHOD

Materials

Commercial chickpea, wheat flour, whole egg and salt were purchased from local markets in Konya, Turkey.

Methods

Production of Aquafaba

Aquafaba was prepared according to the method described by Baik and Han (2012) with some modifications. Firstly, chickpeas were washed and were cleared from dirt, dust and foreign matter. Aquafaba was obtained by cooking 100 g chickpea in 500 mL water (1:5 chickpea/water ratio) for 30 min in boiling water at 98 °C. After the cooking, water and cooked chickpeas waited for 12 hr in a refrigerator at 4 °C. Finally, aquafaba was obtained by removing cooked chickpeas.

Production of Turkish noodle (Erişte)

For production of control Turkish pasta sample, firstly wheat flour (100 g), whole egg (40 g), salt (1 g) and water were mixed and kneaded in a laboratory-type mixer (Hobart N50, Offenburg, Germany) for 8 min. The kneaded dough rested for 20 min and 2 mm thickness/5 mm wide long strips were obtained (1 time in section 6 and 7) by a pasta machine (Shule Pasta Machine; Jiangsu, China). Then, strip-shaped dough were cut to a length of 4 cm. The drying of samples took place in a laboratory-type oven (Nüve KD 200, Ankara, Turkey) at 50 °C for 18 h. In the other samples, aquafaba were replaced at levels of 25, 50, 75 and 100% on the basis of whole egg. The samples were preserved in sealed polyethylene bags at 4 °C.

Cooking properties

Volume increase, weight increase and cooking loss values of pasta were determined according to Bilgiçli et. al. (2011). The weight increase and volume increase were found by differences of dry and cooked erişte samples weights and by the volume difference of water overflow, respectively. For cooking loss determination, cooking water was evaporated to constant weight in an oven and the weight of total solids expressed as a percentage (AACC, 2000).

Texture analysis

The firmness values of erişte samples were measured by TAXT Plus Texture Analyser (Stable Microsystems, Surrey, UK) and A/LKB-F was used as a probe (AACC, 1990). Firstly, 10 g samples were cooked in 200 mL distilled water for optimum cooking time and drained. Then, three strips of erişte sample were placed onto the stand and analyzed.

Color analysis

Color measurement was made by measuring L* value [(0) black- (100) white], a* value [(+) red- (−) green] and b* value [(+) yellow - (−) blue] using the Hunter Lab Color Quest II Minolta CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) (Francis, 1998). Three measurements were taken on each sample. The hue angle and the saturation index value were determined with $\arctan(b^*/a^*)$ and $(a^{*2}+b^{*2})^{1/2}$ equations, respectively.

Sensory analysis

Sensory analysis was carried out for cooked Turkish pasta samples. Turkish pasta samples were evaluated in terms of color, odor, taste, appearance, chewiness and general acceptability

properties by 12 panelists (aged 20–50).Sensory properties of samples were scored using a 7-point hedonic scale in which a score of 7 = like extremely, 1 = dislike extremely.

Statistical analysis

The results were expressed as mean ± standard deviation and were analyzed using the Statistical software JMP 5.0.1 (SAS Institute). The averages of the main variation sources were compared at $p < .05$ level. All measurements were performed in duplicate for each sample.

RESULTS AND DISCUSSION

The data on the effect of different levels of aquafaba on the cooking quality and firmness properties are shown in Table 1. The water uptake values of Turkish pasta samples increased with the use of more than 50%. Compared to control, addition of aquafaba increased volume increase value of Turkish pasta samples from 219.69 to 248.11%. Similar behavior in terms of cooking loss properties was observed Turkish pasta samples produced with different aquafaba. While Turkish pasta prepared with 50-100% legume flour revealed the highest cooking loss, the addition of 25% aquafaba showed similar cooking loss with control sample. The findings of this study demonstrated that the addition of aquafaba increased 1.82-fold with 100% aquafaba the firmness values of pasta samples compared to the control sample (Table 1).

Table 1. Physical properties of Turkish pasta[1]

	Water uptake (%)	Volume increase (%)	Cooking loss (%)	Firmness (g)
Control	215.59±1.89b	219.69±6.03b	4.23±0.11b	642.12±23.35c
25% Aquafaba	220.06±3.44b	229.72±4.81ab	4.75±0.25b	698.98±61.62c
50% Aquafaba	236.70±3.03a	244.86±6.52a	5.43±0.10a	809.39±90.10bc
75% Aquafaba	238.14±1.25a	245.15±5.82a	5.74±0.16a	988.47±35.67ab
100% Aquafaba	243.12±3.16a	248.11±3.95a	5.93±0.13a	1166.62±92.43a

[1]Means followed by the different letters within a column are significantly ($P < 0.05$) different.

Color values of Turkish pasta samples are demonstrated in Table 2. Color L*, a*, and b* results of Turkish pasta samples varied in the range of 65.24-75.11, 0.26-(-2.13), and 24.30-30.56, respectively. Compared to the control sample, the addition of aquafaba into the formulation of Turkish pasta significantly ($p < .05$) increased L*, but decreased a* and b*values. The findings are associated with the natural color properties of eggs. The reason of this result was lower lightness and higher yellowness values of egg compared with aquafaba. The SI value of Turkish pasta sample incorporated with 100% aquafaba were lower than the control and other samples. The hue angle values demonstrated an increase with high aquafaba usage ratio.

Table 2. Color properties of Turkish pasta[1]

	L*	a*	b*	Saturation Index	Hue angle
Control	65.24±1.58c	0.26±0.07a	30.56±0.83a	30.56±0.77a	89.51±0.58b
25% Aquafaba	67.72±1.98bc	-0.37±0.10b	29.94±0.69a	29.94±0.10a	90.71±0.99b
50% Aquafaba	70.52±1.37abc	-0.92±0.14c	29.73±0.59a	29.74±0.84a	91.77±1.02ab
75% Aquafaba	73.12±1.87ab	-2.06±0.03d	28.50±1.92ab	28.58±0.68a	94.27±0.35a
100% Aquafaba	75.11±0.66a	-2.13±0.04d	24.30±1.00b	24.39±0.52b	94.85±0.91a

[1]Means followed by the different letters within a column are significantly ($P < 0.05$) different

Sensory properties of Turkish pasta samples are presented in Figure 1. According to the results; color score shown no any change with incorporation of aquafaba in pasta formulation. When compared with the control sample, the odor, taste, appearance, chewiness and overall acceptability scores of the Turkish pasta samples were found higher with high aquafaba addition levels, statistically. As a result, 50% and more aquafaba usage improved the sensory properties and overall acceptability of Turkish pasta. Mustafa et al. (2018) prepared sponge cake with egg white and aquafaba. The color and texture of sponge cake made with egg white or aquafaba was similar and acceptable, but cakes made with aquafaba were less pliable and less sticky than cakes made with egg whites.

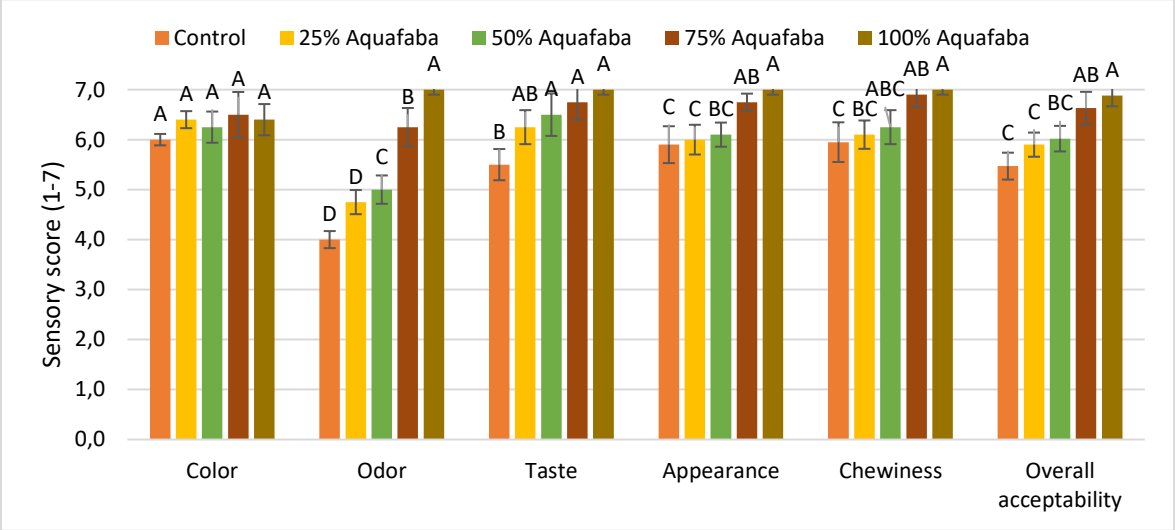

Figure 1. Sensory properties of Turkish pasta

Aslan and Ertaş (2020) used aquafaba as an egg substitute in the cake formulation. According to the results of sensory evaluation, they reported that samples containing 25% aquafaba were preferred more by the panelists.

CONCLUSIONS

In this study, the usability of aquafaba as egg substitute in Turkish pasta (Erişte) production was investigated. According to results, the use of aquafaba as an egg substitute was concluded with an increase in the water uptake, volume increase and cooking loss of pasta samples and so, in terms of cooking quality properties considerably not caused a negative effect up to 50% ratio. Also, the use of aquafaba increased the firmness values compared to the control samples. The incorporation of aquafaba in the pasta samples positively affected the sensory profile in terms of all sensory scores except to color scores.

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DETERMINATION OF THE HARVEST TIME OF SILAGE CORN IN HIGH ALTITUDE REGIONS

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ABSTRACT

Animal feeding with silage has become an indispensable technique all over the world. However, the cultivation of maize, which is the most important crop of silage in high altitude regions, is risky. For this reason, it is necessary to determine the varieties to be grown according to the altitude and their harvest times. This research is about the harvesting of corn varieties (SZE TC-513, Prestige and OSSK-644) with 3 different maturation periods on 3 different dates (1 September, 10 September and 20 September) in Erzurum, which has an altitude of 1860 m. The research was carried out in the experimental area of Atatürk University Plant Production and Research Center in 2012 and 2103. The field trial was set up as 3 replications according to randomized complete blocks experimental design, and the mean scores found to be significant were lettered according to the 5% probability level with the LSD multiple comparison test. Silage yield, some plant characteristics and silage quality characteristics were investigated during two years. According to the two-year average results; under current ecological conditions, Prestige and OSSK-644 varieties have higher silage yields (78.6 and 75.6 t/ha respectively). Between the harvest dates, September 20 (81.2 t/ha) was determined to give higher yields. According to the results of the research, it can be recommended to use mid-early varieties in high altitude regions and similar ecologies and to harvest them at the end of September.

Keywords: Silage maize, high altitude, variety, harvest time

INTRODUCTION

Corn (*Zea mays* L.) is the plant most used in making silage all over the world, due to its high yield, rich in soluble carbohydrates and dry matter, and easy cultivation. However, maize is a tropical grass, and it is productive in warm climates with a long development period and sufficient rainfall or irrigation. A frost-free growing period of at least 3 months is needed to grow corn safely. For this reason, the last frosts of spring and the first frosts of autumn in high altitude regions shorten the corn growing season and put corn agriculture at risk. For this reason, it is important to choose the appropriate variety and determine the harvest time for corn cultivation in high altitude regions.

For the silage corn harvest, the plants must form the cob, set the grain and reach the milk-dough stage. In the sources related to silage, it is reported that 50% of the total silage yield and 70% of the feeding value come from the cob (Açıkgöz, 2002). For this reason, varieties that do

not form sufficient cobs are deficient in terms of both yield and quality. It is also a known fact that the yields of very early varieties are low (Güney *et al*., 2010). For this reason, it should be determined which corn varieties with which growing period are suitable in a region. Güney *et al.* (2010) found that among the corn varieties they examined under Erzurum conditions, those with an FAO value between 400 and 500 reached silage maturity early, but their yield was low. They found that those with an FAO value between 500 and 600 are more suitable for this region, while those with an FAO value above 600 give good results for some years, and for some years they cannot reach the desired maturity.

Adjusting the harvest time in silage corn is important for both the yield of the plant and the quality of the silage. When the plants are harvested early, they are rich in water and low in carbohydrates; when it is harvested late, a very hard grained and dried forage is obtained. If the grains become too hard, their evaluation by animals becomes difficult, and the silage gains a straw appearance (Kılıç, 1986). If the water content of the plant is high, the risk of leakage increases. In the appropriate harvest period, the cobs should be mature enough. Demarguilly (1973) stated that if the corn to be ensiled is harvested earlier than the dough formation period, the amount of dry matter produced per hectare decreases and the nutrient losses increase by leaching during ensiling. Kılıç and Gül (2007) determined that the most suitable harvest time for obtaining high dry matter and silage quality in Diyarbakır is the hard dough stage. However, this maturation takes place as warm weather permits. As a matter of fact, in studies conducted at high altitudes in the Eastern Anatolia Region, there are reports that silage corn is damaged by cold in the first week of September in some years (Güney, 2005). This shows that it will not be advantageous to wait longer in similar ecologies. For this reason, it is also necessary to determine the harvest dates in high altitude regions.

This study deals with the harvest dates of corn varieties with different developmental periods at different times in high altitude regions, which are risky for silage maize cultivation. In the study, it was tried to determine the appropriate ripening value and harvest dates for maize in a region with a high altitude and continental climate.

MATERIALS AND METHODS

The research was carried out in the irrigated trial area of Atatürk University Faculty of Agriculture in 2012 and 2013. In the study, 3 different varieties of corn (*Zea mays* L.) (SZE TC-513, Prestige and OSSK-644) and 3 different harvest dates (1 September, 10 September and 20 September) were used. The varieties used were selected from materials with different FAO values (early-FAO: 500, mid-early-FAO: 550 and mid-late-FAO: 640). The research was established in the randomized complete blocks experimental design with 3 blocks according to the factorial arrangement.

Sowing was done in a pre-prepared seed bed with 70 cm row spacing and 15 cm row spacing. There were 4 rows in the parcels, the width of the parcel was 2,8 m, the length of the parcel was 3 m and the area was 8,4 m^2. As fertilizer, 150 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1} were applied. All of the phosphorus fertilizer was mixed by sprinkling on the plots during the seed bed preparation, and the nitrogen fertilizer was divided into two parts, half of which was applied during planting and the other half when the plants were 40-50 cm tall. After the planted plants completed the emergence, the first weed control was carried out in the form of hoeing at a height of approximately 20-25 cm. In this hoeing, the plants were diluted to be 15 cm above the row. The second hoe was made in the form of throat filling when the plants were about half a meter tall. The second part of the nitrogen fertilizer was given before this application. Taking into account the rainfall and the morphological structures of the plants, flood irrigation was done according to the need (Tan, 2018).

In the research, plant height and ear ratio were determined by cutting 5 plants from the root collar of the middle rows during harvest. In the harvests, one row at the edges of the parcel and 0,5 m from the heads were discarded as the edge effect, and the remaining area (2,8 m^2) was harvested. After the harvested plants were weighed as wet, they were first dried in the open air for a week and then dried in a drying oven set at 60 °C for 48 hours, and dry matter ratio and dry matter yield were determined. The methods followed by Güney (2005) and Geren *et al.* (2003) were used to determine the morphological and agricultural characteristics. Crude protein ratios were determined by Mikro Kjeldahl method (Kacar, 1984), ADF (Acid Detergent Fiber) and NDF (Neutral Detergent Fiber) ratios were determined with the help of ANKOM Fiber Analyzer according to the principles stated by Van Soest (1963). The relative feed value (RFV) is Rohweder *et al*. (1978), dry matter digestion and dry matter consumption were determined by calculation.

The two-year data obtained in the research were subjected to variance analysis according to the randomized complete blocks experimental design. Analyzes were made with the help of MSTAT-C package program. The differences between the means were compared and grouped at the 5% probability level according to the LSD Multiple Comparison Test.

Erzurum province, where the research was conducted, has an altitude of 1869 m and is located on 39° 51' north latitude and 41° 61 ' east longitude. The continental climate prevails in the province, with cold and snowy winters and cool and dry summers. Autumn and spring, which are the transitional seasons, are short, and the winter period is long. Some climate data of Erzurum province for the years 2012 and 2013 and the long-term average are shown in Table 1. In the first year of the experiment (2012), the total precipitation amount (313,4 mm) was below the long-term average, the monthly average temperature (5,6 °C) was at the same level as the long-term average. In the second year of the experiment (2013), precipitation values were lower than both 2012 and the long-term average. However, the monthly average temperatures in the second year of the experiment are close to both 2012 and long-term averages. In May-August, when plants are actively growing, temperatures were close to each other in both years, except for June 2012, it was more rainy.

Table 1. Some climatic data of Erzurum province for 2012, 2013 and the long-term average (LTA)[1]

Months	Total Precipitation (mm)			Mean Temperature (°C)		
	2012	2013	LTA	2012	2013	LTA
January	6,7	28,7	19,6	-8,8	-9,5	-9,3
February	22,2	28,5	23,1	-14,6	-7,4	-7,9
March	8,4	30,9	32,0	-6,7	-0,8	-2,3
April	37,2	36,3	51,5	7,2	7,2	5,5
May	73,0	32,3	70,3	11,0	11,5	10,6
June	7,0	25,1	46,7	15,7	15,0	14,9
July	19,8	7,8	25,8	19,0	19,4	19,3
August	22,8	5,2	16,5	22,0	19,5	19,4
September	11,0	11,5	22,5	15,0	13,6	14,6
October	41,7	17,2	46,8	9,4	6,0	8,0
November	34,2	19,6	30,7	3,8	2,3	0,7
December	29,4	8,3	20,5	-5,9	-13,4	-6,1
Total/Mean	313,4	251,4	406,0	5,6	5,3	5,6

1 It was taken from the data of Erzurum Meteorology Regional Directorate.

The texture class of the soils of the study is clay-loam. According to the EC and % salt values of the soil, it is seen that there is no salinity problem and it is in the salt-free class. It has a pH value of 7,56 and is slightly alkaline, with a lime rate of 1,14% and a slightly calcareous structure. P_2O_5 and K_2O values suitable for plants in the soil are 44.1 kg ha^{-1} and 1710 kg ha^{-1}, respectively, phosphorus amount is low and potassium amount is sufficient. The organic matter content in the soil is insufficient (1,01%; Anonymous, 2019).

RESULTS AND DISCUSSIONS

In the second year of the study, silage maize plants were found to be taller, cob ratios and dry matter were higher. Accordingly, their silage yields are higher (Table 2). Differences in climatic characteristics between years can lead to significant differences in characteristics such as plant height (Öztürk *et al*., 2008). This may be due to the fact that precipitation was higher in 2013, especially in the months in which the experiment was conducted.

In the study, variety selection significantly affected plant height, cob ratio, dry matter ratio and silage yield of silage mass Sorting, cob ratio and dry matter content are genetic characteristics of plants and emerge when environmental conditions allow. In this study, the earliest cultivar, TC-513, was shorter (179,0 cm), while the cultivar with the highest ear rate (42,13%) and dry matter rate (24,83%). Later maturing varieties have longer plant heights, but lower ear and dry matter ratios. Many researchers working with different corn varieties pointed to similar results (Kim *et al*., 2001; Kılıç and Gül, 2007; Güney *et al.*, 2010; Kaya and Kuşaksız 2012; Guyader *et al.,* 2018).

Since harvesting at different dates affected the development times of the plants, it led to an increase in length, an increase in the cob and dry matter ratio, and an increase in silage yield (Table 2). The lowest yield was determined at the harvest on September 1 with 68,9 t ha^{-1}, while the highest yield was obtained from the last harvest date with 81,2 t ha^{-1} With the delay of the harvest date; Kaya and Kuşaksız (2012) reported that plant height, Rabelo *et al.* (2015) determined that the ear rate and Çağrı (2020) determined silage yield increased.

Table 2. Silage yield and some characteristics of corn varieties harvested on different dates*

Applications	Plant Height (cm)	Ear Ratio (%)	Dry Matter Ratio (%)	Silage Yield (t ha^{-1})
Variety				
SZE TC-513	179,0 C	42,13 A	24,83 A	70,0 B
Prestige	195,7 B	31,91 B	24,53 AB	78,6 A
OSSK-644	211,0 A	30,05 C	24,04 B	75,6 A
Harvest Date				
1 September	186,9 B	30,19 C	21,69 C	68,9 C
10 September	194,2 B	35,12 B	24,77 B	74,0 B
20 September	204,6 A	38,77 A	26,94 A	81,2 A
Year				
2012	182,5 B	31,74 B	22,78 B	73,7
2013	207,9 A	37,66 A	26,16 A	75,8
Mean	195,2	34,70	24,47	74,7
Variety x H. Date	ns	0.05	ns	0.05

*Means marked with the same letter are statistically similar. Statistically significant at the 5% level, ns: non-significant

In the study, harvest dates had the greatest effect on the quality parameters of silage maize, and the effect of cultivars was found to be insignificant (Table 3). In 2012, the first year of the study, crude protein ratio and RFV value were found to be higher than the other year. This may be related to the lower content of ADF and NDF in silage material in 2012.

The effects of cultivars used in the study on silage quality parameters were not found significant. Depending on the cultivars, crude protein ratio was 9,58-9,83%, NDF ratio was 39,83-40,24%, ADF ratio was 34,00-34,21% and RFV value showed insignificant changes between 144,4-145,9%.

Delayed harvest dates significantly affected feed quality in silage maize. As the harvest time was delayed, crude protein ratio decreased, irregular changes were observed in ADF and NDF ratios, and RFV value increased. These irregular changes may have occurred due to the increase in the cob ratio in the forage, although the structural materials increased with over time. Horst *et a.l* (2021) also determined that crude protein ratio decreases with maturation.

Table 3. Some nutritional value characteristics of silage maize varieties harvested on different dates*

Applications	Crude Protein (%)	NDF (%)	ADF (%)	RFV
Variety				
SZE TC-513	9,58	39,83	34,11	145,9
Prestige	9,83	40,24	34,21	144,4
OSSK-644	9,63	40,24	34,00	144,7
Harvest Date				
1 September	9,98 A	40,40 A	34,45 A	143,3 B
10 September	9,82 A	40,83 A	33,72 B	142,9 B
20 September	9,23 B	39,07 B	34,16 AB	148,7 A
Year				
2012	10,08 A	39,89	33,48 B	146,9 A
2013	9,29 B	40,32	34,73 A	143,0 B
Mean	9,68	40,10	34,30	145,0
Variety x H. Date	ns	ns	ns	ns

*Means marked with the same letter are statistically similar. Statistically significant at the 5% level, ns: non-significant

CONCLUSION

It has been revealed in many studies that the variety and harvest time have an effect on the yield and feed quality of silage maize. This research focused on the determination of the corn varieties to be grown for silage and the harvesting times in high altitude regions such as Erzurum. According to the results of the research, harvesting at a later date resulted in a higher yield as it provided a longer development period for the plants. It also led to an increase in the cob ratio and dry matter ratio, which are of great importance for silage. Filya (2002) states that the dry matter ratio in silage corn should be more than 20%, and even around 35% gives better results. Harvests done at the wrong time cause high losses with leakages after silage, or decrease silage quality (Hunt *et al*., 1989). In this study, since the low temperature that causes freezing did not occur in September in the years in which the research was carried out, it was revealed that September 20 was more suitable for harvesting. Prestige and OSSK-644 varieties with longer growth times were found to be more productive because an early autumn low temperature did not occur.

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EVOLUTION OF AMPELOGRAPHIC TECHNIQUES FOR CHARACTERIZATION BETWEEN VINE GRAPE VARIETIES

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ABSTRACT

Viticulture has a long history. The species, Vitis vinifera includes thousands of varieties with similarities, hence the birth of a discipline called ampelography whose objective is to distinguish and identify the different grape varieties. The characterization of vine varieties by ampelography was based on description using morphological characters called descriptors concerning the organs of the plant (vegetative, floral, and fruit), each parameter is codified for a single universal language established by the International Vine Office OIV. The application of this visual and subjective method on many individuals is quite difficult and takes years. The morphological description was supplemented by ampelometric studies by carrying out measurements of interest mainly to the adult leaf, these measurements are combined with advanced statistics for differentiation between varieties.

The influence of environmental factors severely limited these methods, forcing laboratory studies with biochemical internal markers, such as isoenzymes, polyphenols, flavonoids, proteins, and carbohydrates. To use the most precise tools, genome sequencing projects become the techniques of choice; especially with high-multiplication technologies; for the search for relationships between varieties. However, the application of these techniques had the disadvantage of expensive cost, the dependence on laboratory techniques and products.

Despite the high precision of these biochemical and molecular methods, ampelographers have subsequently used technological means such as electronic imaging, artificial intelligence, and machine learning due to the large amount of data that these tools can contain and the large number of samples that can be studied. This makes it possible to structure the information in the form of ampelographic data banks that lead to the construction of computerized identification systems and to use them in an updated way.

An ampelographer will obviously be able to couple several methods, to highlight the discrimination between similar varieties.

Key words: ampelography, similarity, vine varieties, identification.

INTRODUCTION:

The grape ; the fruit of the vine; belongs to the Vitaceae family. It is among the oldest cultures. There are three groups of cultivated vines: Asian vines, American vines and Euro-Asian vines which include only one species, Vitis vinifera, including the cultivated archetype, Vitis vinifera sativa, giving rise to thousands of varieties , or grape varieties (HUGLIN and SCHNEIDER, 1998).

According to the Food and Agriculture Organization (FAO, 2018) the total viticulture area is 7.15 million hectares with an annual production of 79.12 million Ton (MT) across the world(Zheng et al., 2020). This fruit is appreciated thanks to its nutritional value due to the presence of different compounds such as oses (fructose and glucose), pectins, organic acids, minerals, vitamins, proteins and amino acids, fibers and its richness in polyphenols and resveratrol (Conde et al., 2007).

Ampelography (from the Greek ampélos = vine and graphy = description) is a discipline which began in the second half of the 19th century with GOETHE (1878) who was the first to think about the study of leaf morphology for recognition and classification of vine stocks (BOURSIQUOT et al. (1989).

It therefore aims to distinguish and identify the different grape varieties based on morphological or internal characters revealed by biochemical and molecular markers. She is also interested in the classification and requirements of vines as well as their evolution and therefore she also studies the botanical and agronomic side (REYNIER, 2003).

AMPELOGRAPHIC METHODS.

1/- Descriptive methods

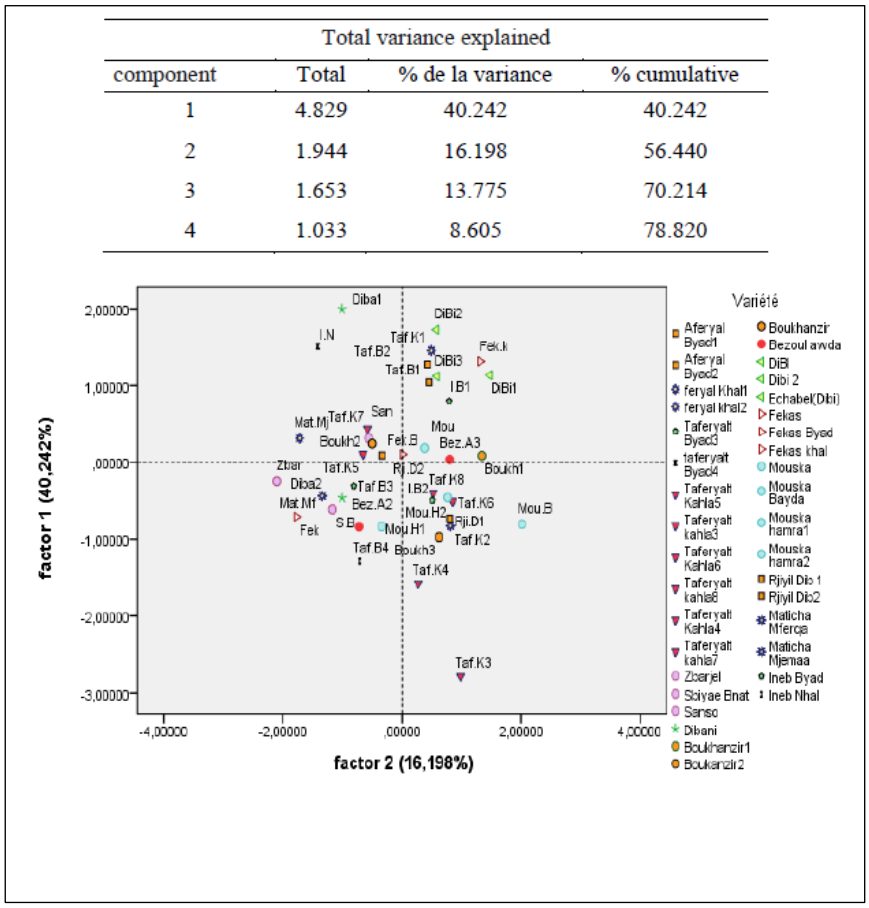
Since the 19th century the methods used were essentially descriptive, using qualitative and quantitative morphological characters, the grape varieties are collected and grouped to be described. The description concerns each organ of the vine: buds, leaves, branches, clusters, these morphological characters are subsequently classified and codified (fig 01). To achieve the practical recognition of grape varieties and rootstocks, observations must be repeated several times (SWANEPOEL and DE VILLIERS, 1987; SCHNEIDER, 1996; SOTES et al., 1996 and REYNIER, 2003). However, such studies based on visual description necessarily have shortcomings, even if they are interesting (GALET, 1998).(FERNANDES et al., 2019).

These descriptive parameters are described by codes established by the OIV (Figure 01) representing the degree of expression for each parameter.

les paramêtres ampélographique, agronomiques et biochimiques *O. Bounab, Z. Laiadi / South African Journal of Botany 124 (2019) 71–79*

les organes	les parametres ampélographiques	les parametres agronomiques	les parametres biochimiques
Woody shoot	OIV101, OIV102, OIV103, OIV105, OIV 106	OIV305	
Shoot	OIV353, OIV006, OIV007, OIV008, OIV014, OIV009, OIV010, OIV013, OIV354,	OIV351	
Bud		OIV301	
Inflorescences	OIV153, OIV151	OIV501, OIV302	
Seed	OIV243, OIV242, OIV241		
Bunch	OIV203, OIV202, OIV209, OIV207, OIV208, OIV204, OIV206	OIV502, OIV303, OIV504, OIV304,	
Berry	OIV220, OIV221, OIV238, OIV222, OIV223, OIV225, OIV226, OIV231, OIV232, OIV240, OIV235	OIV503	OIV233, OIV505, OIV508, OIV506
Tip	OIV003, OIV002, OIV005, OIV004		
Young leaf	OIV056, OIV055, OIV054, OIV053, OIV051		
Mature leaf	OIV306, OIV94, OIV83-2, OIV,83-1, OIV82, OIV80, OIV79, OIV93, OIV72, OIV71, OIV70, OIV69, OIV67, OIV76, OIV75,OIV74, OIV73, OIV88, OIV89, OIV84, OIV85, OIV86, OIV87,OIV90, OIV91, OIV92, OIV610, OIV607, OIV608, OIV609, OIV617, OIV616, OIV618, OIV615, OIV614, OIV77, OIV601,OIV602,OIV613, OIV612, OIV611, OIV603, OIV604, OIV605, OIV606, OIV78, OIV065		
Tendril	OIV017		
Total	94	10	04

El OUALKADI et al tried to apply quantitative ampelographic parameters to differentiate between the clusters of 39 autochthonous vine varieties surveyed in the North-West Moroccan region with repetitions of 10 clusters for each variety. The samples are collected during the fruiting period and the description is made according to the descriptors of the OIV 2001.
The results were analyzed by the principal component analysis technique using the SPSS Version 10 software, they showed a very large variability between the samples and even with the principal component analysis the results were not structured (Figure 02) .
The author was able to notice that none of the characteristics studied makes it possible to distinguish one variety from the others, at least from the data obtained from these samples despite the discriminatory power of the parameters used (El OUALKADI 2019).



Total variance explained

component	Total	% de la variance	% cumulative
1	4.829	40.242	40.242
2	1.944	16.198	56.440
3	1.653	13.775	70.214
4	1.033	8.605	78.820

Figure 02: The association of variances with the axes of the PCA of the characteristics of the clusters. (EL OUALKADI, 2019).

Other disadvantages of this classic descriptive method are the impact of environmental factors, for example "the differences that the clusters can present between them lack constant" RAVAZ in 1902. The glitch although it is important for the distinction but does not allow us to characterize the varieties making up the species Vitis vinifera (BALMELLE et al., 2001). The serration of the adult leaf (rectilinear, ogival, concave, hooked) which is a very good visual ampelographic character, but very difficult to be coded because on the same sheet we find teeth of different shapes and unequal depths (GALET, 1998). This method is based on visual description which must be carried out by a well-trained expert (DIAGO MP et al, 2013).

2/ Ampelometric methods

The descriptive study is complemented by a quantitative study based on measurements called ampelometry or phyllometry. These measurements focus on the lengths of the main veins and the angles that these veins make between them, the values are also coded, which brings an interesting element to the characterization of the grape varieties (GALET, 1998 and TOMAŽIČ and KOROŠEC-KORUZA, 2003) . The bases and principles of the codifications of ampelometry were launched in 1902 by RAVAZ and it was GALET who continued this path by completing it and making improvements (BOURSIQUOT, 1989).

Amplometry is illustrated by computerized analytical and electronic imaging techniques which facilitate the realization of measurements as well as the processing of results. This method is also subject to environmental factors, such as the dimensions of the leaf which depend on many factors such as soil fertility, the vigor of the strain, the training method and the latitude (LAKHRIF z., 2011).

3/ Biochemical and physiological methods

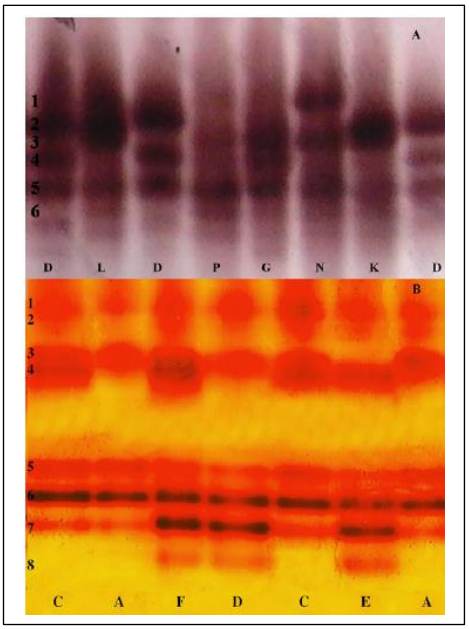
The importance of biochemical characterization was proven for the identification of grape varieties by the work of SATISHA et al. (2007) and RUSJAN and KOROŠEC-KORUZA (2007). The parameters studied are polyphenols, flavonoids, proteins and carbohydrates (LAKHRIF z., 2011).

These methods are also influenced by climatic conditions and therefore environmental factors. For example: Disruption of the immediate cyanogenic response in tissues will likely be linked to interactions with invading microorganisms (FRANKS TK et al, 2005).

The phenolic compounds contained in grape berries are of great importance as ampelographic and taxonomic characteristics for the classification of cultivars. But their concentration in red vines depends on several environmental factors and cultivation practices (LETAIEF H. et al, 2007).

The use of isoenzymes and biochemical techniques in general can only be applied by specialized personnel, require time and are dependent on laboratories and do not allow the identification of a high number of varieties in a rapid manner (DIAGO MP and al, 2013).

The separation of the isoenzymes (Figure 03) is carried out by vertical electrophoresis on polyacrylamide gel. These enzymes are: catechol oxidase (CO), peroxidase (PER), glutamate-oxalacetate transaminase (GOT) and acid phosphatase (AcP) as described by Royo et al. (1997). After electrophoresis, gels were stained for AcP, GOT, and PER with staining solutions as described by Arulsekar and Parfitt (1986); or for CO as described by Sa'nchehez-Ye'lamo (1992). Isoenzyme models were assessed visually(JAHNKE et al., 2009).



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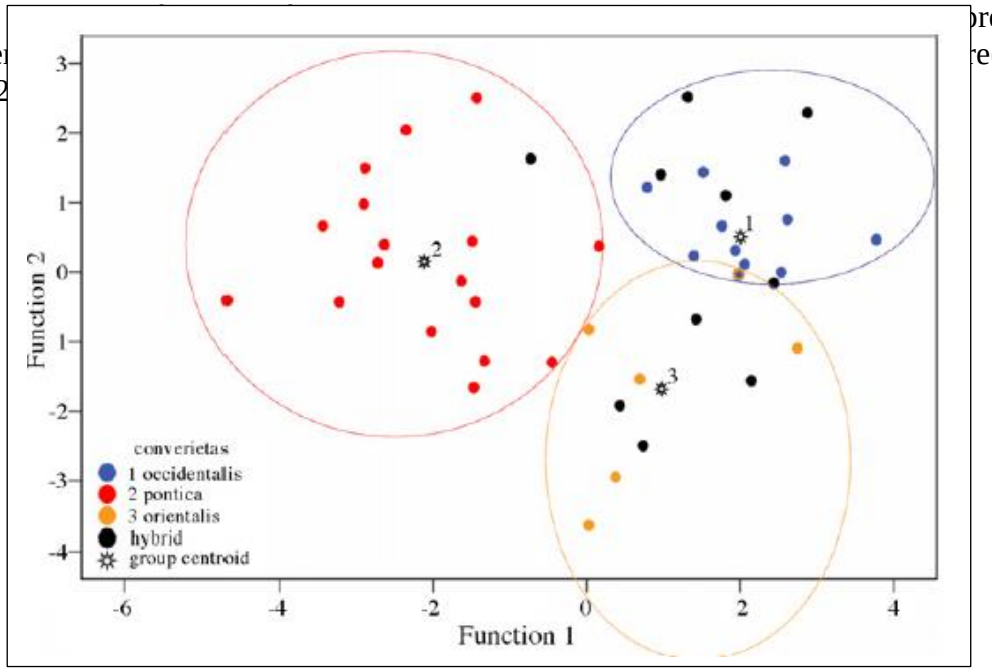

Figure 04: separation of varieties of*Vitis viniferaL*. according to their origin and the origin of their zymogram of isoenzymes (JAHNKE et al., 2009)

The separation between these varieties (Figure 04) is based on isozyme band patterns, for cathecol oxidase, glutamate-oxaloacetate transaminase, acid phosphatase and peroxidase the results are reproducible, and the varieties are characterized and sorted. More than 40 varieties among the 48 which were the subject of this investigation were identified, they are classified into three groups according to their origin. This analysis attempts to investigate whether there are established links between phenotypic characteristics and patterns obtained by isozyme analysis. The enzyme that showed high polymorphism was CO while GOT had the lowest rate of polymorphism, but these enzymes are influenced by the environment (JAHNKE et al., 2009)

4 / Molecular methods

In order to seek more precise and more efficient methods independently of the impact of the environment, researchers are moving towards molecular techniques and isoenzymatic analysis which provide complementarity of ampelographic information both for characterization and identification only to reveal the relationships between grape varieties (MEREDITH and BOWERS, 1996; DE MICHELI et al., 1997; GOLINO, 2000; COSTACURTA et al., 2001; MEREDITH, 2001; REYNIER, 2003; COBAN, 2004; MOSS et al., 2005; THIS et al., 2006; ALMADANIM et al., 2007; LE CUNFF et al., 2008; SPRING et al., 2008; IŞÇI et al., 2009; LAIADI et al., 2009, SABIR et al., 2009 and ZINELABIDINE et al., 2010).
The molecular testing. are enable to determine the genetic structure, gene flow, and spatial structuring of genetic content in table grape populations (Kajkolah 2023). AND molecular markers are introduced in recent years for genetic studies, characterization of cultivars and their identification, RFLP analysis is used for the identification of 16 commercialized rootstock cultivars(Bourquin et al., 1992) and RAPD analysis was applied to reveal genetic relationships (JAHNKE G. et al 2009). The first SSR marker for grapevine was published by Thomas and

Scott (1993) and after a few primary SSR sequences were described (Di Gaspero et al., 2000; Scott et al., 2000; Lefort et al., 2002; Arroyo- Garcia and Martinez-Zapater, 2004).
Molecular techniques use many DNA markers called microsatellite markers such as Simple Sequence Repeats (SSR), also called Sequence-tagged Microsatellite Site (STMS) used to identify collections of cultivars, they are suitable for the genotype of grape varieties (SÁNCHEZ-ESCRIBANO et al., 1999). In addition, these microsatellite markers have enabled the acquisition of more in-depth knowledge on naming problems such as synonymies and homonyms as well as the origin of grape varieties (RIAHI et al., 2010).
Several methods have shown their effectiveness such as the Random Amplification Polymorphism of DNA by PCR (RAPD) method for the identification and molecular analysis of grape varieties (BINIARI and STAVRAKAKIS, 1999; TESSIER et al., 1999; VIDAL et al. al., 1999; HERRERA et al., 2002; ARAS et al., 2005; KOCSIS et al., 2005; GÖKBAYRAK et al., 2006; IŞÇI et al., 2009 and BUTIUC-KEUL et al., 2010), Amplified Fragment Length Polymorphism (AFLP) has been used to identify genetic relationships within grapevine accessions and to detect these intervarietal variations (CERVERA et al., 1998; CERVERA et al., 2001; LABRA et al. ., 2001; IMAZIO et al., 2002; VIGNANI et al., 2002; SIRET et al., 2002; MARTINEZ et al., 2003; ERGÜL et al., 2006; MONCADA and HINRICHSEN, 2007 and IŞÇI et al., 2009).
These methods are slow and expensive, which prevents their widespread use.(FERNANDES et al., 2019), require time, intensively dependent on the laboratory and the intervention of expert technicians(ÁLVAREZ et al., 2020).
Other types of molecular markers such as RAPD (ZOGHLAMI et al. 2003), nuclear SSR (ZOGHLAMI et al. 2009; RIAHI et al. 2010, 2012) and chloroplast SSR (RIAHI et al. 2011) were used to estimate the genetic diversity and characterization of indigenous Tunisian grape varieties, and more recently single nucleotide polymorphism SNPs have become the most popular genetic marker for plants(Riahi et al., 2013).
SSR markers are used as genetic markers to reveal genetic diversity, relationships among cultivars among them, relationships, thus constructing genetic maps.
ZOGHLAMI et al used these markers to study 61 Tunisian indigenous varieties (Figure 05), this analysis allowed the identification of 5 possible parents and that the Tunisian vine derives from an Out crossing between these 5 possible parents. The combination between the alleles is treated and analyzed (Figure 06) by the method (UPGMA), the DNA is extracted according to the Mini Kit protocol from plants by Qiagen DNeasy and Quantified by visual comparison by Lambda DNA: molecular marker on ethidium (branid stained) on agarose gel, 10 SSR loci are selected and quantification is done by PCR using TaQ polymerase(ZOGHLAMI et al., 2009)

Locus	Allele number	Genotype patterns	He	Ho	PI	Q	r
VVMD28	11	17	0.778	0.96	0.142	0.578	−0.106
VVMD27	6	12	0.668	0.803	0.285	0.411	−0.08
VVMD21	6	11	0.742	0.885	0.179	0.522	−0.082
VVIP60	7	10	0.621	0.819	0.365	0.347	−0.122
VVMD5	7	18	0.829	0.95	0.096	0.659	−0.066
VVIP31	10	21	0.833	0.901	0.09	0.669	−0.037
VVMD32	11	15	0.773	0.885	0.135	0.58	−0.062
VVMD24	8	18	0.778	0.786	0.137	0.581	−0.004
VVS2	10	19	0.855	0.918	0.07	0.71	−0.003
VVMD7	8	19	0.791	0.688	0.112	0.614	0.057
Overall	84	160	0.766	0.859	3.39×10^{-9}	0.9999	−0.05

Figure 05: Genetic polymorphism parameters obtained with 10 marked SSRs of 61 indigenous Tunisian cultivars He: Expected heterozygosity, Ho: Observed heterozygosity, PI: probability of identity, Q: exclusion of paternity, r: zero allele frequency(ZOGHLAMI et al., 2009).

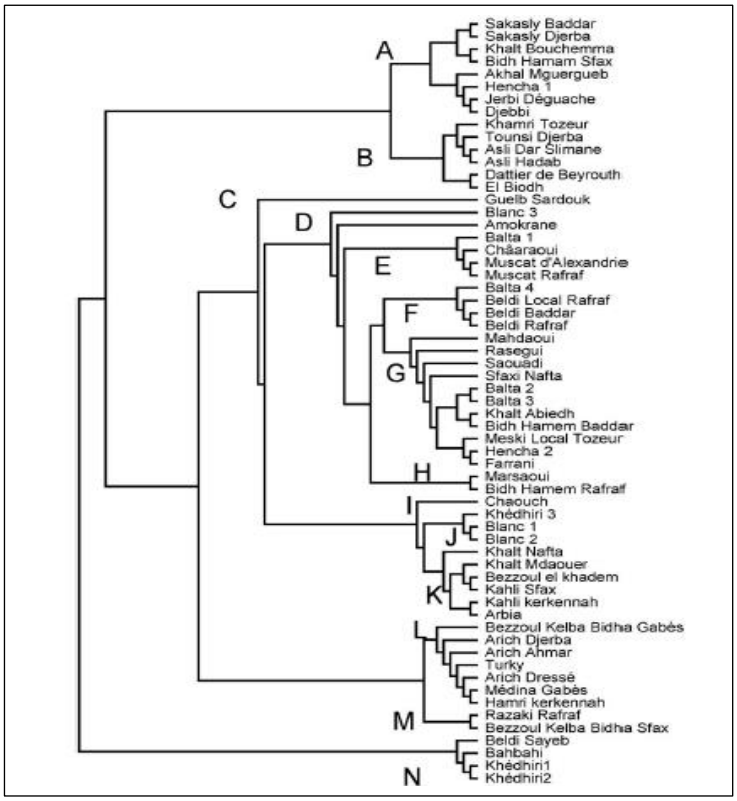

Figure 06: UPGMA dendrogram obtained from microsatellite data from 61 Tunisian indigenous clusters.(Zoghlami et al., 2009)

The abundance of SNPs in genome sequencing projects and the wide choice of high multiplication technologies make them a marker of choice for research (FAN et al. 2006). In addition, the evolution of the natural conservation of SNPs reduces their exposure to homoplasy problems (BRUMFIELD et al. 2003).

With the aim of investigating the variation of alleles and the occurrence of the effects of domestication on the keys to genes controlling adaptation to the environment (RIAHI, 2013).

Molecular methods are generally combined with biochemical separation and identification techniques such as PCR and electrophoresis for better processing of results. Riahi et al attempted to study the genetic diversity and differentiation of cluster samples from the North African region using nuclear microsatellites, DNA is extracted from young leaves or cambium following the DNeasy protocol Plant Mini Kit (Qiagen, Hilden, Germany) then amplified by PCR and separated by electrophoresis.

The use of microsatellites as a complement to descriptors and variety protection became important after the acceptance of allelic SSR by the USDA Plant Variety Protection Office (DIWAN, 1997).

5-Methods based on IT resources

With the development of information technology, new tools are available to the ampelographer to help him, facilitate processing, analyze the results obtained, save effort and time in studies

and structure the data in the form of ampelographic databasesthus using them in an updated way(BOURSIQUOT et al., 1987).Researchers have made it possible to obtain the coordinates of specific points on the leaves and image analysis (REYNIER, 2003). By comparing data according to variety, we can determine whether a new variety introduced into the collection is not already there under another name (BOURSIQUOT et al., 1987). The statistical analysis of a computerized ampelographic file also made it possible to propose groupings of grape varieties which share some particularities (BOURSIQUOT et al., 1987). A factorial correspondence analysis (CFA) will highlight the most important ampelographic characters and obtain an image of the Vitis vinifera population (LAKHRIF Z.2011). However,

With the aim of creating simple methods, in recent years the spectroscopy method has been combined with "machine learning" (Gutiérrez et al., 2015a; Gutiérrez et al., 2016; Gutiérrez et al., 2015b; Cao et al., 2010; Arana et al., 2005; Diago et al., 2013; Yang et al., 2012). The need for the use of these combinations comes from the large quantity of data that this software can contain, the large number of samples that can be studied, which leads to the construction of computerized systems for identifying vine varieties and which can be marketed(FERNANDES et al., 2019)such as classification systems like Support Vector Machines (SVM) and Convolutional Neural Networks (CNN) which is applied for the first time for the identification of vine varieties (Qiu et al., 2018).

New methods such as: attenuated total reflectance (ATR) and Fourier Transform Infrared spectroscopy (FTIR) combined with advanced statistics for differentiation of genotypes of vines are easier and lower priced, faster with computerized data acquisition except that they are non-destructive, these methods measure the absorption of radiation from the surfaces of the samples to obtain an IR spectrum(ÁLVAREZ et al., 2020), the vibrations of this spectrum are considered a fingerprint of any biological or botanical sample (DA Luz, 2006; Milosevic, 2004; Schmidtke, Smith, Müller, & Holzapfel, 2012; Shah, Cynkar, Smith, & Cozzolino , 2010).

The reproducibility of this method must be evaluated on samples of leaves of the same genotypes (variety and clone) obtained in different environments and with different management and trellis systems such as samples which have different physiological conditions, different health conditions and different development stages with the aim of determining its total confidentiality, technical strengths and weaknesses(ÁLVAREZ et al., 2020)

CONCLUSION

The characterization of the high number of vine varieties by ampelography was based first on the description using morphological characters. Applying this visual and subjective method to many individuals is quite difficult and takes years. The morphological description was then completed by ampelometric studies. The influence of environmental factors has strongly limited these methods, which has imposed studies in laboratories with internal biochemical and molecular markers.

Despite the high precision of these biochemical and molecular methods, the dependence on laboratory techniques and products subsequently imposed the use of technological means of artificial intelligence and machine learning. An ampelographer will obviously have to combine several methods, with the aim of highlighting discrimination between similar varieties. However, the classic ampelographic description remains an essential tool in grape variety identification and characterization studies. All other means are therefore complementary.

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DETERMINATION OF YIELD PROTEIN CHARACTERISTICS IN DIFFERENT BEAN CULTIVARS

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ABSTRACT

The experiment was conducted in the 2021-2022 growing season under the conditions of Aydın Province. The study aimed to determine the performance of three different bean varieties (Dermason-Efsane-Maş). The experiment was carried out in the producer field of Köşk district of Aydın province with three replications according to the coincidence blocks experimental design. Grain yield and protein ratios of the varieties were analyzed. It was determined that grain yield values varied between 150-200 kg/da and grain protein ratios varied between 22-25%. As a result of the study, it was determined that genotype factor was effective on the studied traits.

Keywords: Bean, grain yield, grain protein ratio.,

INTRODUCTION

Bean (*Phaseolus vulgaris*) is widely used for direct human consumption, especially in tropical and subtropical countries of America, Europe, Africa and Asia (He et al., 2018). It is cultivated in 126 countries in the world (Mızrak, 2020). Dried beans, which are grown in the temperate climate zone, have a wide adaptation area and can be produced in areas close to sea level in America and Europe and in areas higher than 3000 meters in South America (Sözen and Karadavut, 2020). Although dry bean ranks first in the world among edible grain legumes with a cultivation area of 34,495,662 ha and a production of 30,434,280 tons, it ranks second after chickpea in our country with a cultivation area of 84,786 ha and a production of 220,000 tons. While the average yield in the world countries growing dry beans is 88 kg per decare, this value is around 259 kg in our country (Anonymous, 2018).

Beans are widely consumed in the world and in our country as fresh, canned, fresh grain and dried grain due to their high protein content and delicious taste. Typically, most dried beans contain 15% to 25% protein on a dry weight basis. Water-soluble albumins and salt-soluble globulins account for 10% to 30% and 45% to 70% of total proteins, respectively Globulin in dry beans is salt-soluble and accounts for 50 to 55% of total proteins in dry beans (Sathe, 2002). Its dry grains contain 23-34% protein, 60% carbohydrate, 5% crude cellulose, 1.7% fat and 3.6% ash (Abacı and Kaya, 2018). According to Gepts (2001), beans contain 22-27% protein and 39-47% carbohydrates, making them a valuable foodstuff for more than half a billion people. The digestibility of dry bean proteins ranges from 71-94% (Barampama and Simard, 1994). The main functional components of beans are carbohydrates, vitamins, phytate, lectins, soluble fiber and phenolic. Phenolic, which include phenolic acids, flavonoids and proanthocyanidins, are particularly noteworthy due to their strong antioxidant properties (García-Lafuente et al., 2014). Beans are also rich in proteins that complement the amino acid profile of cereals.

Low yields in beans are associated with both biotic and abiotic stresses. Biotic stresses include diseases, insects and weeds, and the low nitrogen (N) fixing capacity of bean plants. Abiotic stresses include drought, acidic and infertile soils, and reduced use of chemical

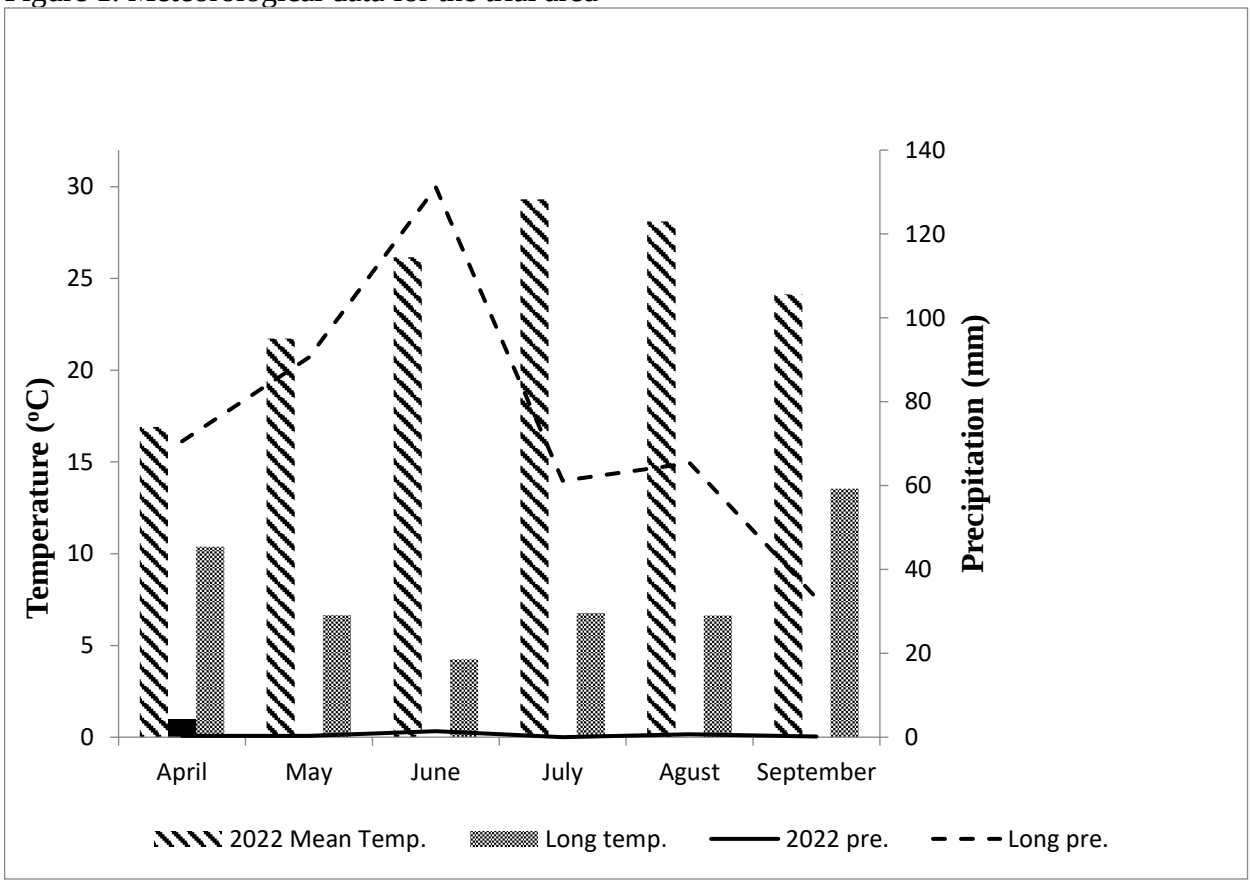
fertilizers, especially in developing countries. Plant parameters such as shoot dry weight, pods per plant or per unit area, 100-grain weight, grain harvest index, N harvest index and leaf area index affect bean yield (Fageria and Santos, 2008). In addition, due to increasing global warming in recent years, drought yield losses are generally reported to be between 30% and 90%; (Hussain et al., 2019).

Dry beans have many varieties including black beans, bush beans, pea beans, pink beans, kidney beans, etc. and village varieties. In this study, grain yield and protein values of some bean varieties were determined.

MATERIAL and METHOD

The experiment was conducted in the growing season of 2022 under the conditions of Köşk District of Aydın Province. Grain yield and some yield components of three different bean cultivars (Dermason-Efsane-Local cultivars) were analyzed. The experiment was sown on 2.08.2022 with three replications. After sowing and maintenance operations were carried out during the growing season, the harvest was realized on 29.11.2022. Hoeing was done when the plants reached 15 cm in height**.**

Figure 1. Meteorological data for the trial area



Climatic data are presented in Table 1. According to these data, it is observed that the average air temperatures during the bean growing period are higher than the long-term average and the total rainfall is lower than the long-term average.

RESULTS and DISCUSSION

Table 1 shows the results of ANOVA with the data obtained from the experiment. According to the results, the effect of cultivars on the traits other than pod number was found significant.

Table 1. Analysis of variance for the traits examined in the experiment

Cultivars	Plant Height	First Pod Height	Branch Number	Pod Number	Pod Lenght	Seed per Pod	100 Seed Weight	Seed Yield	Protein
Blocks	10,05	1,09	0,058	0,671	0,041	0,008	3,821	4,941	0,724
Cultivars	63,41*	118,62*	1,361*	0,618	13,541*	0,383*	57,858*	227,68**	6,168*
General	6,69	1,508	0,091	1,404	0,720	0,0513	2,770	8,971	0,564

*Significant at P<0.05 level, ** Significant at P<0.01 level

Mean values of the traits are presented in Table 2. The average plant height values of the varieties varied between 31.5-40.5 cm. The highest plant height was obtained from Dermason variety with 40.5 cm. Elkoca and Cinar (2015) observed plant height ranging between 37.7-50.5 cm.

Table 2. Averages of the data obtained from the trial

Cultivars	Plant Height (cm)	First Pod Height (cm)	Branch Number	Pod Number	Pod Lenght (cm)	Seed per Pod	100 Seed Weight (gr)	Seed Yield (kg/da)	Protein (%)
Dermason	40,5 a	17,2 b	3,8 a	8,1	8,0 a	3,4 a	34,0 a	64,5 a	25,0 a
Efsane	34,3 b	14,5 b	3,3 a	6,3	10,9 b	2,7 b	28,1 b	56,6 b	22,5 b
Local	31,5 b	26,5 a	2,4 b	6,7	6,8 b	3,0 ab	23,2 c	47,1 c	22,5 b
Mean	35,4	19,4	3,2	7,0	8,6	3,0	28,4	56,1	23,3
$LSD_{cultivar}$	5,84	2,78	0,67	ns	0,19	0,49	3,74	6,76	1,68

ns:non significant

The mean values of the first pod height varied between 14.5-26.5 cm. The highest first pod height was obtained from Local (26,5 cm). The varieties were ranked as local>dermason>efsane.

Varieties were found to be significant in terms of the number of lateral branches. mean values varied between 2.4-3.8. the highest number of branches was obtained from dermason (3.8). The number of side branches is one of the factors affecting yield. In previous studies, İyigün and Kayan (2019) measured 6.3-10.2 and Elkoca and Çınar (2015) measured 2.1-3.6 branches per plant.

The difference between the varieties in terms of the number of pods was not found to be significant. However, in average values, dermason variety (8.1 pieces) had a higher number of pods per plant compared to other varieties. However, Güneş (2015) reported that the effect of genotypes on the number of pods in bean was significant and the mean values varied between 14.9-46.1. Bozoğlu and Gülümser (2000) and Babagil et al. (2011) reported that the number of pods per plant varies depending on genotype and environmental conditions.

The difference between the varieties in terms of the number of grains in pods was found to be significant. The highest number of grains was obtained from dermason (3,4). This was followed by local>efsane. Yilmaz (2008) measured the number of grains in pods as 2.50-3.87 and Babagil et al. (2011) measured 8.63 grains. Akçin (1974) reported that yield components in beans differ according to growing conditions and genetic structure. Some other researchers

reported that there may be significant differences in the number of grains in pods, which is a character with high heritability, according to varieties (Ülker and Ceyhan, 2008; Güneş, 2011).

The difference between the varieties in terms of hundred grain weight was found to be significant. dermason variety (34.0 g) had the highest hundred grain weight followed by efsane>local. In previous studies, Kahraman and Önder (2009) measured face grain weights between 23.98 - 41.62 g, Bozoğlu and Gülümser (2000) measured face grain weights between 15.96-52.09 g.

The effect of varieties on grain yield was found to be significant. The variety with the highest grain yield was dermason (64,5 kg/da). This was followed by efsane (56,6 kg/da)>local (47,1 kg/da). Düzdemir (1998) and Pekşen and Gülümser (2005) reported that many factors affect the productivity of bean varieties and genetic structure is one of the most important traits affecting yield. In previous studies, Bozoğlu and Gülümser (2000) reported that grain yield varied between 162.7 and 237.7 kg/ha, Elkoca and Çınar (2015) reported that grain yield varied between 92.0 kg/ha and 195.4 kg/ha.

The effect of varieties on grain protein values was found to be significant. The highest protein content was measured in dermason (25%), while the protein contents of legend and local were the same. In some of the studies, the grain protein content of the genotypes was lower than our results (17.96-22.07%) (Shimelis and Rakshit, 2005,) in some of the studies it was similar to our results (22.03-24.86%) (Barros and Prudencio, 2016) and in some of the studies it was higher than our results (21.0-30.0%) (Pinheiro et al., 2010). It is reported that genotypes differ in terms of protein content and this difference is influenced by genetic structure and environmental conditions (Vural et al., 1986; Santalla, et al., 1995).

CONCLUSIONS

According to the results of the study, dermason variety was high yielding among the varieties. While the number of pods was not found to be effective on the varieties, the difference between the varieties in terms of plant height, first pod height, number of branches, pod length, number of grains in pods, grain yield and protein ratio was found to be significant.

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THE IMPORTANCE OF LENTIL GRAIN QUALITY IN HUMAN NUTRITION

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ABSTRACT

The lentil is one of the edible grain legume plants that have been cultivated and used in nutrition from ancient times to the present day. In our country, the cultivation of commercial lentils is second only to chickpea among edible legume crops. The lentil production, which has an important position in the agriculture of our country, consists of 87.8% red lentils and 12.2% green lentils. Besides having an important place in human nutrition due to its high protein content, lentils also have an important effect on animal nutrition. Its dry grains contain 25% protein. In addition, it also contains important amino acids such as isoleucine and lysine. When compared to cereals, it is an important legume plant in terms of nutritional value with its high amount of protein and rich amino acid content. In human nutrition, lentils are preferred more than other edible grain legumes due to its low amount of anti-nutritional factors, high protein content and short cooking time compared to other legumes. The lentil is a legume that provides additional income to both the producer and the national economy by entering the cultivation shift in arid regions.

Key words: Lentil, grain protein content, quality, amino acid,

INTRODUCTION

Lentils are an important nutritious crop grown around the world due to their high macro and micronutrient content, including all minerals. Canada and India account for 58% of total production in the world. It can replace animal proteins, especially in countries where poor people live. In developed countries, it takes its place as a part of vegetarian diets (Sharma et al., 2022).

Lentil protein and its amino acid composition maintain amino acid balance for physiological functions. It can positively affect human health by preventing protein-energy malnutrition and non-communicable diseases. Therefore, studies aim to improve the protein quality of lentils (Salaria et al., 2022). Factors affecting protein quality include protein content, amino acid composition and protein digestibility. Although lentil has a protein content of 23.22-31.7%, it contains higher protein than cereals and this protein content can be affected by variety and environmental factors (Lee et al., 2007; Zeidan, 2007; Niri et al., 2010; Nosworthy et al., 2018; Amirnia et all, 2019; Öktem 2019; Köse et al., 2019; Küçükay et al., 2019; Subedi et all, 2021)

Influence of Some Factors on Lentil Grain Quality

Harvest Date

Lentil grain quality also depends on harvest time. Choosing the right harvest time helps to harvest the best quality grains. Lentil plants are usually sown in the fall and green and red lentils are harvested with moisture contents around 16-18% and 14-16%, respectively (Chelladurai and Erkinbaev 2020). It is recommended to harvest lentils at higher moisture levels (16-20% weight) in order to reduce grain losses during harvest and reduce post-harvest drying

losses to 13-14% to extend shelf life. This can also increase susceptibility to damage and breakage during transportation, storage and post-harvest handling (Opoku et all, 2009).

Seed Size

The size and shape of lentil grains also affect grain quality. More regular and homogeneous grains are generally considered to be of higher quality. In terms of market preference, cooking time and hull separation are greatly influenced by seed size and shape. Moreover, seed size and seed number determine the overall seed yield of any crop; this trait is determined at early seed development stages (Dutta et all, 2022). Large grain varieties favor higher emergence and lower mortality, increased seedling, root: and shoot rates (Sing et all, 2019). Among various quality traits, seed size is an important trait that defines overall lentil quality. Lentil cultivars with round seed shape have reduced damage during processing compared to thin, sharp-edged cultivars (Singh et al., 2022). In a study conducted on neither lentils nor chickpeas, it was found that the effect of seed size on yield and yield components was not significant (Biçer, 2009) Lentils generally cook faster than other legumes due to their seed size and thin seed coat (Choukri, et all, 2023).

Grain Density

The density of lentil grains has an impact on their commercial and processing value. Higher grain density is considered to be higher quality lentil grains. Lentils contain high amounts of dietary fiber. Dietary fiber is widely recognized in plants to comprise mainly the plant cell wall, complex substances of indigestible polysaccharides (e.g. cellulose, hemicellulose, oligosaccharides, pectins, gums), waxes and lignin, In addition, pulse fibers can be used to improve or modify the texture of food products through fat or water retention Important health benefits associated with dietary fiber intake include reduced risk of heart disease, diabetes, obesity, and some types of cancer (Tosh and Yada 2010)

Grain Color

Lentil grains can be of different colors. Color is a factor affecting grain quality. Seed coat color is an important visual trait that significantly affects the market value of pulse grains (Mishili et all, 2009).There are different colored lentil species in the market such as red, green, yellow and black. It is noteworthy that the color of lentil grain is an important quality parameter (Shahin and Symons, 2001). The color of lentil seeds can range from light to brown or darker brown; (Jackson et all, 2021) Red and green lentil species are the most widely consumed varieties of these edible legumes (Oduro-Yeboah et all, 2023) The color of lentils has been reported to vary from black, brown, green, orange, red or yellow depending on the variety and the composition of the cotyledons and seed coats. The cotyledon color, which can be red or yellow, is the main factor determining the color of the groats, while the color of the seeds can be black, brown, green, gray or tan depending on the color of the seed coat. (Mokrani, 2023)

Factors Important for Human Nutrition in Lentil Grain

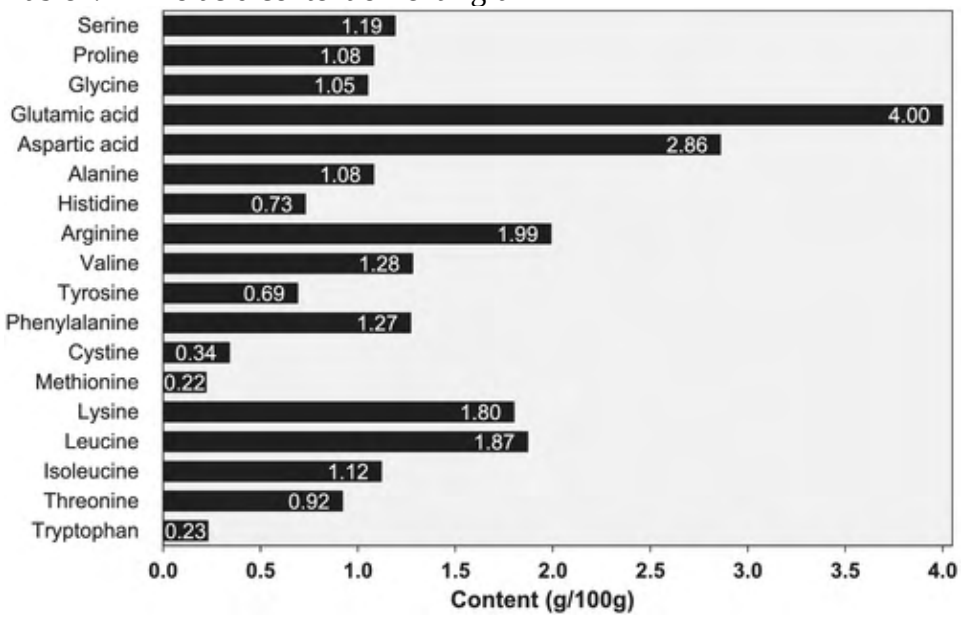
Proteins and Amino acids

Lentil proteins consist of storage proteins classified according to their solubility properties, including globulins (salt soluble), glutelins (dilute acid/base soluble) and prolamins (alcohol soluble). The protein content consists of 16% albumin, 70% globulins, 11% glutelins and 3% prolamins (Boye et al., 2010; Joehnke et al. 2021). Lentil proteins are stored in protein bodies called 'storage proteins' in cotyledon cells (Duranti and Gius, 1997). These seed proteins provide carbon (C), nitrogen (N) and sulfur (S) and account for 80% of the total protein available for plant growth and disease resistance after germination (Khazaei et al., 2019). Furthermore, lentils can be an alternative to animal and soybean proteins in food processing industries due to their broad functional properties (Khazaei et al., 2019). As functional properties of proteins in foods; solubility, water retention and fat binding capacities, foaming properties are of great importance in determining the quality of the protein (Boye et al., 2010).

Lentils are a source of high quality protein. High protein content is an important factor in terms of nutritional value.

The synthesis of sufficient protein in cells for the body to remain healthy requires a good balance of amino acids. Of the 20 amino acids needed to synthesize protein, nine are essential for adults and must be ingested through food (Bonke et al., 2020). The most abundant amino acid in lentils is glutamic acid, followed by aspartic acid, arginine, leucine and lysine, while cystine, tryptophan and methionine are at the bottom of the list according to their content. Therefore, methionine and tryptophan are the first and second limiting amino acids, respectively. Lentil proteins contain both essential and non-essential amino acids, but the sulfur-containing amino acids methionine (Met) and cysteine amino acids are less abundant (Salaria et all, 2022).

Table1.Amino acid content of lentil grain



Source: (Dhull et al.,2023)

Lentils contain all essential amino acids (table 1). When assessing grain quality, the amino acid profile of lentil grains should also be considered. Especially the content of important amino acids such as lysine and methionine is important. It is also rich in leucine, arginine, aspartic and glutamic amino acids. It contains limited amounts of sulfur-containing amino acids (methionine and cysteine) and tryptophan (Monnet et al., 2019). Due to the high content of amino acids such as lysine and arginine, lentils complement cereal proteins (Paucean et al., 2018). Amino acid amounts in lentil varieties were examined. They measured that Arginine, one of the essential amino acids, was the most abundant amino acid in most of the lentil genotypes and ranged from 6.6 to 10 g/kg. It was followed by leucine, valine, lysine, phenylalanine, threonine, histidine and isoleucine amino acids. In a study, tryptophan and methionine were found to be the limiting amino acids and ranged from 0.61 to 0.92 and 0.96 to 2.1 g/kg, respectively (Alghamdi et al., 2013).

Vitamin and Mineral Content

In studies conducted in the USA, Canada and Egypt, the mineral content of raw, dehulled and cooked grains varies. This content may vary according to the chemical composition of the grain and the type of soil in which the lentil is grown.

Table 2. Lentil mineral composition

	Ca	K	P	Mg	S	Fe	Zn	Cu	Mn	Na	I	B	Se	Mo
g/kg							mg/kg							
Raw Whole seed	0.20-1.60	5.4-14.4	0.72-6.30	0.70-2.98	1.2-2.56	54-505	181.8-330	2.0-18.0	8.4-20.0	13-1100	0	1.5	2.3-6.0	0.15-1.60
Kernel	0.47-0.88	7.80-80.62	2.86-5.22	0.91	-	40-101	31.5	8.9	14.2	25-840	-	-	0.56	-
Cooked														
Whole seed	0.16-4.0	0.2-2.0	1.15-4.68	0.5-0.90	1.0-1.2	22-24	29-33	8.0-28.0	17-8.0	60-200	-	-	-	0.16-1.82
kernel	0.18-0.84	3.92-8.11	1.86-3.17	0.3	-	39.8-370		2.5-9.0	2.5-9.0	21.1	-	-	-	-

Source: Sharma et al., 2022.

Lentils are richer in calcium than cereals (table 2). It is found in equal amounts in the husk and seed. It has the lowest calcium content among legumes. Phosphorus is accumulated in the grain. 40.5-42.9 % of the phosphorus in lentil grain is found in phytic acid. The level of phytic acid phosphorus in kabuki and grain may decrease during cooking. The sodium content is in a wide range of 13-849 mg/kg in whole grain and 25-840 mg/kg in grain.

In general, legumes are rich in B group vitamins and generally low in A, C and E group vitamins. While cooking causes a loss of vitamins in legumes, peeling the skins may increase the vitamin content (Pekşen and Artık, 2005).

Other Nutrients

Lentils also contain other nutrients such as fiber, vitamins, minerals and antioxidants. These nutrients also affect grain quality. Lentils are a nutritionally high-quality legume with high protein content, slowly digestible starch and carbohydrate with lower crude fiber content, essential minerals, vitamins and high energy value. Moreover, lentils also contain significant amounts of bioactive phytochemicals such as antioxidants and phytoestrogens (Kaale et al., 2023). Bioactive compounds found in lentils include polyphenols (flavonols, tannins, phenolic compounds), phytate, phytosterols, minerals, vitamins, oligosaccharides, resistant starch, proteins, bioactive peptides and saponins and have beneficial health effects. Scientific research explains that consumption of lentils has beneficial effects on cardiovascular diseases, diabetes, and various types of cancer (Zhang et al., 2014). Lentils have the highest total phenolic content compared to six other legumes such as green peas, chickpeas, cowpeas, yellow peas, mung beans and peanuts

CONCLUSIONS

Studies have partially revealed the nutritional content of lentil grain. Lentils are frequently used in meals because they are easy to eat and have high digestibility. It is a preferred food with low calories and high protein content.

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THE EVALUATION OF SOME DROUGHT INDICES IN SUNFLOWER HYBRIDS IN DRY CONDITIONS

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ABSTRACT

The biodynamic farming system was built in 1924 by Rudolf Steiner (philosopher) and E. Pfeiffer (agronomist) and an anthropological theory based on the concept of human-nature-universe. Biodynamic farming is familiar to organic farming. Fundamentally, both stem from opposing perspectives on the use of chemical inputs (fertilizers, pesticides, herbicides, hormones, etc.). The basic ecological principle of biodynamic agriculture is that it considers the farm as an organism and a self-sufficient entity.

Organic agriculture, home-made products, safe and traceable foods and ancient teachings (old knowledge) have come to the fore in societies that have turned inward with the effect of the Covit 19 pandemic. It was prepared to compile the teachings.

Keywords: Biodynamic Agriculture, Plant Life, Demeter

INTRODUCTION

With the existence of humanity, nutrition has taken the first place among basic needs. From the research carried out in different geographies of the world, where the first findings of people living together were made, to the ruins of Göbeklitepe in our country, to today's modern age, the existence of agriculture cannot be ignored. Production of plant and animal products, improving their quality and efficiency, preserving, processing, evaluating and marketing these products under appropriate conditions are the basic issues of today's agriculture. Proof of the negative effects of pesticides used in the past years on human health and the fact that the damage to the environment constitutes the most important input cost in production has enabled the development and dissemination of production models such as GAP, Organic Agriculture and EKUY. With the 1990s, "ORGANIC AGRICULTURE" came to the fore. Organic agriculture includes plant rotation, green manure, compost, "biological pest control" and relies on mechanical processing to ensure soil productivity; It is a method of agriculture that rejects or limits the use of synthetic fertilizers, pesticides, hormones, animal feed additives and genetically modified organisms (Anonim., 2023).

Biodynamic agriculture is; Increasing popularity with the industrial revolution in the 18th century and global warming caused people to realize the ecological degradation on the world, and accordingly, saving ecology and the world became one of the main topics of current affairs. The biodynamic farming system was built in 1924 by Rudolf Steiner (philosopher) and E. Pfeiffer (agronomist) and an anthropological theory based on the concept of human-nature-universe. Biodynamic farming is familiar to organic farming. Fundamentally, both stem from opposing perspectives on the use of chemical inputs (fertilizers, pesticides, herbicides, hormones, etc.) (Anonim., 2023a) The basic ecological principle of biodynamic agriculture is that it considers the farm as an organism and a self-sufficient entity. It is accepted that each farm has its own characteristics, that is, an individuality. The aim is to recycle everything produced on the farm land, sustainability of the soil, and the healthy continuity of the crops and sheltered animals. Farmers are also a part of this whole. As a result of the farmer acting by

taking into account the interactions in his ecosystem, the environmental, social and financial aspects of the farm are highlighted and a holistic management is implemented. The farmer produces with minimal external inputs and uses materials from his own farm whenever possible. With this feature, biodynamic agriculture is the most economical production and processing method for farmers.

Biodynamic agriculture came to life with the ancient knowledge that Rudolf Steiner, a philosopher, scientist, educator, artist and founder of the school of anthroposophy, born in Austria in the second half of the 19th century, gathered under the title 'Agricultural Lessons' in Koberwitz in 1924 and conveyed it to farmers in eight separate seminars (Anonim., 2023b).

Biodynamic agriculture is a spiritual, ecological and holistic organic living system. The aim is to heal the soil.

In biodynamic agriculture, defined as nature-friendly agriculture, celestial dynamism and agriculture are used together. In biodynamic agriculture, environmental pollution is minimized by preserving soil and water. In this agricultural method based on integrated pest management, the aim is not to eliminate the pest completely, but the basic principles are to protect soil and water resources by preserving biodiversity, collecting rainwater and ensuring that the organic matter and water retention capacity in the soil is at the desired

level. In biodynamic agriculture;

- Minimal cultivation of the soil
- Minimizing chemical entry
- Protecting biodiversity
- Ensuring that the soil is covered with products
- It is based on the basic principles of adaptation to the local environment.

In biodynamic agriculture, it is known that in plant production, as the moon grows, the ones growing upwards are decreasing while they shrink downwards, that is, the plants with rhizomes and tubercles are affected by planting, harvesting or collecting ripe fruits in these periods, and that the plants are exposed to insect attacks during this period because the amount of liquid that attracts insects increases in the plant at the new moon. . The water in the soil rises with the gravity of the moon. Therefore, capillarity in the soil also increases during the full moon. Planting is completed a few days before the full moon. It is known that logs left on the new moon (when the moon is waxing) attract insects, while logs left on the old moon (when the moon is waning) do not harm insects.

Completing work and operations according to the moon movements is a part of farming. While all biodynamic agricultural products are organic, not every organic product is a biodynamic product.

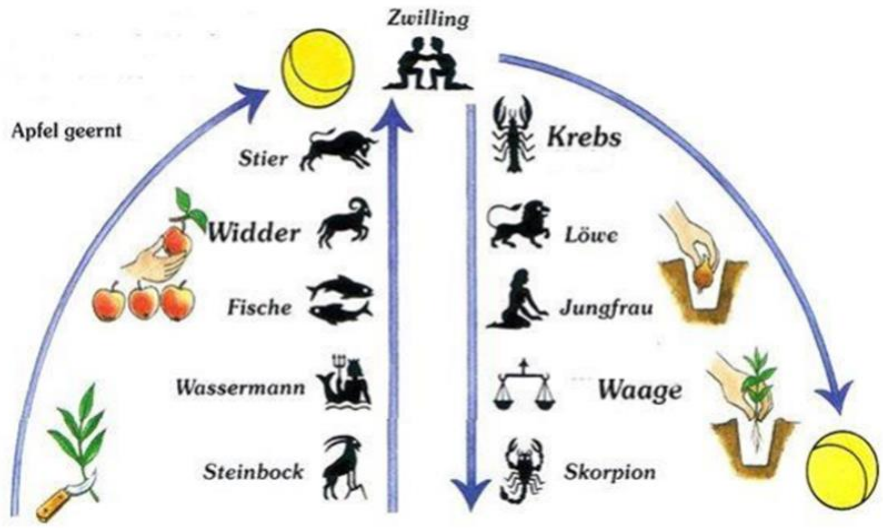

Figure 9. Effect of moon phases on agriculture

All technical, economic and legal measures taken to protect agricultural products from diseases and damages, to treat them or to minimize the damage that may arise from them are called plant protection (plant health) (Anonim 2023ı). Plant protection activities are quite comprehensive and form the basis of agricultural activities. It should not be forgotten that growing the plant healthy is much more economically and environmentally important than saving it after it becomes sick / damaged.

The distinguishing feature of biodynamic agriculture is the use of nine biodynamic preparations defined by Steiner to improve soil quality and stimulate plant life. They consist of mineral, plant or animal manure extracts, usually fermented and administered in small proportions to compost, fertilizers, soil or directly to plants, after dilution and mixing procedures called dynamisation. Original biodynamic (BD) preparations are identified by numbers to distinguish each plant. BD 500 preparation (horn manure), also known as land preparation (spray preparation), is made from cow manure (obtained by fermenting cow manure by burying it in the ground for six months during autumn and winter) and is sprayed into the soil to stimulate solid root development and humus formation. . BD 501 preparation (horn silica) is obtained by filling powdered quartz into cow horn and burying it in the ground for six months during spring and summer. It is applied as a foliar spray to stimulate growth, regulate and enhance flavour. The next six preparations, BD 502-507, are used in preparing Compost. Finally, there is the BD preparation 508, which is prepared from the silica-rich horsetail plant (Equisetum arvense) and used as a foliar spray to suppress fungal diseases in plants. (1).

Medicinal plants used in biodynamic agriculture: Biodynamic compost, together with biodynamic preparations, are the cornerstones of this practice. It is an effective way to transform animal manure and organic waste on the farm, keep the nitrogen level in balance, increase humus production and ensure the healthy sustainability of the soil. BD Compost and its preparations prepared with BD preparations are listed below:

- No. 502 Yarrow flowers (Achillea millefolium)
- No. 503 Chamomile flower (Chamomilla officinalis)
- No. 504 Nettle (Urtica dioca)
- No. 505 Oak bark (Quercus robur)
- No. 506 Dandelion flowers (Taraxacum offtcinale)
- No. 507 Valerian (Valeriana officinalis)

Biodynamic preparations are thought to help to improve and strengthen life on the farm (etheric), as well as to moderate and regulate biological processes. Preparations are used in homeopathic amounts, that is, they produce an effect in extremely dilute amounts. (Anonim., 2023c).

Medicinal plants used in biodynamic agriculture:
1- Yarrow flowers (Achillea millefolium):
2- Daisy flower (Chamomilla officinalis)
3- Nettle (in full bloom on the whole plant) (Urtica dioca)
4- Oak bark (Quercus robur)
5- Dandelion flowers (Taraxacum offtcinale)
6- Valerian (Valeriana officinalis)

Characteristics of medicinal plants used in biodynamic agriculture:
Yarrow flowers (Achillea millefolium): Yarrow is also known as the Kandil flower and the thousand-leaf herb, which grows spontaneously in our country. It has been known and used since ancient times for its different therapeutic properties. According to legend, in ancient Greece, Achilles used yarrow to heal the wounds of his friends during the siege of Troy (Anonim., 2021). Achillea is a mythological plant, belonging to the Asteraceae family, growing especially in the northern hemisphere, with more than 100 species in the world and a total of 52 taxa in our country, 30 of which are endemic. is represented. Achillea species are used in folk medicine to reduce fever, relieve colds, relieve digestive complaints and heal wounds (Anonim., 2023l). As a medicinal plant, yarrow has a plant height of 25-95 cm, depending on the ecological conditions in which it is found. In general, yarrow can show its growth and development ability up to an average altitude of 2500 meters (Anonim 2023m). In biodynamic agriculture, the preparation prepared in the deer bladder increases the adaptation of the plant used to its location and regulates the potassium metabolism, nitrogen, carbon, sulfur and potassium processes in the plant.

Figure1. Yarrow and Deer Bladder (Anonim 2023r).

Chamomile flower (Chamomilla officinalis) Matricaria chamomilla (Medical Chamomile, May daisy) is from the Asteraceae (Compositae) family, and most of the plants of this family are herbaceous, a few of them are shrubs or trees. The family is the richest family of flowering plants, with nearly 1000 genera and nearly 20,000 species. (Anonim.,2023d). Chamomile has been used in herbal medicine for thousands of years and is known in ancient Egypt, Greece and Rome. Anglo-Saxons believe that this plant is one of the 9 sacred plants given to humans by God. Chamomile medicine is included in the pharmacopoeia of 26 countries. As a medicine for flatulence, colic, hysteria and intermittent fever.M. The flowers of chamomilla contain between 0.2% and 1.9% blue essential oil. Chamomile is used mainly as an anti-inflammatory and antiseptic, but also as an antispasmodic and mild diaphoretic. True daisy is an annual plant with thin spindle-shaped roots that penetrate only straight into the soil. The flower heads with long

and narrow leaves are individually located, 10-30 mm in diameter, stalked and heterogamous. The 5-toothed golden-yellow tubular flowers are 1.5-2.5 mm long and always end in a glandular tube. The chamber is 6–8 mm wide, initially flat, later conical, cone-shaped, hollow (the latter being a very important distinguishing feature of Matricaria) and paleaeless (Anonim., 2023n). In biodynamic agriculture, this sulfur-containing preparation prepared in the cow intestine reacts with calcium and regulates the health of the soil.

Nettle (in full flower on the whole plant) (Urtica dioca) is a large group within the Urticales order of the Nettle family (Urticaceae), widespread in tropical and subtropical areas of both hemispheres. He listed 48 genera and 1050 species in the Nettle family. Cronquist (1981) described the nettle family as having features such as stinging hairs, individual seeds, most of them lacking milky pulp, simple leaves and showing foreign pollination (Anonim., 2023e).

The rhizomes of the nettle plant spread more than 150 cm underground, and the fringe roots develop by exiting along the underground rhizomes. It is covered with burning pointed soft hairs on a thin and branchless stem that can grow up to 90-300 cm high. Since the flowers are separate sexual, male and female clusters are found on the same plant but in different leaf axils, or there may be completely male or female flowers on different plants. Its state is longer than the female flower state. The plant has many uses in medicine, as a fiber, in cosmetics, as a dye, pollen source, animal food, energy plant, insecticide, herbicide and fertilizer (Ayan et al., 2020). In biodynamic agriculture, it is used to improve the soil structure.

Oak bark (Quercus robur) has a wide distribution in Europe, Turkey and the Caucasus. Its general distribution in portions is in Thrace, the Black Sea Region and Eastern Anatolia. It is a multidimensional forest tree that can live 400-500 years, sometimes 1,000 years. It is found in small groups or singly in forests, on foothills of mountains, on plains with high water table, and in streams. It can spread up to altitudes of 100-2,300 meters. It is quietly sensitive to mild autumn and winter frosts. It is resistant to cold climate conditions. It thrives more in a temperate and humid climate (Anonim., 2023f). Oak is a large deciduous tree. The diameter of its body can reach up to 4-12 meters. The oak is 7–14 centimeters (2.8–5.5 in) tall, with lobes and very short stems. It flowers in mid-spring and its acorns ripen in mid-autumn. Mature trees are flood resistant. It is long lasting. Ensuring the transformation of trees with large branches like a wide crown. Although they can live for several centuries in nature, the longest-lived ones are those pruned by humans. (19)In biodynamic agriculture, animal

(such as cows, goats, horses, sheep) The preparation prepared in its pages promotes calcium processes and protects fungi from infecting and pests. Configure exactly that data wherever the plant is likely to be

Dandelion (Taraxacum officinale) is a common plant species from the Asteraceae family. Even though its flowers are yellow and its leaves are green, the name of the plant is called "dandelion". This plant, known as "katagan" by the Egyptian and Kipchak Turks and "saçratku" by the Chagatai Turks, has survived to this day as "dandelion". Chicory is a word of Arabic origin. It is claimed that the eye disease it is used to treat is caused by trachoma. Although it is known as "acıgıcı", "acıgünek", "güneyik", "çıtlık", "cırtlık" and "arslandişi" in Anatolia, its most commonly used name is "radika". Dandelion grows in meadows and roadsides all over the field in April and May. is a perennial herbaceous plant with yellow flowers. The long taproot, filled with a bitter milk called "kengel", bears deeply toothed leaves gathered at the base in rosettes and flower stalks that are longer than the leaves (Anonim., 2023g). In biodynamic agriculture, the preparation prepared from cow shirt fat (Cow intestinal hanger) promotes calcium processes and protects the fungus from diseases and pests. It strengthens the exact area of the plant where there is a possibility of disease.

Catnip:(Valeriana officinalis,) is a perennial herbaceous plant with fragrant leaves, stems, flowers and roots. The plant, which grows up to 30-150 cm, has deeply lobed basal leaves and white to pale pink flowers in a paniculate flower state. Its roots have a strong scent that cats love. The used parts, such as the rhizome, root and stolon, are harvested in September and must be carefully dried at temperatures below 40◦C. The plants, which usually grow naturally on roadsides and fields, are native to Europe and Western Asia. The plant grows naturally in Europe, Asia, Northeast America and Turkey. Valeriana genus is represented by 12 species including this species in our country. The genus name comes from the medieval name derived from the Latin word “valere” meaning “to be wholesome,” possibly in reference to the plant's medicinal uses in nervousness and hysteria (Anonim., 2023h). In biodynamic treatment, it increases the processes of phosphorus and is used to regulate the relationship between temperature, soil, fertilizer and plants.

Fıgure 3. (Anonim 2023s).

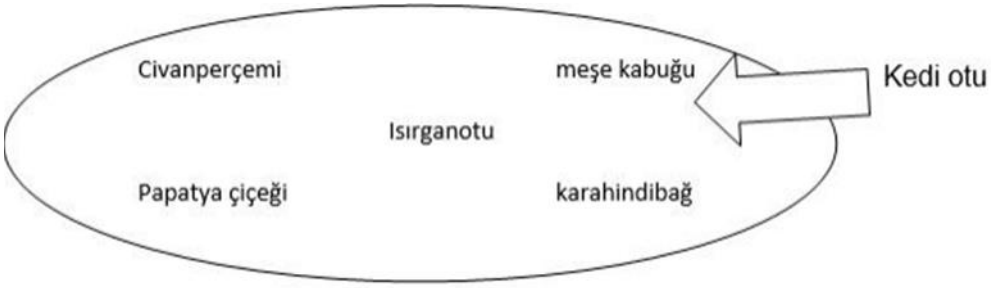

Fıgure 4. (Anonim 2023t).

Biodynamic compost, together with biodynamic preparations, are the cornerstones of this practice. It is an effective way to transform animal manure and organic waste on the farm, keep the nitrogen level in balance, increase humus production and ensure the healthy sustainability of the soil. Biodynamic preparations are thought to help to improve and strengthen life on the

farm (etheric), as well as to moderate and regulate biological processes. Preparations are used in homeopathic amounts, meaning that they produce an effect in extremely dilute amounts. For example, just one teaspoon of each preparation is added to a seven to ten-ton compost pile (Anonim 2023k).

Table 1. Table 1. Preparation and usage of MAP used in Biodynamic Agriculture (Anonim., 2023j).

PLANT OF PREPARATIONS (502-507)			
of the preparation Its number	name	what what it is prepared in or filled with	effect
502P	Yarrow flowers (Achillea millefolium)	Deer in his bladder	It increases the adaptation of the plant to its location and regulates potassium metabolism, nitrogen, carbon, sulfur and potassium processes in the plant.
503P	Daisy blossom (Chamomilla officinalis)	Cow in your gut	This prp. containing sulfur reacts with calcium and regulates the health of the soil.
504P	Urtica (Urtica dioca)		Improves the structure of the soil
505P	Oak peel (Quercus robur)	Animal (such as cow, goat, horse, sheep) to the skull	It promotes calcium processes and protects the fungus from diseases and pests. It strengthens the exact area of the plant where there is a possibility of disease.
506P	Dandelion flowers (Taraxacum offtcinale)	Cow shirt oil (Cow intestine hanger)	It promotes potassium and silicate processes, increases the plant's adaptation to external conditions and its ability to absorb nutrients.
507P	Valerian (Valeriana officinalis)	–	It increases phosphorus processes and regulates the relationship between temperature, soil, fertilizer and plants.

CONCLUSION and RECOMMENDATIONS

As a result, in response to the increasing world population, in order to increase plant and animal production at the same pace, to keep it at a certain standard and efficiency, to ensure sustainability in agriculture by causing the least harm to the environment, soil, water and other living things, people have engaged in agricultural activities in different ways and methods from the Industrial Revolution to the present day.

With Biodynamic Agriculture;

1. By harvesting rain water, existing water resources will be protected and water will not be wasted. Noting that our water resources are rapidly decreasing and we are among the water poor countries, it is important to protect water resources in Biodynamic agriculture.
2. Considering the negative effects of the pesticides used on soil, water, air and other living things, the global and national costs are very high.
3. BD 502 yarrow contains potassium and sulfur BD 503 medicinal chamomile stabilizes nitrogen in biodynamic compost piles. It activates the soil and revitalizes the plant. BD 504 provides the nitrogen, iron, potassium and calcium that the plant needs and revitalizes the soil. BD 505 is used against oak bark fungal diseases. BD 506 strengthens the relationship between dandelion flowers silicylic acid and potassium. Silica attracts cosmic power to the soil. BD 507 valerian flowers stimulate photosynthesis. It increases flowering and fruit set. BD 508 horsetail prevents fungal diseases (Pakkener.,2023)
4. In the application time and repetition of the above-mentioned compost and preparations, the plant used should be used in accordance with the "Biodynamic Agricultural Calendar", taking into account the element of water, air, fire or earth, and the state of the planets and the moon.
5. Biodynamic agriculture is ahead of organic agriculture in ensuring sustainability in agriculture by protecting soil, water, the environment and other living things.
6. They often decide when to plant and harvest crops, when to prune fruit trees, when insects will infest the crops, when to trim the wool of animals, and when to make bulgur, tomato paste, pickles, cheese and bread by looking at the moon in the sky at night.
7. Considering the current state of the agricultural areas used by the Egyptians, who practiced irrigated agriculture by taking advantage of the tides in the Nile River hundreds of years ago, the reuse of ancient teachings, the transfer of the wisdom of the ancestors from generation to generation, BIODYNAMIC agriculture, which is an environmentally friendly agriculture model compatible with celestial and earth movements, should be implemented in suitable locations. and should be an example to the new generation and the world by opening up to ecotourism.
8. The basis of the biodynamic philosophy is based on the DEMETER certificate. Demeter takes its name from Demeter, the goddess of agriculture, fertility, seasons and maternal love in Greek mythology. It symbolizes crops, especially wheat. Biodynamic agriculture;

• The farmer must first have his business certified by a control and certification body (KSK) in accordance with the relevant regulations of the European Union (EU): 834/2007 and 889/2008.

• The farmer starts bio-dynamic agriculture by working with a consultant accredited for Turkey by “Demeter”.

• The transition of the producer certified according to the EU to biological dynamic agriculture is made by the consultant according to the "Demeter" regulation.

• The agricultural enterprise is controlled by a KSK approved by Demeter and its report is sent to Demeter. Certification is done by Demeter based on the report.

The yield trials were conducted in Edirne and Tekirdag location in 2017 to determine yield performances of candidate sunflower hybrids. There were 23 hybrids including 4 controls from commercial hybrids (ITALICA, SY GIBRALTAR, P 64 LL 62, LG 5582) in the market. The experimental design was a Randomized Complete Block Design with four replicates. The four rows plots were 7,50-m long with the 70 x 35 cm plant spacing. Total plot area at planting was 7,5*2,8 as 21 m^2. The middle two rows were harvested and the border rows were discarded, and plot size was 9.66 m^2 at harvest. The compose fertilizers (20-20-0, Zn) were applied 200 kg/ha dose at planting. Statistical analysis was performed with JMP statistical program.

Tekirdag location was conducted in Beyazkoy village fields, Saray County and the trials were planted by hand in 15 April 2017. Emergence date of sunflower plants was in 22 April 2017 and left only one plant each as mentioned plant density above. The trials were harvested by hand in 25 August 2017 as middle two rows except one plant at the beginning of the middle rows. Edirne location was conducted in Sarayakpinar village fields and the trials were planted by hand in 28 April 2017. Emergence date of sunflower plants was in 5 May 2017 and the trials were harvested by hand in 5 September 2017. The plant height and head diameter of hybrids were measured from 3 plants at mid rows of the plots in each replication at PM stage. Oil content of the hybrids were determined utilizing Nuclear Magnetic Resonance (NMR) analysis.

Some sunflower hybrids both from classical and IMI types were planted in the pots to measure responses to drought conditions with measuring of dry and wet root weight as well as total chlorophyll content as the most known drought indices in sunflower drought tests (Figure 1, 2 and 3). The ratio of Chlorophyll content of sunflower hybrids was recorded by Portable Florescence Device (HandyPEA, Hansatech Ltd.) at R5-1 vegetative stages (Figure 4). Furthermore, plant height, plant number per area, leaf number per plant, leaf area, anthocyanin existence, head inclination, hairiness at stem, total chlorophyll content, leaf width and leaf length were measured at the yield trials conducted in the field to determine their responses to drought stress.

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FEASIBILITY OF SAFFRON (*Crocus sativus* L.) CULTIVATION IN AEGEAN REGION, TÜRKİYE

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ABSTRACT

In the past few years, an increasing interest in saffron (*Crocus sativus* L.) cultivation has been observed due to its high economic value and low requirements to agronomical inputs. Based on this, a study was done to investigate the climatic parameters in Aegean region (Türkiye) for the development of saffron cultivation using spatial analysis of geographic information system (GIS). Some climatic parameters including annual rainfall, annual average temperature, minimum temperature, maximum temperature, relative humidity and number of frost days were chosen for saffron-land suitability analysis in Aegean region. This climatic data was obtained from metrological stations located within the study area. The total data was averaged from 1991 to 2020. In this research, the climatic requirements of saffron were determined and classified based on scientific sources and the opinions of local experts. The final land suitability map indicated that the most suitable areas for growing saffron were located in the south, central and western Aegean region. Also, in the viewpoint of the number of frost days, the north of this region was located in non-suitable (NS) zone especially Kütahya. The results highlighted that in the areas with limited saffron production potential across the region, the main limiting features were number of frost days and minimum temperature. In general, development of saffron cultivation is possible in the southern part of Aegean region especially İzmir and Denizli. In addition, Aydın, Manisa, Uşak and Muğla provinces were classified as moderately suitable (S2) zone. These results can be useful as a planning support tool for decision makers and farmers to determine feasibility of saffron cultivation in Aegean region.

Keywords: Aegean, Climatic parameters, GIS, Saffron

INTRODUCTION

Saffron (*Crocus sativus* L.) is the most expensive plant in the world. Although other types of saffron are also used as ornamental plants due to having beautiful flowers, but its cultivated species has a special place economically. The distinctive characteristics of saffron is the appearance of flowers before the vegetative organs, the beginning of vegetative growth in the autumn, end of growth in the spring, the lack of fertile seed production, and harvesting flowers in the morning (Kafi, 2001).

Saffron (*C. sativus* L.) is an economically important species of the Crocus genus within the Iridaceae family. It is known that there are approximately 85 species of crocus (Crocus) in the world. Approximately 70 species of these grow naturally in Asia Minor and Mediterranean countries (Vurdu, 2004; Yıldırım et al., 2016; Yıldırım and Hatipoğlu, 2020). It is stated that a total of 72 taxa, including 36 species and 36 subspecies, grow naturally in Turkey, and 19 of

these species and 21 of the subspecies, a total of 40 taxa, are endemic in Turkey (Yıldırım et al., 2016; 2017; Yıldırım and Hatipoğlu, 2020).

Climatic parameters and their effects on plants are one of the most important effective factors in increasing yield and agricultural production. With agro-climatological evaluation, it is possible to determine the current facilities of different regions in terms of growing different plants and make maximum use of these facilities. In this regard, Rashid Sorkhabadi et al., (2014) determined the suitable area for saffron cultivation based on climatic and soil variables using the hierarchical analysis method in Torbet-Haidarieh city (east of Iran). The climatic capability of saffron cultivation in Kermanshah (west of Iran) was investigated by Mojarad and Ghaforizadeh (2014). The results of the research showed that nearly 30.48% of region had the moderate capacity for saffron cultivation. In other study, Maleki et al. (2017) developed a land use suitability model for saffron cultivation by multi-criteria evaluation and spatial analysis in northeast of Iran (Azadshahr township). The results demonstrated that climate and soil conditions play major role in potential saffron cultivation.

In the last few years, an increasing interest in saffron expansion has been observed due to the high economical value and low requirements to agronomical inputs. The present study was therefore carried out with the objective land suitability analysis for feasibility saffron cropping using geographical information system (GIS), and evaluation of climatic variables in Aegean region, Türkiye.

MATERIAL AND METHOD

Study area

The study carried out in Aegean region, Türkiye, during 2023. The Aegean region is one of the 7 geographical regions of Türkiye (Figure 1). This region covers approximately 11% of Türkiye's territory, with a surface area of around 85,000 km^2. It is neighbor on the Marmara Region in the north, Central Anatolia Region in the east, and the Mediterranean Region in the southeast and surrounded by the Aegean Sea in the west. The provinces in the Aegean Region are Kütahya, Uşak, Aydın, Manisa, Denizli, Muğla, İzmir, and Afyonkarahisar. The climate type in the Aegean region is the Mediterranean climate. Summers are hot and dry in the region, while winters are rainy and warm. The Mediterranean climate is more common on the coast than inland. Cold weather is seen in the northern parts of the region. January is the coldest month, and July is the hottest. The amount of precipitation can vary between 500 and 999 mm (Anonymous, 2021).

Figure 1. Location of the study area in Aegean region, Türkiye

Climatic data

In this study, some climatic parameters include annual rainfall, annual average temperature, minimum temperature, maximum temperature, relative humidity and number of frost days *are chosen for saffron-land suitability analysis and thematic maps are developed for each of the parameters.* These climatic data are obtained from 8 meteorological stations located within the study region. The data are averaged from 1991 to 2022. In this research, the spatial distribution of climatic variables is evaluated using interpolation methods.

Land suitability analysis

In GIS environment, all the spatial data convert into raster layers and georeferred to UTM coordinate system. In order to assess the land suitability of Aegean region, are used to match the climatic requirements of saffron and the land characteristics. Schematic diagram of land suitability is shown in Figure 2. The first step in delineating suitable areas is to identify the relevant climatic variables. In this research, the climatic requirements are identify from scientific resources and local expert's opinion then classified (Table 1) and thematic maps are provided by ArcGIS 10.3 software. Then land suitability model carried out based on matching between land qualities/characteristics and crop requirements by weighted overlay technique (WOT) in GIS media**.** In final, the land suitability map for this crop is generated in 3 classes including: suitable (S1), moderately suitable (S2), and non-suitable (NS). Thus, S1 class represents that the land unit is suitable to saffron production with no limitations, S2 class represents that the land unit is moderately suitable with some limitations; and non-suitable(NS) land was assumed to have limitations (Zhang et al., 2015; Nasrollahi et al., 2017).

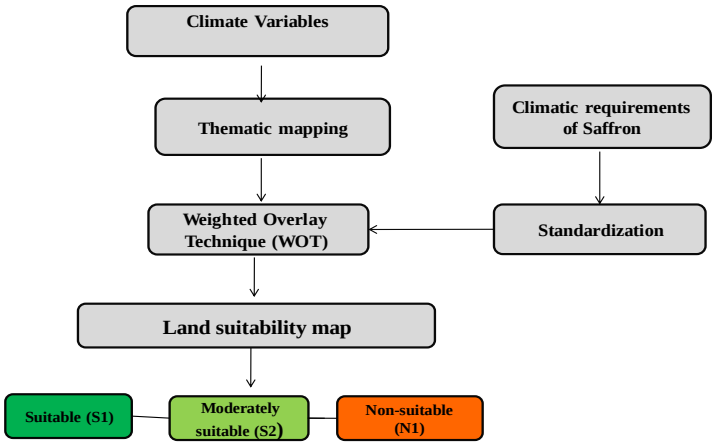

Figure 2. Schematic diagram of land use suitability for saffron in Aegean region, Türkiye**.**

Table 1. Criteria for delineating land suitability of saffron

Climatic factors	**Suitable (S1)**	**Moderately suitable (S2)**	**Non-suitable (NS)**
Annual rainfall (mm)	300-400	180-300 >400	<180
Rainfall of reproductive period (mm)	17-20	17-25	>25
Rainfall of growth period (mm)	>58	37-58	<37
Annual average temperature (°c)	>14.5	9.5-14.5	>9.5
Maximum temperature of reproductive period (°c)	>23	14.5-23	<14.5
Maximum temperature of growth period (°c)	23	15-23	<15
Minimum temperature of reproductive period (°c)	>9	5-9	<5
Minimum temperature of growth period (°c)	>(-14)	(-14)-(-22)	<(-22)
Number of frost days	<20	20-40	>40
Relative humidity (%)	40-50	50-70	>70

RESULTS AND DISCUSSION

The final land suitability map indicated that the most suitable area for growing saffron were located in the south, central and western of Aegean region (Figure 3). Also, in the viewpoint of the number of frost days, the north of this region was located in non-suitable (NS) zone particularly Kütahya. The low number of frost days especially during flowering, is an advantage for growing saffron (Arsalani et al, 2015). Basically, the occurrence of autumn frosts during the flowering time of saffron has a very harmful effect on crop (Nokandi, 1999). Mojarad and Ghaforizadeh (2014) concluded that the average number of frost days in the reproductive growth period for saffron to be 16.5 days or less. Also, our results highlighted that the main limiting features in non-suitable class were number of frost days, minimum temperature, high relative humidity, and high annual rainfall.

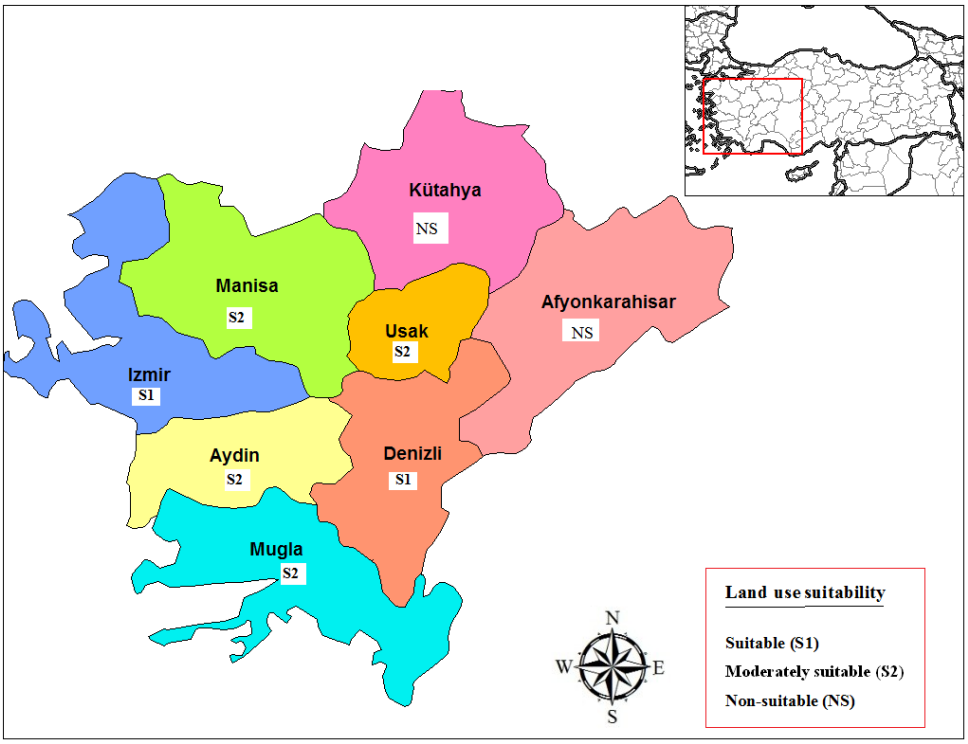

Figure 3. Land suitability map for saffron cultivation in Aegean region Türkiye.

Results showed that development of saffron cultivation is possible (S1 class) in the south part of Aegean region, especially Izmir and Denizli (Figure 3). In addition, Aydın, Manisa, Uşak and Muğla provinces were classified in moderately suitable (S2) zone. Maleki et al. (2017) demonstrate that climate and soil conditions play major role in potential saffron expansion in northeast of Iran (Azadshahr). Their results highlighted that the main limiting features in these region were high rainfall in reproductive stage of saffron (25-30 mm), slope 12% <, high relative humidity, low annual rainfall and high elevation in north (2700 m <). In general, these results can be useful for decision markers and farmers to determine feasibility of saffron cultivation in Aegean region, as a planning support tool.

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HEAVY METAL RESISTANCE OF *Staphylococcus aureus* ISOLATES FROM SEAWATER FISH

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ABSTRACT

Staphylococcus aureus is an important foodborne pathogen present in the aquatic environment. Seafoods, including fish and fish products, are frequently contaminated with this pathogen due to its high tolerance to salt stress. Heavy metals are widespread environmental pollutants. Heavy metal pollution in seawater fish has become a major global concern. Therefore, resistance to various heavy metals was investigated in the *S. aureus* isolates of seawater fish origin in this study. The heavy metals used in the present research included mercury ($HgCl_2$), copper ($CuCl_2$), zinc ($ZnCl_2$), lead (Pb $(NO_3)_2$), chromium (Cr $(NO_3)_2$ $9H_2O$), and cadmium (Cd $(NO_3)_2$ $4H_2O$). The minimum inhibitory concentration (MIC) of the tested heavy metals against the isolates was determined quantitatively using a broth microdilution test. Among the seawater fish isolates, the highest resistances to copper (Cu) (91.7%) at a MIC value of 1600 µg/ mL, chromium (Cr) (58.3%) at a MIC value of 3200 µg/ mL were found, followed by mercury (Hg) (25%) at a MIC value of 12.5 µg/ mL. However, none of the isolates were resistant to lead (Pb), cadmium (Cd), and zinc (Zn). This study documented the presence of resistance to heavy metals in some *S. aureus* isolates from seawater fish. As a result, it is important to monitor heavy metal resistance, which poses a significant risk to ecosystems and human health.

Keywords: *Staphylococcus aureus*, heavy metal resistance, seawater fish, minimum inhibitory concentration (MIC)

INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium that is the most common pathogen in the genus *Staphylococcus*. It is normally a commensal member of the human microbiota but can also become an opportunistic pathogen. It is one of the main pathogens and causes various diseases, including skin infections, respiratory infections, soft tissue infections, wound infections, urinary tract infections, osteomyelitis, septicemia, endocarditis, and food poisoning (Bhunia, 2008; Götz et al., 2006). It can grow in the presence of high salt concentrations, such as 10% NaCl, and at temperatures ranging from 18 to 40 °C (Schleifer and Bell, 2009). *S. aureus* has been isolated from a wide range of foods, including meats, fish, shellfish, poultry and egg products, milk and dairy products, and natural environments such as sea water, fresh water, soil, and plant surfaces (Bhunia, 2008; Schleifer and Bell, 2009).

Fish as a food is considered a nutritionally valuable part of the human diet due to its excellent protein source, vitamins (vitamin A, vitamin B2, vitamin B6), omega-3 fatty acids, minerals (calcium, potassium, iron, and other minerals), carbohydrates, and other nutrients. It has health benefits, especially for cardiovascular disease, high blood pressure, age-related vision loss, and a lower risk of cancer of the prostate and dementia (Abraha et al., 2018).

Bacteria, enzymes, and oxygen are the primary causes of a variety of physiological and microbiological deteriorations in fish and seafood products. *S. aureus* does not belong to the

natural flora of fish or related marine products. These bacteria, however, can be isolated from fish (Bhunia, 2008; Abraha et al., 2018). Seafood contamination can occur at any point along the chain, from production or harvesting to processing and transportation, due to several factors, such as improper handling and processing or storage of food, inadequate cooking temperatures, poor hygiene, and cross-contamination by workers (Bhunia, 2008; Kukulowicz, 2021). Many studies have reported contamination of fish and fish products with foodborne pathogens, including *S. aureus* (Vazquez-Sanchez et al., 2012, Arslan and Özdemir, 2017; Kukulowicz, 2021; Külahcı and Gündoğan 2021, Mumbo et al., 2023).

Foods can act as vehicles of transmission for pathogenic bacteria, such as antimicrobial resistant *S. aureus* to humans (Bhunia, 2008). Previous studies indicated that *S. aureus* isolated from fish and fishery products could be resistant to various antimicrobial agents (Vazquez-Sanchez et al., 2012; Sergelidis et al., 2014; Arslan and Özdemir, 2017; Mumbo et al., 2023). Fish is considered a potential source for the emergence and spread of antimicrobial-resistant pathogens, posing a threat to human health and safety (Kukulowicz, 2021). Antimicrobial resistance is affected not just by the presence of antimicrobials; it has also been demonstrated that environmental factors, such as heavy metals, can promote the development of antimicrobial-resistant bacteria (Seiler and Berendonk, 2012; Yazdankhah et al., 2018). Particularly, heavy metals can increase resistance to antimicrobials through cross-resistance or co-resistance (Vats et al., 2022; Anedda et al., 2023). The link between antimicrobial resistance and heavy metals has been reported in many studies (Seiler and Berendonk, 2012; He et al., 2017; Hu et al., 2017; Duan et al., 2019; Hao et al., 2021).

Heavy metals naturally occur in the environment. Heavy metal contamination is widespread in aquaculture as well as agriculture. Furthermore, heavy metals are used as nutritional additives in animal and fish feed to enhance animal health and growth. For instance, higher concentrations of copper (Cu) and zinc (Zn) are normally used in animal feed for the prevention of various bacterial infections, such as diarrheal illnesses, and as an alternative to on-feed antimicrobials for promoting growth (Yazdankhah et al., 2018; Vats et al., 2022; Anedda et al., 2023).

Heavy metals are among the most dangerous environmental pollutants of anthropogenic origin due to their toxic effects, persistence in the environment and bioaccumulative nature (Anedda et al., 2023). Heavy metal contamination of fish and seafood leads to serious problems to human health and ecological integrity. Therefore, resistance to various toxic heavy metals, including mercury (Hg), copper (Cu), cadmium (Cd), lead (Pb), zinc (Zn), and chromium (Cr) was investigated in the *S. aureus* isolates of seawater fish origin in this study.

MATERIALS AND METHODS

Bacterial isolates

Twelve *S. aureus* isolates from seawater fish samples were examined in the current study. Of them, 11 were gilthead sea bream (*Sparus aurata*) and 1 was European sea bass (*Dicentrarchus labrax*). Seawater fish samples were purchased from several public bazaars and supermarkets in Bolu, Turkey's northwest region. The biochemical tests and PCR for the thermonuclease gene (*nucA*) and species-specific fragment (Sa442) were previously used to identify the *S. aureus* isolates (Brakstad et al., 1992; Martineau et al., 1998; Götz et al., 2006; Becker and von Eiff, 2011). All *S. aureus* isolates from seawater fish were grown overnight at 37ºC in Brain Heart Infusion broth (BHI) (Merck, Germany).

Determination of MIC by broth microdilution

The heavy metal resistance of *S. aureus* isolates from seawater fish samples was investigated using six heavy metals, including chromium (Cr $(NO_3)_2$), copper ($CuCl_2$), cadmium (Cd $(NO_3)_2$), mercury ($HgCl_2$), lead (Pb $(NO_3)_2$), and zinc ($ZnCl_2$). All heavy metals were purchased from Sigma-Aldrich (Sinopharm Chemical Reagent Co., Shanghai, China). The minimum inhibitory concentrations (MICs) of heavy metals against the isolates were quantified in 96-well microplates using the broth microdilution method (CLSI, 2012; He et al., 2016; Dahanayake et al., 2019). Heavy metal concentrations ranged from 3200 to 62.5 µg/mL for Cr, Cu, Cd, Pb, and Zn, whereas Hg concentration ranged from 400 to 0.78 µg/mL. MICs were determined as the lowest concentration of heavy metal that completely inhibited the growth of the organism after 18-20 hours of incubation at 37 ºC. The tests were carried out in triplicates. *Escherichia coli* K-12 was used as a quality control strain in heavy metal resistance test (Dahanayake et al., 2019).

RESULTS AND DISCUSSION

Resistance of the *S. aureus* isolates seawater fish to toxic heavy metals, such as mercury (Hg), copper (Cu), lead (Pb), chromium (Cr), zinc (Zn), and cadmium (Cd) was investigated in the current study. As presented in Table 1, a maximum MIC of 3200 µg/mL for Cr, 1600 µg/mL for Cu, and 12.5 µg/mL for Hg were detected as compared to the *E. coli* K-12 control strain (Dahanayake et al., 2019).

Table 1. Heavy metal resistance of the *S. aureus* isolates from seawater fish

Seawater isolates	MICs for heavy metals in µg/mL					
	Hg	Cd	Pb	Cu	Cr	Zn
S1	12.5	100	200	800	1600	200
S2	12.5	400	400	1600	1600	200
S3	6.25	100	400	1600	1600	800
S4	6.25	400	400	1600	3200	800
S5	6.25	200	200	1600	3200	200
S6	6.25	100	800	1600	3200	800
S7	12.5	100	400	1600	3200	800
S8	1.56	200	200	1600	3200	50
S9	1.56	100	200	1600	3200	50
S10	1.56	100	200	1600	3200	800
S11	1.56	50	400	1600	1600	800
S12	6.25	100	400	1600	1600	800
Escherichia coli K-12 strain	6.25	400	800	800	1600	800

MIC, Minimum inhibitory concentration

Among the seawater fish isolates, the highest resistances to copper (Cu) (91.7%) at a MIC value of 3200 µg/ mL were found, followed by chromium (Cr) (58.3%) at a MIC value of 3200

µg/ mL and mercury (Hg) (25%) at a MIC value of 12.5 µg/ mL. However, none of the isolates were resistant to lead (Pb), cadmium (Cd), and zinc (Zn) (Table 1 and Figure 1).

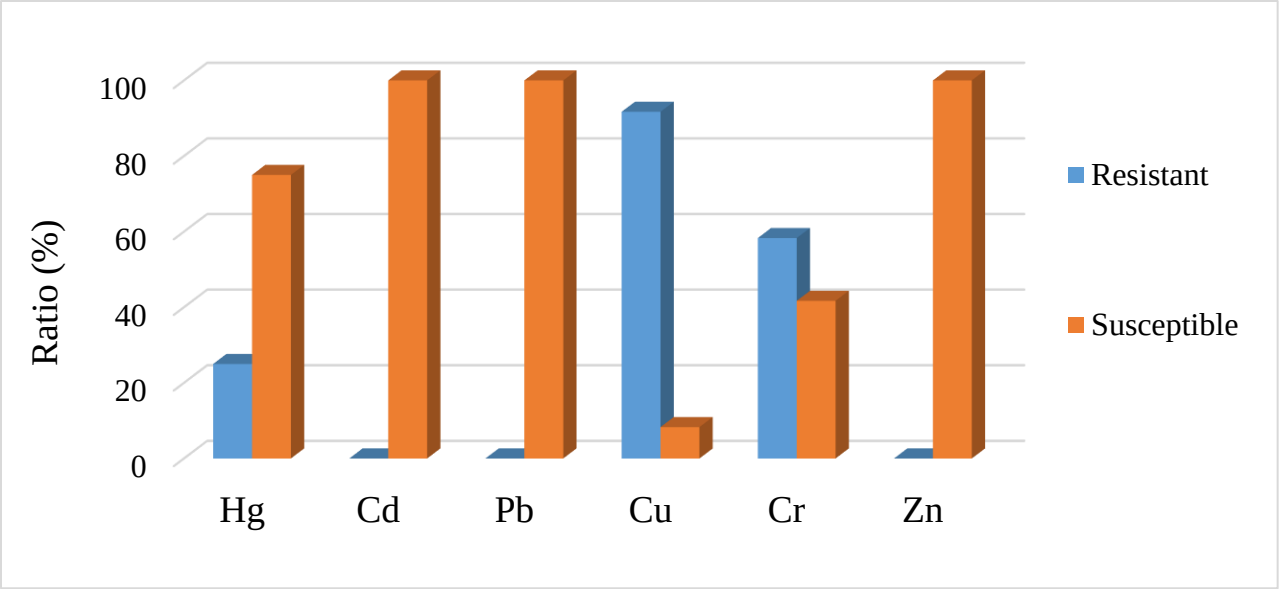

Figure 1. Prevalence of resistance to heavy metals in the *S. aureus* isolates from seawater fish

Heavy metal resistance has been documented in several studies related to fresh shrimp (He et al., 2016), crustaceans and shellfish (Hu and Chen, 2016), seafood (Dahanayake et al., 2019), and Nile tilapia fish (Gufe et al., 2022). He et al. (2016) reported the majority of *Vibrio parahaemolyticus* isolated from fresh shrimps in Shanghai fish markets displayed resistance to Cu (93.3%), similar to our results. High resistances to Cu (89.4%), Pb (80.3%), and Cd (80.3%) were also found in *V. parahaemolyticus* isolated from crustaceans and shellfish (Hu and Chen, 2016). In contrast to our findings, Gufe et al. (2022) were reported that lead (Pb) resistance ranging from 30.8-69.2% among the various bacterial species including *S. aureus* isolated from fish samples in anthropogenically polluted Lake Chivero, Zimbabwe.

In this study, *S. aureus* isolates originated from seawater fish indicated five different heavy metal resistance profiles (Figure 2). Among the detected resistance phenotypes, “Cr, Cu” was the most predominant (50%). In the current study, only one isolate (8.3%) exhibited the “Cr, Cu, Hg”, resistance phenotype, against three heavy metals. Moreover, only three of the isolates (25%) showed resistance to a single metal “Cu”. In this research, 75% of the isolates had resistance two or more heavy metals, might be pose a potential threat to human health.

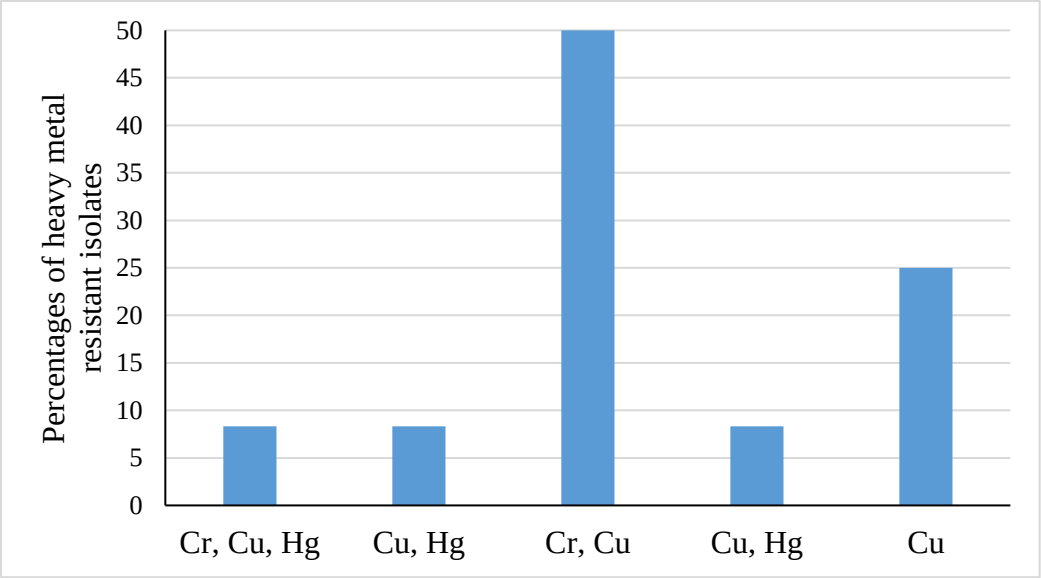

Figure 2. Incidences of heavy metal-resistant phenotypes among the *S. aureus* isolates from seawater fish

CONCLUSIONS

This study documented the presence of resistance to toxic heavy metals such as mercury, copper, and chromium, which are commonly associated with poisoning in humans, in some *S. aureus* isolates from seawater fish. As a result, it is important to monitor heavy metal resistance, which poses a significant risk to ecosystems and human health.

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THE FLORISTIC VALUES OF 'NARTË - PISHË PORO' PROPOSED NATURA 2000 SITE IN ALBANIA

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ABSTRACT

The Nartë-Pishë Poro is proposed as a Natura 2000 site in Albania with a surface of 235.4 km^2, situated along the Adriatic Sea shore, on both sides of Vjosa River and its delta. Two existing national protected areas are included in the site, namely the 'Vjosë-Nartë Protected Landscape" and the "Pishë Poro Managed Nature Reserve" (Category V and IV according to IUCN criteria). In this study we aim to describe its floristic richness, important species distribution, and the existing pressures and threats with mapping display of their normalized values. Thus values will testify of the site's uniqueness which fulfills the scientific requirement for sites of interest of the European Community. As result, 770 species were identified of which 757 species phanerogams, 6 species ferns and 6 species algae.120 species of this floristic richness have a conservation status according Albanian Red List and/or IUCN. Specifically, 41 species are part of the Albanian Red List, 99 species of IUCN, and 20 species have a conservation status according to the Albanian Red List and IUCN. There are reported 4 species of annex II, IV and V of Habitats Directive and two species of annex I of Berne Convention. *Galatella albanica, Achillea baldaccii* and *Silene cephallenia* are three subendemic species found in the area; *Halopeplis amplexicaulis, Isoetes histrix, Arthrocnemum perenne, Chamaemelum fuscatum, Euphorbia pinea, Glycyrrhiza glabra, Sphenopus divaricatus, Ononis variegate* and *Thymelaea hirsuta* occur only in this site in the whole country. The estimated floristic richness of conservation interest species are given numerical values to carry out statistical processing which are reflected in maps of normalized species values, according to the request and instructions of Article 17 of the Habitats Directive. Also, it was estimated 4 main pressures and threats for floristic values of the study area, which are: intensive public maintenance parks/cleaning of beaches, forestry clearance, actively burning down existing vegetation and invasive non-native species. The maps of normalized threats values were designed.

Keywords: Nartë-Pishë Poro, Natura 2000, floristic values, species of conservation interest, normalized maps

INTRODUCTION

Nartë-Pishë Poro study area extends approximately 40 km along the Albanian Riviera with a surface of 235.4 km^2. It borders with Hoxhara channel in the north, Vjosa River in north-east, Panaja-Mifol hills chain in the east, Soda forest in the south and the Adriatic sea coastline in the west (Fig.1).

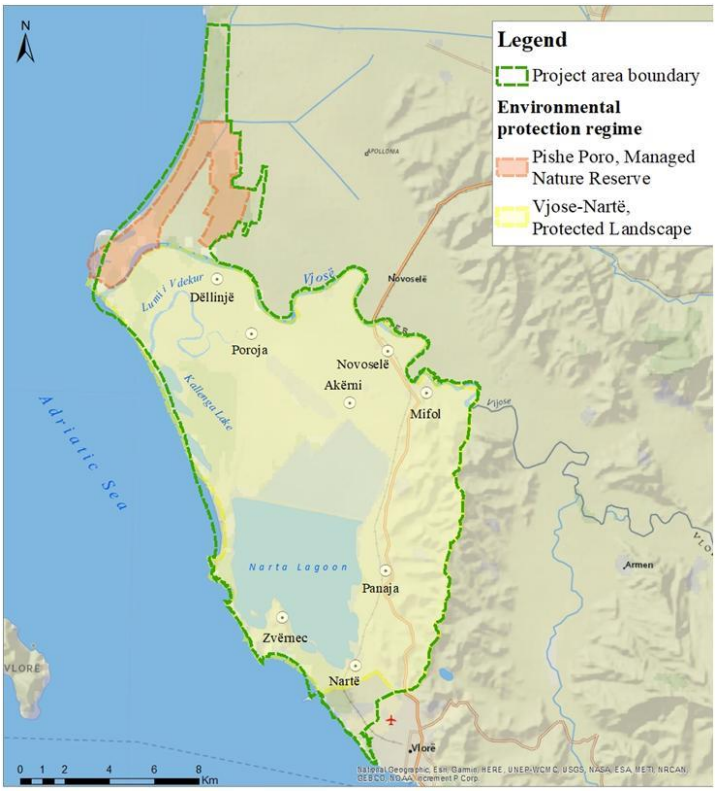

Figure 1. The map of the study area.

The boundaries of the study area are defined following ecological criteria and mainly natural boundaries such as: vegetation community, water bodies, coastlines, hill slopes, etc., determined by satellite images of the area.

Two protected areas, Vjosë-Nartë Protected Landscape (Category V according to IUCN criteria) and the Pishë Poro Managed Nature Reserve (Category IV according to IUCN criteria) are included in the proposed site, as well as many important and well-preserved habitats, but at the same time many areas under high pressure and threats which affect the long-term preservation of its natural values.

There are more than 20 villages with a population of more than 24,000 inhabitants in the proposed site. The nearest city is Vlora, one of the largest cities in Albania with a population of 106,000 (Anonymous, 2019). The life of these inhabitants is closely related to nature and their activities are livestock, agriculture, fishing, coastal tourism, collection of medicinal plants, beekeeping, orchards, etc.

The Nartë-Pishë Poro is widely reported for its biodiversity values. Among the most important ecosystems are wetlands, agricultural areas and forests. Wetlands cover 37% of the surface, agricultural areas 33% and forests 6%. The rest is occupied by urbanized areas or other forms of land use (PPNEA, 2013).The high floristic diversity of this territory has attracted researchers' attention and several papers and reports are published already (Buzo, 2000; Xhulaj and Mullaj, 2002; Pano and Frashëri, 2007; Imeri et al., 2018, etc.).

Based on the existing reported data and new updated field data, this paper aims to estimate the floristic richness of the area, by analyzing and mapping floristic values of species with conservation concern, theirs threats, and presentation of these values and threats on normalized maps according to the request and instructions of Article 17 of the Habitats Directive for Natura 2000 sites.

MATERIAL AND METHOD

Field data collection was carried out through 18 botanical expeditions during the period May 2020 – May 2021, mostly during flowering seasons, in spring and autumn. Plant species were collected as living material and were dried in electric presses. After the final determination they were deposited in the National Herbarium (TIR), in the Museum of Natural Sciences, Faculty of Natural Sciences, University of Tirana. For the full list of flora, data from the literature of previous publications, the register of TIR, and unpublished data were used as well (Buzo, 2000; Anonymous, 2005; Pano and Frashëri, 2007; PPNEA, 2013; Barina et al., 2017; Imeri et al., 2018). The location of important species found in the field was georeferenced with GPS Gramin.

All identified and confirmed plant species were entered into a Microsoft Excel 2010 database. Plant identification was carried out mainly based on the Flora of Albania, Vol. 1- 4 (Paparisto et al., 1988; Qosja et al., 1992; et al., 1996; Vangjeli et al., 2000), Flora Europaea, Vol. 1-5 (Tutin et al., 1964; et al., 1968; et al., 1972; et al., 1976; et al., 1980) and the flora of neighboring countries such as the Flora of Italy (Pignatti 1982). For the national and European conservation status, were consulted the Albanian Red List of vascular plants (VKM, 2013), the IUCN Red List of Threatened Species (IUCN, 2016), Habitats Directive (Appendix II, IV), Bern Convention and Appendix II of CITES.

The important species for the genofond and for conservation at the national, European and global level, accompanied by ecological data, were uploaded into the BIONNA format (Pacifici et al., 2018), the unified database for important species for the genofond at national, European and global level. In this database, the species is uploaded as many times as it has been encountered in the field, accompanied by other data such as: location, coordinates, habitat, plant community, threats and pressures, date of data collection, altitude above the sea level, slope, presence in the conservation lists, etc. From here, data processing and statistical analyzes are carried out. Species distribution maps were designed by downloading the georeferenced BIONNA data into ArcMap 10.7.

For each species of conservation interest, 'values' and normalized values have been estimated. ‘Value’ is called the integration of all the elements that determine the balance of biodiversity, the importance and uniqueness that appears (Viola et al., 2002). The ‘value’ in this method is not a theoretical determination but a quantitative one. The values of the species are calculated from the sum of their quantitative affiliations in the important national and international lists, according to the numerical system in the table below (Tab. 1).

Table 1. Quantitative values of species of conservation interest according toViola et al., 2002.

Values category	Values
Annex II of the Habitats Directive	10
Annex IV of the Habitats Directive	5
Endemic	8
Steno – Endemic	10
IUCN conservation status (Global)	
Cr	*10*
En	*8*
VU	*6*
NT	*4*
LC	*2*
Albanian Red List conservation status	
Cr	*10*
En	*8*
VU	*6*
NT	*4*
LR	*2*

For map designation in the GIS, the evaluated values have been reclassified to achieve a quantitative and qualitative homogeneity, between values and species of conservation interests. According to the most accurate method the values were normalized from 0 to 1 (or from 1 to 100), through the formula: $zi = (xi - \min(x)) / (\max(x) - \min(x))$ (Costantini, 2005) .
The existing and potential pressures and threats were assessed according to the European list of threats, defined in Article 17 of the Habitats Directive (https://cdr.eionet.europa.eu/help/natura2000). From this list, the experts identified 10 biggest risks and threats in the area. The vulnerability was determined for each important species and vegetation group. It was estimated by the combination of threats, relative value and vulnerability. For each species with conservation interest of the BIONNA database and for plant communities, 4 of the threats and pressures were estimated that affect directly. The threat levels were determined according to the scale from 0-3, where 0- no threats, 1- low intensity threats, 2- medium intensity threats and 3- high intensity threats. The scaling of vulnerability provided by Prosser and Sitzia (2001) are: 0 - no damage, 1 - low vulnerability, 2 - medium vulnerability and 3 - high vulnerability. It means the overall possibility for a species to suffer degradation or loss of relevant values as a result of external pressures.

RESULTS AND DISCUSSION

1) Floristic richness

The results showed a great floristic diversity in the Nartë-Pishë Poro. A total of 764 species (20.9% of the Albanian Flora) belonging to 450 genera (46.3% of the genera of Albanian Flora) and 110 families (62.2% of the families of Albania Flora) were identified. Analyzed according to taxonomic divisions results that 98% (757 species) of this flora is represented by phanerogams, 1% (6 species) of ferns and 1% (6 species) of algae.

20 plant species were reported for the first time in the area from the data collected during may 2021 - may 2022 (Appendix, Tab.1), while the rest of the floristic richness has been reported before by different authors (Buzo, 2000; Anonymous, 2005; Pano & Frashëri, 2007; PPNEA, 2013; Barina et al., 2017; Imeri et al., 2018;) and listed in unpublished data.

Human activity is almost everywhere in the area and isolated natural habitats are very rare, but still there are some rare plant species for the country. *Galatella albanica*, a sub endemic species to Albania was recorded and reported for the first time. In Barina et. al, 2017,

two other sub endemic species such as *Achillea baldacci*i and *Silene cephallenia* are reported as well.

The area is also very important for sheltering plant species which don't have any conservation status or have in general a wide areal but in Albania according to Barina et. al, 2017, these species occur only in this proposed Natura 2000 site which makes it very important for national biodiversity conservation. Such species are: *Halopeplis amplexicaulis, Isoetes histrix, Arthrocnemum perenne, Chamaemelum fuscatum, Euphorbia pinea, Glycyrrhiza glabra, Sphenopus divaricatus, Ononis variegata, Thymelaea hirsuta*.

The study contributed with accurate data for the distribution of the aquatic phanerogam *Althenia filiformis* and the green algae *Lamrothamium papulosum*, two rare aquatic macrophytes found only in the study area and Divjakë-Karavasta National Park. Temporary coastal wetlands with semi-salty water bodies are the main habitats for these species. The damage of these habitats or intervention to turn them into lagoons for fishing purposes, such has happened with Kallanga, could seriously endanger the extinction of these species.

2) *Flora of conservation interest*

In Nartë-Pishë Poro were identified 120 species with a conservation status (Appendix, Tab.2) according to the Albanian Red List (VKM, 2013) and/or IUCN (2016). Of these 120 species, 41 are included in the Albanian Red List, 99 species have a conservation status according to the IUCN, and 20 have conservation status according to the Albanian Red List and the IUCN.

Among the 41 species of the Albanian Red List, *Petrosimonia oppositifolia*, listed for the area from unpublished data, has the status 'critically endangered' (CR), 9 species have the status 'endangered' (EN), 30 species have the status 'vulnerable' (VU) , 2 species have the status 'low risk' (LR). Almost all species with a IUCN conservation status belong to 'low risk' (LC) or 'data deficient' (DD) such as *Gladiolus palustris, Luzula forsteri, Daucus carota, D. guttatus* and *Malus sylvestris*. *Platanus orientalis* and *Marsilea quadrifolia* have the conservation status 'vulnerable' (VU) (Appendix, Tab.2).

4 plant species are part of the annexes of the Habitats Directive and 2 species are part of the Bern Convention. Specificaly, *Anacamptis pyramidalis* is part of Annex II of the Habitats Directive, *Marsilea quadrifolia* is part of Annexes II and IV, *Gladiolus palustris* is part of Annex IV and *Ruscus aculeatus* is included in Annex V. Aquatic phanerogams *Cymodocea nodosa* and *Zostera noltii* are part of the Bern Convention. About 15 species of orchids are part of the CITES Convention (Appendix, Tab.2).

For each species with conservation interests, their quantitative value was calculated and the results are presented in the value map of figure 2, which also shows the distribution of species with conservation interests. The size of the circles on this map is proportional to the natural values that the species have. The larger the circle drawn at the location of a species of conservation interest, the greater the sum of the values of this species, which means that it can be an endemic/sub endemic species and at the same time has a high national conservation status and/or international (see methodology).

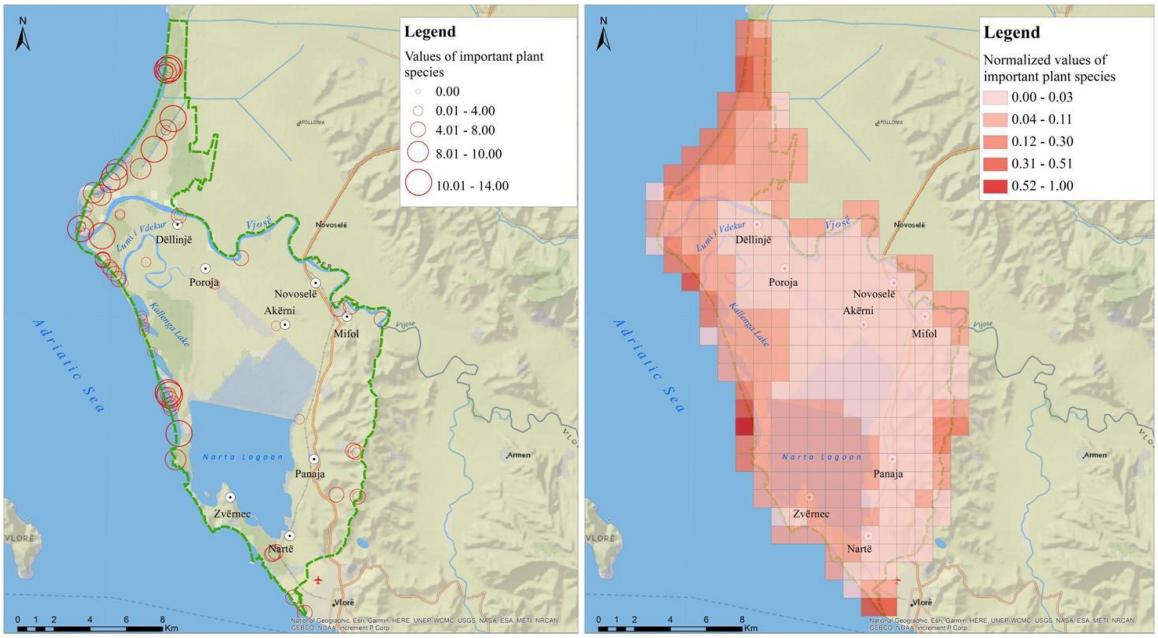

Figure 2. The values map (left) and normalized map of values (right) of the species with conservation interests.

From the administration and management point of view of a Natura 2000 site, as we proposed the Nartë-Pishë Poro, it is important that all values of the species are identified,monitored and preserved together with the area where they are located and not only as individual species. For this purpose, the normalized map of values (Fig. 2) shows the geographical areas with the highest values of the study, presented in a 1 x 1 km system as required by Article 17 of the Habitats Directive. The stronger the color of these squares, the greater is the sum of the values that this 1 km^2 area shelters.

We clarify that according to this methodology: 1) if a squared area (1 km2) has values (species with conservation interests) even a small part of it, the entire square of 1 km2 receives a value that is visually indicated by color on the map; 2) if a quadrat due to spatial/dimensional reasons extends to two or more habitats/environments (e.g. in coastal sands and part of it includes marine water surfaces) its value is determined by that area of the quadrat that has species with conservation value (e.g. coastal dunes with *Pancratium maritimum*, *Ammophila arenaria*, etc., and not the water surface).

Following the map of normalized values, the most critical areas, which host 1 or several important species, are the most natural ones, such as the sandy seacoast area, the pine forest and the area along the Vjosa River. In these areas there are individuals or populations of species with national and/or international conservation interest. *Pancratium maritimum* (sea lily) is one of the species of conservation interests that is found in almost every 1 km^2 of the fens and old pine wooded dunes. The sea lily is often found close (within a 1 km^2 quadrate) to *Ammophila arenaria*, *Galatella albainca* or other species of conservation interest causing some quadrate areas to take on an intense color as the sum of the values is high. Along the Vjosa River, the most common species of conservation interest that gives value and colors the map squares is *Populus alba*. The squared areas of the Narta lagoon and small coastal marshes receive values (between 0.03 and 0.1) because in these habitats there are present some macrophytes species of conservation interest such as *Ruppia cirrhosa*, found almost everywhere. Although with a limited area, the Mediterranean shrubs on the hilly area have medium to high values for housing plant species of conservation interest.

On the map of figure 2 it is obvious that even the areas of intensive agriculture or the villages have values, although lower compared to natural ones. The squared areas of these semi-

natural or artificial habitats gain value as a result of the reeds that are found almost everywhere in the drainage channels of agricultural lands. These reeds are dominated by *Phragmites australis* and *Typha angustifolia*, both with conservation status LC according to the IUCN. Also, along the banks of the drainage and irrigation canals there are belts of *Tamarix hampeana*, which has also a conservation status (LC) according to the IUCN. In these canals or abandoned agricultural land there are several other plant species with conservation status LC according to the IUCN (*Lemna minor, Lolium perenne, Lotus corniculatus, Lythrum salicaria, Ranunculus baudotii, Trifolium repens*, etc.) which give color to almost each square 1 km^2 of the map of normalized values.

3) Threats and pressures of floristic values

4 main pressures and threats for floristic values were prioritized in the area as expert judgment, consultation with stakeholders and communication with inhabitants: Intensive maintenance of public parks/cleaning of beaches:

1. Intensive maintenance of public parks/cleaning of beaches:

The sandy coastline of the proposed site is used for sunbathing and beach activities. Wrong practices such as flattening or plowing dunes to clear or open new beaches are a major extinction risk for species of conservation interest there. In the map of figure 3 (left), squares with intense color show those areas where the risk is high because in those areas there are species of conservation interest that are directly threatened by this factor. This pressure is more intense and serious in the recently opened beaches because the sandline which is used for years for sunbathing unfortunately is not any more affected because species of conservation interest have already gone extinct. This important species extinction in the old beach areas is clear evidence of how the long-term of this human activity is a serious threatening factor.

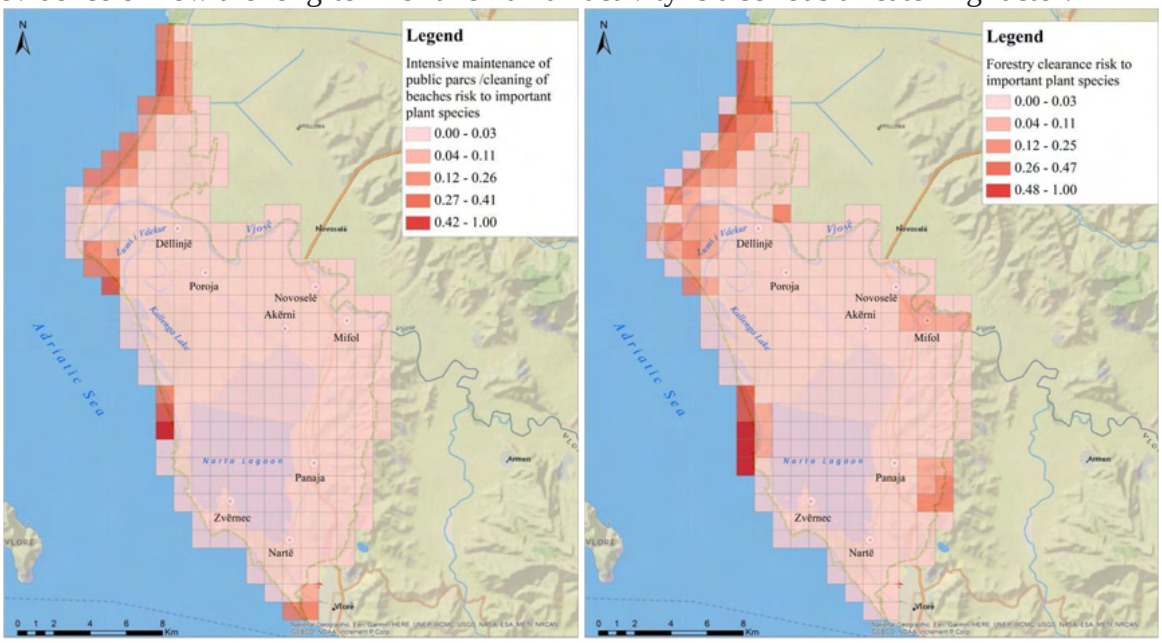

Figure 3. Normalized map of risk level assessment 'Intensive maintenance of public parks/cleaning of beaches' (left) and normalized map of risk level assessment 'actively burning down existing vegetation' (right) on plant species of conservation interests.

2. Forestry clearance:

The wood cuttings and deforestation are a direct threat factor for wood species with conservation interests such as *Populus alba, Ulmus minor, Tamarix hampeana*, etc., and for other herbaceous species of the forest layers: *Galatella albanica, Orchis sp., Ophrys sp.*, etc.

Along Vjosa River the threat is more moderate and it is attributed to the cutting of *P. alba* and *U. minor*, species with national and international conservation status.

The squared areas of 1 km^2 with intense color on the map show those areas where forestry clearance risks the population of the species with conservation interests which represent higher natural values than the sparse individuals of the species. This is why the squares that cover the area of *T. hampeana* galleries along the Limpua lagoon have intense color (the belt between the sea and Narta lagoon, Fig.3, right).

3. Actively burning down existing vegetation:

The forest and scrublands of the study area are under the risk of fires. Species of conservation interest such as *Tamarix hampeana, Populus alba, Juniperus oxycedrus subsp. macrocarpa, Ulmus minor,* etc., are under the direct pressure of this risk. When fires threaten plant formations formed by species of conservation interest the damage caused is higher and serious. TExactly these areas of the Nart-Pishë Poro are strongly colored on the 'actively burning down existing vegetation' map of figures 4 (left).

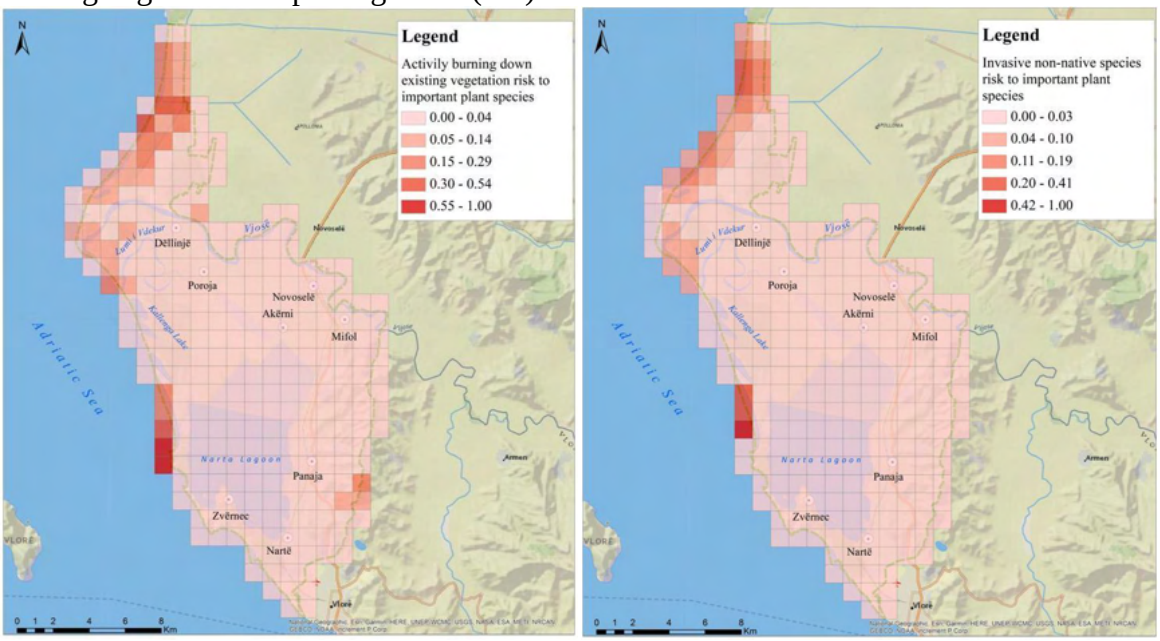

Figure 4. Normalized map of the risk level assessment 'actively burning down existing vegetation' (left) and normalized map of the risk level assessment of 'invasive non-native species' (right) on plant species of conservation interests.

4. Invasive non-native species.

5.8% of the plant species in the study area are alien species (45) (Appendix, Tab.3). The majority of alien species (27.5%) originate from North America and East Asia (12.5%). The species *Carpobrotus edulis* and *Robinia pseudacacia* are part of the list of the 100 most dangerous invasive species in Europe (Nentwig et al., 2018), but in Nartë-Pishë Poro it is *Acacia saligna* which is the most dangerous. This species, originally from Australia, was planted in the area before the 1990s. Today it is one of the most aggressive invasive plant species that is putting directly in pressure the *Juniperus oxycedrus subsp. macrocarpa* which has national and international conservation status and forms the annex one priority habitat 2250*: Coastal dunes with Juniperus spp.. *Oenothera parodianaee*, is a newly reported invasive species in the area, as a direct result of this study. Previously it was known only for the Velipoja area (Rakaj and Rostanski, 2009).

The map of figure 4 (right) shows the squared areas where the invasive plants directly threaten native species with conservation interests. In general, it is obvious that sandy coastal areas are most threatened by the invasion of alien species. The information of this map is very

valuable even from the conservation and management point of view. It should be taken into consideration even by any activity or management plan for invasive species and for the native species preservation, among them many of conservation interest.

CONCLUSIONS

Nartë-Pishë Poro is characterized by a high floristic richness. This area represents less than 1% of Albanian territory but there are found 764 plant species or 20.9% of the Albanian floristic richness. Among the identified species, 757 species of this flora are represented by phanerogams, 6 ferns species and 6 algae species .

20 species are reported for the first time in the area.*Galatella albanica*, a sub endemic species to Albanian i reported for first time in this area and together with *Achillea baldaccii* and *Silene cephallenia*, reported previously from this area, increase the total number of sub endemic species in the area in three.

9 species such are *Halopeplis amplexicaulis, Isoetes histrix, Arthrocnemum perenne, Chamaemelum fuscatum, Euphorbia pinea, Glycyrrhiza glabra, Sphenopus divaricatus, Ononis variegata* and *Thymelaea hirsuta* are found only in Narte Pishe Poro, in the whole Albanian territory, making it an important site for plant species genofond. *Althenia filiformis* and *Lamrothamium papulosum* are two rare aquatic macrophytes found only in the study area and Divjakë-Karavasta National Park.

The Nartë-Pishë Poro has many species with conservation status: 41 species are included in the Albanian Red List, 99 species have a conservation status according to the IUCN, and 20 have a conservation status according to the Albanian Red List and the IUCN.

Among the species with conservation interest of the Albanian Red List (VKM, 2013), *Petrosimonia oppositifolia* has conservation status ‘critically endangered’ (CR), 9 species ‘endangered’ (EN), 30 species ‘vulnerable’ (VU) , 2 species ‘low risk’ (LR). The most of the species with a conservation status according to the IUCN belong to the lowest category 'low risk' (LC) or 'data deficient' (DD). *Platanus orientalis* and *Marsilea quadrifolia* are two threatened species according to IUCN with conservation status 'vulnerable' (VU).

Among the species with international conservation interest *Anacamptis pyramidalis* is part of Annex II of the Habitats Directive, *Marsilea quadrifolia* part of Annexes II and IV, *Gladiolus palustris* part of Annex IV and *Ruscus aculeatus* is included in Annex V. Aquatic phanerogams *Cymodocea nodosa* and *Zostera noltii* are part of the Bern Convention an about 15 species of Orchids are part of the CITES Convention.

Based on the normalized map values, the most natural areas which host the species of national and global conservation interest are the sandy seacoast, the pine forest and the area along the Vjosa River.

Four main pressures and threats to the floristic values are prioritized following the definition at the Article 17 of the Habitats Directive:: intensive maintenance of public parks/cleaning of beaches forestry clearance, actively burning down existing vegetation and invasive non-native species. These directly or indirectly affect the floristic values. Their most negative impact is when they affect the population of species with conservation interests, shown in the maps of normalized threats values with squares colored more intensely.

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APPENDIX

Table 1. The plant species reported for the first time in the study area.

No.	Species name	Family
1.	*Silene velutina Loisel.*	Caryophyllaceae
2.	*Crepis foetida L. subsp. foetida*	Compositae
3.	*Eleocharis ovata (Roth) Roem. & Schult.*	Cyperaceae
4.	*Melilotus neapolitanus Ten.*	Fabaceae
5.	*Bellardia trixago (L.) All.*	Lamiaceae
6.	*Linum trigynum L.*	Linaceae
7.	*Oenothera parodiana Munz subsp. parodiana*	Onagraceae
8.	*Ranunculus peltatus Schrank*	Ranunculaceae
9.	*Kochia prostrata (L.) Schrader*	Chenopodiaceae
10.	*Chamaemelum mixtum (L.) All.*	Compositae
11.	*Symphyotrichum squamatum (Spreng.) G. L. Nesom*	Compositae
12.	*Juniperus phoenicea subsp. turbinata (Guss.) Nyman*	Cupresaceae
13.	*Carex cuprina (Heuff.) A. Kern.*	Cyperaceae
14.	*Trifolium echinatum M. Bieb.*	Fabaceae
15.	*Micromeria cristata (Hampe) Griseb.*	Lamiaceae
16.	*Aegilops neglecta Req. ex Bertol.*	Poaceae
17.	*Bromus arvensis L.*	Poaceae
18.	*Aremonia agrimonoides (L.) DC.*	Rosaceae
19.	*Populus × canescens (Aiton) Sm.*	Salicaceae
20.	*Sixalix atropurpurea (L.) Greuter & Burdet*	Caprifoliaceae

Table 2. The species with conservation interests according to Albanian Red List (2013) and IUCN (2016).

Species name	Al. Red List	IUCN	Species name	Al. Red List	IUCN
Ruscus aculeatus	-	LC	*Lythrum salicaria*	-	LC
Arundo donax	-	LC	*Agrimonia eupatoria*	-	LR
Eriophorum angustifolia	-	LC	*Urtica dioica*	-	LC
Equisetum arvense	-	LC	*Butomus umbellatus*	VU A1b	-
Equisetum palustre	-	LC	*Nymphoides peltata*	VU A1b	-
Equisetum telmateia	-	LC	*Sparganium erectum*	-	LC
Juncus articulatus	-	LC	*Zostera marina*	VU A2d	-
Typha angustifolia	-	LC	*Posidonia oceanica*	VU A2d	-
Iris pseudacorus	VU A2b	LC	*Alisma lanceolatum*	-	LC
Orchis albanica	EN A1b	-	*Alisma plantago-aquatica*	-	LC
Orchis x paparisti	Vu A1b	-	*Lemna minor*	-	LC
Nymphaea alba	VU A1b	LC	*Potamogeton crispus*	-	LC
Orchis morio	VU A1b	LC	*Potamogeton natans*	-	LC
Orchis coriophora	VU A1b	LC	*Spyrodela polyrhiza*	-	LC
Anacamptis laxiflora	VU A1b	LC	*Vitis sylvestris*	-	LC
Anacamptis pyramydalis	VU A1b	LC	*Salix amplexicaulis*	-	LC
Ophrys apifera	VU A1b	LC	*Tamarix hampeana*	VU A2b	-
Ophrys scolopax	VU A1b	LC	*Alnus incana*	-	LC
Ophrys sphegodes	VU A1b	LC	*Capparis spinosa*	VU A1b	-
Serapias vomeracea	-	LC	*Juniperus oxycedrus ssp. macrocarpa*	-	LC
Ammophila arenaria	EN A1b	-	*Quercus robur*	VU A1b	-
Asphodelus macrocarpus	-	LC	*Malus sylvestris*	-	DD
Gladiolus palustris	LR nt	DD	*Prunus spinosa*	-	LC
Juncus effusus	-	LC	*Ulmus glabra*	VU A1c	-
Typha latifolia	-	LC	*Ulmus campestris*	VU A2b	-
Asparagus acutifolius	-	LC	*Sambucus nigra*	VU A1b	-
Allium roseum	-	LC	*Quercus coccifera*	-	LR
Pancratium maritimum	EN A1b	LC	*Tamarix parviflora*	-	LC
Ophrys bertolonii	VU A1b	LC	*Quercus ilex*	EN A1b	-
Ophrys fusca	VU A1b	LC	*Laurus nobilis*	EN A1b	-
Ophrys bombyliflora	VU A1b	LC	*Populus alba*	VU A2b	-
Ophrys lutea	VU A1b	LC	*Populus nigra*	-	LC
Ophrys speculum	VU A1b	LC	*Salix alba*	-	LC
Ophrys umbilicata	VU A1b	LC	*Platanus orientalis*	VU A2b	LC
Serapias parviflora	-	LC	*Bidens tripartita*	-	LC
Arundo plinii	-	LC	*Avena fatua*	-	LC
Cyperus longus	-	LC	*Trifolium nigrescens*	-	LC
Adiantum capillus-veneris	-	LC	*Medicago minima*	-	LC
Ophrys ferrum-equinum	VU A1b	LC	*Aegilops triuncialis*	-	LC
Eleocharis palustris	-	LC	*Vulpia ciliata*	-	LC
Phragmites australis	-	LC	*Trifolium angustifolium*	-	LC
Cephalanthera rubra	-	LC	*Vicia bithynica*	-	LC

Agrostis stolonifera	-	LC	*Vicia lutea*	-	LC
Holcus lanatus	-	LC	*Lepidium ruderale*	-	LC
Poa pratensis	-	LC	*Petrosimonia oppositifolia*	CR A1c	-
Melilotus officinalis	-	LC	*Trifolium patens*	-	LC
Origanum vulgare	EN A1b	-	*Juncus bufonius*	-	LC
Trifolium repens	-	LC	*Daucus guttatus*	-	DD
Veronica beccabunga	-	LC	*Desmazeria marina*	VU A1b	-
Carex distans	-	LC	*Vicia sativa*	-	LC
Luzula forsteri	-	DD	*Cyperus fuscus*	-	LC
Mentha pulegium	-	LC	*Centaurium erythraea*	-	LC
Teucrium scordium	-	LC	*Centaurium pulchellum*	-	LC
Nasturtium officinale	-	LC	*Cyperus flavescens*	-	LC
Cichorium intybus	-	LC	*Daucus carota*	-	DD
Veronica anagallis-aquatica	-	LC	*Digitalis lanata*	LR cd	LR
Lotus cytisoides	EN A1b	-	*Silene vulgaris*	-	LC
Hordeum bulbosum	-	LC	*Mentha aquatica*	-	LC
Apium graveolens	-	LC	*Lycopus europaeus*	-	LC
Hypericum perforatum	EN A1b	-	*Medicago lupulina*	-	LC

Table 3. Alien species of the study area.

No.	Species name	Family	Corology
1	*Carpobrotus edulis*	Aizoaceae	S Africa
2	*Amaranthus hybridus*	Amaranthaceae	America Trop.
3	*Amaranthus retroflexus*	Amaranthaceae	N America
4	*Amaranthus albus*	Amaranthaceae	S America
5	*Agave Americana*	Asparagaceae	N America
6	*Heliotropium supinum*	Boraginaceae	Paleosubtropic
7	*Heliotropium curassavicum*	Boraginaceae	Neotropic.
8	*Coronopus didymus*	Brassicaceae	S America
9	*Chenopodium ambrosioides*	Chenopodiaceae	Cosmopolit.
10	*Conyza bonariensis*	Compositae	America Trop.
11	*Conyza canadensis*	Compositae	N America
12	*Xanthium strumarium*	Compositae	Cosmopolit.
13	*Cuscuta campestris*	Convolvulaceae	N America
14	*Cupressus sempervirens*	Cupressaceae	E Asia
15	*Lemna minuta*	Lemnaceae	N America
16	*Euphorbia maculate*	Euphorbiaceae	N America
17	*Amorpha fruticosa*	Fabaceae	N America
18	*Robinia pseudacacia*	Fabaceae	N America
19	*Sisyrinchium angustifolium*	Iridaceae	America
20	*Linum usitatissimum*	Linaceae	Euromedit.
21	*Punica granatum*	Lythraceae	Euromedit -Asia
22	*Ficus carica*	Moraceae	E Asia
23	*Alcea rosea*	Malvaceae	Asia
24	*Morus alba*	Moraceae	E Asia
25	*Oenathera parodiana*	Onagraceae	N America
26	*Oenathera biennis*	Onagraceae	America
27	*Oxalis pes-caprae*	Oxalidaceae	S America
28	*Arundo donax*	Poaceae	Asia
29	*Paspalum paspalodes*	Poaceae	Sub-Cosmopolit.
30	*Sorghum halepense*	Poaceae	Cosmopolit.
31	*Cydonia oblonga*	Rosaceae	E Asia
32	*Cydonia oblonga*	Rosaceae	E Asia
33	*Acer negundo*	Sapindaceae	N America
34	*Ailanthus altissima*	Simaroubaceae	E Asia
35	*Portulaca oleracea*	Portulacaceae	Tropic.
36	*Capsicum annum*	Solanaceae	S America
37	*Physalis angulata*	Solanaceae	America Trop.
38	*Datura stramonium*	Solanaceae	Cosmopolit.
39	*Allium sativum*	Amaryllidaceae	Asia
40	*Symphyotrichum squamatum*	Compositae	N America
41	*Xanthium spinosum*	Compositae	S America
42	*Pinus pinaster*	Pinaceae	Steno Mesdit.
43	*Acacia saligna*	Fabaceae	W Australia
44	*Ficus carica*	Moraceae	E Asia
45	*Zizifus jujuba*	Rhamnaceae	EuroAsia

ALTERNATIVE OILSEED CROPS IN TURKEY

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ABSTRACT

Despite its sufficient production in many agricultural crops, our country has a large foreign trade deficit, especially in the production of oilseeds and edible oil. Alternative oilseeds are valuable crops that are grown in marginal areas in Turkey and have a crucial contribution to oil production. Alternative oilseed crops cultivated in our country are rapeseed (canola), safflower, sesame, linseed, camelina and cephalaria. While these oilseeds can be successfully grown in Turkey, the markets and supply chains some of them are not necessarily developed. Especially camelina and cephalaria production is too low to be recorded in the statistical database. In 2022, 150.000 tons of rapeseed, 30.000 tons of safflower, 17.366 tons of sesame and 8 tons of linseed were produced in our country. The production of these crops is highly low in Turkey where edible oil consumption is high. Although almost every region of our country is suitable for the production of major and alternative oilseed crops, the increasing vegetable oil deficit is a major problem. In this study, the availability, production and future of alternative oilseed crops in Turkey are considered as a whole.

Keywords: Alternative oilseed crops, Rapeseed, Safflower, Sesame, Linseed, Camelina

INTRODUCTION

In parallel with the increase in the world population, the consumption of foodstuffs is increasing and at the same time the consumption of edible oil is also increasing. This consumption forces producers to increase production on available agricultural area. In addition, the fact that vegetable oils have become the raw material of the energy sector such as biodiesel increases the need for these oils even more (Culpan, 2015).

Edible oils, a staple food, are obtained from oilseed crops. Some of these crops are wild and some of them are cultivated (Arslan and Culpan, 2023). Vegetable or edible oils, which are basic foods, are obtained from oilseed crops. Oilseed crops rich in primary and secondary metabolites (fat, protein, carbohydrates, vitamins etc.) constitute an essential source of raw material for human and animal nutrition as well as for the industrial sector (Yılmaz et al., 2021).

Despite its sufficient production in many agricultural crops, our country has a large foreign trade deficit, especially in the production of oilseeds and edible oil. Alternative oilseeds are valuable crops that are grown in marginal areas in Turkey and have a crucial contribution to oil production. Alternative oilseed crops cultivated in our country are rapeseed (canola), safflower, sesame, linseed, camelina and cephalaria. While these oilseeds can be successfully grown in Turkey, the markets and supply chains some of them are not necessarily developed. Although almost every region of our country is suitable for the production of major and alternative oilseed crops, the increasing vegetable oil deficit is a major problem. In this study, the availability, production and future of alternative oilseed crops in Turkey are considered as a whole.

ALTERNATIVE OILSEED CROPS

Rapeseed (*Brassica napus* L.)

Rapeseed, a crop of Mediterranean origin, is important for human and animal nutrition (Gürsoy, 2019) and biodiesel production. It is the third most produced annual oil crop in the world (71.3 million tons as of 2021), behind soybean and seed cotton. The countries with the highest production are Canada, China, India, Germany and France (FAOSTAT, 2021). According to the data of the Turkish Statistical Institute for the year 2022, 150.000 tons of rapeseed was produced from 41.145 ha and the average seed yield was 3650 kg/ha (Table 1). Rapeseed is intensively cultivated in Thrace region in Turkey and its contribution to the oil production is highly important (Culpan et al., 2022).

Table 1. Rapeseed production in Turkey in the last 10 years

Years*	**Area Harvested (ha)**	**Yield (kg/ha)**	**Production Quantity (tons)**
2013	31.127	3280	102.000
2014	32.133	3420	110.000
2015	35.081	3440	120.000
2016	35.453	3530	125.000
2017	16.520	3640	60.000
2018	37.845	3300	125.000
2019	52.514	3430	180.000
2020	34.989	3470	121.542
2021	37.601	3720	140.000
2022	41.145	3650	150.000

* Turkish Statistical Institute, 2022

Rapeseed cultivation is highly similar to wheat cultivation practices (Tıraş, 2011); it is sown and harvested in almost the same period (September-July). The reasons that make rapeseed valuable are that it winter survival when it enters winter during the rosette period, does not require additional irrigation by being content with natural rainfall, reaches harvest maturity in July at the latest, can be easily harvested with a wheat harvester and yields more than cool climate cereals under dry farming conditions (Arslan, 2016). Due to the 40-50% quality oil in the seeds of rapeseed (Murphy, 1995; Gürsoy and Kolsarıcı, 2017), high oil yield from per hectare is an advantage compared to other spring oilseed crops such as safflower. The most important problem of rapeseed in Turkey is the poor availability of moisture in the soil in September and early October, the time of winter sowing in Central Anatolia and Thrace. This can lead to poor emergence and perhaps to repeat sowing.

Safflower (*Carthamus tinctorius* L.)

Safflower (*Carthamus tinctorius* L.) is a multipurpose oilseed crop that can grow in arid and semi-arid environments because of its tolerance for drought stress (Mosupiemang et al., 2022). *Carthamus tinctorius* L. which belongs to the Asteraceae, is one of the oldest cultivated plants that started to be cultivated 3000 years ago. It contains 25-45% oil in its seeds, has two different types as linoleic (ω-6) and oleic (ω-9), has high quality edible oil, is suitable for biodiesel production, is cultivated in the form of residue and mixture and is considered as animal feed (Arslan et al., 2012; Culpan and Arslan, 2022). On the other hand, drought tolerant and cultivation without irrigation enable especially availability of fallow areas (Arslan and Culpan, 2018). According to the data of the Turkish Statistical Institute for the year 2022, 15.000 tons of safflower was produced from 26.237 ha and the average seed yield was 1140 kg/ha (Table 2). There was a significant increase in safflower cultivation areas until 2014 and 2015 growing seasons, but then a decrease was observed again.

Table 2. Safflower production in Turkey in the last 10 years

Years*	Area Harvested (ha)	Yield (kg/ha)	Production Quantity (tons)
2013	29.292	1540	45.000
2014	44.305	1410	62.000
2015	43.107	1640	70.000
2016	39.571	1470	58.000
2017	27.376	1830	50.000
2018	24.693	1420	35.000
2019	15.860	1380	21.883
2020	15.115	1410	21.325
2021	14.588	1110	14.000
2022	26.237	1140	15.000

* Turkish Statistical Institute, 2022

The most important advantage of safflower is that it does not require much agricultural practices such as irrigation and fertilization. However, since safflower is known as a drought tolerant crop, it is cultivated without irrigation conditions and therefore its seed yield is low. According to the researches, seed yield of safflower increases up to 2 times with irrigation under appropriate growing conditions in dry areas (Öztürk et al. 2009; Arslan and Culpan, 2023). In the arid and semi-arid areas of the Central Anatolia Region, where the wheat-fallow system is widely practiced in Turkey, safflower is the most important alternative oilseed crop that can take place in crop rotation with wheat.

Sesame (*Sesamum indicum* L.)

Sesame is one of the first oil crops to be cultivated and is very important for human nutrition due to its valuable nutrients. It seeds contain 50-60% oil and 25% protein and are sown as spring crops. Sesame oil contains high levels of unsaturated fatty acids such as oleic (40-50%) and linoleic (45-50%) and saturated fatty acids such as palmitic (7-9%), stearic (4-5%) and arachidic (0.4-1.0%) (Bakal and Arıoğlu, 2020).

In 2022, 17.366 tons of sesame was produced from 24.285 ha and the average seed yield was 720 kg/ha (Table 2). While sesame production in Turkey was 40 thousand tons in the early 1990s, it has been below 20 thousand tons in recent years. This is mainly because its seed yield is low and the price of oil is quite expensive. Accordingly, sesame has no contribution to our vegetable oil industry. It is used in cakes, pastries and breads in our country, in addition to tahini production, which takes the crop away from oil production. By introducing varieties with high seed yields in the Aegean, Mediterranean and GAP regions, the oil production potential can be increased if mechanization problems are solved under irrigation conditions (Arslan, 2016).

Table 3. Sesame production in Turkey in the last 10 years

Years*	Area Harvested (ha)	Yield (kg/ha)	Production Quantity (tons)
2013	24.807	620	15.457
2014	26.349	670	17.716
2015	28.088	660	18.530
2016	28.933	670	19.521
2017	28.031	660	18.410
2018	25.985	670	17.437
2019	24.860	680	16.893
2020	25.666	730	18.648
2021	25.486	690	17.657
2022	24.285	720	17.366

* Turkish Statistical Institute, 2022

Linseed (*Linum usitatissimum* L.)

Linseed (*Linum usitatissimum* L.) is a traditional oilseed crop that represents a valuable alternative for fallow areas due to its adaptability to unfavorable soils and its high economic value relative to the high quality of the seed oil (Zanetti et al., 2013). It is grown either for its fiber (fiber flax) or for its oil (oilseed flax) (Hall et al., 2016). Its oil is the best source of the n-3 fatty acid, α-linolenic acid, which constitutes nearly 55 % of its total fatty acids. This value is 5.5 times more than the next best sources of α-linolenic acid (Bloedon and Szapary, 2004).

Linseed is an alternative oilseed crop and has unique drought tolerance; in extreme conditions, it can complete its life cycle in climates in which annual rainfall is only 200 mm (Li and Wang, 2016). Genotype × environment interactions have been shown to be high for linseed (Diepenbrock et al., 1995), and seed yield change significantly between production years, depending on location and climate conditions. Linseed, like safflower that can be used to utilize fallow and poor soil fields in Turkey. It is an important source of vegetable oil that can be utilized especially in conditions where rain and irrigation water is limited and therefore other oil crops cannot be grown (Arslan, 2016). Linseed cultivation has resumed in our country in the last few years and production has reached 8 tons according to 2022 data (TSI, 2022).

Camelina (*Camelina sativa* (L.) Crantz)

Camelina (*Camelina sativa* (L.) Crantz) is ancient oilseed that blongs to *Brassicaceae* family that is grown worldwide (Righini et al., 2019; Schillinger, 2019). Several characteristics of camelina make it an alternative oilseed crop, indeed a potential oilseed crop. In recent years, camelina has started to gain importance in the international arena again and many new researches have been carried out on it (Sevilmiş et al., 2019). Many researchers documented that camelina is drought and heat tolerant (Angelini et al., 1997; Blackshaw et al., 2011). It (*Camelina sativa* (L.) Crantz) is more adaptive to drought conditions than other oil seeds crops such as canola (Raza et al., 2015). The seed yield of camelina ranged from 1177 kg/ha under drought conditions in Saskatchewan to 3012 kg/ha in northern Alberta (Francis and Campbell, 2003). Zubr (1997) reported seed yields of 2600 kg/ha and 3300 kg/ha for spring and winter varieties, respectively. In Turkey, on the other hand, camelina is not cultivated at present. However, with the expansion of the cultivation area in the future, the potential to provide raw materials to the oil industry can be reached.

Cephalaria (*Cephalaria syriaca* L.)

Cephalaria (*Cephalaria syriaca* L.) is an annual plant in the Dipsacacea family. Studies have revealed that the oil content of cephalaria seeds varies between 21-26% and protein content between 14-20% (Çağlar, 1968). Its oil can be used directly as edible oil or mixed with other oils. However, the 7-8% epoxy acid in oil indicates that this oil should not be used as edible oil in this form (Sezgin et al., 2017). It is a crop that is widespread in Anatolia, grows wild in wheat fields and although its growth form is not similar to wheat, it is quite similar in terms of seed structure, size and shape (Boz and Karaoğlu, 2013; Sezgin et al., 2017). Cephalaria is a potential alternative oilseed crop for the future as it is cold and drought resistant. It grows very well in clay and loamy soils. As in camelina, cephalaria can reach the potential to provide raw materials to the oil industry by expanding its cultivation area in the future.

CONCLUSIONS

In 2022, 150.000 tons of rapeseed, 30.000 tons of safflower, 17.366 tons of sesame and 8 tons of linseed were produced in our country. The production of these crops is highly low in Turkey where edible oil consumption is high. Although almost every region of Turkey is suitable for the production of major and alternative oilseed crops, the increasing vegetable oil deficit is a major problem. Our country will be able to make use this advantage ideally and plan and program it with a number of precautions so that it will be able to exert itself in the production of oil seed and vegetable oil and export the production excess. However, the necessary measures and precautions listed below must be taken to unlock this potential and increase production quantities;

1. Long-term planning and sustainable policies should be implemented in the vegetable oil industry and alternative oilseed crops production.
2. In oilseed production, local seed breeding and production should be accelerated and supported by the state.
3. Alternative oilseed crops production should be included in the alternative crop project in fallow areas.
4. Irrigation investments should be accelerated and oilseed crop cultivation should be emphasized in new irrigable cultivation areas.
5. The Ministry of Agriculture should play an active role in seed supply and technical support for alternative oilseeds (rapeseed, safflower and linseed etc.) and should be encouraged to purchase the produced product.
6. In order to eliminate the low seed yield of safflower and to use it more effectively in crop rotation, winter-tolerant varieties should be developed.
7. Measures should be taken to expand mechanization in sesame agriculture.
8. Production of alternative oilseeds should be supported with low interest loans and premium amounts per kg should be increased.

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BIODIVERSITY AND TROPHIC STRUCTURE OF INVERTEBRATE ASSEMBLAGES ASSOCIATED WITH RED ALGAE *TITANODERMA TROCHANTER* AND *ELLISOLANDIA ELONGATA* BEDS

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ABSTRACT

The modern biodiversity crisis, referred to as the "seventh extinction," sets itself apart from previous mass extinctions due to its primary cause - human activities. Human actions, such as deforestation, pollution, overexploitation, and climate change, are central factors driving this crisis, with marine ecosystems bearing the brunt of the consequences. Despite the importance of marine species and ecosystems, the conservation efforts of organizations like the IUCN Red List often tend to prioritize terrestrial species, possibly due to the dominance of terrestrial-focused groups within these organizations.

Addressing the neglect of marine species' conservation needs is crucial. Ensuring comprehensive protection for both terrestrial and marine ecosystems is vital, as both play essential roles in maintaining global biodiversity and ecological balance. Bridging the gap between terrestrial and marine conservation efforts requires increased awareness, international cooperation, and more inclusive policies to effectively combat the ongoing biodiversity crisis.

This research aims to evaluate the importance of biodiversity, shelter, and reproduction habitats of two significant species of bioconstructors in the marine intertidal area. The study focuses on two red algae species, *Titanoderma trochanter* and *Ellisolandia elongata*, which are included in Annex II ('endangered and threatened species') of the Barcelona Convention's Mediterranean Action Plan by the United Nations. These calcareous rhodophytes' structures enhance substrate complexity, support diverse assemblages, and play a crucial role in CO2 sequestration. Additionally, they host many endemic species of the Mediterranean Sea, making their evident structures rare in the Basin.

The research examines the associated fauna of these two calcareous rhodophytes through sampling in 20 x 20 cm squares in four areas of the Karaburuni peninsula, within the Karaburun Sazani Marine Protected Area. The sampling was conducted during March, April, September, and October of 2021 and 2022.

In the spring season, a total of 67 invertebrate species were identified among 126 sampled invertebrates in *Ellisolandia elongata*. On the other hand, *Titanoderma trochanter* revealed 59 identified species out of 136 sampled invertebrates. During the autumn season, a total of 75 invertebrate species were identified in *Ellisolandia elongata*, while *Titanoderma trochanter* hosted 72 species.

Both species showed a dominance of the phylum Polychaeta, especially in *Ellisolandia elongata*, which had the highest number of present families. The researchers conducted spatiotemporal and comparative analyses to determine the diversity of the associated fauna of these two calcareous rhodophytes.

Keywords: *Ellisolandia elongata, Titanoderma trochanter*, macrozoobenthos, marine invertebrates, polychaeta, Karaburuni peninsula

INTRODUCTION

The Mediterranean Sea, comprising only 0.82% of the world's ocean surface area and 0.32% of its volume (Bianchi and Morri, 2000), stands as a renowned hotspot for marine biodiversity, boasting a documented species count exceeding 20,000 (Pascual et al., 2017; Rindi et al., 2019). Within coastal ecosystems, macroalgal beds are widely acknowledged for their pivotal roles in biodiversity maintenance and carbon fluxes (Dayton 1985). However, the coastal marine environments of the Mediterranean have been subjected to continuous exploitation for millennia (Rindi et al., 2019), resulting in pervasive transformations. Presently, these changes have given rise to urbanized, heavily polluted, and densely populated coastlines. These anthropogenic activities exert a disproportionately greater influence on the Mediterranean compared to other global seas (Coll et al., 2010; Rindi et al., 2019). The primary drivers of these changes include habitat loss, degradation, pollution, overexploitation of marine resources, and the introduction of invasive species. Moreover, these drivers are expected to intersect and interact with climate-induced changes in the coming decades. Consequently, understanding the biology of Mediterranean coastal habitats has emerged as a pressing priority in recent years. Coralline algae, which have thrived in the Mediterranean for approximately 140 million years (Chatalov et al., 2015), continue to be widely distributed in the region (Chatalov et al., 2015). Certain species of coralline algae form communities and habitats that are recognized in the 2000 habitat list.

This research aims to elucidate the significance of two coralline algae species as habitat builders while providing insights into the associated invertebrate fauna's biodiversity and trophic structure. Notably, *Ellisolandia elongata* and *Titanoderma trochanter* are both designated as 'endangered and threatened species' within Annex II of the Barcelona Convention's Mediterranean Action Plan, as designated by the United Nations (Verlaque et al., 2019). These two species often play a crucial role as ecosystem engineers. They modify the substrate through their three-dimensional structure consisting of calcareous thalli and trapped sediment (Bressan et al., 2009; Ingrosso et al., 2018; Rindi et al., 2019). These calcareous algae bioconstructions are recognized as biodiversity hotspots of the Mediterranean sea (Ballesteros, 2006).

Recent efforts to quantify energy flows within Mediterranean algal forests and coralligenous outcrops have highlighted their significant contribution to the energy balance of coastal ecosystems (Buonocore et al., 2020; De la Fuente et al., 2019). However, despite their importance, there remains a lack of comprehensive and precise assessment regarding the ecosystem services offered by rocky reefs throughout the Mediterranean Sea. Such an evaluation would be of utmost significance for policymakers and environmental practitioners, aiding in the development of suitable conservation and management strategies (Bevilacqua et al., 2021).

MATERIAL AND METHOD

The study area

The Karaburuni Peninsula, spanning 62 km2 in Vlora Bay, separates the Albanian coast along the Adriatic and Ionian Seas. It connects to Sazani Island via the Mezokanali channel. Geologically, it's primarily Cretaceous limestone with terrigenous deposits in the northwest (Kashta et al., 2011). The terrain has hills, with peaks like Maja e Ilqes (733 m), Maja e Flamurit (826 m), and Çadëri (839 m). The peninsula's coastline is rugged with cliffs, especially on the western side, making access to some areas challenging without a boat. In contrast, the eastern coast is less fragmented. The northwestern tip is Cape Gjuhezes, Albania's westernmost point. Vegetation is sparse, except for some maquis and grass, and there are no freshwater sources. The peninsula encloses bays like Raguza, St. Jan, Bristan, and Dafina (Kashta et al., 2011). Peninsula was declared a natural reserve in 1966 and in April 2010, the coastal and marine area of Sazani Island and the Karaburuni Peninsula were raised to the status of Marine Protected Area (MPA), which is the first MPA in Albania (Kashta et al., 2011).

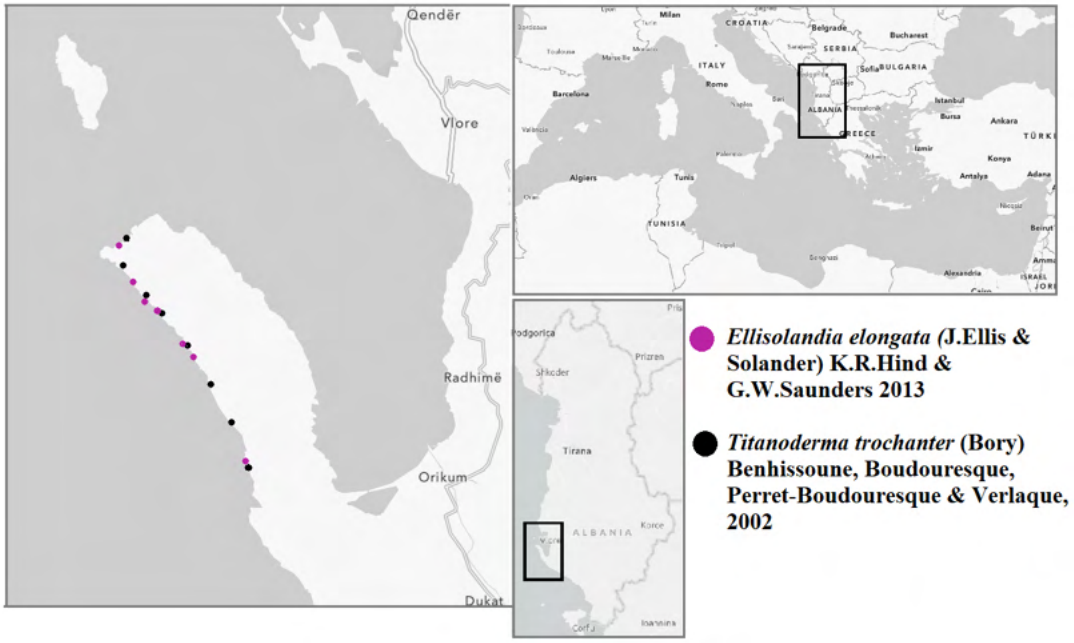

Figure 10. Location of the study area: a) and b) Karaburuni penisula part of Sazan Karaburuni MPA.

In the months of May, June, September, and October 2021, we carried out benthic sampling on hard bottoms, adhering to established protocols outlined by Cattaneo et al. (1978), Drago et al. (1980), and Salomidi (2003). Our primary objective for this sampling endeavor was to conduct a quantitative assessment of benthic populations within diverse algal assemblages and to assess the variety of benthic fauna associated with each specific assemblage. For every algal grouping, we collected three random samples, each covering a 20 cm x 20 cm area. In total, we sampled 15 distinct algal associations, which are itemized in the table provided. Our sampling location was the Karaburun Peninsula, chosen based on guidance from the distribution and vitality map of Lithophyllum byssoides rims, as documented by Blanfuné et al. in (2016) (Figure 1).
Our rationale for selecting the 20x20 cm sample size was twofold; it allowed us to focus on the dominant species that constituted over 90% of the algal composition in each sample, and it helped minimize any potential impact on the habitat resulting from the sampling process. We conducted the collection by carefully scraping the substrate using metallic tools and subsequently preserved the collected material in a solution of 4% formaldehyde. These samples were then transported to the laboratory for identification, employing appropriate instruments such as stereomicroscopes and determination keys.

Data analyses

The analysis of epifaunal community data aimed to compare community structure and diversity across two host algal species, and sites. To assess differences in assemblages hosted by different macroalgal samples in terms of morphologies and spatiotemporal variations, we utilized the Bray–Curtis dissimilarity index (Bray & Curtis, 1957) and visualized these dissimilarities through non-metric multidimensional scaling (NMDS). Additionally, we employed Analysis of Similarities (ANOSIM) to test for variations in community structure among different algal species. These analyses were conducted using the PERMANOVA statistical method (Anderson 2001).
To investigate the influence of algal morphology and algal species on the diversity of epifauna associated with macroalgae, we employed a multiple linear regression model. We quantified diversity using the Shannon–Wiener diversity index for each epifaunal community within a macroalgal sample, which served as the response variable. Furthermore, we conducted an

analysis to identify the trophic structure of each algal species. A comprehensive model encompassing the mentioned effects and their potential interactions was fitted for these analyses (Gan et al., 2019).

RESULTS AND DISCUSSION

Biodiversity and trophic structure of *Titanoderma trochanter* beds

In the samples of *Titanoderma trochanter* collected during both Spring and Autumn, we identified a total of 109 taxa across the two seasons. During the Summer season, the dominant phylum, in terms of species numbers, is Polychaeta (31 taxa), followed by Mollusca (11 taxa) and Arthropoda (9 taxa). Concerning the abundance of invertebrates during this season, Polychaeta stands out, constituting an average of 32.59% of the invertebrates in the analyzed samples. Mollusca accounts for 24.75%, and Arthropoda for 20.1% (refer to Figure 2).

The proportions of different phyla in terms of both the number of identified taxa and the number of individuals in the samples change notably in the Autumn season. Polychaeta still exhibits the highest species diversity with 24 taxa, followed by Arthropoda (20 taxa) and Mollusca (18 taxa). However, when considering the average number of individuals present in the samples, Mollusca becomes more abundant, making up an average of 55.84% of the counted invertebrates. Arthropoda follows as the second most abundant phylum at 24.83% (figure 2).

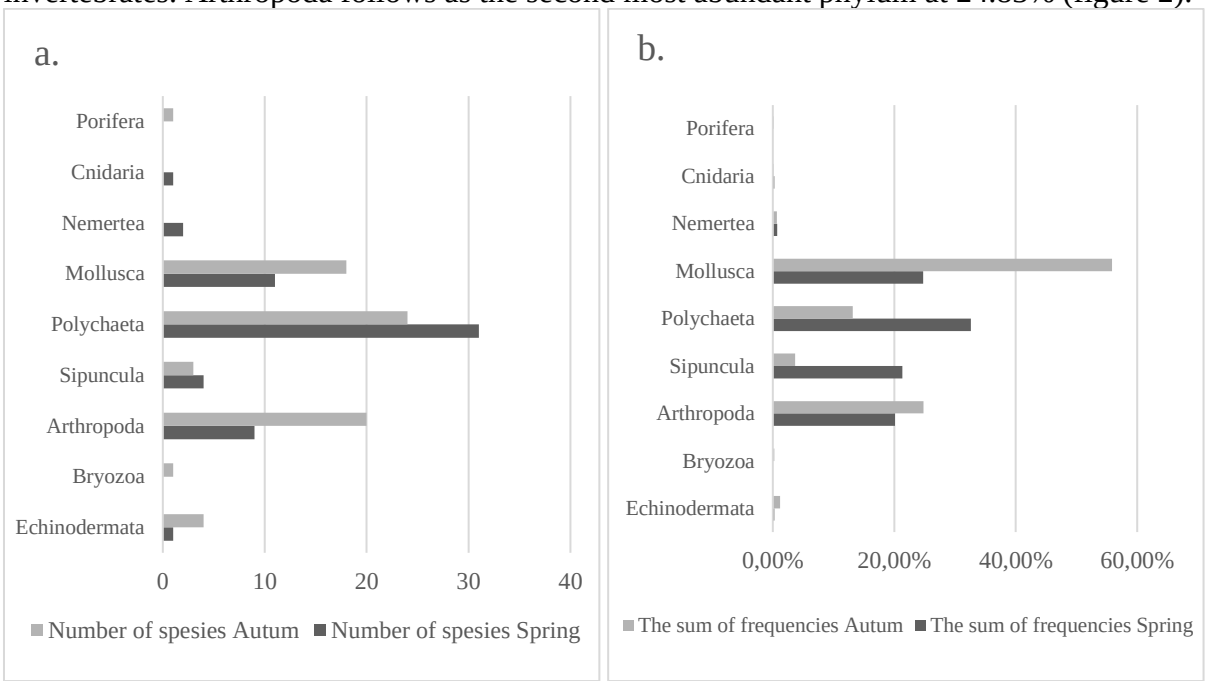

Figure 11. (%DQ - a) Number of taxa present in Titanoderma trochanter algal beds. (%DI - b) Percentages of cumulative abundance of each phylum.

Trophic Analysis and Marine Biotic Index AMBI ecological group analysis

The trophic analysis of the fauna associated with *Titanoderma trochanter* algae has classified them into eight feeding type groups, as outlined in the literature. In instances w.here data was lacking, some groups were denoted as "NE" (Figure 3, Table 1).

The most populous trophic group is the Predators, encompassing 48 identified species, with a significant representation of polychaetes. Regarding the abundance of individuals within the analyzed samples, the Filter Feeders constitute 27.36% of the invertebrates during the spring season and 52.52% during the autumn season. This is primarily attributed to the substantial presence of *Mytilus galloprovincialis* and various sedentary polychaetes. Consequently, there is a notable density of sessile fauna, including barnacles, sedentary polychaetes, and bivalves, which attach themselves to the calcareous structures of these algae thalli.

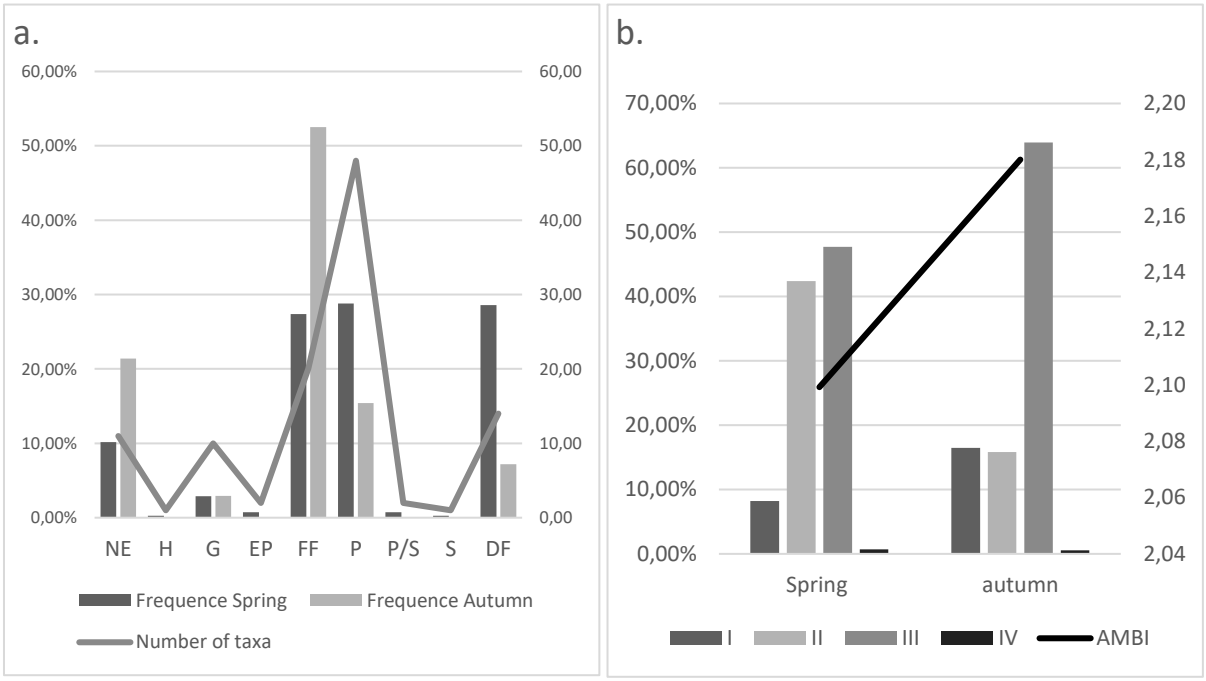

Figure 12. Trophic analysis of Titanoderma trochanter algal beds (a). Percentages of abundance (%DI) according to the feeding guilds feeding guilds (Filter feeder-FF; Predator- P; Grazer-G; Deposit feeder-DF; Not Evaluated-NE; Predator/Scavenger-P/S; Scavenger-S; Ectoparasitic-EP; Herbivore-H) and b. AMBI values at each season and cumulative frequences of AMBI ecological group ((I)- very sensitive to disturbance; (II)- indifferent to disturbance; (III)- tolerant to disturbance; (IV)- second-order opportunistic; Not Evaluated-NE).

To assess the ecological conditions of the sampled waters, we utilized the Marine Biotic Index AMBI and conducted an ecological group analysis. Table 1 presents the AMBI ecological groups, and Figure 3 displays the analysis results. Threshold values for AMBI classification were determined based on recommendations from Muxika et al. (2005) and Borja & Tunberg (2011): 'high quality' <1.2; 'Poor quality' 1.2-3.3; 'Moderate quality' 3.3-4.3; 'Poor quality' 4.3-5.5; 'Bad quality' >5.5.

According to Muxika et al.'s (2005) classification, the Marine Biotic Index AMBI value ranges from 2.10 in spring to 2.18 in autumn. Both of these values fall within the 'Good quality' category for the marine waters where Titanoderma trochanter substrates were sampled.

Table 1. Taxonomic list of species, occurring in *Titanoderma trochanter* beds in 2 seasons (Spr- Spring; Aut-Autumn), with their abundance (N), feeding guilds (Filter feeder-FF; Predator- P; Grazer-G; Deposit feeder-DF; Not Evaluated-NE; Predator/Scavenger-P/S; Scavenger-S; Ectoparasitic-EP; Herbivore-H) and AMBI ecological group ((I)- very sensitive to disturbance; (II)-indifferent to disturbance; (III)- tolerant to disturbance; (IV)- second-order opportunistic; Not Evaluated-NE).

Titanoderma trochanter			**Spring**		**Autumn**	
Species	**Trophic group**	**AMBI group**	**Abudance**	**Frequence**	**Abudance**	**Frequence**
Porifera						
Scalarispongia scalaris (Schmidt, 1862)	FF	(II)	0.00	0.00%	0.33	0.13%
Cnidaria						
Bunodactis verrucosa (Pennant, 1777)	P	(I)	0.33	0.24%	0.00	0.00%
Nemertea						
Leucocephalonemertes aurantiaca (Grube, 1855)	P/S	(II)	0.33	0.24%	0.00	0.00%
Notospermus geniculatus (Delle Chiaje, 1822)	P/S	(II)	0.67	0.48%	0.00	0.00%
Mollusca						
Rhyssoplax corallina (Risso, 1826)	G	NE	0.67	0.48%	4.00	1.60%
Rhyssoplax olivacea (Spengler, 1797)	G	(II)	1.33	0.97%	0.00	0.00%
Acanthochitona crinita (Pennant, 1777)	G	(I)	0.33	0.24%	0.00	0.00%
Acanthochitona fascicularis (Linnaeus, 1767)	G	(I)	1.33	0.97%	0.67	0.27%
Diodora italica (Defrance, 1820)	P	(I)	0.00	0.00%	0.67	0.27%
Tritia unifasciata (Kiener, 1834)	S	(I)	0.33	0.24%	0.00	0.00%
Pisania striata (Gmelin, 1791)	P	NE	0.33	0.24%	0.00	0.00%
Trophonopsis muricata (Montagu, 1803)	P	(I)	0.33	0.24%	0.00	0.00%
Pyrgostylus striatulus (Linnaeus, 1758)	EP	(I)	0.67	0.48%	0.00	0.00%
Bittium reticulatum (da Costa, 1778)	G	(I)	0.00	0.00%	0.33	0.13%
Cerithium vulgatum russoi T. Cossignani, 2021	P	(II)	0.00	0.00%	0.67	0.27%
Rissoa variabilis (Megerle von Mühlfeld, 1824)	G	(I)	0.00	0.00%	0.33	0.13%
Pisania striata (Gmelin, 1791)	P	(IV)	0.00	0.00%	0.67	0.27%
Ocenebra edwardsii (Payraudeau, 1826)	P	(II)	0.00	0.00%	0.33	0.13%
Patella ulyssiponensis Gmelin, 1791	G	(I)	0.00	0.00%	0.33	0.13%
Gibbula turbinoides (Deshayes, 1835)	DF	(I)	0.00	0.00%	2.67	1.06%
Azorinus chamasolen (da Costa, 1778)	DF	(I)	0.00	0.00%	0.33	0.13%
Scaphopoda	DF	(II)	1.33	0.97%	0.33	0.13%
Kellia suborbicularis (Montagu, 1803)	FF	(I)	2.00	1.45%	0.00	0.00%
Striarca lactea (Linnaeus, 1758)	FF	(I)	0.67	0.48%	0.00	0.00%
Musculus costulatus (Risso, 1826)	FF	(I)	0.00	0.00%	9.00	3.59%
Mytilus galloprovincialis Lamarck, 1819	FF	(III)	25.67	18.64%	113.00	45.08%
Pinctada radiata (Leach, 1814)	FF	(II)	0.00	0.00%	5.67	2.26%
Annelida						
Lumbrineris sp Blainville, 1828	P	(III)	0.33	0.24%	0.00	0.00%
Aphrodita perarmata Roule, 1898	P	(III)	0.33	0.24%	0.00	0.00%
Pontogenia chrysocoma (Baird, 1865)	P	(III)	0.33	0.24%	1.00	0.40%
Eunoe hubrechti (McIntosh, 1900)	P	(III)	0.00	0.00%	0.33	0.13%
Lepidonotus clava (Montagu, 1808)	P	(III)	10.67	7.75%	10.33	4.12%
Ceratonereis (Composetia) costae (Grube, 1840)	P	(II)	3.67	2.66%	0.00	0.00%
Hediste diversicolor (O.F. Müller, 1776)	P	(III)	1.00	0.73%	0.00	0.00%
Alitta succinea (Leuckart, 1847)	P	(III)	0.00	0.00%	1.33	0.53%
Neanthes fucata (Savigny, 1822)	P	(III)	1.33	0.97%	0.00	0.00%
Neanthes nubila (Savigny, 1822)	P	(III)	0.33	0.24%	0.00	0.00%
Neanthes acuminata (Ehlers, 1868)	P	(III)	0.00	0.00%	2.33	0.93%
Nereis sp. Linnaeus, 1758	P	(III)	0.00	0.00%	0.33	0.13%
Nereis pelagica Linnaeus, 1758	P	(III)	1.00	0.73%	4.67	1.86%
Hesione splendida Lamarck, 1818	P	(II)	0.67	0.48%	0.00	0.00%
Nereis persica Fauvel, 1913	P	(III)	8.67	6.30%	2.00	0.80%
Perinereis Kinberg, 1865	P	(III)	0.33	0.24%	0.00	0.00%
Perinereis macropus (Claparède, 1870)	P	(III)	0.33	0.24%	0.00	0.00%
Perinereis oliveirae (Horst, 1889)	P	(III)	0.00	0.00%	0.67	0.27%
Platynereis coccinea (Delle Chiaje, 1822)	P	(III)	0.33	0.24%	0.00	0.00%
Syllidae Grube, 1850	P	(III)	0.33	0.24%	0.67	0.27%
Odontosyllis Claparède, 1863	P	(III)	0.67	0.48%	0.00	0.00%

Syllis amicarmillaris Simon, San Martín & Robinson, 2014	P	(II)	0.00	0.00%	1.00	0.40%
Syllis hyalina Grube, 1863	P	(II)	1.67	1.21%	0.00	0.00%
Syllis krohnii Ehlers, 1864	P	(II)	3.00	2.18%	2.33	0.93%
Syllis prolifera Krohn, 1852	P	(II)	1.00	0.73%	0.00	0.00%
Syllis variegata Grube, 1860	P	(II)	0.33	0.24%	0.67	0.27%
Syllis vittata Grube, 1840	P	(II)	0.33	0.24%	0.00	0.00%
Syllis gracilis Grube, 1840	P	(II)	0.00	0.00%	0.33	0.13%
Trypanosyllis zebra (Grube, 1860)	P	(II)	0.67	0.48%	0.00	0.00%
Nephtys Cuvier, 1817	P	(II)	0.33	0.24%	0.00	0.00%
Syllis variegata Grube, 1860	P	(II)	0.00	0.00%	0.67	0.27%
Nereiphylla rubiginosa (de Saint-Joseph, 1888)	P	(II)	0.00	0.00%	0.67	0.27%
Phyllodoce laminosa Savigny in Lamarck, 1818	P	(II)	0.00	0.00%	0.33	0.13%
Phyllodoce schmardaei Day, 1963	P	(II)	0.00	0.00%	0.33	0.13%
Sabellidae Latreille, 1825	P	(II)	0.00	0.00%	1.33	0.53%
Eulalia pusilla Ørsted, 1843	P	(II)	0.67	0.48%	0.00	0.00%
Bathyvermilia langerhansi (Fauvel, 1909)	FF	(II)	1.33	0.97%	0.00	0.00%
Filograna sp. Berkeley, 1835	FF	(II)	0.33	0.24%	0.00	0.00%
Serpula Linnaeus, 1758	FF	(II)	1.33	0.97%	0.00	0.00%
Serpula vermicularis Linnaeus, 1767	FF	(II)	1.33	0.97%	0.33	0.13%
Spirobranchus Blainville, 1818	FF	(II)	0.33	0.24%	0.00	0.00%
Vermiliopsis Saint-Joseph, 1894	FF	(II)	0.00	0.00%	0.33	0.13%
Vermiliopsis infundibulum (Philippi, 1844)	FF	(II)	0.00	0.00%	1.00	0.40%
Dodecaceria concharum Örsted, 1843	FF	(IV)	0.00	0.00%	0.33	0.13%
Polycirrus Grube, 1850	FF	(IV)	0.00	0.00%	0.33	0.13%
Janua heterostropha (Montagu, 1803)	FF	(II)	0.33	0.24%	0.00	0.00%
Spirorbis sp. Daudin, 1800	FF	(IV)	1.00	0.73%	0.00	0.00%
Sipuncula						
Aspidosiphon muelleri Diesing, 1851	DF	(I)	1.00	0.73%	0.67	0.27%
Phascolosoma granulatum Leuckart, 1828	DF	(II)	24.00	17.43%	6.00	2.39%
Golfingia vulgaris (de Blainville, 1827)	DF	(I)	1.33	0.97%	0.00	0.00%
Phascolion strombus (Montagu, 1804)	DF	(II)	2.67	1.94%	2.67	1.06%
Crustacea						
Megabalanus tintinnabulum (Linnaeus, 1758)	FF	(II)	3.33	2.42%	0.00	0.00%
Achelia echinata Hodge, 1864	EP	(I)	0.33	0.24%	0.00	0.00%
Nototropis massiliensis (Bellan-Santini, 1975)	P	(I)	0.33	0.24%	0.00	0.00%
Caprellidae Leach, 1814	H	(I)	0.33	0.24%	0.00	0.00%
Autonoe spiniventris Della Valle, 1893	DF	(I)	0.33	0.24%	0.00	0.00%
Unciolella lunata Chevreux, 1911	DF	(I)	1.33	0.97%	0.00	0.00%
Amphithopsis depressa Schiecke, 1976	NE	(I)	0.00	0.00%	0.33	0.13%
Elasmopus brasiliensis (Dana, 1853)	NE	(III)	14.00	10.17%	18.67	7.45%
Elasmopus pectenicrus (Spence Bate, 1862)	NE	(III)	0.00	0.00%	3.00	1.20%
Stenothoe marina (Spence Bate, 1857)	NE	(II)	0.00	0.00%	0.33	0.13%
Ampelisca anophthalma Bellan-Santini, Kaim-Malka, 1977	NE	(II)	0.00	0.00%	1.00	0.40%
Microprotopus maculatus Norman, 1867	NE	(I)	0.00	0.00%	1.33	0.53%
Apohyale crassipes (Heller, 1866)	NE	(II)	0.00	0.00%	3.67	1.46%
Ptilohyale eburnea (Krapp-Schickel, 1974)	NE	(I)	0.00	0.00%	22.00	8.78%
Joeropsis brevicornis brevicornis Koehler, 1885	NE	(II)	0.00	0.00%	1.33	0.53%
Joeropsis sp. Koehler, 1885	NE	NE	0.00	0.00%	1.33	0.53%
Anthura gracilis (Montagu, 1808)	NE	(I)	0.00	0.00%	0.67	0.27%
Gnathia phallonajopsis Monod, 1925	DF	NE	0.00	0.00%	0.33	0.13%
Microdeutopus Costa, 1853	DF	(II)	0.00	0.00%	0.33	0.13%
Dynamene edwardsi (Lucas, 1849)	DF	(II)	2.00	1.45%	0.67	0.27%
Dynamenella sheareri (Hatch, 1947)	DF	(II)	5.33	3.87%	1.67	0.66%
Paracerceis sp. Hansen, 1905	DF	(II)	0.00	0.00%	2.33	0.93%
Eriphia verrucosa (Forskål, 1775)	P	(III)	0.00	0.00%	2.00	0.80%
Thia scutellata (JC Fabricius, 1793)	P	(II)	0.00	0.00%	0.33	0.13%
Pachygrapsus marmoratus (J.C. Fabricius, 1787)	P	(II)	0.00	0.00%	1.33	0.53%
Bryozoa						
Patinella radiata (Audouin, 1826)	FF	(II)	0.00	0.00%	0.67	0.27%
Perforatus perforatus (Bruguière, 1789)	FF	(II)	0.00	0.00%	0.67	0.27%
Echinodermata						
Echinoidea	G	NE	0.00	0.00%	1.00	0.40%
Arbaciella elegans Mortensen, 1910	G	(I)	0.33	0.24%	0.00	0.00%

Psammechinus microtuberculatus (Blainville, 1825)	G	(I)	0.00	0.00%	0.67	0.27%
Amphipholis squamata (Delle Chiaje, 1828)	P	(I)	0.00	0.00%	1.33	0.53%

Biodiversity and trophic structure of *Ellisolandia elongata* beds

The analysis of substrate samples containing *Ellisolandia elongata* beds revealed a total of 110 taxa, with 67 taxa identified during the spring season and 76 taxa during the autumn season. Polychaetas exhibited the highest diversity in terms of the number of species, with 32 species present during spring and 37 species during autumn. In contrast, the diversity of Molluska and Arthropoda associated with this algae was relatively low, comprising only 12 species for each phylum. However, it's worth noting that the number of Echinodermata species increased significantly, with 7 species identified in this habitat (Table 2; Figure 4).

These findings underscore the seasonal variations in species diversity within *Ellisolandia elongat*a beds, with Polychaetas dominating and Echinodermata showing an uptick in species richness. The contrast in diversity levels among different phyla highlights the complex ecological dynamics at play in this particular substrate environment across different seasons.

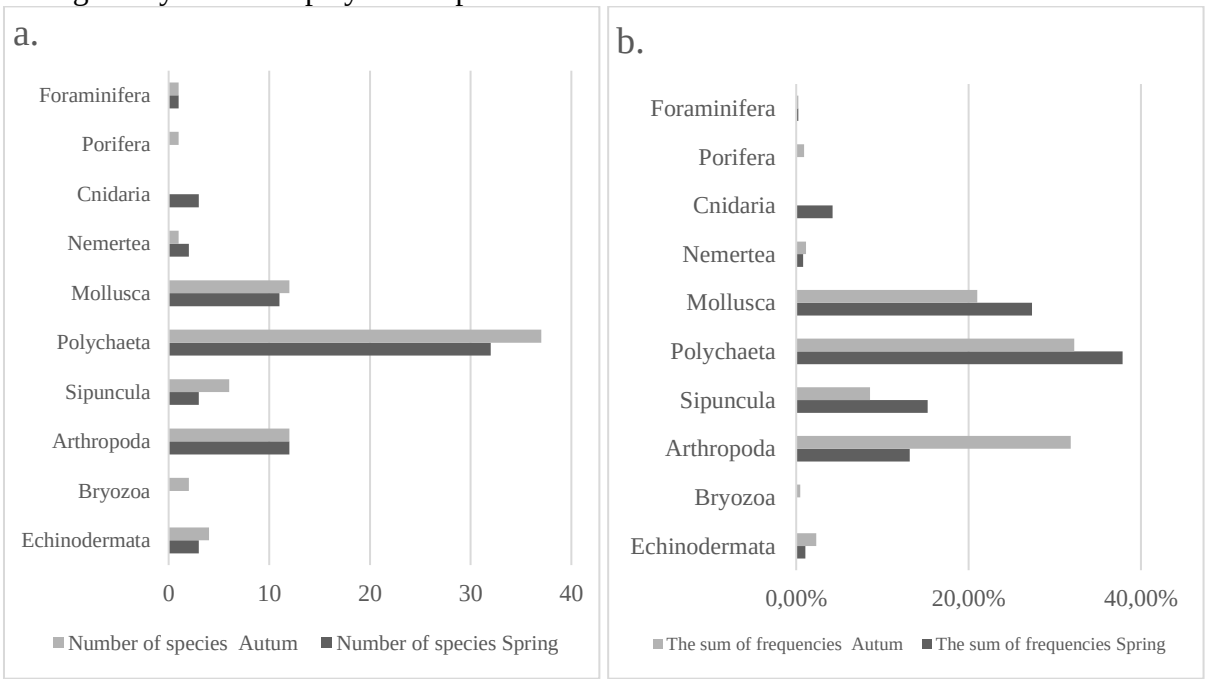

Figure 13. (%DQ - a) Number of taxa present in Ellisolandia elongata algal beds. (%DI - b) Percentages of cumulative abundance of each phylum.

The analysis of the average frequency of invertebrates present in the samples of *Ellisolandia elongata* reveals intriguing seasonal variations in the composition. During the spring season, approximately 38% of the invertebrates comprise polychaeta, whereas in the autumn season, this percentage decreases slightly to around 33%. This shift is primarily attributed to the notable abundance of species belonging to the Nereididae and Syllidae families, both of which exhibit the highest number of individuals within the examined samples.

Moving on to Mollusca, their abundance makes up about 27% of the samples during the summer season, and this percentage decreases to 21% in the autumn season. This fluctuation can be attributed to the high-density presence of two bivalve mollusks, namely *Musculus costulatus* and *Mytilus galloprovincialis*, which contribute significantly to the overall composition.

Crustaceans, on the other hand, show an interesting pattern. Their average abundance experiences a peak of approximately 31%, driven primarily by the substantial presence of two amphipoda species, *Elasmopus rapax* and *Apohyale crassipes*, during the periods under consideration.

These findings illustrate the dynamic nature of the *Ellisolandia elongata* habitat, with distinct invertebrate groups exhibiting varying degrees of abundance and dominance across different seasons. This highlights the intricate interplay between ecological factors and the community structure of this substrate environment throughout the year.

Trophic Analysis and Marine Biotic Index AMBI ecological group analysis

The trophic analysis of *Ellisondia elongata* unveils a noteworthy dominance of Predators (41.35% in spring and 30.72% in autumn) in terms of both species diversity and average abundance during the sampled seasons (Figure 5). This prevalence is attributed to the rich diversity of polychaetes and echinodermates present in the examined epiphytic fauna. Following Predators, the Filter Feeders group (30.26% in spring and 21.02% in autumn) takes center stage, primarily composed of sedentary bivalves, barnacles, and polychaetes. Additionally, there is a notable abundance of Deposit Feeders, mainly consisting of mollusks and arthropods.

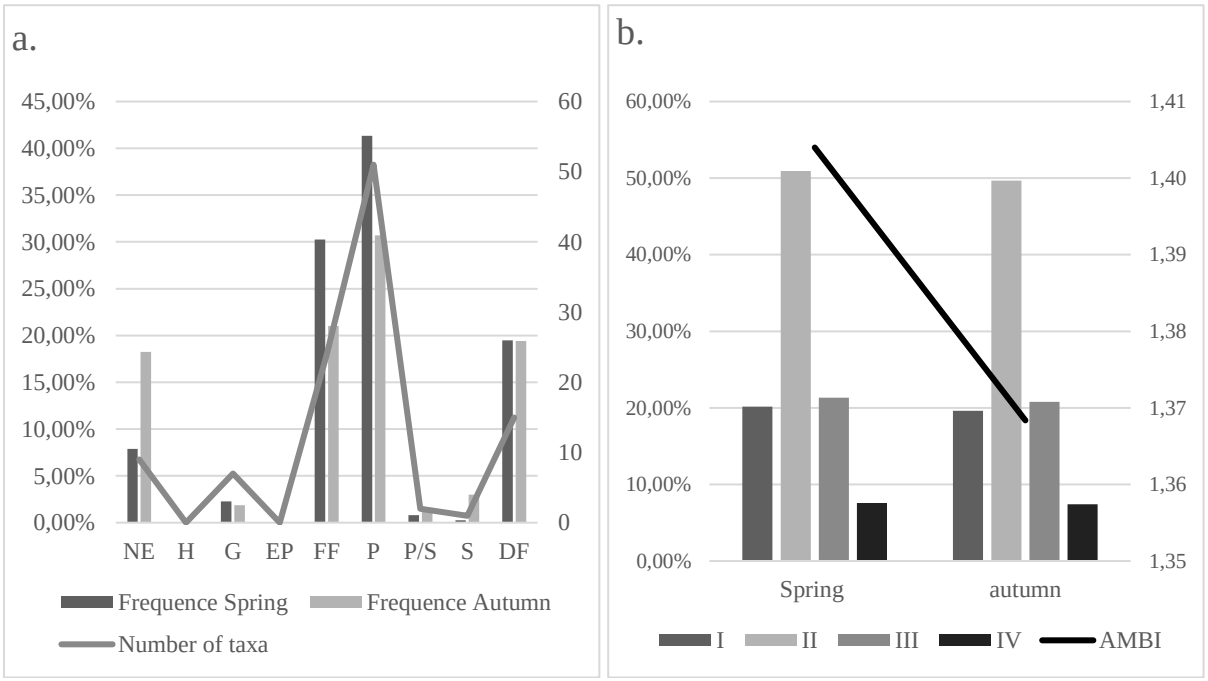

Figure 14. Trophic analysis of Ellisolandia elongata algal beds (a). Percentages of abundance (%DI) according to the feeding guilds feeding guilds (Filter feeder-FF; Predator- P; Grazer-G; Deposit feeder-DF; Not Evaluated-NE; Predator/Scavenger-P/S; Scavenger-S; Ectoparasitic-EP; Herbivore-H) and b. AMBI values at each season and cumulative frequences of AMBI ecological group ((I)- very sensitive to disturbance; (II)-indifferent to disturbance; (III)- tolerant to disturbance; (IV)- second-order opportunistic; Not Evaluated-NE).

In evaluating the ecological conditions of the sampled waters, we employed the Marine Biotic Index AMBI and conducted an analysis of ecological groups. Table 1 provides an overview of the AMBI ecological groups, while Figure 3 visually represents the analysis results. The cumulative frequency of AMBI ecological groups highlights the dominance of group II, often categorized as "indifferent to disturbance." This predominance is a consequence of the high density of polychaetes, sipunculids, and mollusks within the composition.

According to the classification proposed by Muxika et al. (2005), the Marine Biotic Index AMBI ranges from 1.40 in spring to 1.36 in autumn. Notably, both of these values fall within the 'Good quality' category for the marine waters where Ellisondia elongata substrates were

sampled. These findings indicate a favorable ecological state in the studied marine environment during the seasons under investigation.

Table 2. Taxonomic list of species, occurring in *Ellisolandia elongata* beds in 2 seasons (Spr-Spring; Aut-Autumn), with their abundance (N), feeding guilds (Filter feeder-FF; Predator- P; Grazer-G; Deposit feeder-DF; Not Evaluated-NE; Predator/Scavenger-P/S; Scavenger-S; Ectoparasitic-EP; Herbivore-H) and AMBI ecological group ((I)- very sensitive to disturbance; (II)-indifferent to disturbance; (III)- tolerant to disturbance; (IV)- second-order opportunistic; Not Evaluated-NE).

Ellisolandia elongata			**Spring**		**Autumn**	
Species Foraminifera	**Trophic group**	**AMBI group**	**Abudance**	**Frequence**	**Abudance**	**Frequence**
Miniacina miniacea (Pallas, 1766)	FF	(I)	0.33	0.26%	0.33	0.23%
Porifera						
Scalarispongia scalaris (Schmidt, 1862)	FF	(II)	0.00	0.00%	1.33	0.92%
Cnidaria						
Actinia equina (Linnaeus, 1758)	FF	(I)	0.33	0.26%	0.00	0.00%
Bunodactis verrucosa (Pennant, 1777)	P	(I)	0.33	0.26%	0.00	0.00%
Exaiptasia diaphana (Rapp, 1829)	FF	(II)	4.67	3.68%	0.00	0.00%
Nemertea						
Nemertea	P/S	(II)	0.33	0.26%	1.67	1.15%
Tubulanus annulatus (Montagu, 1804)	P/S	(II)	0.67	0.53%	0.00	0.00%
Mollusca						
Acanthochitona fascicularis (Linnaeus, 1767)	G	(I)	0.33	0.26%	0.00	0.00%
Rhyssoplax olivacea (Spengler, 1797)	G	(II)	0.67	0.53%	0.00	0.00%
Cymbula safiana (Lamarck, 1819)	G	(I)	0.33	0.26%	0.00	0.00%
Bittium reticulatum (da Costa, 1778)	G	(I)	0.67	0.53%	0.33	0.23%
Alvania lineata Risso, 1826	DF	(I)	0.00	0.00%	2.00	1.39%
Pseudofusus margaritae (Buzzurro & Russo, 2007)	P	(I)	0.00	0.00%	7.33	5.08%
Tritia louisi (Pallary, 1912)	S	NE	0.33	0.26%	4.33	3.00%
Pisania striata (Gmelin, 1791)	P	(IV)	1.33	1.05%	0.00	0.00%
Muricopsis cristata (Brocchi, 1814)	P	(I)	0.00	0.00%	0.33	0.23%
Pusia granum (Forbes, 1844)	P	(I)	0.00	0.00%	0.33	0.23%
Patella ulyssiponensis Gmelin, 1791	G	(I)	0.00	0.00%	0.33	0.23%
Steromphala rarilineata (Michaud, 1829)	G	(I)	0.00	0.00%	2.00	1.39%
Ocinebrina edwardsii (Payraudeau, 1826)	P	(II)	0.67	0.53%	0.00	0.00%
Scaphopoda	DF	(II)	1.33	1.05%	0.00	0.00%
Parvicardium trapezium Cecalupo & Quadri, 1996	FF	NE	0.00	0.00%	0.33	0.23%
Parvicardium scriptum (Bucquoy & Dollfus, 1892)	FF	(I)	1.33	1.05%	0.00	0.00%
Musculus costulatus (Risso, 1826)	FF	(I)	18.67	14.74%	0.33	0.23%
Mytilus galloprovincialis Lamarck, 1819	FF	(III)	9.00	7.11%	12.33	8.55%
Polycheta						
Polychaeta	P	(III)	0.67	0.53%	0.00	0.00%
Pontogenia chrysocoma (Baird, 1865)	P	(III)	0.00	0.00%	0.33	0.23%
Lepidonotus clava (Montagu, 1808)	P	(III)	8.33	6.58%	0.33	0.23%
Lepidonotus squamatus (Linnaeus, 1758)	P	(III)	0.33	0.26%	0.00	0.00%
Harmothoe Kinberg, 1856	P	(II)	0.00	0.00%	0.33	0.23%
Harmothoe impar (Johnston, 1839)	P	(II)	0.00	0.00%	0.33	0.23%
Dorvillea Parfitt, 1866	P	(II)	1.00	0.79%	0.00	0.00%
Dorvillea rubrovittata (Grube, 1855)	P	(II)	0.33	0.26%	0.00	0.00%
Lysidice ninetta Audouin & H Milne Edwards, 1833	P	(II)	0.67	0.53%	2.33	1.62%
Glycera tridactyla Schmarda, 1861	P	(II)	0.00	0.00%	0.33	0.23%
Hesionidae Grube, 1850	P	(II)	0.00	0.00%	0.67	0.46%
Lumbrineris Blainville, 1828	P	(III)	0.67	0.53%	0.00	0.00%
Arabella iricolor (Montagu, 1804)	P	(I)	0.33	0.26%	0.00	0.00%
Nereididae Blainville, 1818	P	(III)	0.33	0.26%	5.67	3.93%
Ceratonereis (Composetia) costae (Grube, 1840)	P	(III)	0.33	0.26%	0.33	0.23%
Hediste diversicolor (O.F. Müller, 1776)	P	(III)	1.00	0.79%	0.00	0.00%
Neanthes acuminata (Ehlers, 1868)	P	(III)	0.67	0.53%	0.67	0.46%
Neanthes nubila (Savigny, 1822)	P	(III)	3.00	2.37%	0.33	0.23%
Nereis Linnaeus, 1758	P	(III)	1.67	1.32%	0.00	0.00%
Nereis pelagica Linnaeus, 1758	P	(III)	4.00	3.16%	0.33	0.23%
Nereis splendida Grube, 1840	P	(III)	0.33	0.26%	0.33	0.23%
Nereis zonata persica Fauvel, 1913	P	(III)	5.33	4.21%	3.33	2.31%
Perinereis marionii (Audouin & Milne Edwards, 1833)	P	(III)	1.67	1.32%	4.00	2.77%
Streptosyllis Webster & Benedict, 1884	P	(III)	0.00	0.00%	0.33	0.23%

Odontosyllis cucullata (McIntosh, 1908)	P	(III)	0.00	0.00%	1.33	0.92%
Haplosyllis spongicola (Grube, 1855)	P	(III)	1.67	1.32%	0.00	0.00%
Salvatoria limbata (Claparède, 1868)	P	(II)	0.00	0.00%	7.00	4.85%
Syllis Lamarck, 1818	P	(II)	0.33	0.26%	0.00	0.00%
Syllis amicarmillaris Simon, San Martín, Robinson, 2014	P	(II)	0.33	0.26%	0.33	0.23%
Syllis gracilis Grube, 1840	P	(II)	2.00	1.58%	0.33	0.23%
Syllis krohnii Ehlers, 1864	P	(II)	3.67	2.89%	0.33	0.23%
Syllis prolifera Krohn, 1852	P	(II)	2.00	1.58%	0.33	0.23%
Syllis variegata Grube, 1860	P	(II)	2.33	1.84%	0.67	0.46%
Naiades cantrainii Delle Chiaje, 1830	P	(II)	0.67	0.53%	0.67	0.46%
Eulalia viridis (Linnaeus, 1767)	P	(II)	1.00	0.79%	0.00	0.00%
Myxicola infundibulum (Montagu, 1808)	FF	(II)	0.00	0.00%	0.67	0.46%
Protula anomala Day, 1955	FF	(II)	0.00	0.00%	1.33	0.92%
Phyllodoce Lamarck, 1818	P	(II)	0.67	0.53%	0.33	0.23%
Phyllodoce laminosa Savigny in Lamarck, 1818	P	(II)	0.00	0.00%	0.33	0.23%
Janua heterostropha (Montagu, 1803)	FF	(II)	0.33	0.26%	0.00	0.00%
Maldanidae Malmgren, 1867	FF	(II)	0.67	0.53%	0.00	0.00%
Polyophthalmus pictus (Dujardin, 1839)	DF	(II)	1.33	1.05%	0.67	0.46%
Fam. Scalibregmatidae Malmgren, 1867	DF	(I)	0.33	0.26%	0.00	0.00%
Serpula vermicularis Linnaeus, 1767	FF	(II)	0.00	0.00%	0.33	0.23%
Janua heterostropha (Montagu, 1803)	FF	(II)	0.00	0.00%	0.33	0.23%
Cirriformia tentaculata (Montagu, 1808)	FF	(II)	0.00	0.00%	0.33	0.23%
Flabelligera affinis M. Sars, 1829	FF	(II)	0.00	0.00%	0.33	0.23%
Ampharete Malmgren, 1866	FF	(II)	0.00	0.00%	0.33	0.23%
Anobothrus gracilis (Malmgren, 1866)	FF	(II)	0.00	0.00%	10.00	6.93%
Polycirrus Grube, 1850	FF	(II)	0.00	0.00%	1.00	0.69%
Capitellidae Grube, 1862			0.00	0.00%	1.00	0.69%
Sipuncula						
Phascolosoma granulatum Leuckart, 1828	DF	(II)	17.67	13.95%	0.67	0.46%
Golfingia elongata (Keferstein, 1862)	DF	(I)	0.00	0.00%	2.33	1.62%
Golfingia vulgaris (de Blainville, 1827)	DF	(I)	0.00	0.00%	1.00	0.69%
Phascolion strombus (Montagu, 1804)	DF	(II)	1.67	1.32%	2.67	1.85%
Sipunculus nudus Linnaeus, 1766	DF	(I)	0.00	0.00%	5.33	3.70%
Aspidosiphon muelleri Diesing, 1851	DF	(I)	0.00	0.00%	0.33	0.23%
Arthropoda						
Perforatus perforatus (Bruguière, 1789)	FF	NE	0.67	0.53%	0.00	0.00%
Adna anglica Sowerby, 1823	FF	NE	1.00	0.79%	0.00	0.00%
Leucothoe incisa Robertson, 1892	FF	(I)	1.33	1.05%	0.00	0.00%
Gammaropsis crenulata Krapp-Schickel & Myers, 1979	NE	NE	1.33	1.05%	0.67	0.46%
Autonoe spiniventris Della Valle, 1893	DF	(I)	2.00	1.58%	0.00	0.00%
Monomia sp. Gistel, 1848	NE	NE	0.00	0.00%	0.67	0.46%
Gammarus sp. Fabricius, 1775	NE	NE	3.67	2.89%	2.33	1.62%
Pasiphaea multidentata Esmark, 1866	NE	NE	0.00	0.00%	2.33	1.62%
Apohyale crassipes (Heller, 1866)	NE	(II)	4.33	3.42%	21.67	15.01%
Anthura gracilis (Montagu, 1808)	NE	(I)	0.33	0.26%	0.00	0.00%
Anthuroidea Leach, 1814	NE	(I)	0.00	0.00%	0.33	0.23%
Cymodoce truncata Leach, 1814	NE	(I)	0.00	0.00%	0.33	0.23%
Dynamene curalii Holdich & Harrison, 1980	NE	(I)	0.33	0.26%	0.00	0.00%
Dynamenella sheareri (Hatch, 1947)	DF	(II)	0.33	0.26%	0.00	0.00%
Dynamene edwardsi (Lucas, 1849)	DF	(II)	0.00	0.00%	0.67	0.46%
Elasmopus pectenicrus (Spence Bate, 1862)	DF	(II)	0.00	0.00%	3.33	2.31%
Elasmopus rapax Costa, 1853	DF	(II)	0.00	0.00%	9.00	6.24%
Acanthonyx lunulatus (Risso, 1816)	P	(I)	1.00	0.79%	4.33	3.00%
Eriphia verrucosa (Forskål, 1775)	P	(III)	0.33	0.26%	0.33	0.23%
Bryozoa						
Cellepora sp. Linnaeus, 1767	FF	(II)	0.00	0.00%	0.33	0.23%
Patinella radiata (Audouin, 1826)	FF	(II)	0.00	0.00%	0.67	0.46%
Echinodermata						
Asterina gibbosa (Pennant, 1777)	P	(I)	0.33	0.26%	0.00	0.00%
Amphiura sp. Forbes, 1843	P	(I)	0.33	0.26%	0.00	0.00%
Amphiura chiajei Forbes, 1843	P	(II)	0.67	0.53%	0.00	0.00%
Amphipholis squamata (Delle Chiaje, 1828)	P	(I)	0.67	0.46%	0.00	0.00%
Amphiura filiformis (O.F. Müller, 1776)	P	(I)	0.67	0.46%	0.00	0.00%
Ophiura ophiura (Linnaeus, 1758)	P	(II)	1.00	0.69%	0.00	0.00%
Psammechinus microtuberculatus (Blainville, 1825)	G	(I)	1.00	0.69%	0.00	0.00%

Comparative analysis

In our comprehensive study, we meticulously assessed the Shannon–Wiener index for the two host algae across each of the distinct seasons. The Shannon index exhibited a noteworthy range, spanning from 3.31 to 3.81, which signifies a high biodiversity value. Intriguingly, the values at the upper end of this range were consistently associated with Titanoderma trochanter in both seasons, as depicted in Figure 6. This underscores the prominent role of Titanoderma trochanter in hosting a diverse array of species, contributing to the overall biodiversity of the ecosystem.

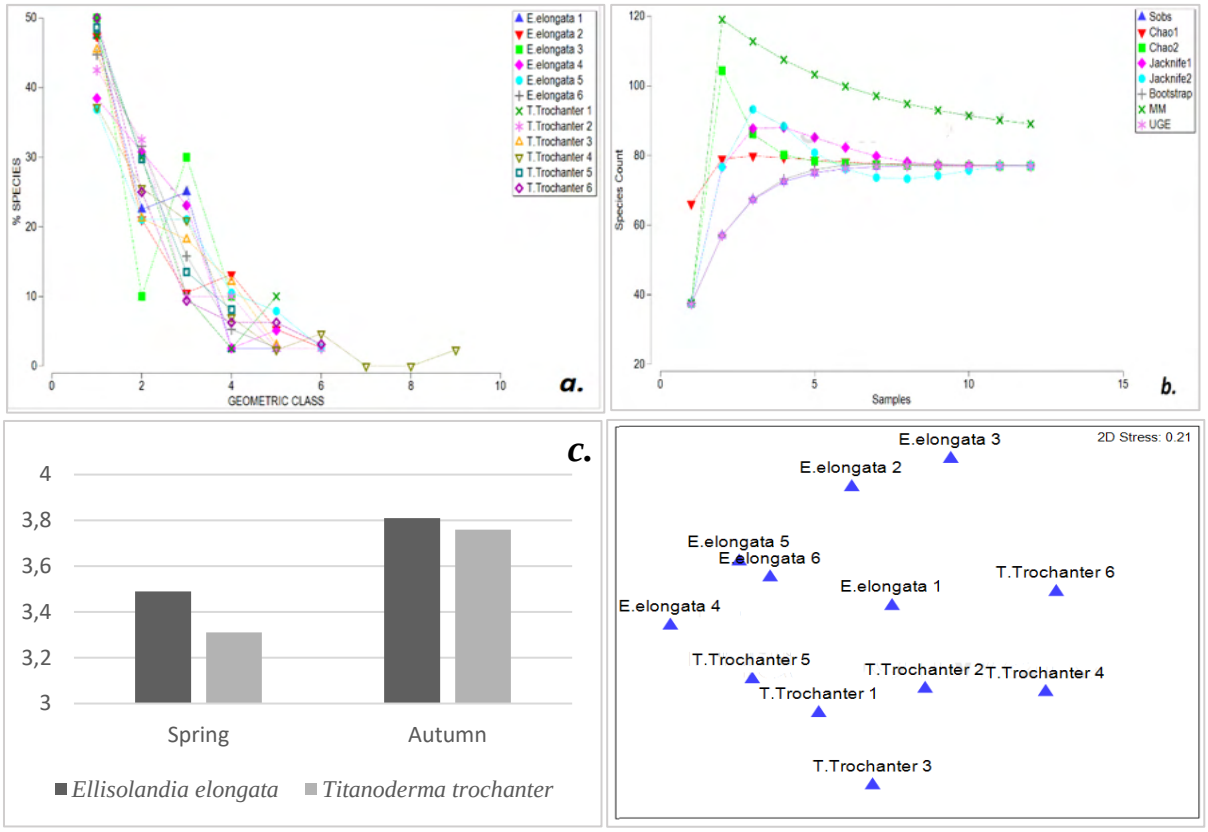

Figure 15. Comparative analysis berween *Ellisolandia elongata* e *Titanoderma trochanter*: a. Geometric class plot; b. Species accumulation plot; c. Shannon–Wiener index; d. Bray-Curtis similarity analisis.

Furthermore, the results obtained from the Bray-Curtis similarity analysis shed light on the specific composition of the associated fauna within the two host algae species. It is evident that the similarity between these compositions remains relatively low. Interestingly, when comparing samples collected in close geographical proximity, a greater degree of similarity is observed in the associated fauna, whereas samples from locations with distinct geographical proximity display a lower level of similarity. This intriguing pattern suggests that geographical proximity plays a pivotal role in shaping the composition of the associated fauna.

Moreover, our analysis employed both the geometric class plot and species accumulation plot to gain deeper insights into the composition of species and the abundance ratios among them. These analyses revealed striking differences in species composition and abundance ratios, further underscoring the complex dynamics and unique ecological characteristics of the studied substrate environments. These findings emphasize the importance of considering not only the diversity of species but also their relative abundance and distribution patterns in the assessment of ecological conditions and habitat quality.

CONCLUSIONS

This research represents a pioneering effort, as it stands among the initial endeavors to comprehensively investigate both the composition of macroalgae-associated assemblages and their trophic structure by leveraging macrozoobenthic fauna assemblages. The amalgamation of these two distinct but interconnected approaches has provided us with a profound understanding of the intricate dynamics governing invertebrate communities associated with each seaweed species.

Remarkably, both *Titanoderma trochanter* and *Ellisollandia elongata*, despite their classification as calcareous algae with relatively modest nutritional profiles, have proven to be thriving hosts for a diverse array of invertebrates. Within these assemblages, annelids and mollusks dominate, contributing significantly to the richness of the associated fauna. This intriguing similarity in the composition of associated assemblages challenges conventional expectations, suggesting that a substantial portion of the associated fauna may not necessarily depend on the host algae for sustenance. Instead, it underscores the pivotal role played by sediment, which becomes trapped within the intricate three-dimensional structures of these algae, serving as a foundational component of the local food web.

Given the paramount influence of nutritional factors in structuring macroalgae-associated assemblages, as noted in prior studies (Norderhaug et al. 2003; Schaal et al. 2010), we advocate for the continued development of integrated approaches, similar to the one employed in this study, to further unravel the ecological intricacies of these ecosystems. Rocky shores, as recognized, are extraordinarily dynamic environments where an array of processes unfolds at varying spatial and temporal scales (Burrows et al. 2008; Benedetti-Cecchi & Trussell 2014; Gauthier et al., 2016). While this study has unveiled statistically significant findings, signifying differences among understory algae-associated invertebrate assemblages at the micro-scale, it's worth acknowledging that our sampling was limited to just three replicates, providing a somewhat confined spatial perspective.

Consequently, there remains an intriguing avenue for future research to explore the multifaceted interplay of factors acting at diverse spatial scales, encompassing hydrodynamics, substrate orientation, temperature, and more (Gauthier et al., 2016). Such investigations will inevitably shed light on the nuanced structure and functioning of these assemblages, ultimately influencing biodiversity patterns within rocky shore ecosystems. The complexity and variability inherent to these coastal habitats beckon for continued exploration and understanding, promising new insights into the intricate relationships that define them.

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PRELIMINARY STUDY ON HEALTH INDICATORS OF UNWEANED CALVES FED WITH A PREBIOTIC BASED ON SACCHAROMYCES CEREVISIAE (AVIATOR®)

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ABSTRACT

The objective of this study was to investigate the effect of supplementation with a prebiotic *saccharomyces cerevisiae* yeast culture (Aviator®) on the health indicators of unweaned calves. The study involved 24 unweaned Holstein calves (average age = 15 days) over a period of 8 weeks (W1 to W8). The calves were assigned into three homogeneous groups. Each group was composed of 8 calves (4 males and 4 females). The first control group (C) received the conventional feed (milk without prebiotic). The second group (T1) received milk supplemented with 7g/calf/day of Aviator®, and the third group (T2) received milk supplemented with 14g/calf/day Aviator®. The Health parameters of the calves were noted such as coat condition, presence or absence of diarrhea and bronchitis. Calf feces were collected for bacteriological analysis. The results showed that 21% of the calves had an abnormal coat, while 46% had a coat soiled by feces. The rates of diarrhea and bronchitis were noted in 15% of and 17% of the calves, respectively. Besides, the rate of diarrhea occurrence in all calves decreased from W1 to W3 (8% *vs* 4%) and it was lower in the T1 and T2 groups compared to the C group (1.5% and 3% *vs* 8%, $p<0.05$). However, the rate of bronchitis cases ranged from 4.5% to 37% over the period of the trial. It was lower in the C group compared to the treated groups T1 and T2 (6.5% *vs* 12.5% and 31.5%). Bacteriological analysis of the calves' feces showed that the number of bacterial colonies was lower in the T2 group compared to the T1 and C groups ($p<0.01$). The number of bacterial colonies varied according to the weeks of the trial ($p<0.01$). Nevertheless, health parameters did not varied between male and female ($p>0.05$). The preliminary results of the study suggest that the supplementation with the prebiotic Aviator® improved some health parameters in unweaned calves, especially the diarrhea rate and the number of bacterial colonies in feces.

Key-words: *saccharomyces cerevisiae*, unweaned calves, diarrhea, bronchitis, bacteriological analysis.

INTRODUCTION

Improved management and nutrition can promote better feed efficiency and health in young calves (Heinrichs and Heinrichs, 2011). Feed additives are usually used in intensive system to enhance the well-being and performance of young calves before weaning (Alugongo et al., 2017). On the other hand, when research has shown that the use of antiobiotics in livestock farming has harmful effects on animal and human health, in addition to antibiotic resistance

(Langford et al., 2003), probiotics and prebiotics have been seen as the best alternative to antibiotic use in livestock (Signorini et al., 2012).
Yeast of *Saccharomyces cerevisiae* has been incorporated into domestic animal diets, particularly ruminant animals and their young ones. The use of *Saccharomyces cerevisiae has improved the immune system by stimulating the antioxidative system* of young and adult animals (Jensen et al., 2008 ; Zaworski et al., 2014).

The objective of this study was to investigate the effect of supplementation with a prebiotic *saccharomyces cerevisiae* yeast culture (Aviator®) on the health indicators of unweaned calves.

MATERIAL AND METHODS

Study location

The study took place in the BEN CHIBOUB FARM, situated in the north of Tunisia and 48km from the capital Tunis. The region belongs to the sub humid bioclimatic stage. The region has a temperate Mediterranean climate with hot and dry summers according to the Köppen-Geiger classification. Over the year, the average temperature is 18.6°C and rainfall averages is 473.9mm. The farm is known for its Holstein dairy cattle.

Experiment

The trial involved Twenty four (24) unweaned Holstein calves. The average age at the starting of the trial was 15 days. The calves were assigned into three homogeneous groups. Each group was composed of 8 calves (4 males and 4 females). The first control group (C) received the conventional feed (milk without prebiotic). The second group (T1) received milk supplemented with 7g/calf/day of Aviator®, and the third group (T2) received milk supplemented with 14g/calf/day of Aviator®. The groups were fed during 8 weeks (W1 to W8).

Aviator® is a heat-stable feed additive consisting of a preparation of refined functional carbohydrates, namely Mannan-oligosaccharides (MOS), D-mannose and β-glucan, derived from the cell wall of the yeast Saccharomyces cerevisiae. It's a blend of hydrolyzed yeast, yeast culture and yeast extracts.

Heath indicators

The Health parameters of the calves were noted in the three groups once a week.

- Coat condition: the coat is noted whether it is damaged, altered or dehydrated, abnormally colored or textured or heavily soiled with feces, mud or other soiling, and either whether it has parasites (OIE, 2019).
- Diarrhea: the tail and hindquarters were controlled whether are soiled with liquid stools. The stools give indications of the origin of the disease, either an infection or a feeding error, based on color (yellow, bloody, dark), quantity and consistency (watery, pasty).
- Bronchitis: the animal was monitored whether it displays signs such as accelerated respiratory rate, panting and coughing.
- Bacteriological analysis: In the treated groups, fecal samples of calves were taken from the rectums every week during the trial period (total number of samples/group= 56). For the control group, a single sample was taken during the first week of the trial (n= 8). From each sample, 0.5 g of faecal material was diluted and vortexed. 100 µL of the dilution suspensions were spread on VRBL (or TBX) medium and incubated overnight at 37°C (VRBL agar selective and differential medium used for the detection and enumeration of enterobacteria). After incubation, plates were examined and presumptive colony counts were performed. Colonies of *E. coli* were selected and subcultured on VRBL medium to obtain pure cultures.

The antibiotic sensitivity of the strains identified was studied using 8 antibiotics: amoxicillin, amxicillin+clavulanic acid, ceftazidime, cefotaxime, tetracycline, gentamicin, tobramycin, clavulanic acid, trimethoprim/sulfamethoxazole, tobramycin.

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Statistical analysis

Statistical analysis were carried out using SAS software (SAS Institute, Inc). The General Linear model (GLM) procedure was used to study the effects of the group, sex and week on calves' heath parameters. The level of signification was fixed at $p<0.05$.

RESULTS AND DISCUSSION

The general condition of the calves' coats showed that 21% had abnormal coats, while 46% had coats soiled with faeces. Diarrhea was noted in 15% of calves, due to digestive problems. Bronchitis was detected in 17% of calves, indicating respiratory problems. Respiratory problems were mainly observed in calves housed in group stalls and in humid areas (Hr = 54%) with draughts.

Besides, the rate of diarrhea occurrence in all calves decreased from W1 to W3 (8% *vs* 4%, Figure 1) and it was lower in the T1 and T2 groups compared to the C group (1.5% and 3% *vs* 8%, $p<0.05$, Figure 2).

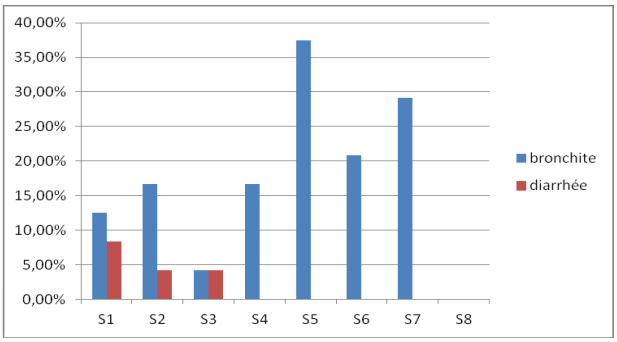

Figure 1. Variation of the percentages of diarrhea and Bronchitis during the experiment.

However, the rate of bronchitis cases ranged from 4.5% to 37% over the period of the trial (Figure 1). It was lower in the C group compared to the treated groups T1 and T2 (6.5% *vs* 12.5% and 31.5%, Figure 2).

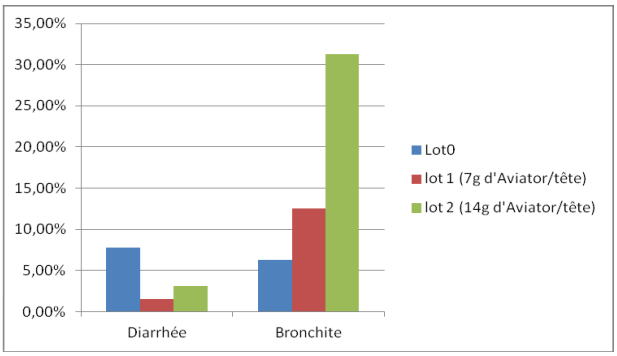

Figure 2. Variation of the percentages of diarrhea and Bronchitis according to the groups.

ANOVA (Table 1) showed that bacteriological flora varied according to groups and weeks of the trial. Nevertheless, health parameters did not varied between male and female ($p>0.05$).

Table 1. Results of the ANOVA.

	ddl	**Number of bacterial colonies**
group	2	**
Sex	1	ns
Week	6	***
R^2		0,26

*ns : not significant; ** : p<0,05 ; *** : p<0,01*
ddl= degree of freedom ; R^2= model coefficient of determination

Bacteriological analysis of the calves' feces showed that the number of bacterial colonies was lower in the T2 group compared to the T1 and C groups ($p<0.01$). The number of bacterial colonies varied according to the weeks of the trial ($p<0.01$, Table 2).

Table 2. Variation of the number of bacterial colonies according to the groups during the experiment (UFC/g).

	W1	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**
C	0.2±0,1	-	-	-	-	-	-
T1 (7g/head)	0.12±0.07	41.74±16.99	111.12±77.64	774.56±462.82	65.80±57.85	61.68±60.15	170.96±102.50
T2 (14g/head)	0.04±0.02	1.29±1.26	15.89±10.84	700.23±611.41	47.15±43.15	80.69±44.03	395.15±373.38

Our results are in agreement with those found by Askri et al. (2018) who reported a significant decrease in the numbers of pathogenic bacteria: in groups receiving prebiotic in diet. The decrease of *E. coli* could be explained by the competition between mannan-oligosaccharides (mos), components of the yeast wall, and the antigenic determinants of certain pathogens containing mannan residues which limits the possibility of pathogen attachment to the intestinal wall and therefore their development (Castro et al. 1994; De ruiter et al. 1994). On the other hand, Askri et al. (2018) found that the number of lactobacilli in chickens receiving the prebiotic was higher than in the control group on days 10, 30 and 42. A study by Baurhoo et al. (2007)

showed that MOS intake (0.2%) in chickens led to an increase in Lactobacilli and Bifidobacteria in their caecal contents, compared with to the control diet. Another study showed that mannan-oligosaccharides are able to improve gastrointestinal health by increasing beneficial bacteria such as lactobacilli in the gut (Patterson and Burkholder 2003). Generally, administration of the Aviator® prebiotic could selectively improve lactobacillus populations and reduce pathogenic bacteria.

The results in table 3 showed that only 7 antibiotic-resistant to *E.coli* strains were present in the group C. While in T1, the total number of antibiotic-resistant *E.coli* colonies was around 38, with the highest proportion 34/38 for the Amx antibiotic. Similarly, for T2, the total number of antibiotic-resistant to *E.coli* was around 37, with the highest proportion 30/38 for the Amx antibiotic.

Table 3. Variation of the number of antibiotic-resistant *E. coli* strains in batches of treated calves.

	Amx	Amc	Caz	Tet	An	Gen	Tob	Ctx
T1	34/38	22/38	10 /38	31/38	3/38	5/38	6/38	4/38
T2	30/37	18/37	8/37	29/37	2/37	11/37	5/37	2/37

Amx: amoxicillin, amc: amxicillin+clavulanic acid, caz: ceftazidime, ctx: cefotaxime, tet: tetracycline, gen: gentamicin, tob: tobramycin, An: clavulanic acid, sxt: trimethoprim/sulfamethoxazole, tob: tobramycin

CONCLUSIONS

The preliminary results of this study suggest that the supplementation with the prebiotic Aviator® improved some health parameters in unweaned calves, especially the diarrhea rate and the number of bacterial colonies in feces.

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INFLUENCE OF DIETARY SUPPLEMENTATION OF SACCHAROMYCES CEREVISIAE (A-Max Ultra®) ON GROWTH AND DIGESTIBILITY OF WEANED CALVES

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ABSTRACT

The study aimed to determinate the effect of diatery supplementation with prebiotic *Saccharomyces cerevisiae* (A-MAX Ultra®) on Holstein calves growth parameters. Twenty three weaned calves aged 3 months were assigned into 3 groups : the control group (C, n=8) was fed with the conventional diet, and the 2 treated group (T1, n=8 and T2, n=7) with the conventional diet supplemented with 7g and 14g per calf and per day of *Saccharomyces cerevisiae* respectively, during 8 weeks. The height at withers (HT), chest circonfrence (CC) and the weight (W) were determined once a week using a tape. Then, the average daily gain (ADG) was determinated in each group. The digestibility (D) was studied at the end of the experiment at the week 8. ANOVA was carried out using the SAS software. The results showed that the ADG did not varied between groups. However, the HT, CC and W were higher in T1 compared to C and T2 groups ($p<0.01$). Moreover, the D was higher in T1 and T2 groups compared to C group ($p<0.05$). The findings suggest that the dietary supplementation with 7g of *Saccharomyces cerevisiae* (A-MAX Ultra®) improved growth and digestibility in Holstein weaned calves.

Key-words : *Saccharomyces cerevisiae*, weaned calves, growth parameters, digestibility.

INTRODUCTION

Most of dairy calves performances depend on rumen environment and conditions. Feed additives have therefore been applied to stimulate rumen activity and improve digestibility efficiency that could increase feed intake and growth (Newbold et al., 1996). The yeast culture *Saccharomyces cerevisiae* is one of the alternatives that has been applied to the feed of growing calves. The results of its application have been positive according to some authors (Stefańska et al., 2018) and negative (Mitchell and Heinrichs, 2020) or even no significant variation according to others (Saldana et al., 2019). These differences could be attributed to the livestock conditions in which the animals are reared, the method of administration of the yeast and the animal itself.

The study aimed to determinate the effect of diatery supplementation with prebiotic *Saccharomyces cerevisiae* (A-MAX Ultra®) on Holstein calves growth parameters and digestibility.

MATERIAL AND METHODS

Twenty three weaned calves aged three months were assigned into 3 groups : the control group (C, n=8) was fed with the conventional diet, and the 2 treated group (T1, n=8 and T2, n=7) with the conventional diet supplemented with 7g and 14g per calf and per day of *Saccharomyces cerevisiae* (A-MAX Ultra®) respectively, during 8 weeks.

The weaned calves receive 3kg of concentrate per calf and unlimited straw. The concentrate is based on soya, barley, maize, crushed declassified dates, mineral elements and CMV. The drinking water used is well water, which is permanently and automatically available to the animals. The recommended doses of A-MAX Ultra® for the treated groups were mixed with the concentrate and were distributed every day at 8 am.

The different groups of calves were housed in collective boxes (density : 8). The boxes are located in the open air. The boxes are arranged side by side, with a metal roof and a cemented floor covered with straw bedding.

The height at withers (HT), chest circonfrence (CC) and the weight (W) were determined once a week using a tape. Then, the average daily gain (ADG) was determinated in each group.

The digestibility (D) was studied at the end of the experiment at the week 8, and was measured *in vivo* for each group using two sieves placed one on top of the other. The first with a diameter of 5 mm and the second with a diameter of 2 mm. The method consisted in collecting 250g of faeces from each group separately, then pouring them into the upper sieve, and washing them with water until all the particles with a diameter of less than 5 mm pass to the second sieve, which has a diameter equal to 2 mm. Finally, the particles recovered from the 2nd sieve were weighed. It represented the undigested particles of the group.

The digested portion was calculated (Carjot, 2013): $dX = (Xi - Xf) / Xi$

(X: proportion of component; Xi: proportion of initial component: faeces;Xf: proportion of final component: undigested part).

ANOVA was carried out using the SAS software (SAS Institute Inc®). The General Linear model (GLM) procedure was used to study the effects of the group, sex and week on calves' growth and digestibility. The level of signification was fixed at $p<0.05$.

RESULTS AND DISCUSSION

The results of the ANOVA were presented in table 1. The ADG did not varied between groups. No effect of sex on all the studied parameters was shown.

Table 1. Results of the ANOVA.

	ddl	ADG	ddl	HT	CC	W
Group	2	ns	2	***	***	***
sex	1	ns	1	ns	ns	ns
week	6	***	7	***	***	***
R^2		0,18		0,35	0,39	0,39

*ns : not significant ; *** : p<0,01*

The HT, CC and W were higher in T1 compared to C and T2 groups ($p<0.01$, Table 2). Hiss and Sauerwein (2003) and Rozeboom et al. (2005) reported that dietary supplementation with

Saccharomyces cerevisiae improved weight in farm animals. Zhang et al. (2005) showed that supplementation with yeast wall extracts gave the best growth results.

Table 2. Variation of the height at withers (HT), chest circonfrence (CC) and weight (W) according to the groups.

	C	T1	T2
HT (cm)	104 ± 1.3^{a}	106 ± 1.1^{b}	101 ± 2.7^{c}
CC (cm)	145 ± 3.5^{a}	147 ± 3.5^{b}	135 ± 5.2^{c}
W (Kg)	208 ± 6.7^{a}	212 ± 6.7^{b}	189 ± 9.7^{c}

Digestibility (D) was higher in T1 and T2 groups compared to C group ($p<0.05$, table 3). Fomenky et al, (2019) showed that adding probiotics to calves' rations increased beneficial bacteria populations and disadvantaged other harmful populations in their digestive systems.

Table 3. Variation of digestibility (D) according to the groups.

	C	T1 (7g/head)	T2 (14g/head)
Digestibility (D)	$0,76^{a}$	$0,83^{b}$	$0,82^{b}$

C : conventional feed ; T1 : conventionnal feed supplemented with 7g A-max Ultra /head ; T2 : conventionnal feed supplemented with 14g A-max Ultra /head

CONCLUSIONS

The findings suggest that the dietary supplementation with 7g of *Saccharomyces cerevisiae* (A-MAX Ultra®) improved growth and digestibility in Holstein weaned calves.

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DIVERSITY OF BENTHIC MACROMOLLUSCAN COMMUNITIES ON THE ROCKY SHORES OF EASTERN KARABURUNI PENINSULA, VLORE, ALBANIA

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ABSTRACT

Variations in species richness within ecosystems are influenced by natural processes and can be further affected by both natural and human activities. Our research focuses on the Karaburuni Peninsula, situated in the southern part of the Albanian coast. This peninsula features a diverse rocky coastline interspersed with small pebble beaches and gulfs, such as the gulf of Raguzë, Shën Vasil, and Shën Jan. The outer region of the peninsula is encompassed within the Karaburun Sazani Marine Protected Area.

For our study, we specifically investigate the eastern part of the Karaburuni peninsula, which was divided into three study stations: Raguzë, Shën Vasil, and Shën Jan. These stations comprise rocky coasts that serve as our primary areas of interest for the research.

The main objective of this research was to investigate these variations. The study focused on rocky intertidal mollusks and aimed to achieve three specific goals: 1) to determine the species richness of these mollusks; 2) to track their geographical distribution at the State level; and 3) to understand how species richness changes in response to aquaculture activities.

To accomplish these objectives, data were collected at different time points throughout two seasons: May, July and October of 2020. The sampling area comprised three transects for each site, and within each transect, three samples were taken. The sampling area was delimited using a PVC rectangle frame measuring 50 x 50 cm per side. During the sampling process, all mollusks present within these designated units were meticulously collected, identified, and counted.

The analysis of species distribution in the study was based on different sites seasonal species richness and biodiversity composition. Overall, the research identified 45 mollusk species between 1396 individuals. Their richness was found to be associated with factors such as substrate stability, wave intensity at each site, and trophic level. Among the mollusk classes, Gastropods exhibited the highest species richness.

When examining the sites distribution, the researchers observed a consistent pattern of species richness in areas with marine vegetation. The dominance of gastropods in species composition and density could be attributed to their broad food range, which includes carnivorous, necrophagous, phytophagous, and detriphagous species. Notably, certain species like *Phorcus sp* and *Patella rustica* contributed to the high ecological value and thus, the dominance of these species across all stations.

Surprisingly, the overall species richness in the rocky intertidal zone was significantly increased by aquaculture activities in the Ragusa area. However, upon closer analysis, the malacofauna exhibited changes in species richness influenced by the constant expansion of marine barens and the retreat of marine forests of *Cystoseira sensu lato*. These changes in habitat appear to have a direct impact on the diversity of mollusk species in the region.

Keywords: Karaburuni, macrozoobenthos, gastropod, malacofauna, Bivalvia, Vlora Bay

INTRODUCTION

The coastal ecosystems, especially the hard-bottomed ones of Albania, are rich in habitat types, communities and animal and plant species important for the natural heritage of the country and the Mediterranean region (Kashta et al., 2011). In the last twenty years, Albania has undergone profound changes, including huge investments along the coast. The effects of these investments have become visible on coastal ecosystems in terms of changes in natural habitat fragmentation, eutrophication, increase in sea urchin barriers (Fraschetti et al., 2011; Maiorano et al., 2011). The waters of the gulf of Vlora, in particular, has been subject in the last few progressive natural and anthropic impacts (Maiorano et al., 2011). Regular studies of the structure and composition of species in the coastal marine communities of this gulf represent a register of important data for the assessment of the environmental impact that these activities have on the ecosystem of the gulf.

The existing data on the macrozoobenthos of the rocky areas of the Albanian Adriatic coast of Vlora are relatively recent and often concentrated on the malacofauna (Kasemi et al., 2008; Kasemi & Haxhiraj, 2009; Kasemi et al., 2008; Ruci et al., 2013; Nasto et al., 2022 a, b). Studies focused on the macrozoobenthos of these areas aim to evaluate the species composition, abundance, environmental status of macrozoobenthic populations and their seasonal comparisons are limited. The most recent studies on the macrozoobenthos of the rocky coasts of the Gulf of Vlora date back to 2008 (Kasemi et al. 2008; Selmani et al., 2015; Nasto et al., 2022b). The studies in question cover the eastern part of the gulf of Vlora and the island of Sazani.

The southwestern area of the gulf of Vlora includes a large portion of the gulf coast. The area is characterized by the presence of rocky coasts mainly made by limestone; a defining characteristic of this marine basin often interrupted by small pebble beaches. The area has some bays such as Raguza 1 and Raguza 2, Shen Vasil, Shen Jan. Being on the border of the first Marine Protected Area in Albania, Karaburun Sazani MPA, the area of our research has an importance for the assessment of anthropogenic environmental impact on ecosystems. In the research area lies an intense marine aquaculture activity at Raguza 1 and 2 bays (Bakiu et al., 2018). The primary aim of this research was to explore these ecological situations of this study area. The investigation centered around mollusks inhabiting rocky intertidal zones, with the intention of accomplishing three distinct objectives: 1) ascertaining the diversity of species among these mollusks; 2) mapping out their geographic prevalence within the State boundaries; and 3) comprehending the fluctuations in species diversity in reaction to aquaculture undertakings. This study attempts to expand the current knowledge on the rocky infralittoral zone of the Vlora gulf by providing occurrence data of molluscan species from three different stations of infralittoral zone.

MATERIAL AND METHOD

The study area

Stretching across the western expanse of Vlora Bay, the Karaburuni Peninsula spans an area of 62 km2, effectively acting as a separator between the Albanian coastline along the Adriatic Sea and the Ionian Sea. This landmass is connected to Sazani Island by a slender sea channel, referred to as Mezokanali, meaning "middle channel" in English. Geologically speaking, Karaburuni is predominantly comprised of Cretaceous carbonic limestone, with the northern-western portion around the Bay of St. Jani being characterized by terrigenous deposits (Kashta et al., 2011).

The terrain consists of a series of hills, reaching elevations of up to 800 meters. Among the highest peaks stand Maja e Ilqes (733 m), Maja e Flamurit (826 m), and Çadëri (839 m). The peninsula's perimeter meets the sea through sheer and unapproachable cliffs. On the western shore, the terrain rises steeply, marked by numerous crevices, caverns, openings, and small shores. Gaining access to several coastal regions and beaches, particularly on the western flank, proves to be quite challenging and at times impossible without a boat due to the coastal cliffs (Kashta et al., 2011).

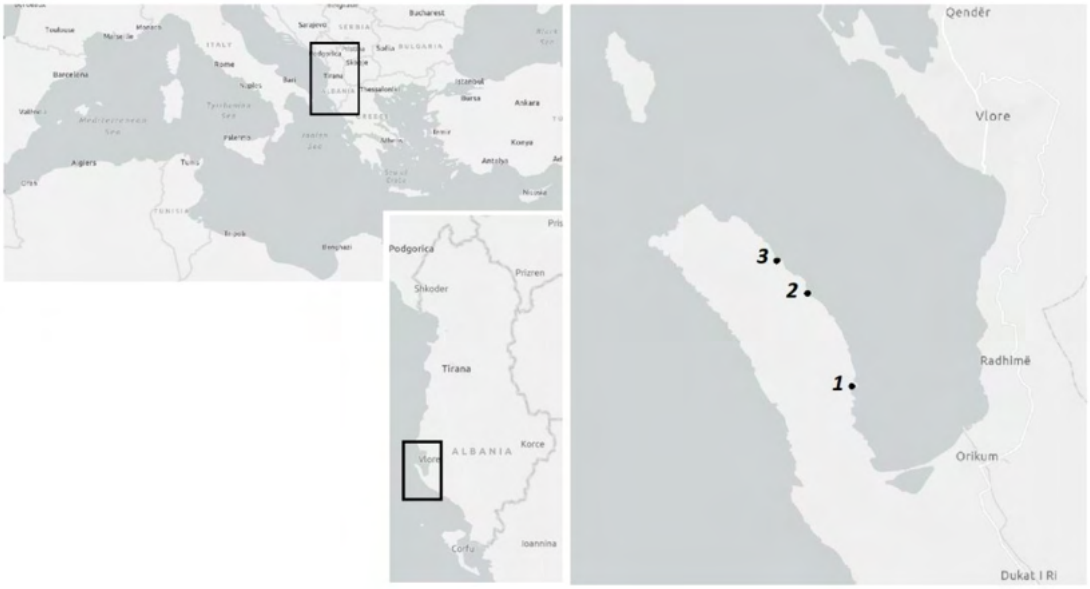

Figure 16. Map of the study stations: 1) Ragusa Station, 2) St. Vasilit Station. 3) St. Jan Station.

In contrast, the eastern coast exhibits less fragmentation. The northwestern tip of the peninsula is marked by Cape Gjuhezes (Kepi i Gjuhezes), positioning itself as the westernmost point of Albania. Woody vegetation is notably scarce throughout the area, except for pockets of sparse maquis and untamed grasses, and freshwater sources are absent. Within the embrace of the Karaburun Peninsula lie several diminutive bays, including the Bay of Raguza, the Bay of St. Jan, the Bay of Bristan, the Bay of Dafina, and more (Kashta et al., 2011). The stations where benthic macroinvertebrates have been collected include the rocky shores of the Adriatic Sea, from Ragusa II to Saint Jan on the border with Cape Gjuheza, specifically 3 stations: Ragusa II, Saint Vasil, Saint Jan (Figure 1). For the collection of material, field expeditions were carried out during 3 months of 2020, more precisely during the months of May, July, and at the beginning of October 2020. Sampling was carried out in three seasons: spring, summer and autumn to verify the variations spatiotemporal of the malacofauna.

Sampling method

Sampling was conducted in accordance with established protocols for benthic data collection on solid substrates (as outlined by Cattaneo et al. 1978, Revkov et al. 1999). The objective was to quantitatively assess benthic populations during the spring, summer, and autumn seasons in supralittoral and medio-littoral zones. Due to challenges in precisely distinguishing between medio-littoral and infralittoral areas, upper infralittoral regions were also included in the sampling.

The collection procedure targeted both surface-dwelling macroinvertebrates on rocks and those sheltered within algae. Consequently, samples were taken from prominent algal cover types to provide a more comprehensive understanding of the biocenoses. Within each station, three transects were sampled, maintaining a linear separation of 50 meters. Within each transect, six samples were collected: three from the supralittoral and three from the medio-littoral and upper

infralittoral zones. This resulted in a total of 18 samples per station per period, amounting to 54 samples for all three stations in each sampling period, or a grand total of 162 samples over all sampling sessions.

Quantitative data acquisition involved using a 50 cm x 50 cm test quadrat for capturing and evaluating macrobenthos. This quadrat was further divided into 16 smaller quadrats to facilitate detailed quantitative assessments of the macrobenthos. Within these smaller sections, counts of individuals or percentage assessments of algal coverage, along with small colonial organisms like *Chthamalus, Mytilaster, Serpula*, etc., were conducted. The collection process was carried out manually. Following sample collection, the material was preserved in 75% ethanol and transported to the laboratory for subsequent identification and analysis.

Data analysis

Species-area curves were generated for each habitat to assess the effectiveness of the sampling process. The analysis of molluscan communities involved the utilization of sin ecological indices, including species richness (SR), the count of individuals (N) per 2 dm3, Pielou's Evenness (J), and the Shannon-Weaver diversity index (H'). Additionally, both quantitative (DI, representing the percentage of individuals of a specific species relative to the total individuals) and qualitative (DQ, denoting the percentage of species within a given taxon relative to the total species) indices were computed.

Comparative analyses of habitats were expanded using sample-based and individual-based interpolation (rarefaction) and extrapolation curves, as outlined by Colwell et al. (2012) and Chao and Jost (2012). To discern variations across habitats, a permutational analysis of variance (PERMANOVA) was performed. This analysis was based on Euclidean distance and carried out as a univariate approach (Anderson, 2012), employing a one-way model with habitat as a fixed factor.

Employing the same design, a PERMANOVA analysis based on Bray-Curtis similarity was conducted to assess distinctions in molluscan communities among the five habitats, each with four replicates. Further investigation was facilitated through pairwise tests, elucidating disparities among the habitats. For visualization, a non-metric multidimensional scaling (n-MDS) ordination (Bray & Curtis, 1957) was employed. To unveil the species that chiefly contributed to habitat similarities and those that distinctly characterized each habitat, a similarity percentage–species contribution analysis (SIMPER) was executed (Clarke, 2014).

RESULTS AND DISCUSSION

Within the scope of this study, the three designated sampling stations revealed a collective tally of 45 distinct species. Among the identified mollusks, they are classified into three primary classes: Polyplacophora, Gastropoda, and Bivalvia. In the Polyplacophora class, a total of 3 families were discerned, namely Leptochitonidae (comprising 2 distinct types), Chitonidae (consisting of 1 type), and Ischnochitonidae (encompassing 1 type). Within the gastropod class, there exists a comprehensive assembly of 32 species, distributed across 11 distinct families: Cerithiidae (inclusive of 2 species), Triphoridae (comprising 1 species), Rissoidae (with 1 species), Columbellidae (consisting of 1 species), Fasciolariidae (comprising 2 species), Conidae (including 1 species), Rissoinidae (with 1 species), Muricidae (encompassing 3 species), Patelidae (comprising 5 species), Tudiclidae (1 species), Pisaniidae (1 species), and Trochidae (encompassing a diverse count of 13 species) (Table 1). The Bivalvia class, on the other hand, encompasses four distinctive families: Mytilidae (accounting for 5 species), Anomiidae (comprising 1 species), Arcidae (inclusive of 2 species), and Carditidae (with 1 species) (Figure 1a).

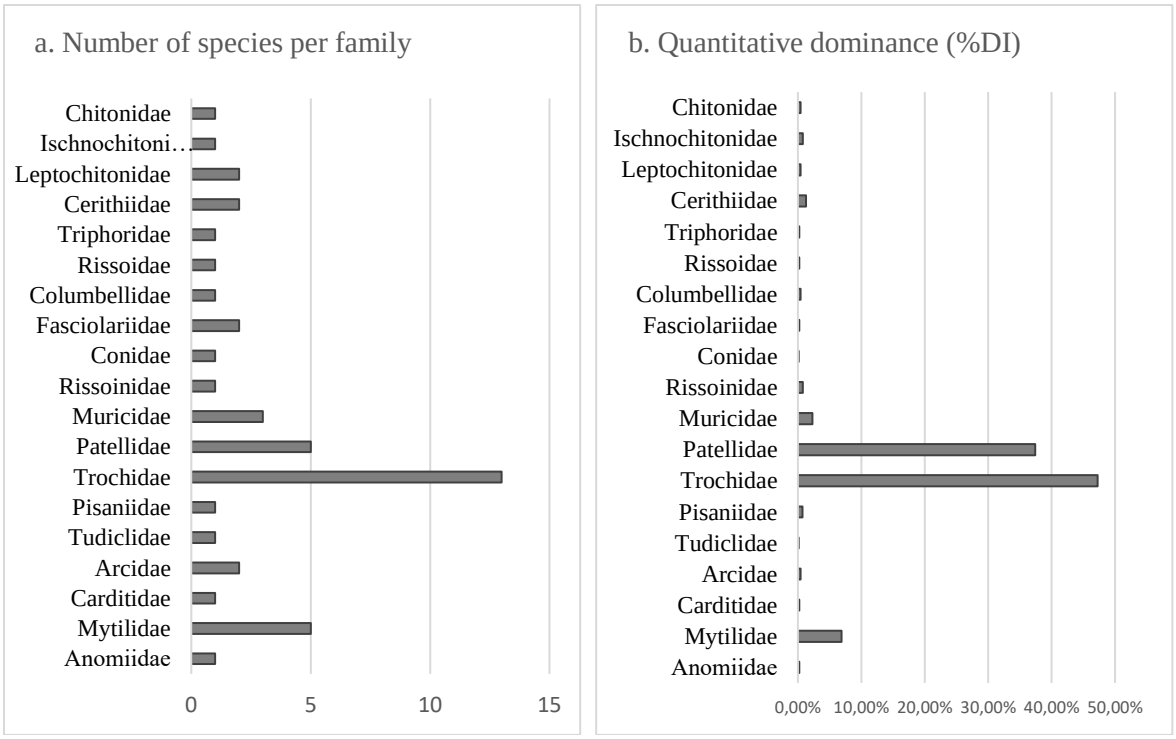

Figure 17. Percentages of abundance (%DI - a) and species richness (%DQ - b) of of the families present in the study.

Both in percentages of abundance and species richness, the family Trochidae dominates with an average of 47.26% of all samples (figure 2b). Another family equally represented is the Patellidae family which, despite having 5 species present, constitutes 37.39% of the collected samples. The ones with the highest frequency values are *Patella rustica* 15.25%, *Patella caerulea* 14.56%, *Steromphala divaricata* 16.35%, *Phorcus turbinatus* 11.17%.

Table 1. Taxonomic list of species, occurring in each sampling station in 3 seasons (Spr-Spring; Su-Summer; Aut-Autumn), with their abundance (N), feeding guilds (G-Grazer; SF-Suspension feeders; DF/G-Deposit feeder /Grazer; DF -Deposit feeder; P-Predators; H-Herbivores; SF-Suspension feeders) and AMBI ecological group ((I)- very sensitive to disturbance; (II)-indifferent to disturbance; (III)- tolerant to disturbance).

FG	AMBI EcoGroup	Scientific Name	Raguza II			St. Vasil			St. Jan		
			Spr	Su	Aut	Spr	Su	Aut	Spr	Su	Aut
		Polyplacophora									
G	(II)	*Rhyssoplax olivacea* (Spengler, 1797)	-	1	-	1	1	-	1	-	-
G	(II)	*Ischnochiton rissoi (Payraudeau, 1826)*	-	-	-	-	1	-	1	6	-
G	(I)	*Leptochiton algesirensis* (Capellini, 1859)	-	-	-	-	-	-		1	-
G	(I)	*Leptochiton scabridus* (Jeffreys, 1880)	-	-	-	-	1	-	1	1	-
		Gastropoda									
P	(II)	*Cerithium vulgatum* Bruguière, 1792	2	1	2	-	4	-	-	1	-
P	(II)	*Cerithium caeruleum* G. B. Sowerby II, 1855	-	1	-	-	-	1	-	-	1
P	(I)	*Monophorus perversus* (Linnaeus, 1758)	-	-	-	-	1	1	-	-	-
G	(I)	*Alvania cimex* (Linnaeus, 1758)	-	1	-	-	-	-	-	1	4
P	(II)	*Pisania striata* (Gmelin, 1791)	-	-	2	1	1	-	2	1	-
H	(I)	*Columbella rustica* (Linnaeus, 1758)	-	-	-	-	1	-	2	1	-
P	(I)	*Euthria cornea* (Linnaeus, 1758)	-	1	-	-	-	-	-	-	-
P	(I)	*Pseudofusus rolani* (Buzzurro & Ovalis, 2005)	-	1	-	-	-	-	-	-	-
P	(I)	*Tarantinaea lignaria* (Linnaeus, 1758)	-	-	-	-	-	-	-	-	1
P	(I)	*Conus ventricosus* Gmelin, 1791	-	-	-	1	-	-	-	-	-
G	(II)	*Rissoina bruguieri* (Payraudeau, 1826)	1	-	-	-	-	-	-	1	-
P	(I)	*Hexaplex trunculus* (Linnaeus, 1758)	-	3	3	-	-	-	-	-	1
P	(II)	*Ocinebrina aciculata* (Lamarck, 1822)	-	1	-	-	-	6	-	-	6
P	(II)	*Muricopsis cristata* (Brocchi, 1814)	-	2	-	-	1	-	-	-	-
G	(III)	*Patella caerulea* Linnaeus, 1758	4	-	3	3	-	53	28	9	46
G	(I)	*Patella depressa* Pennant, 1777	1	-	-	-	-	-	1	-	-
G	(III)	*Patella rustica* Linnaeus, 1758	2	1	12	18	64	4	8	44	-
G	(I)	*Patella ulyssiponensis* Gmelin, 1791	1			-	-	1	-	-	-
G	(II)	*Cymbula safiana* (Lamarck, 1819)	1	2	7	27	3	-	22	8	4
DF	(I)	*Steromphala adriatica* (R. A. Philippi, 1844)	57	14	3	4	-	-	-	-	-
DF/G	(I)	*Steromphala divaricata* (Linnaeus, 1758)	3	72	32	46	1	2	-	6	2
DF/G	(I)	*Steromphala leucophaea* (R. A. Philippi, 1836)	-		1	1	-	-	-	-	-
DF/G	(I)	*Steromphala pennanti* (R. A. Philippi, 1846)	1	7	7	1	-	-	-	-	1
DF/G	(II)	*Steromphala racketti* (Payraudeau, 1826)	1	-	1	-	-	1	-	-	-
DF/G	(I)	*Steromphala umbilicalis* (da Costa, 1778)	-	-	-	1	-	-	-	-	-
DF/G	(I)	*Steromphala varia* (Linnaeus, 1758)	-	-	-	-	-	21	-	-	6
DF/G	(II)	*Gibbula philberti* (Récluz, 1843)		1		-	-	-	-	-	1-
DF/G	(II)	*Gibbula vimontiae* Monterosato, 1884	-	1	-	-	-	-	-	-	-
DF/G	(III)	*Phorcus articulatus* (Lamarck, 1822)	-	24	25	-	2	-	-	14	1
DF/G	(II)	*Phorcus lineatus* (da Costa, 1778)	-		1	-	1	27	-	-	22
DF/G	(I)	*Phorcus richardi* (Payraudeau, 1826)	-	2	7	-	-	-	-	-	-
DF/G	(III)	*Phorcus turbinatus* (Born, 1778)	23	2	4	38	2	-	59	6	1
		Bivalvia									
SF	(I)	*Cardita calyculata* (Linnaeus, 1758)	-	1	-	-	1	-	-	-	-
SF	(I)	*Arca noae* Linnaeus, 1758	-	1	1	-	-	-	-	1	-
SF	(I)	*Barbatia barbata* (Linnaeus, 1758)	-	-	-	-	1	-	-	-	-
SF	(I)	*Lithophaga lithophaga* (Linnaeus, 1758)	6	-	-	-	-	-	-	-	-
SF	(I)	*Modiolus barbatus* (Linnaeus, 1758)	-	1	-	-	-	-	-	2	-
SF	(I)	*Mytilaster minimus* (Poli, 1795)	-		-	5	21	-	-	4	-
SF	(III)	*Mytilus galloprovincialis* Lamarck, 1819	-	4	-	3	11	-	-	1	-
SF	(I)	*Musculus costulatus* (Risso, 1826)					5		4	1	1
SF	(I)	*Anomia ephippium* Linnaeus, 1758	-	1	-	-	-	-	1	-	-

Trophic Analysis

As for trophic analysis, five feeding guilds were identified in the three sampling stations (Figure 3). Considering the ecological conditions at the three research stations, we opted to conduct a trophic analysis to assess whether the presence of aquaculture activity at the Ragusa station impacts the local ecosystem. In our study, these three stations exhibit variations in terms of vegetation, substrate composition, and suspended organic matter levels.

At the Ragusa II station, the notable feature is the accumulation of suspended organic materials, primarily attributable to the presence of aquaculture cages. Here, the substrate lacks significant algae growth, with only a few species sporadically covered by a thin layer of mud and organic substances.

In contrast, the St. Vasil station stands out for its notable presence of Posidonia oceanica meadows, brown algae from the Cystoseira genus, and Corallina algae.

Lastly, the St. Jan's station encompasses an entire coastline characterized by extensive sea barrens, prominently inhabited by sea urchins and gastropods belonging to the Patellidae family.

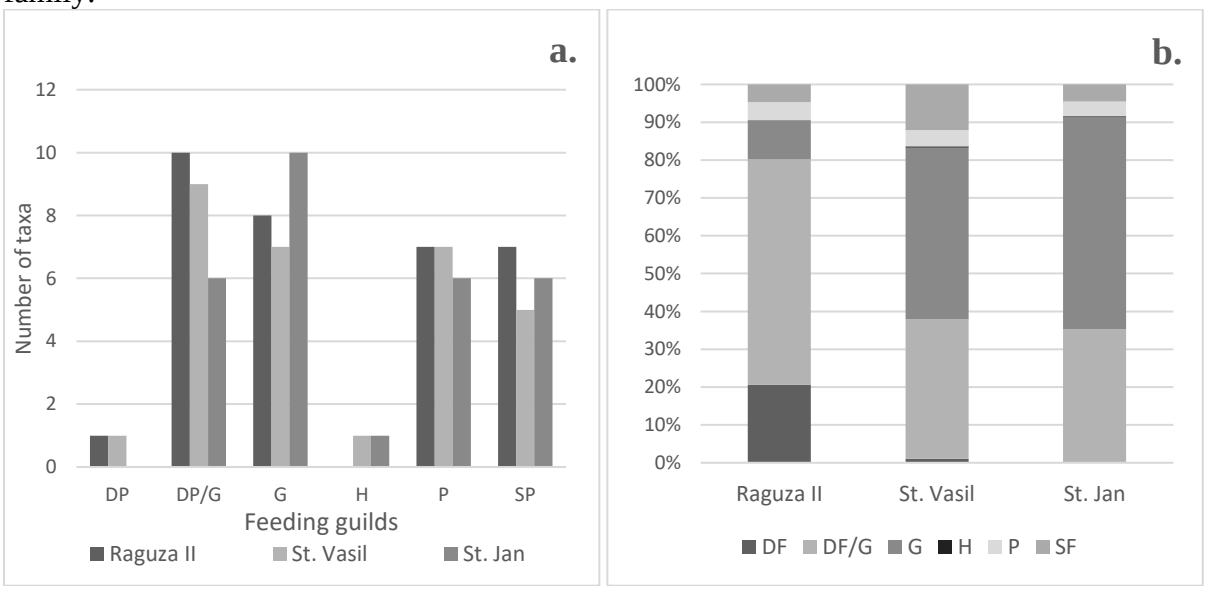

Figure 18. (a). Species richness (Number of taxa) and (b). Percentages of abundance (%DI) according to the feeding guilds feeding guilds (G-Grazer; SF-Suspension feeders; DF/G-Deposit feeder /Grazer; DF -Deposit feeder; P-Predators; H-Herbivores; SF-Suspension feeders).

As depicted in Figure 3, Deposit feeders and Deposit feeders/Grazers are the dominant groups in terms of both species diversity and the frequency of individuals observed at the Raguza II station (DF - 20.6% and DF/G - 59.7%). However, the number of species and the frequency of Deposit feeders/Grazers show a decrease in the other two stations, specifically at St. Vasil (DF - 36.9%) and St. Jan (DF - 35.33%). In terms of species diversity, G-Grazers between these two stations exhibit relatively stable numbers, with 7 species at the St. Vasil station and 10 species at the St. Jan station. Nevertheless, the cumulative frequency of G-Grazers appears to increase as we move away from the aquaculture cages. Notably, in the case of the St. Jan station, which is dominated by sea urchin barrens, G-Grazers are particularly abundant throughout the habitat. Regarding SF-Suspension feeders, the number of species is higher at the Ragusa II station. Even though, the frequency of individuals is notably greater at the St. Vasil station due to the presence of two species from the Mytilidae family, namely Mytilaster minimus and Mytilus galloprovincialis.

Marine Biotic Index AMBI ecological group analysis

The AMBI (AZTI's Marine Biotic Index) was specifically developed to evaluate how macrobenthic assemblages in European coastal waters respond to shifts in environmental quality, as documented by Borja et al. in 2000 and Warwick et al., 2010. It categorizes species into five ecological groups based on their sensitivity to environmental stressors, and the index relies on the relative abundance of species within each group. This index has emerged as a cornerstone for evaluating ecological conditions in accordance with the European Water Framework Directive, as highlighted by Blanchet et al. in 2008. Moreover, this index's effectiveness has been demonstrated by comparing its results across the three sampling stations to identify potential environmental disturbances in one of them.

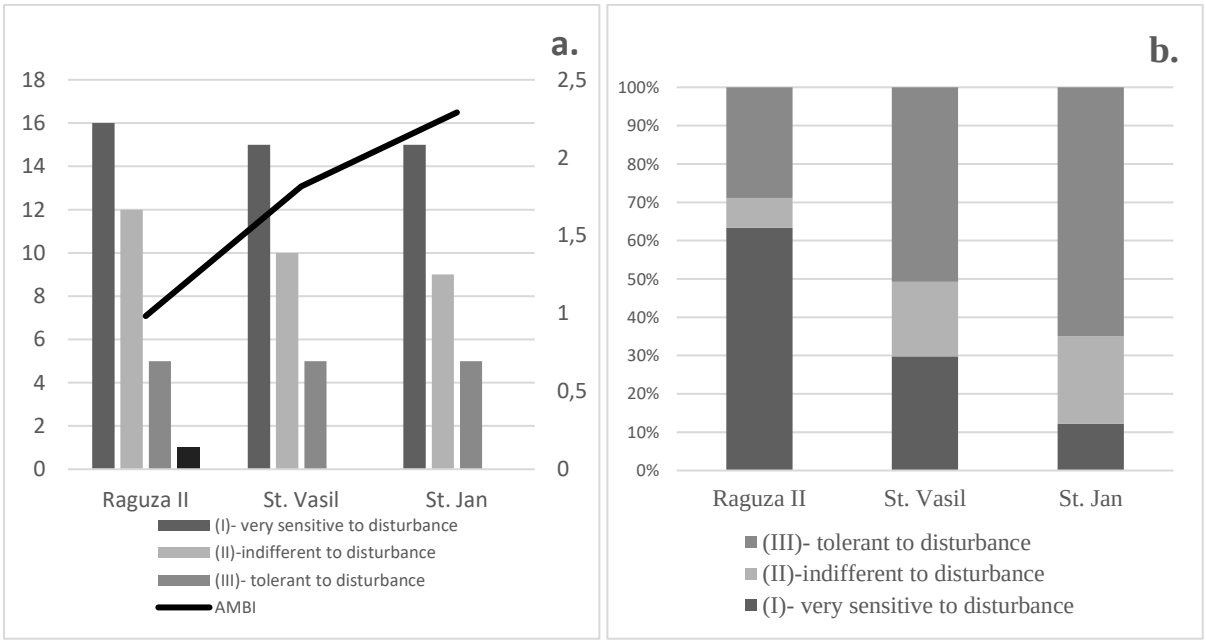

Figure 19. a. Number of species per each ecological group and AMBI values at each one of the tree sampling locations, b. Cumulative frequences of ecological groups (I, II, III)

As reported by Muxika et al. in 2003 and as indicated by the findings depicted in Figure 4, the Marine Biotic Index AMBI exhibits a range of values, ranging from 0.98 at the Raguza station to 2.29 at the St. Jan station. Notably, all three stations fall within the category of slightly polluted values according to the AMBI Index.

Inter-Habitat Comparison of the Molluscan Assemblages

According to Figure 5, three distinct and statistically significant clusters have emerged. Cluster 1 pertains to the Raguza sampling site, situated in proximity to the aquaculture farm. Cluster 3 encompasses the St. Vasil sampling sites, which are located near the Raguza II station. Cluster 3, representing St. Jan, situated to the north of the study area, appears to have experienced less impact from the Fish Farm.

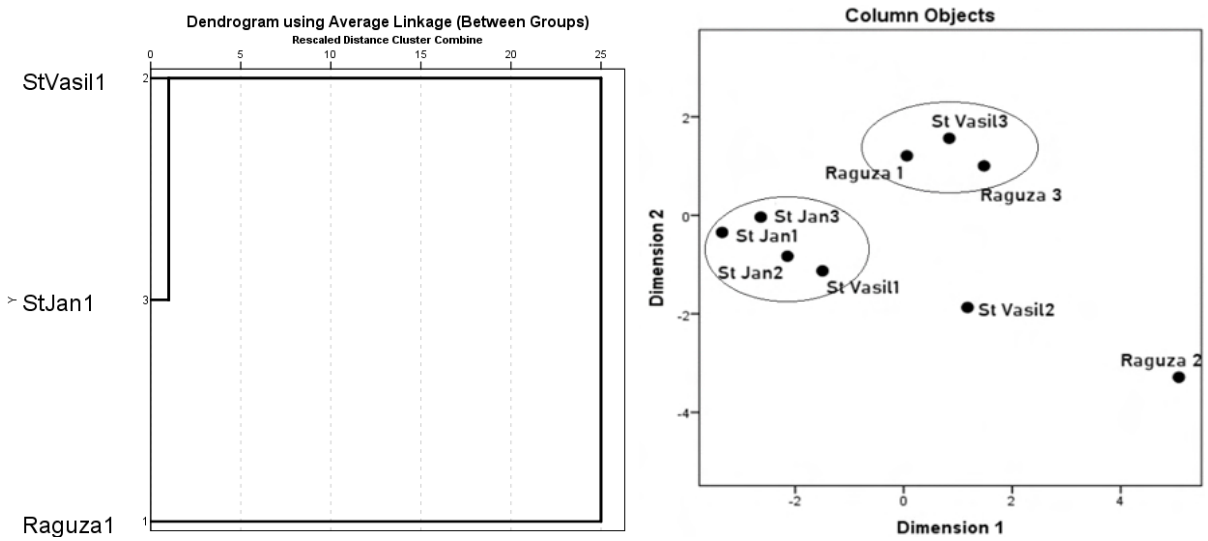

Figure 20. Cluster analysis of sampling stations based on species composition (mean values) during study period.

The dendrogram provides clarity regarding the differences at the Raguza II sampling station, which forms Cluster 1. This cluster appears to receive effluents from various sources, including both point and non-point sources—namely, from the fish farm activities and potentially from urban waters originating from the city of Orikum.

In contrast, the sampling sites at St. Jan and St. Vasil exhibit a lower influence from these polluting activities, resulting in a higher degree of similarity in species composition between these two stations.

CONCLUSIONS

This research has furnished a comprehensive dataset regarding the mollusc fauna inhabiting the study area, coupled with an analysis of the ecological conditions observed during the monitoring period. In summary, our findings unequivocally underscore the pivotal role of food availability and the heightened habitat complexity facilitated by biological structures on substrates in shaping mollusc assemblages. This holds true across the infralittoral to the circalittoral zones of the Mediterranean Sea. Notably, the prevalence of algae and their epiphytes on photophilic hard substrates emerges as a critical factor influencing gastropod-dominated assemblages, predominantly characterized by both micro- and macro-grazers with high mobility.

Looking ahead, the introduction of aquaculture production in these regions may potentially exert adverse impacts on benthic communities. However, it is imperative to conduct further assessments to comprehensively evaluate the potentially deleterious effects of aquaculture practices on the surrounding areas.

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DETERMINATION OF INDICATOR BENTHIC MACROINVERTEBRATES IN LÜLEBURGAZ STREAM (KIRKLARELİ, TÜRKİYE)

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ABSTRACT

In the present study, the benthic macroinvertebrate fauna of the Lüleburgaz Stream located in Kırklareli Province (Turkish Thrace) was examined. Sediment samples were taken from the selected four sampling stations at seasonal intervals in 2022 and 2023 years. The obtained benthic macroinvertebrates were evaluated according to their ecological tolerances. In addition, some environmental variables (dissolved oxygen, calcium, magnesium, total hardness, salinity, nitrite nitrogen, nitrate nitrogen, phosphate, sulfate, pH, temperature, conductivity, and total dissolved matter) were measured in water samples taken from the sampling stations in autumn and spring seasons to support the indicators' ecological tolerance conditions A total of ten benthic macroinvertebrate taxa (*Limnodrilus hoffmeisteri* Claparède, 1862 and *Tubifex* sp. belonging Oligochaeta; *Chironomus riparius* (Meigen, 1803), *Chironomus plumosus* (Linnaeus, 1758), *Polypedilum nubifer* (Skuse, 1889) belonging Diptera; *Physella acuta* (Draparnaud, 1805) belonging Gastropoda, *Gammarus* sp. belonging Amphipoda,*Asellus aquaticus* (Linnaeus, 1758) belonging Isopoda and the individuals belonging Bivalvia and Hirudinae) were identified. While the ecological tolerances of the taxa were presented in this study, the measured environmental variables that may be effective in the distribution of the detected organisms were also evaluated. As a result, it was determined that some physicochemical properties measured in the Lüleburgaz Stream showed seasonal fluctuations and that the stream was exposed to pollution load, while it was determined that the benthic macroinvertebrate species obtained also supported these results.

Keywords: Water Quality, Indicator Organisms, Physicochemical Variables, Stream

INTRODUCTION

Water is vital for humans and all ecosystems. So, the importance of aquatic ecosystems in the world for humans and ecosystems is clear (Kazancı, 2008). Aquatic ecosystems are most affected by climate changes caused by global warming (Yanık and Aslan, 2018). In addition, due to the agricultural and industrial pollution that develops due to the increase in population, the control and protection of these ecosystems has gained importance (Tokatlı, 2019). Limnological studies are necessary to protect water resources and aquatic ecosystems. Because aquatic ecosystems can maintain continuity as long as they contain biodiversity (Kazancı, 2008).

Aquatic ecosystems have a dynamic structure with their living diversity and water quality (Protasov et al., 2019) Especially those living at the bottom of water bodies, benthic macroinvertebrates, are a group of organisms that are effectively used in biological monitoring studies because they are a heterogeneous group, respond differently to environmental pollution, have a longer life cycle than other organism groups, are easy to identify and collect, and are found in water bodies at all times of the year (Akay et al., 2018).These organisms have been used in various monitoring studies to determine biological water quality in our country in recent years. (Akay et al., 2018).

The use of benthic macroinvertebrates as indicators of contamination in waters provides an early warning mechanism for short-term changes that may be missed in chemical analysis. While some of these organisms such as Tubificidae and Chironomidae larvae show high tolerance to pollution, some groups such as Ephemoreptera and Tricoptera are quite sensitive. (Hawkes, 1979; Metcalfe-Smith, 1994).

Lüleburgaz Stream is a stream located in the Thrace Region, flowing into the Ergene River after it rises from the Aktepe foothill in the north of the Poyralı district. (http://docs.neu.edu.tr/library/6298108714.pdf). Lüleburgaz stream flows for 58 km through residential areas and then flows into the Ergene River and it is also exposed to anthropogenic effects.

Among the previous studies on Lüleburgaz Stream, the causes of pollution of the stream were mentioned in the Kırklareli Environmental Status Report 2019 (Çevre, T.K.V., & Müdürlüğü, Ş.İ. 2020). In addition, the ecological status of Lüleburgaz Stream and the status and quality of water were evaluated in the Meriç-Ergene River Basin Management Plan. As a result, it has been stated that the amount and quality of Lüleburgaz Stream groundwater is in poor condition. (https://www.tarimorman.gov.tr/SYGM/Belgeler/NHYP%20DEN%C4%B0Z/MER%C4%B0%C3%87-ERGENE%20NEH%C4%B0R%20HAVZASI%20Y%C3%96NET%C4%B0M%20PLANI.pdf). Although there are taxonomic studies that include the study area, no field-specific study has been found to date, evaluating the Lüleburgaz Stream benthic macroinvertebrates and their relationships with environmental factors.

In this study, it was aimed to evaluate the indicator benthic macroinvertebrates detected from Lüleburgaz Stream together with environmental parameters that may be effective in their distribution.

MATERIAL AND METHOD

The study area, the Lüleburgaz Stream, is located within the borders of Kırklareli Province of the Thrace Region in Turkey and is located at the coordinates 41° 20′ 48″ North and 27° 19′ 4″ East (Figure 1). A total of two stations were determined over the stream, one in the city center (station 1, St. 1) and the other approximately 500 meters before it flows into the Ergene River (station 2, St. 2) (Figure 1).

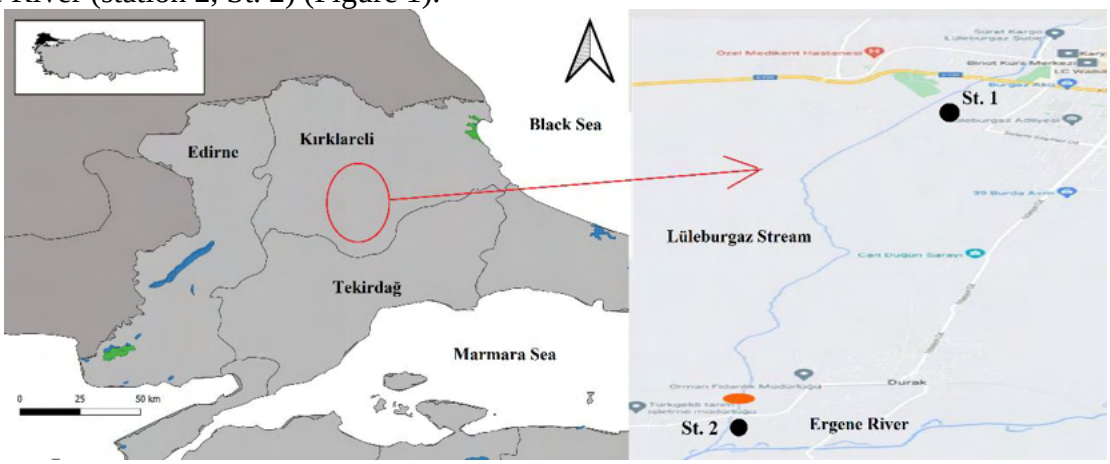

Figure 1. The study area and the sampling stations

The sediment samples taken from the selected sampling stations at seasonal intervals(In 2022, on dates corresponding to the summer and autumn seasons, and in 2023, on dates corresponding to the winter and spring seasons) The ordinary drift nets were used to take sediment sampling. The sediment samples were washed through a 100-µm sieve and the obtained material was transferred to 250 cc bottle containing 70% ethanol. At the laboratory,

samples were determined using a stereomicroscope and specimens identified to the possible taxonomical category. The following literature was used in identification; Brinkhurst (1971), Karaman and Pinkster (1977), Cranston (1982), and Schmelz and Collado (2010).

For the evaluating physicochemical variables, in autumn 2022 and spring 2023, the water samples taken from the water surface were placed in dark glass bottles (1 L). The water temperature, salinity, pH, conductivity, and total dissolved matter were measured using a Consort™ multi-parameter analyzer C5020 were measured in the stations when the field studies. The other parameters (dissolved oxygen, calcium, magnesium, total hardness, nitrite nitrogen, nitrate nitrogen, phosphate, sulfate) were measured in the laboratory by using classical titrimetric and spectrophotometric methods (Egemen, Sunlu 1996).

RESULTS AND DISCUSSION

The obtained data belonging to benthic macroinvertebrates weregiven in Table 1 and Table 2. The species *Limnodrilus hoffmeisteri* and *Tubifex* sp. belonging Oligochaeta group; *Chironomus riparius*, *Chironomus plumosus*, and *Polypedilum nubifer* belonging Diptera group; *Physella acuta* belonging Gastropoda group; *Gammarus* sp. Belonging Amphipoda group; *Asellus aquaticus* belonging Isopoda group, and individuals belonging to Bivalvia and Hirudinae groups were detected.

When the ecological preferences of the benthic macroinvertebrate taxa detected in the Lüleburgaz Stream were examined, it was determined that especially those identified at the species level were individuals with wide ecological tolerance and were also shown in studies as pollution indicators. The species *L. hoffmeisteri* is ecologically known as an indicator species that shows organic pollution and low oxygen in the waters, and is also frequently used in studies to determine the toxicity and bioaccumulation of pollutants in sediment (Shang et al., 2014). The species *C. riparius, C. plumosus, P. nubifer* belonging to Chironomidae survive in places with very low oxygen values and high organic pollution (Epler, 2001). The species *A. aquaticus* and *P. acuta* have a fairly cosmopolitan distribution, they feed on dead organic material in the sediment and can survive under temporary harsh conditions (extreme temperatures and water pollution) (Semenchenko et al., 2008).

Table 1. The benthic macroinvertebrates of the Lüleburgaz Stream obtained from station 1

Taxa/Seasons ➡ ⬇	**Spring**	**Summer**	**Autumn**	**Winter**
Oligochaeta	Tubificidae *L. hoffmeisteri*	Oligochaeta (immature) *L. hoffmeisteri*	-	Oligochaeta (immature) *Tubifex* sp. *L. hoffmeisteri*
Diptera	-	*C. plumosus* *C. riparius*	*C. riparius*	*C. riparius*
Gastropoda	Gastropoda	*Physella acuta*	*Physella acuta*	*Physella acuta*
Bivalvia	Bivalvia	Bivalvia	Bivalvia	-
Amphipoda	Amphipoda	-	Gammarus sp.	Amphipoda
Hirudinea	-	-	-	-
Isopoda	Isopoda	-	*Asellus aquaticus*	-

Table 2. The benthic macroinvertebrates of the Lüleburgaz Stream obtained from station 2

Taxa/Seasons ➡ ⬇	**Spring**	**Summer**	**Autumn**	**Winter**
Oligochaeta	Oligochaeta (immature) Tubificidae *L. hoffmeisteri*	Oligochaeta (immature) *L. hoffmeisteri*	Oligochaeta (immature) *Tubifex* sp.	Oligochaeta *L. hoffmeisteri*
Diptera	-	*C. plumosus* *P. nubifer*	*C. riparius* *C. plumosus* *P. nubifer*	-
Gastropoda	Gastropoda	Gastropoda *Physella acuta*	-	*Physella acuta*
Bivalvia	Bivalvia	Bivalvia	Bivalvia	-
Amphipoda	Amphipoda	Gammarus sp.	-	Amphipoda
Hirudinea	Hirudinea	Hirudinea	Hirudinea	Hirudinea
Isopoda	-	-	*Asellus aquaticus*	-

The values of physicochemical variables belonging to spring and autumn season were given in Table 3. When physicochemical data are examined according to the Surface Water Resources Control Regulation (YSKKY, 2016):

It was observed that while dissolved oxygen values showed second-class water quality values in the autumn, they approached first-class quality in the spring. pH values varied between minimum 7.4 and maximum 7.7 in both periods, and it was determined that the water was close to slightly basic. While it was observed that the stream showed freshwater character in the spring in terms of conductivity values (678-762 µS/cm), it was observed that the conductivity increased very much in the autumn period, especially in the city center (St.1) (1330 µS/cm), and this increase continued at the next station (1100 µS/cm). The temperature values of the stream, which shows freshwater character in terms of salinity values (0.3-0.6 ‰), were measured low (1.5-1.8 ^{0}C) during the sampling periods. It was determined that the stream, which was soft water in the autumn in terms of total hardness, increased to medium/hard water (in the city center) in the spring and the increasing magnesium and calcium ions were effective in this increase. The medium/hard feature of water in terms of pH value was parallel to the medium hard water feature determined by total hardness. While nitrite nitrogen was not found in the stream in the autumn period, it was determined that this value increased and decreased to the fourth class water quality at both sampling stations in the spring period. While an increase was observed in terms of nitrate nitrogen in the spring period, it was determined that the measured values did not exceed first class water quality (0.250-4.72 mg/L). The values measured in terms of sulfate ion increased in the spring compared to the autumn period and the values were of first class water quality. In terms of total phosphate measurements, it was determined that the station near the Ergene River (St. 2) had a second-class water quality in both autumn and spring, while the city center station (St.1) had a third-class water quality in the spring. It was determined that the values measured in the spring period in terms of total dissolved matter doubled in the autumn period, but still did not show a significant deviation from the first class water quality of 0.5 g/L.

Table 3. Physicochemical analysis results of the Lüleburgaz Stream sampling stations for the spring and autumn seasons

Physicochemical Variables	**SPRING SEASON**		**AUTUMN SEASON**	
	St. 1	**St. 2**	**St. 1**	**St. 2**
Dissolved Oxygen (mg/L)	7.03	6.79	5	6
pH	7.71	7.45	7.4	7.6
Conductivity (µS/cm)	678.7	762.5	1330	1100
Temperature (°C)	1.5	1.5	1.8	1.8
Salinity (‰)	0.380	0.422	0.6	0.5
TDS (g/L)	0.333	0.374	0.66	0.59
Calcium (mg/L)	24.04	16.32	14.2	12.3
Magnesium (mg/L)	14.52	9.6	1.9	1.8
Total Hardness (FS^0)	25.04	19.2	3.8	3.6
Nitrite nitrogen (mg/L)	0.09	0.08	*	*
Nitrate nitrogen (mg/L)	4.13	4.172	*	0.25
Phosphate (mg/L)	1.385	1.298	0.717	1.014
Sulfate (mg/L)	0.42	0.08	*	0.156

* Below measurable value

In terms of measured physicochemical variables, especially low oxygen values (indicating second-class water quality) and seasonally increasing nutrient salts, as well as seasonal increase in conductivity, indicate that organic and inorganic pollutants have entered the stream.

In addition, some of the benthic macroinvertebrate species detected in the study (such as *C. plumosus, C. riparius, P. nubifer*) belong to Chironomids, which are indicators of organic pollution, and Oligocheats, which are also highly tolerant to pollution (such as *L. hoffmeisteri* and *Tubifex* sp.), apart from *P. acuta*. The fact that they contain Gastropod species, which can live in polluted waters, and Hirudinae individuals, which are pollution indicators, supports our determination that the Lüleburgaz Stream is exposed to polluting elements.

CONCLUSIONS

As a result, it was determined that the Lüleburgaz Stream, which was determined as the study area, was occasionally exposed to pollutants and therefore pollution indicator species settled more in the stream. Considering that the physicochemical variables fluctuate seasonally in the study, it is recommended to carry out periodic studies in the stream in question and prevent the entry of pollutants that will deteriorate the water quality into the stream.

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PRELIMINARY CHARACTERIZATION OF *METSCHNIKOWIA PULCHERRIMA* STRAINS FOR FUTURE APPLICATIONS IN WINE BIOTECHNOLOGY

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ABSTRACT

In recent years, in the oenological sector there has been a re-evaluation of non-*Saccharomyces* oenological yeasts, considered in the past as unwanted or deteriorating yeasts, for a positive contribution they can make in improving the analytical composition and aromatic profile of the wine. Therefore, the selection and use of non-*Saccharomyces* yeasts with peculiar technological and enzymatic characteristics could represent a key point for the production of wines with good and distinctive chemical and organoleptic characteristics. The main objective of this work was to evaluate a possible use of *Metschnikowia pulcherrima* as starters in the production of wines obtained from native grape varieties of Albania. Therefore, three *M. pulcherrima* strains (ASB3R, AS3C1, 14AS), isolated from grape must, have been tested for their antimicrobial and enzymatic activities, biogenic amine production and some fermentative properties. The results showed an antimicrobial activity of these yeasts that suggests their possible use as biocontrol agents in winemaking. In addition, their β-glucosidase activity was detected, which could contribute to the release of varietal aromas from aromatic precursors present in grapes. Furthermore, these strains were safe for health because they did not produce biogenic amines. These results, although preliminary, open the way to further investigations aimed at a possible use of these yeasts as a starter in the alcoholic fermentation of grape juice and the contribution they can give in the definition of the physical-chemical and organoleptic characteristics of regional wines

Keywords: *Winemaking,* non-*Saccharomyces, Metschnikowia pulcherrima, Biocontrol, β-glucosidase activity*

INTRODUCTION

The increasing interest in natural wines has spurred researchers to investigate strategies that promote sustainability, environmental impact reduction, and wine quality enhancement. Recent scientific studies have focused on the oenological properties, enzymatic activity and antimicrobial capacity of non-Saccharomyces yeasts, aiming to utilize their winemaking potential in improving aromatic complexity and the stability of wine (Bruno Testa, 2021). To be considered as 'suitable', selected non-Saccharomyces yeasts should show desirable enological characteristics, including good fermentation rates, ethanol tolerance and complete consumption of reducing sugars, resistance to SO_2, absence of H_2S or off-flavors, as well as killer character or resistance to toxin activity. Among these yeasts, preference should be given to those that enrich the wines with superior sensorial attributes.

Wine yeasts play a crucial role in converting grape sugars into ethanol, carbon dioxide and various secondary compounds. In this context, the use of mixed and multi starters in fermentation process, involving both Saccharomyces and non-Saccharomyces wine yeasts, is proposed as a new strategy for winemakers to enhance the chemical composition and sensorial characteristics of wine (Ciani1, 2009). Extensive research is conducted on non-Saccharomyces yeasts, such as *Torulaspora delbrueckii, Lachancea thermotolerans, Metschnikowia pulcherrima, Hanseniaspora uvarum, Schizosaccharomyces pombe,* and *Pichia kluyveri*, commonly founded on grape skins. The utilization of these non-Saccharomyces yeasts is associated with significant contributions to the metabolic impact and aroma complexity of the final wine product (Benito, 2019; Bruno Testa, 2021; Maurizio Ciani, 2009). Non-Saccharomyces yeasts exhibit higher enzyme production compared to Saccharomyces species, including β-glucosidase, proteases, lipases, esterase, among others, which play a pivotal role in the production of aroma-active compounds in wine (Charoenchai C, 2009). For instance, β-glucosidase enzymes hydrolyze β-D-glycosidic bonds, leading to the volatile compound formation, such as terpenes, aliphatic alcohols, and esters. Additionally, certain non-Saccharomyces yeasts can produce proteolytic and pectinase enzymes that aid in reducing protein haze and extracting polyphenols from grape skins (Ubeda-Iranzo JF, 1998; Charoenchai C, 2009; Javier Vicente, 2020). Non-Saccharomyces yeasts dominate the early stage of alcoholic fermentation; notably, M. pulcherrima exhibits high β-glucosidase activity and its presence in mixed cultures can lead to wine improvements, such as reduced volatile acidity, increased production of medium-chain fatty acids, higher alcohol content, enhanced esters and terpenoids, and elevated glycerol levels (García, 2016; María Eugenia Rodríguez, 2007).

Furthermore, non-Saccharomyces yeasts possessing antimicrobial activity can contribute to the preservation of must and wine by protecting them from spoilage bacteria and yeasts. As such, these yeasts can be utilized as bio protective agents at different stages of winemaking. The protective mechanisms employed by these yeasts include competition for nutrients and the production of killer toxins or inhibitory compounds (Morata A. , 2021; Simonin, 2020). For instance, certain species of non-Saccharomyces, such as *M. pulcherrima*, can produce pulcherrimin acid, a red-brown pigment that exhibits antimicrobial effects. The union of pulcherrimin acid with Fe3+ ions results in inhibition of other microorganisms that require high levels of Fe ions for their cellular processes. Nutrient competition represents another widespread mechanism through which one microorganism affects the growth of another. Additionally, the secretion of extracellular lytic enzymes, such as chitinase and glucanase, by non-Saccharomyces yeasts can inhibit the growth of other microorganisms by damaging their cell walls or other components (Sipiczki, 2020; Javier Vicente, 2020). Moreover, studies have demonstrated that non-Saccharomyces yeasts exhibit good adaptability when combined with *Saccharomyces cerevisiae* yeast for completing alcoholic fermentation. Sequential inoculation of non-Saccharomyces and Saccharomyces yeasts has been identified as an optimal approach for producing wines with distinctive aromatic profiles and increased complexity (Maëlys Puyo, 2023; Kai Chen, 2018). Importantly, scientific research indicates that non-Saccharomyces yeasts do not produce toxic compounds such as biogenic amines, thereby ensuring their safe use in winemaking.

The focus of this study is to characterize a specific strain of *M pulcherrima* in terms of its oenological properties and enzymatic activities relevant to winemaking. By examining these characteristics, we aim to evaluate the strain's potential as a valuable mean in enhancing wine quality, improving sensorial attributes and mitigating microbial risks during fermentation and aging process.

To accomplish this, the chosen strain will be subjected to comprehensive analyses, including assessments of its fermentation performance, sensory impact on wines, enzymatic activities and antimicrobial properties. By understanding the specific traits and capabilities of this strain, we

can ascertain its suitability for integration into winemaking practices and identify potential avenues for optimizing wine production.
In conclusion, the strains exploration and characterization present a promising opportunity to enhance winemaking toward sustainable wine production practices. This study aims to contribute to the existing knowledge by evaluating the oenological properties and enzymatic activities of a specific strain, shedding light on its potential application in the pursuit of high-quality, environmentally conscious wines.

MATERIAL AND METHODS

2.1 Yeast Strains

This study aimed to characterize three strains of *M pulcherrima* and assess their suitability for the production of high-quality wine. The strains, namely *AS3C1, 14AS,* and *ASB3R*, were isolated, identified from grape must and preserved in the culture collection of the DiAAA (Department of Agricultural, Environmental and Food Sciences, University of Molise). The experiments were conducted at the Microbiological Laboratory of the University of Molise.

The selected strains were subject of various tests to assess their enzymatic activity, antimicrobial activity and oenological properties. These tests included evaluating their *β-glucosidase β-lyases*, and *proteases* activities and antimicrobial activity as well. Also are examined their oenological properties such as production of acetic acid, alcohol, reducing sugar in fermentation on synthetic must.

To conduct the experiments, strains were refreshed in YPD medium (consisting of 1% yeast extract, 2% peptone, and 2% dextrose) under aerobic conditions at a temperature of 30°C. The cultivation period lasted for 48 hours.

2.2 Antimicrobial activity

The antimicrobial activity of the strains was tested in accordance with the (Massimo Iorizzo, 2022) refreshed in YPD medium (1% w/v yeast extract, 2% w/v peptone and 2% w/v dextrose) at 30°C under aerobic condition for 48h. The antimicrobial activity of yeast strains assessment followed these steps:

Prepare the YPD Agar medium with the following components for 100ml: Peptone: 2g, Glucose: 2g, Agar: 2g, Yeast extract: 1g, sterilized in an autoclave at 121°C for 15 minutes. If the concentration of the refreshed wild yeast culture is higher than 10^5cells/ml, perform dilutions. Vortex all the wild yeast samples to ensure homogeneity. Prepare the plates by adding 2ml of the diluted yeast culture and 18ml of YPD Agar medium, totaling 20ml. When the medium has solidified, create wells and pipette 50-70µL of yeast strains sample into each well. The plates incubate at 28°C for 24 hours. After incubation, examine the plates and record the results to evaluate the antimicrobial activity of each yeast strains, measuring the diameter (mm) of the clear zone of inhibition (ZOI) around the inoculated wells.

V2.3 Enzymatic activity

β-glucosidase activity

In the characterization of β-glucosidase activity in yeast of oenological origin, a screening method was prepared to assess the activity of this enzyme. The following components were

used to prepare a 100ml nutrient agar medium: (Nutrient agar: 2.8g, Arbutin: 0.5g, Ammonium ferric citrate [$(NH_4)_5[Fe(C_6H_4O_7)_2]$]: 1g (1% w/v), Distilled water: 100ml, Final pH: 5.0).
The Ammonium ferric citrate was weighed before and sterilized in an Eppendorf pipette at 121°C for 15 minutes. After sterilization, it was added to the medium in sterile condition under. The yeast strains of interests, which were refreshed in YPD agar broth the day before, were then streaked onto the surface of the prepared plates using a sterile pipette, with 10µL of the yeast strains culture in each plate. The plates were incubated at 28°C for 48 hours and after are observer the results. Yeast with β-glucosidase activity grow on the medium and are surrounded by a dark color, the darker the color, the stronger the enzymatic activity of the strains.

β- lyases activity

In the characterization of β-lyases activity, a medium was prepared to assess the activity of this enzyme. The following components were used to prepare a 100ml medium: (Carbon yeast base: 1.1g, Methionine: 0.1g, Pyridoxal 5' phosphatase (LP) (Vitamin B6): 0.02g, Final pH: 3.50). To prepare the medium, 80ml of water was added to the above components. Separately, 2g of bacteriological agar was mixed with 20ml of water, and after adjusting the pH of the agar solution, it was added to the medium. The entire mixture was then autoclaved for sterilization. Once the medium was sterilized, it was poured into separate plates. Each yeast strain was then plated onto the surface of the medium using sterile pipette10µL of the yeast strains culture. The plates were incubated at 28°C for 48 hours. After the incubation period, the results of the β-lyases activity analysis were obtained. The yeasts that have β-*lyase* activity grow in the medium and form a white halo around them, whereas those that lack the activity of this enzyme do not grow.

Protease activity

To assess protease activity in yeast, the following medium can be prepared:
Components for 100ml: (YPD Peptone: 2g, Agar: 2g,Glucose: 2g, Yeast extract: 1g are dissolved in 50ml of water, and the mixture is sterilized and additionally, 2g of skimmed milk is mixed with 50ml of water and pasteurized at 75°C for 15 minutes).
After both the YPD medium and the pasteurized skimmed milk are prepared, they are combined under sterile conditions. The mixture is then poured into plates, and in each solidified plate, 10µL of yeast culture is dropped.
The plates are then incubated under appropriate conditions suitable for the yeast being tested. This medium allows the assessment of protease activity in yeast by observing the degradation or clearing of the skimmed milk protein caused by the protease enzymes produced by the yeast strains and the formation of halos surround them, the results express by measure the diameter of halo.

2.4 Fermentation ability

The fermentation ability of strains was evaluated using standard fermentation assays. A synthetic must grape is prepared with a composition for g/L (Tartaric acid 5g, Citric acid 0.3g, DL-Malic acid 5g, Fructose 100g, Yeast extract 1g, Glucose / dextrose 100g, Sodium chloride NaCl 0.2g, Dipotassium hydrogen phosphate trihydrate KH2PO4* 3H2O 5g, (NH4)SO4 2g, Magnesium sulfate heptahydrate (MgSO4.7H2O) 0.4g, Manganese sulfate (MnSO4) 0.05g, and the final Ph 3.50), is placed in the 100ml flask, capped with cotton and sterilized. In each of the flasks was inoculated a strain of yeast and fermentation was allowed to proceed under controlled conditions. Parameters such as sugar consumption, alcohol production and pH changes, were monitored at regular intervals to assess the fermentation performance of the strains.

2.5 Statistical Analysis

The data are express as mean of standard deviation (n=3) using analysis of variance (ANOVA). Statistical significance was attributed to values of $p \leq 0.05$.

3. Results and discussion

The characterization of the strains (ASB3R, AS3C1, 14AS) in terms of their oenological properties and enzymatic activities revealed several remarkable findings.

3.1. Antimicrobial Activity

The results of the antagonistic activity of the strains toward the wild yeast indicator are presented in **Figure 1** as the mean diameter (mm) of the zone of inhibition (**ZOI**), with water as the control. The strains inhibited the growth of the wild yeast indicator by producing a ZOI between 2 and 4 mm. The strain *AS3C1* has the ability to inhibit all indicator wild yeasts with different power expression as ZOI. The *14AS* strain was not able to inhibit the growth of the *S. Ludwigi* indicator wild yeast. While the strain *ASB3R* was only able to partially inhibit the development of *S. Pompea*.

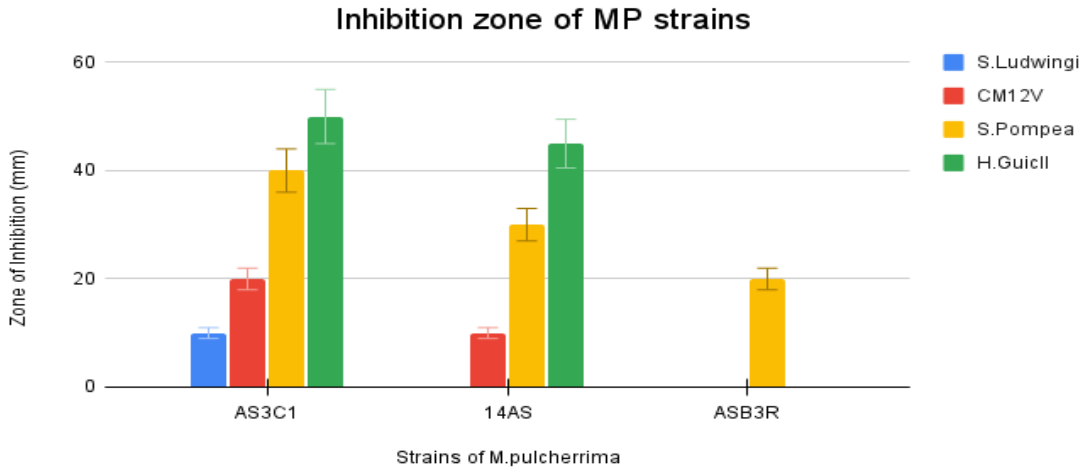

Figure 1 the mean diameter (mm) of the growth inhibition zone (ZOI)

3.2 Enzymatic Activity:

Significant β-glycosidase activity was observed in the strains. This enzymatic activity is essential for the release of varietal aromas and contributes to the improved sensory profile of wines. Good protease activity was observed in strain ASB3R. While the β-lyase activity was better expressed by the strain AS3C1 than two other strains, the results are shown in Table1.

Yeast strains MP	B-Glucosidase	B-Lyases	Protease activity (halo mm)
ASB3R	++	w	8mm
AS3C1	+++	++	4mm
14AS	+++	w	3mm

Table 1 The results of the enzymatic activity of the strains express – (negative), +(positive).w(weak), mm (diameter of halo).

3.3 Fermentation Ability:

The strains are refreshed in YPD broth for 48 hours. The synthetic grape must is prepared, separated in 200 ml elutriators and sterilized at 121ºC for 15 minutes. Before inoculation, the strains are centrifuged at 8000 rpm after washing with physiological water (0.9%). Fermentation is carried out under control temperature and is monitored until constant weight is

reached. At the end of fermentation a physico-chemical analysis is carried out on three of the strains. The data obtained show a good fermentation capacity for both strains. The changes in pH were significantly for AS3C1 as were the pH and volatile acidity (0.12g/l acid acetic). The consumed sugar is efficiently and produced desirable levels of alcohol during the fermentation process. In Table 2, the data are expressed as means with standard deviations. The analysis is performed in triplicate and results correspond to the average; the letter (a) indicates significant with 95% confidence differences.

Strains of MP	pH	Total acidity (g/l)	Vol. Acidity(g/l)	Alcohol %(v/v)	Reducing sugar Brix
14AS	3.08±0.02	7.15±0.03	0.15±0.02	3.1±0.10	12.07±0.12
ASB3R	3.07±0.06	7.25±0.02	0.18±0.01	3.37±0.06	11.13±0.15
AS3C1	3.1±0.01[a]	7.34±0.02	0.12±0.01[a]	4.6±0.20	9.77±0.21

Table 2 Data are expressed as mean values ± standard deviations (n = 3) with ($p \leq$ 0.05);

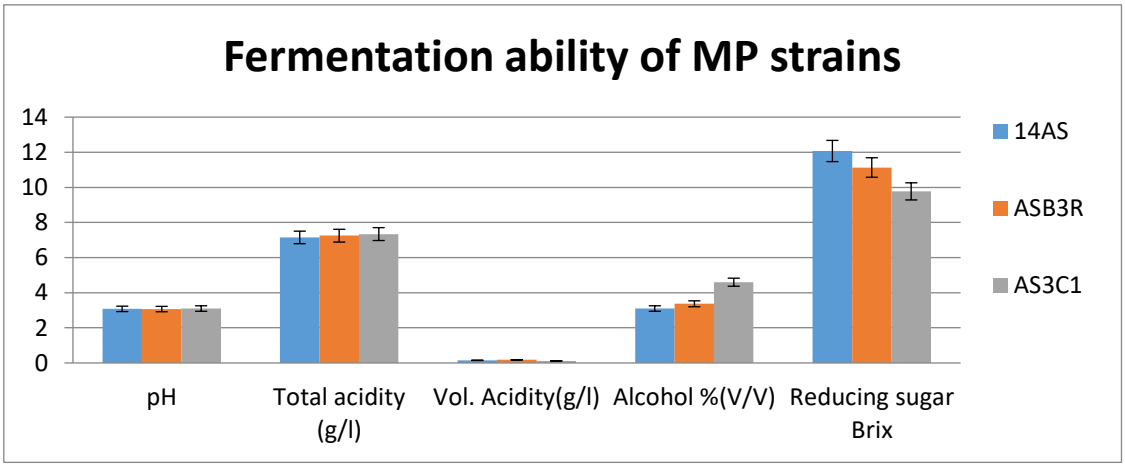

Figure 2, Fermentation ability of *M pulcherrima* strains in synthetic grape must

4. Conclusion

In conclusion, the characterization of the strains (*ASB3R, AS3C1, 14AS*) for their oenological properties and enzymatic activities has provided valuable insights into their potential applications in winemaking. The results demonstrate that the AS3C1 possesses strong antimicrobial activity against wild yeasts, highlighting their effectiveness in microbial control during fermentation.

The significant *β-glycosidase* activity observed in the strains indicates the ability to release grape varietal flavors, contributing to enhanced sensorics profiles and the development of complex aromas and flavors in wines.

Furthermore, the fermentation ability displayed by the strain AS3C1, with efficient pH, volatile acidity, sugar consumption and alcohol production, support the suitability for winemaking processes.

Overall, these findings suggest that the strains (*ASB3R, AS3C1, 14AS*) have great potential as beneficial components in winemaking, offering improved microbial control, enhanced sensorial characteristics promising the production of high-quality wines.
Further research and application trials are warranted to explore the full potential of these strains and to optimize their integration into winemaking practices. With continued investigation, *M pulcherrima* strains could contribute to the advancement of sustainable and quality-focused winemaking techniques.

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THE IMPORTANCE OF BIODEGRADABILITY OF PLASTICS USED IN AGRICULTURE

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ABSTRACT

Plastic products find use in the agricultural sector as well as in many areas. Covering films successfully perform essential tasks such as maintaining moisture and soil temperature and preventing weeds. Granular fertilizer coating films supplies slow or controlled realese of fertilizers by preventing the environment from the resudies and provides sustanibility.

Biodegradability is a sought-after feature in agricultural applications, especially starch-based biodegradable plastics are the most widely used ones. In agriculture, biodegradable polymers are also used as planting tapes, which contain plant seeds and fertilizer together. After harvesting, these films are destroyed by the degradation process.

Biodeterioration plastics break down spontaneously as a result of microbiological and chemical processes and remain in the environment as microplastics. Biodegradable plastics, on the other hand, are destroyed in the environment by turning into products such as carbon dioxide, water and methane as a result of the process called mineralization.

In this study, the ASTM D 5988-03 test method and the equivalent ISO 17556.2019 test method, which allows determining the degree of aerobic biodegradation of plastic materials by using soil or soil-compost mixture under laboratory conditions, were followed. The measurements of CO_2 formed in the presence of microorganisms after a period of exposure of the plastic material to the soil are provided the evaluation of the degree of biodegradability. Biodegradability tests are not carried out in our country, and very few laboratories around the world perform this test and issue a biodegradability certificate. This method has been validated in our laboratory and biodegradability tests have been analyzed on some polymer materials, which are used in agriculture. With the results obtained, it was checked whether the European Union met the criteria set forth or not.

Keywords: Biodegradability, Sustainability, Agriculture, Test method

INTRODUCTION

With the advancement of technology and the increase in the world population, plastic materials have found wide application areas in all areas of life and industry. Most conventional plastics such as polyethylene, polypropylene, polystyrene, poly(vinyl chloride) and poly(ethylene terephthalate) are not biodegradable, and their increasing accumulation in the environment poses a threat to the planet. Bioplastics consist of either biodegradable plastics (i.e. plastics produced from fossil materials) or bio-based plastics (i.e. plastics synthesized from biomass or renewable resources). Polyhydroxybutyrate (PHB), polylactide (PLA) and starch mixtures are produced from biomass or renewable resources and are therefore biodegradable. Although Polyethylene (PE) and Nylon 11 (NY11) can be produced from biomass or renewable resources, they are not biodegradable. Environmentally friendly biodegradable plastics reduce greenhouse gas emissions because they are obtained from renewable raw materials. For example, polyhydroxyalkanoates (PHA) and lactic acid (raw materials of PLA) can be produced by fermentative biotechnological processes using agricultural products and microorganisms [1–3]. It offers many benefits, including increased soil fertility, reduced accumulation of bulky plastic materials in the environment (minimizing animal injury) and reduced waste management

costs. Additionally, biodegradable plastics can be converted into useful metabolites (monomers and oligomers) by microorganisms and enzymes. It should be evaluated in terms of biodegradability, microbial (enzyme) properties and plastic properties. Microbial (enzyme) properties refer to the distribution and types of microorganisms, as well as growth conditions (pH, temperature, moisture content, oxygen, nutrients, etc.) and enzyme types (intracellular and extracellular enzyme, exo- or endo-cleavage types). The chemical structure of polymers is important for the biodegradability of water-soluble polymeric materials. When evaluating the biodegradability of solid polymers, attention should be paid to their physical properties as polymer aggregates as well as their chemical properties. In addition, the surface conditions of plastics (surface area, hydrophilic, hydrophobic properties) generally affect the biodegradation mechanism of plastics [2].

The biological diversity and formation of microorganisms vary depending on environmental factors such as soil, sea, compost and activated sludge. In general, adhesion of microorganisms to the plastic surface and subsequent colonization of the exposed surface are the main mechanisms involved in microbial degradation of plastics. Enzymatic degradation of plastics by hydrolysis is a two-step process: first the enzyme binds to the polymer substrate, then catalyzes a hydrolytic cleavage. Polymers are broken down into low molecular weight oligomers, dimers and monomers and finally mineralized to CO_2 and H_2O [4-5]. Two standard test methods were followed to determine the degree and rate of aerobic biodegradation of plastic materials in contact with soil relative to the reference material under laboratory conditions. These test methods are designed to be applicable to all plastic materials that do not inhibit bacteria and fungi found in soil.

Soil medium (natural soil or laboratory mixture of natural soils collected from selected locations or "natural soil/mature compost mixtures for standard soil"), shape of the test sample (large film samples or fragmented or pulverized film), soil pH (natural or adjusted), C/N ratio (natural or adjusted to 10:1-20:1 in the sample or with added organic C or the sample adjusted to at least 40:1 for the ratio of organic C to soil N) parameters must be set [6-7-9]. When performed by different laboratories, such permissible variations in the application of the test method may lead to poor reproducibility of results. Reproducibility is one of the most important issues in biodegradation standard testing methods [10-11]. Many factors such as soil type, soil biodiversity, testing conditions (temperature, water content, nutrients) and measurement method can affect the repeatability of results. Eliminating some of these sources of uncertainty and assessing the range of validity of test methods are important issues for establishing robust and reliable biodegradation test methods [8].

MATERIAL AND METHOD

By following the ISO 17556 test method and its equivalent ASTM 5988 test method, biodegradability test trials were carried out by taking some plastic samples with different carbon contents used in agriculture to determine the amount of carbon dioxide produced by microorganisms. The test setup is an environment where soil and test material are mixed at the bottom of the desiccator, and there is barium hydroxide ($Ba(OH)_2$) solution in one beaker and water in the other beaker on a perforated plate. Samples were sent to the METU University Central Laboratory to determine the initial %C content of each test sample. The test was carried out in a dark environment in an air-conditioning cabinet and the ambient temperature was kept between 20°C and 28°C. Then, to start the first series of biodegradability trials, a soil sample was taken from the forest and analyzed. Care was taken to ensure that the soil pH value was between 6 and 8. By studying 21 test samples that were different from each other in terms of content, 3 parallel experiments were started from each of them to see the repeatability. A total of 72 tests were started simultaneously. Cellulose was used as the reference material, and 3 parallel tests were started for blank and technical trials. The water in the environment was changed at certain time intervals and the amount of CO_2 produced by microorganisms was

calculated by back titrating the unreacted barium hydroxide solution ($Ba(OH)_2$) with hydrochloric acid solution. The values are compared with the theoretically expected amount of CO_2. Then, for the 2nd series of biodegradability trials, standard soil was used, unlike the 1st series. This standard soil contains 700g/kg industrial quartz sand, 100g/kg clay, 160g/kg natural soil and 40g/kg mature compost. To standard soil, Potassium dihydrogen phosphate (0.2 g/kg soil), Magnesium sulfate (0.1 g/kg soil), Sodium nitrate (0.4 g/kg soil), Urea (0.2 g/kg soil) and Ammonium chloride (0.4 g/kg soil) salts were added dissolved in water. After the standard soil was prepared, it was analyzed to know its initial values such as %C content, lime, pH and EC. Samples with different contents from the first series were added and an applicator was used to keep the film thickness constant. The samples were passed through a grinder and turned into powder. To see their repeatability, 3 parallel tests were started. Cellulose was used as the reference material, and tests were started for blank and technical trials. The water in the environment was changed at certain time intervals and the amount of mg CO_2 produced by microorganisms was calculated by back titrating the unreacted barium hydroxide solution with HCl. Percentage biodegradability values were calculated by proportioning the amount of CO_2 released as a result of the measurement with the amount of CO_2 theoretically required to be found.

Figure 1. Applicator used to adjust film thickness

Figure 2. Adjusting the thickness of various film samples using an applicator

Figure 3. Film sample pulverized by cryogenic crusher

Figure 4. Experimental set up

RESULTS AND DISCUSSION

Deviations in the 1st series biodegradability studies (Example 1, Example 2, Example 7, Example 8 and Blank samples in Graph 1.) were eliminated by conducting the 2nd series studies. Unlike the 2nd series, standard soil (as seen in Table 1, organic matter content was reduced from 10.64% to 2.43%) was used and the amount of soil and sample was increased. The chemical and physical structure of the soil (mineral, lime amount, organic matter, C/N ratio, pH, etc.) is one of the most important parameter. The pH value should be between 6-8. When pH is above 8.0, the soil retains more CO2 developed by microorganisms than a neutral soil. A soil with a pH below 6.0 has the potential to contain an atypical microbial population. As seen in Table 1, the pH of the soil used in the 1st series is 7.8 (slightly alkaline) and the pH of the soil used in the 2nd series is 7.75. Viability, number of microorganisms, diversity of microorganisms, inhomogeneity of film thickness and bubbles on the film surface affect the result of the test. An applicator was used to keep the film thickness constant, and the test materials were pulverized with a cryogenic crusher. One of the most important reasons for the deviation in the 1st trial is that the blank trial (no sample, only soil) constantly produces CO_2 due to its high organic matter content (as seen in Table 1).

ANALYSIS RESULTS				
Test	**Unit**	**Test method**	**Serie-1**	**Serie-2**
Body (Sand)	%	Hydrometer	15	51
Body (Clay)	%	Hyrometer	47	5
Body (Silt)	%	Hydrometer	38	44
pH (25°C)		1:2,5	7,8	7,75
EC	mS/cm	1:2,5	0,24	1,68
Limestone	%	Calcimetric	21,3	11,8
Organic Matter	%	Walkey-Black	10,64	2,43
Nitrogen (N)	%	Theoretical	0,59	0,12
Phosphor (P)	ppm	Spectrofotometric	77,4	64,85
Potassium (K)	ppm	A.A/ICP-OES	1,088	623
Calcium (Ca)	ppm	A.A/ICP-OES	5,868	3,378
Magnessium (Mg)	ppm	A.A/ICP-OES	301	181
Sodium (Na)	ppm	A.A/ICP-OES	59,1	279
Change Na	%	Theoretical		5,71
Iron (Fe)	ppm	DTPA/ICP-OES	38,84	11,5
Manganese (Mn)	ppm	DTPA/ICP-OES	10,9	10,48
Zinc (Zn)	ppm	DTPA/ICP-OES	6,64	3,34
Copper (Cu)	ppm	DTPA/ICP-OES	1,37	0,72

Table 1. Analysis results of soil samples used in the 1st series and 2st series biodegradability trials

According to the standard method the validation criterias are given below;

a) the degree of biodegradation of the reference material is more than 60 % at the plateau phase or at the end of the test;

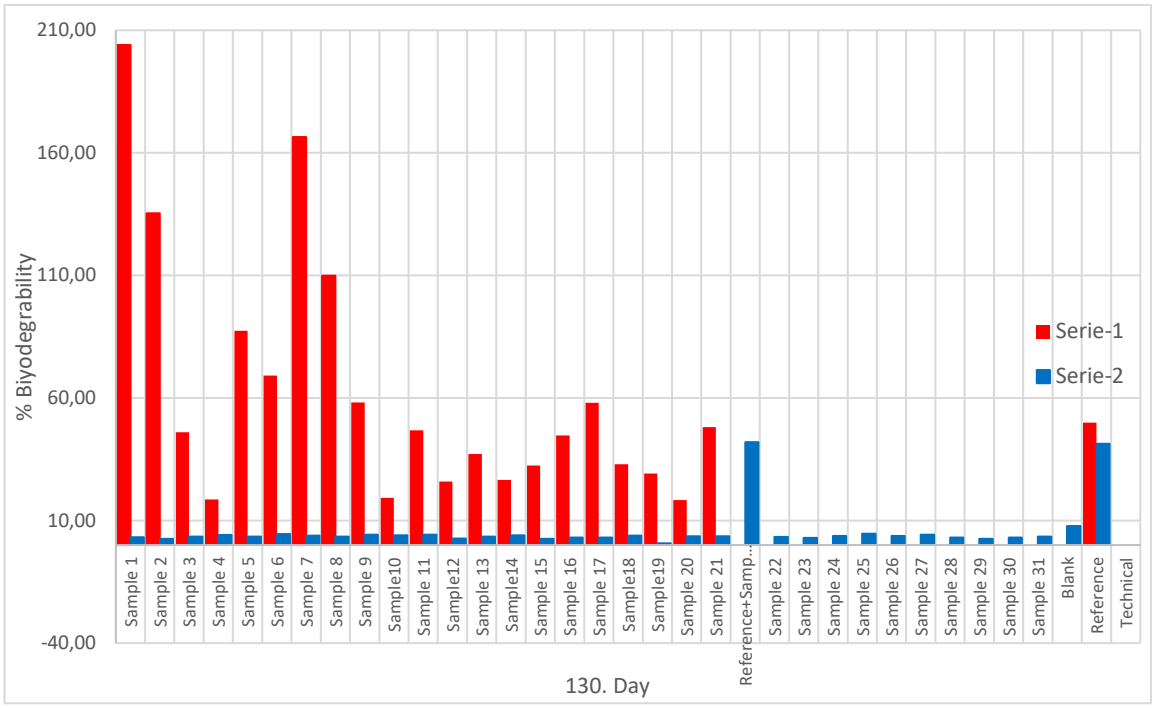
b) amount of carbon dioxide evolved from, the three blanks are within 20 % of the mean at the plateau phase or at the end of the test.

As seen in Table 3, the deviation value of the three blank trials at the end of the test was within 20% of the average.

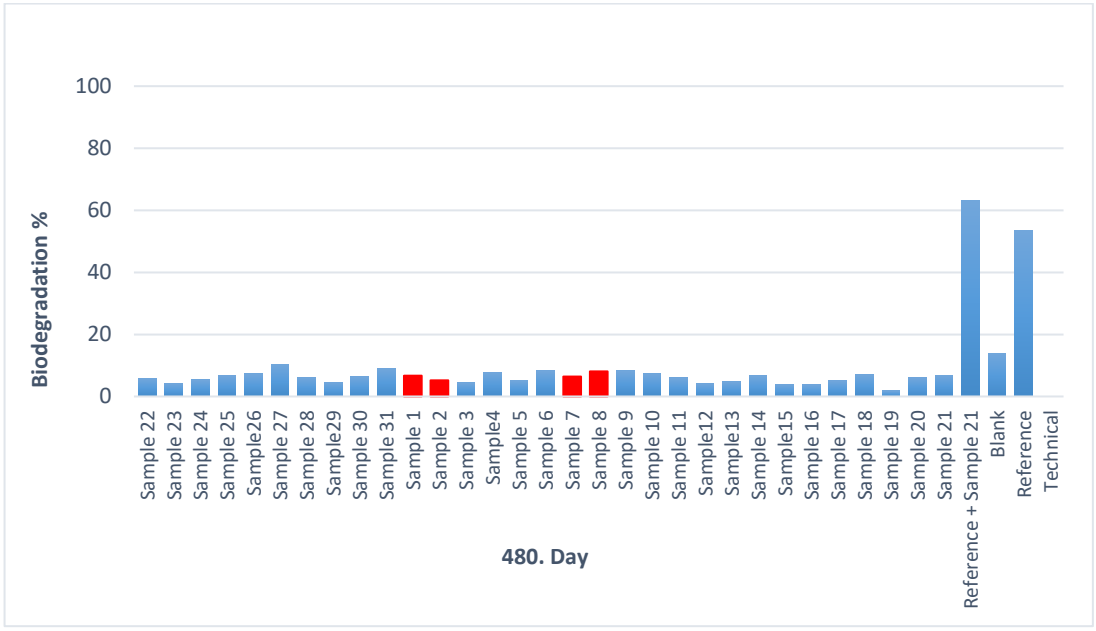
	1. measurement %deg	**2. measurement %deg**	**3. measurement %deg**	**Average %**	**Range %**
Blank	11,23	10,29	13,89	11,8	9,44-14,16

Table 3. Deviation values of three repeated blank experiments

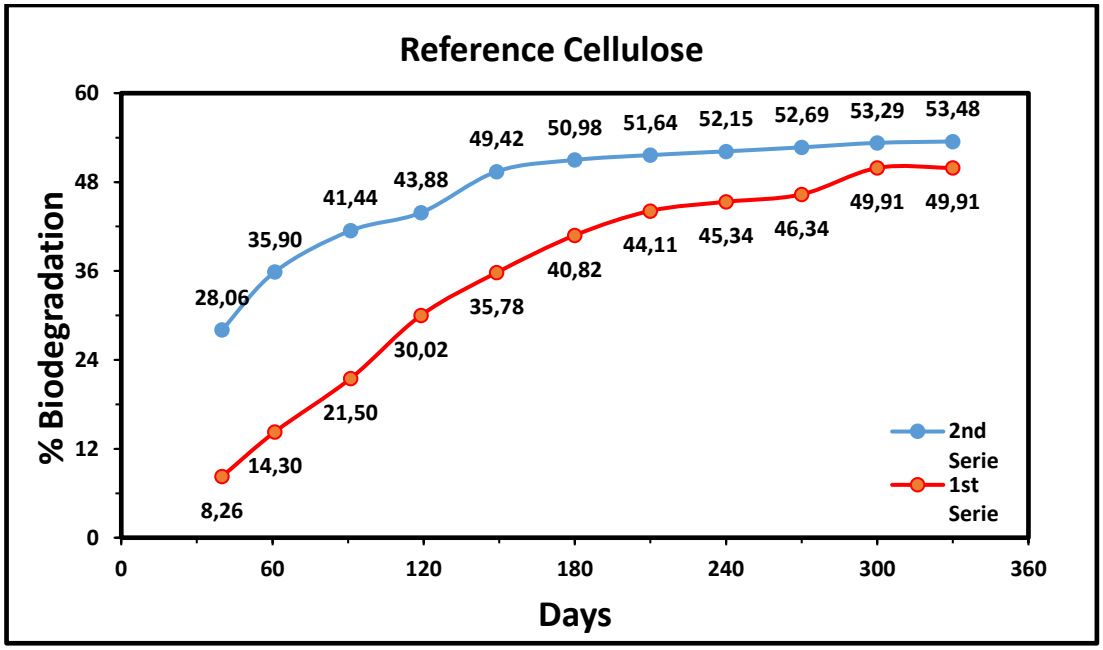
Even in the 2nd series trial, most of the estimated reason of the errors seen in the 1st series were eliminated, the biodegradability value of cellulose remained at 53,56% and there are many factors that may cause this. The purpose of this trial is to see whether the measures taken against the problems in the 1st series worked in the 2nd series.



Graph 1. Biodegradability values of the samples in the 1st and 2.st series at the end of 130 days



Graph 2. Biodegradability values of the samples in the 2st series at the end of 480 days



Graph 3. Decomposition trend of the same substance at different times

CONCLUSIONS

In this study, biodegradability values were determined by applying standard test methods. However, parameters such as viability, number and diversity of microorganisms, soil properties, soil amount, %C content of the sample, sample amount, temperature and humidity affect the course of the test. Errors observed in the 1. Series were eliminated by changing these parameters. The 1st and 2nd series trials require a lot of experience, and the studies shed light on the next steps.

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NOVEL APPLICATION OF POMEGRANATE PEELS-CHITOSAN AS A PRETREATMENT FLOCCULANT FOR ENHANCED SAND FILTRATION AND EFFICIENT REMOVAL OF HEAVY METALS FROM WASTEWATE

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ABSTRACT

This work explores the potential of using pomegranate peels and chitosan as a natural flocculant for pre-treating wastewater to enhance sand filtration and remove heavy metals (Ni^{2+}, Cu^{2+} and Zn^{2+}) effectively. Active compounds like tannin are extracted and purified from the pomegranate peels, then chitosan and tannin are modified to create a novel flocculant. The results of this project provide a safe, easy, eco-friendly and cheap method of wastewater treatment. The parameters investigated include flocculant dosage, contact time, pH, and heavy metal concentration. To ensure the effective execution of this research, a series of flocculation jar tests were performed under varying conditions. The natural compounds exhibit synergistic effects, combining the adsorption capabilities of extract product of pomegranate peels - tannin and the coagulation properties of chitosan. The findings of this study contribute to the development of sustainable and cost-effective solutions for heavy metal removal from water. The utilization of natural compounds offers an eco-friendly approach, reducing the reliance on synthetic flocculants and minimizing the environmental impact associated with heavy metal contamination. The flocculation-sand filtration system offers a viable solution for treating wastewater with dissolved metal ions, operating at low pressures, and enabling environmentally safe discharge.

Keywords: Wastewater treatment, Natural flocculant, Chitosan, Pomegranate peels, Eco-friendly

INTRODUCTION

Water is a vital resource that is essential for sustaining life and supporting various industrial processes. However, the rapid growth of industrialization and urbanization has led to a significant increase in the volume of wastewater, which is often contaminated with harmful pollutants, including heavy metals. Heavy metal pollution resulting from industrial activities in ferrous and nonferrous metallurgy and chemical industries poses a significant threat to the environment. The presence of heavy metals such as nickel (Ni^{2+}), copper (Cu^{2+}), and zinc (Zn^{2+}) in wastewater poses a serious threat to the environment, affecting ecosystems and human health [Verma A.K., et al., 2012]. To mitigate these challenges and promote sustainable water management, there is an urgent need for innovative and environmentally friendly wastewater treatment methods. Traditional treatment approaches often rely on chemical flocculants, which can be expensive and pose additional environmental problems [Freitas T.K.F.S, et al., 2018].

Heavy metals such as chromium, copper, iron and lead are ubiquitous pollutants that, even in low concentrations, can cause serious damage to living organisms. Traditional methods of

wastewater treatment often use inorganic and synthetic polymer flocculants, which can contain toxic and harmful chemical compounds that have a negative impact on the environment.

The use of iron and aluminum salts as inorganic coagulants inwater and wastewatertreatment has been widespread due to theireffectiveness in pollutant removal, ease of mixing, user-friendly handling and storage, and cost-effectiveness [Chai W.S., et al., 2021]. However, despite theiradvantages, the usage of these coagulants is not withoutdrawbacks, leading tocertain concerns inwater treatment processes. One of the maindrawbacks associated with the use of ironand aluminum saltsis the generation of a substantial volume of sludgeduring the treatment process. This sludgecan pose challenges for disposal andcan contribute to environmentalconcerns if notmanaged properly. Furthermore, the application of these coagulants often requiresthe addition of alkalinity and pHadjustment to achieve optimal treatment results. This additional stepcan increase the complexity of the treatment process and mayresult in increased operational costs. Anothersignificant concern is the potential high concentration ofresidual metals, particularly aluminum, in the treated wateror sludge. High levels of aluminum in water sourcescan have adverse effects on human health and the environment. Studies have raised concerns about the potential link between the neurotoxicity of aluminum found in wastewater sludge andthe pathogenesis of Alzheimer's disease [Beyene H.D., et al., 2016]. While the direct cause-effect relationship between aluminum exposure and Alzheimer's diseaseremains a subject of ongoing research, the potential risk underscores the need for careful consideration and monitoring of metal concentrations in treated water and sludge.

Plant-based coagulants havegarnered significant attention in the field of water and wastewater treatment andhave been the subject offrequent research. Some of the plant-based coagulants that have been extensively studied include Moringa oleifera (M. oleifera), Strychnos potatorum (nirmali), tannin, and cactus [Sellami M., et al., 2014], [Hameed Y.T., et al. 2018].

The use of natural flocculants such as chitosan and tannin offer an environmentally friendly alternative to wastewater treatment. Recent research has shown the potential of cationic tannins as effective coagulants or flocculants for wastewater treatment. However, previous studies mainly focused on the removal of colloidal substances and the influence of heavy metals on hardness has not yet been fully studied. The structure of tannin is presented schematically in Figure 1.

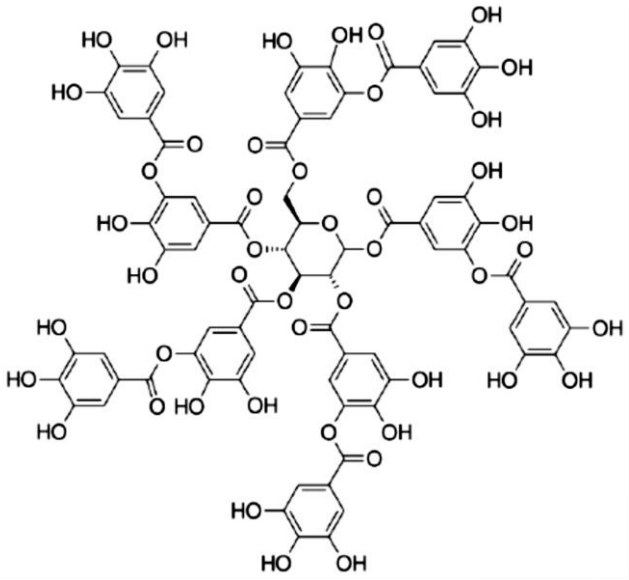

Figure 1. Structure of Tannin

Tannin extracted from various sources, such as valonia oak and Schinopsisbalansae, has beenapplied in wastewater treatment forturbidity removal [Simón U.-F., et al., 2021], [Saad H., et al., 2012].

This project investigates the potential of using natural resources, namely pomegranate peel and chitosan, as a novel and environmentally friendly flocculant for the pre-treatment of wastewater. Pomegranate peel, an abundant agricultural waste, has been found to contain active compounds such as tannin known for their flocculating properties [Li X., et al., 2019]. By extracting and purifying these compounds, we can harness their potential for wastewater treatment. For over 4000 years, pomegranate (Punica granatum L.) has been cultivated by humans due to its medicinal and nutritional properties. This fruit holds significant cultural importance in ancient Mediterranean civilizations. In 2018 alone, California produced approximately 218,000 tons of pomegranates, making roughly 118,000 tons of pomegranate rind and seed waste. On a global scale, there are three-million tons of total pomegranate production, resulting in approximately 1.62 million tons of waste [Pantoja-Castro M. A., 2019]. The sheer amount of waste that is produced for each edible percentage of pomegranate makes it important to look for proper methods of optimizing the nutritional and bioactive components of pomegranate waste and then convert this waste into value-added products to save energy, sustain resources, and protect the environment.

Chitosan, a biopolymer derived from chitin, complements the natural flocculating properties of pomegranate peel. Through modification, chitosan can be enhanced to further enhance its effectiveness as a flocculant, making it an ideal candidate for combination with tannin.

Chitosan, a non-toxic polysaccharide composed of repeating N-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN) monomers, is widely used as a cationic coagulant pretreatment to assist in the removal of microbial and heavy metal contamination from drinking water. The structure of chitosan is presented schematically in Figure 2.

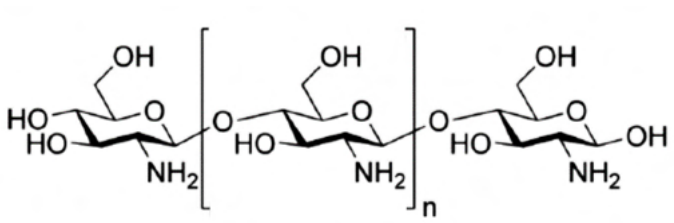

Figure 2. Structure of chitosan

Chitosan can neutralize the negative charges and also bridge the aggregate of destabilized particles. Chitosan can be produced locally as it is derived from the chitin found in the shells of shrimp and crustaceans, which are abundant in many resource-limited settings. This local production of chitosan presents a viable business opportunity for entrepreneurs in those regions. Presently, commercial production of chitin and chitosan is primarily conducted in several countries, including Japan, the United States, India, Poland, Australia, and Norway. Additionally, to a lesser extent, these materials are produced in Canada, Italy, Chile, and Brazil. The cost of chitosan manufacturing will vary depending on the specific region and the availability of feedstocks. According to Roberts, G., the average manufacturing cost of chitosan is estimated to be around $11.5/kg [Lipps W.C., et al., 2011].

Indeed, the urgent need toaddress the challenge of heavymetal removal from wastewatercalls for the development of an efficient flocculantthat can effectivelytreat such pollutants before conventional sandfiltration. While natural coagulants have been extensively studiedfor water and wastewater treatment, the potential of chitosanmodified with tannin as a

natural coagulantremains unexplored. Thispresents a valuable opportunityfor researchers toinvestigate the effectiveness and applicability of this novel coagulantin environmentalremediation and sustainable watertreatment practices. The study of chitosanmodified tannin as a new flocculantholds great promise andmay lead to significantadvancements in wastewater treatmentmethodologies.

MATERIAL AND METHOD

Chitosan was is supplied by Merck (Sigma-Aldrich, USA CAS Number: 9012-76-4). Tannin extract from pomegranate peels is obtained using the Soxhlet extraction method.

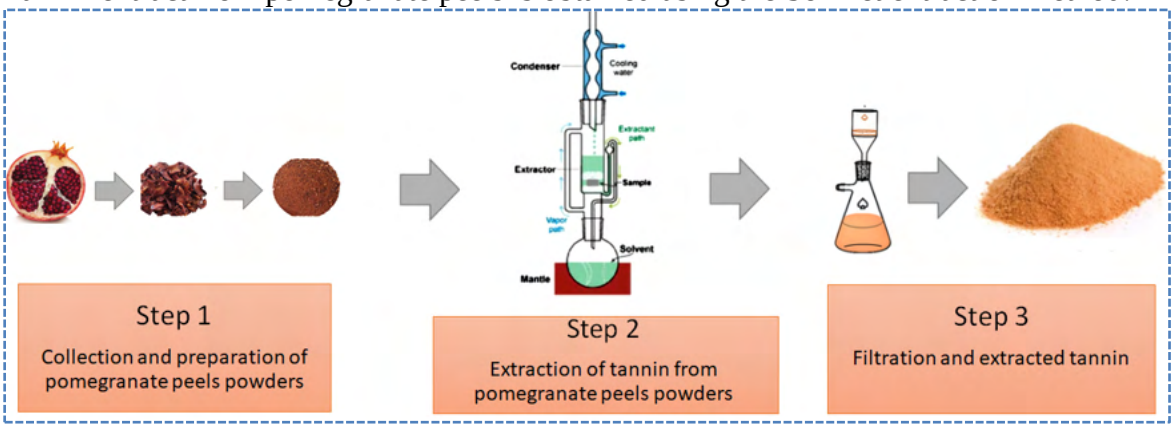

Figure 3. Soxhlet extraction of tannin

The Soxhlet extraction is a technique that involves continuously cycling a solvent (such as ethanol or water) through a sample material to extract desired compounds (Figure 3.). In this case, pomegranate peels are placed in a thimble and subjected to repeated solvent extraction and condensation cycles. This process allows for the efficient extraction of tannins from the peels, which can then be used as a coagulant in combination with chitosan for the pretreatment of wastewater and removal of heavy metals.

To extract tannin from pomegranate peels, the cleaned peels were first cut into pieces, thoroughly washed with distilled water, and then dried in an oven fora duration of 4 hours. Once dried, the peels were ground into a fine powder using a grinder. Subsequently, the powdered samples were sieved through a 40-mesh sieve to achieve a uniform particle size. To maximize tannin extraction, the process was carried out at elevated temperatures. This involved four rounds of extraction using a water-ethanol mixture (1:1) in a Soxhlet apparatus, following a known method [Lipps W.C., et al.,]. The tannin extract obtained from the extraction process was collected in a ceramic bowl and further dried in a thermostat until its weight reached a stable state. To verify the presence of tannin in the pomegranate peel extract, a test was conducted. A mixture of 5 ml of the extract, 5 ml of distilled water, and 3-4 drops of 0.1%ferric chloride was prepared in a test tube. If tannin was present, a color change to blue would be observed in the reaction mixture, indicating the presence of tannin.

To analyze the chemical structure of the extracted tannin, was using the Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu® Japan) within the wave range of 4000-500 cm^{-1} in ± 60 seconds. This spectroscopic analysis allowed for the identification and characterization of the chemical bonds present in the obtained tannin, providing valuable insights into its molecular structure and properties.

Figure 4. shows that the spectrum of tannic acid where it can find a strong absorbtion around 3402 cm^{-1}. This band is assigned to the hydroxyl groups (-OH) H-bonded broad. At 1521-1517 a band due to the C-C aromatic compounds are observed. A weak signal at 1611 cm^{-1} is related to carbonyl groups. Peaks determining during 1600-1400 cm^{-1} are characteristics of aromatic compounds.

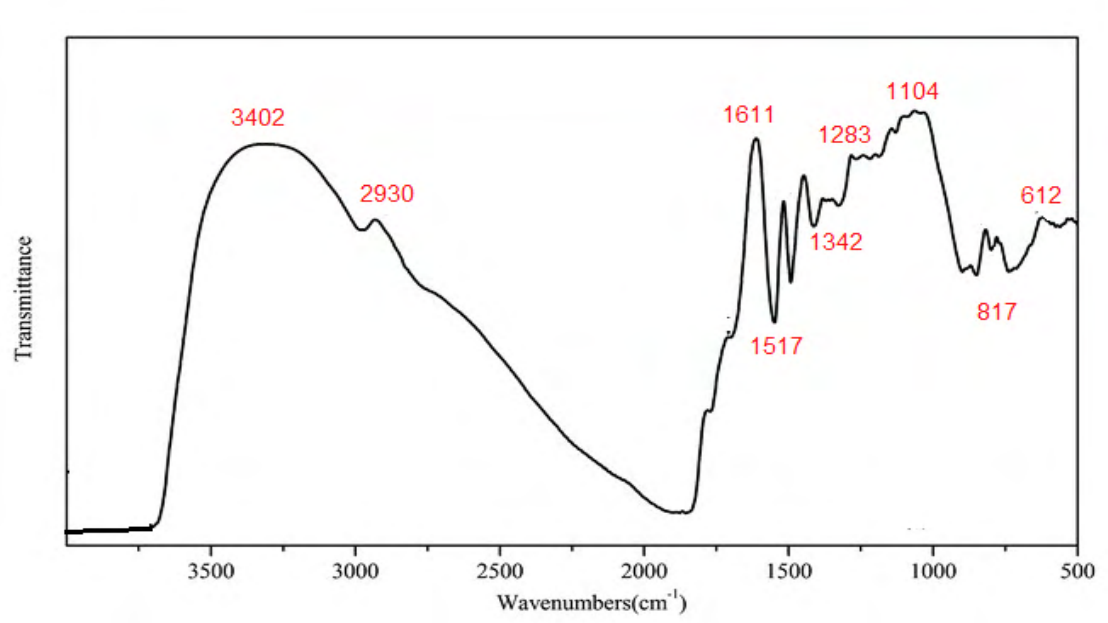

Figure 4. IR Fourier spectrum of tannin compound extracted from pomegranate peel

The composite utilized in the study in corporates a 25% glutaraldehyde or 1,5-pentanodial (OHC-$(CH_2)_3$-COH) compound, which is locally produced in Russia and serves as a binding agent in the composition. This compound is cost-effective and plays a significant role inaltering the properties of thepolymer through cross-linking via amino groups present in chitosan. Byutilizing 25% glutaraldehyde or 1,5-pentanodial in the composite, the researchers can create strong bonds between the chitosan molecules, enhancing the overall structural integrity and stability of the material. This cross-linking process results in improved properties such as increased mechanical strength, enhanced chemical resistance, and better tolerance to environmental conditions. The low cost and advantageous properties of this binding agent make ita suitable choice forthe synthesis of the composite, further supporting the development of a cost-effective and efficient flocculant for water and wastewater treatment applications.

1.1. Modification and blending of tannin and chitosan.

In the experimental procedure, 4 g of chitosan was introduced into a 500 mL 2-neck round-bottom flask containing 1% acetic acid. The mixture was then stirred at a rate of 100 rpm using a magnetic stirrer for 1 hour. Concurrently, 4 g of tannin was added to25 ml of distilled water and mixed thoroughly. The two aqueous solutions were then combined and stirred together at a temperature of 25°C for a duration of 6 hours. Subsequently, 1 mL of glutaraldehyde (25%) was introduced into the suspended mixture. The stirring process continued initially at 25°C for 4 hours and then at an elevated temperature of 40°C for another 4 hours. Through out the experiment, the pH of the medium was maintained at 2 by the addition of hydrochloric acid. The resulting product was apale yellow material, which was then filtered, washed with distilled water, and dried in an oven at 40°C for 20 hours. Once dried, this modified tannin-chitosan composite was applied as apretreatment coagulant in wastewater treatment. By combining tannin and chitosan with the modification process, a synergistic coagulant mixture is created, offering improved capabilities for the efficient removal of heavy metals from wastewater. This innovative approachhas the potential to significantly enhance the overall efficiency and effectiveness of wastewater treatment procedures, contributing to a more sustainable and environmentally friendly water management system.

1.2. Collection of wastewater samples and analysis methods

Surface water was collected from Vilash river in the South of Azerbaijan on February 22, 2023 and was artificially contaminated with various metal solutions. The purpose of this approach was to examine the issue from a realistic perspective. The river water was treated immediately after collection. Metal concentration analysis was carried out by a spectrophotometric method. The characteristics of the raw water sample were analyzed following the APHA standard methods to assess both water and wastewater properties [Lipps W.C., et al., 2023].

The treatment process involved the following steps: 1 liter of the turbid surface water was placed in a beaker. Approximately 20 ppm of metal was added, and the pH of the experiment was adjusted using a 1 M HCl solution and a saturated NaOH solution. The JAR-test procedure was performed using a VELP-Scientifica JLT4 apparatus. Subsequently, a specific dose of flocculant was added, the pH was readjusted, and the jar test procedure was repeated in the same manner. After 1 hour of settling, the loss in metal concentration was determined. Flocculation process at laboratory shown in Fugure 5.

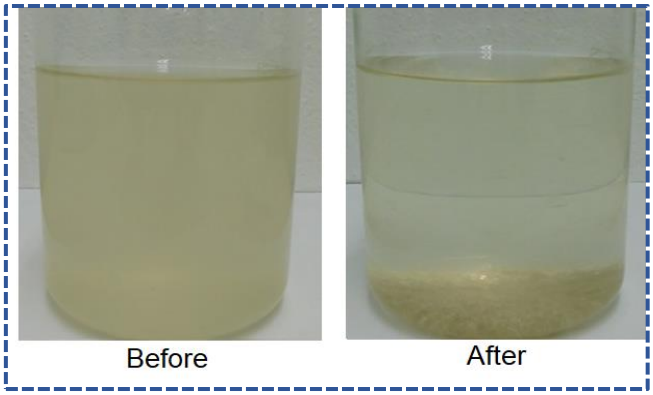

Figure 5. Flocculation process at laboratory

1.3. Chemical treatment and sand filtration

The schematic diagram of our proposed flocculation and sand filtration system consists of various components arranged in a sequential flow to purify water.

Firstly, coagulant adds to the wastewater. Coagulants help destabilize and aggregate small suspended particles in the water. Then the water with added coagulant enters the rapid mix tank. In this tank, high-speed mechanical mixing agitation is employed to promote the rapid and uniform mixing of the coagulant with the water. The detailed setup of the offer treatment filter system is shown in *Figure* 6.

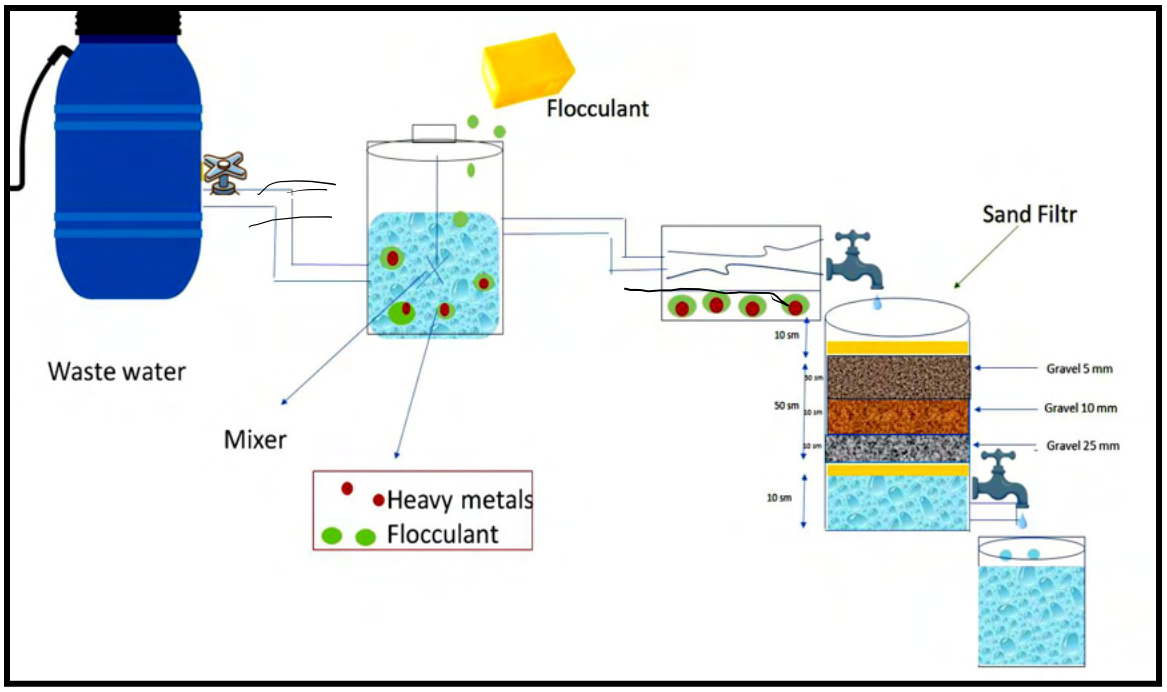

Figure 6. Schematic diagram of water treatment filtration system

As it clearly seen the water from the flocculation tank moves into the clarifier, which facilitates the settling of the larger floc particles. The clarifier is designed to provide a quiescent zone where the floc particles can settle under the force of gravity. The settled particles form a sludge layer at the bottom, while the clarified water moves on to the next stage. The clarified water then enters the sand filter, which consists of a bed of granular media. The sand acts as a physical barrier, trapping remaining suspended particles as well as some dissolved substances. The water percolates through the sand bed, allowing the clean water to pass through while retaining the contaminants.

1. RESULTS AND DISCUSSIONS

During the electroplating process, the wastewater generated can contain complex heavy metals, including Cu^{2+}, Zn^{2+} and Ni^{2+}. These heavy metals pose significant risks to human health and the ecological environment if discharged without proper treatment [Asrafuzzaman M., et al., 2011]. The utilization of pomegranate peel extract and chitosan as a novel flocculant for wastewater pretreatment shows great potential in enhancing sand filtration and effectively removing heavy metals.

The combination of pomegranate peel extract tannin and chitosan as a flocculant offers several advantages, including being natural and eco-friendly. Application of this flocculant has successfully demonstrated the removal of heavy metals such as Cu^{2+}, Zn^{2+}, and Ni^{2+} from wastewater, achieving significant reductions in metal concentrations, with Cu^{2+} reduced by up to 90%, Zn^{2+} by up to 75%, and Ni^{2+} by up to 70%.

Figure 7 shows the removal of Cu^{2+}, Zn^{2+}, and Ni^{2+} ions from water, depending on the dosage of the flocculant and the pH of the water.

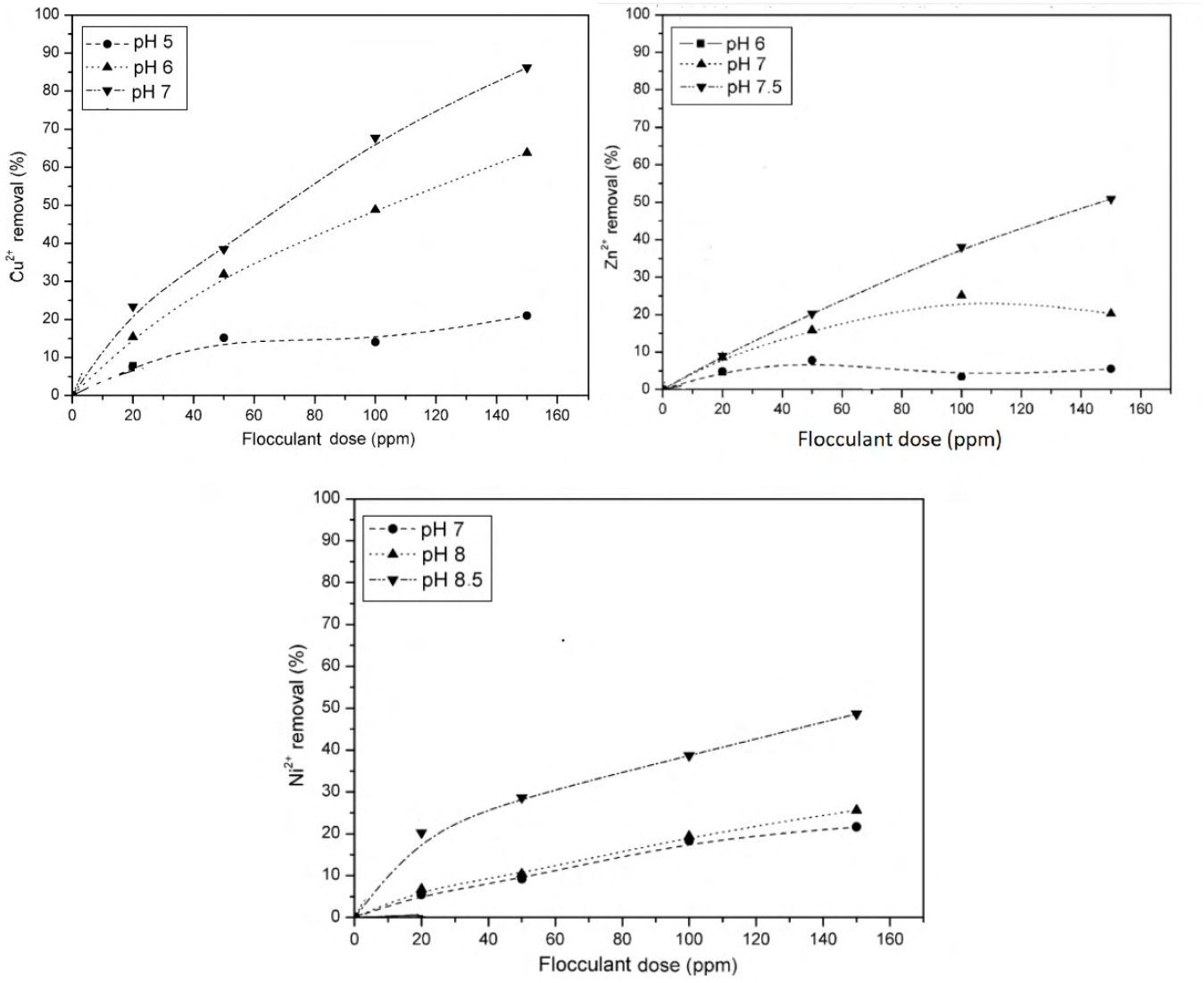

Figure 7. Removal of Cu^{2+}, Zn^{2+}, and Ni^{2+} ions from water depending on the dosage of the flocculant and the pH of the water

The addition of the flocculant, along with proper pH adjustment, has improved the efficiency of the metal removal processes. pH was identified as a critical variable, with specific optimum values determined for different metals. Compared to traditional methods such as chemical precipitation and conventional coagulation-flocculation processes, the pomegranate peel-chitosan flocculant offers advantages due to its natural origin, ease of production, and simplified pH adjustment requirements. Further investigations are warranted to explore the efficacy of the pomegranate peel-chitosan flocculant with other challenging-to-remove metals using conventional methods.

2. CONCLUSION

The implemented wastewater treatment system, comprising of flocculation and sand filtration processes, effectively treated metal-ion-containing wastewater from a chemistry research laboratory, meeting the recommended discharge standards. The novel bioflocculant process significantly improved various water characterization parameters, including pH and turbidity. The application of bioflocculant successfully removed heavy metals such as Cu^{2+},

Zn^{2+}, and Ni^{2+} from the wastewater, achieving substantial reductions in their concentrations, with Cu^{2+} reduced by up to 90%, Zn^{2+} by up to 75%, and Ni^{2+} by up to 70%. The flocculation-sand filtration system offers a viable solution for treating wastewater with dissolved metal ions, operating at low pressures, and enabling environmentally safe discharge.

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SEROLOGICAL ANALYSES OF VIRUSES PRESENCE ON TOMATO COLLECTION FROM THE GENE BANK OF THE REPUBLIC OF SRPSKA

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Abstract

Testing for virus presence on tomato (*Solanum lycopersicum* L.) collection from the Gene Bank of the Republic of Srpska was conducted during 2023 in greenhouse of the Institute of Genetic Resources. Thirty samples were taken and preliminary tested for presence of 3 viruses: TSWV (Tomato spotted wilt virus, Tospovirus), TBRV (Tomato black ring virus, Nepovirus), ToBRFV (Tomato brown rugose fruit virus, Tobamoviruses) with ELISA (Bioreba) test. Fourteen samples were positive for TSWV presence and negative for other two viruses. The previous investigations have been conducted on the presence of TSWV on conventional tomato varieties in the open field and in the greenhouse, but never on the tomato accessions from the Gene Bank that represent domesticated germplasm.

Keywords: TSWV, TBRV, ToBRFV, tomato, Gene Bank

INTRODUCTION

Tomato spotted wilt virus (TSWV) is one of the most widespread plant viruses and also have the largest host-range. The current list of TSWV hosts consists of 1090 plants species (Parrella, et al. 2003) both monocotyledonous and dicotyledonous plants (Moyer, 1999) and weed species. Tomato spotted wilt virus (TSWV) belongs to the genus Tospovirus of the family Bunyaviridae. TSWV is transmitted by thrips in a circulative and propagative manner (Pappu, 2008). This virus is one of the most destructive virus, responsible for numerous epidemics in different regions of the world, and cause heavy economic losses (Parrella et al., 2003). In Republic of Srpska (district of Bosnia and Herzegovina), until now TSWV was detected in pepper plants from greenhouses and tobacco plants from open field (Delić et al., 2017) and also confirmed the presence of western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) and tobacco thrips, *Thrips tabaci* (Linderman) in greenhouses in Herzegovina region (Trkulja et al., 2013; Kohnić et al., 2006). The highly polyphagous nature, the efficiency of virus transmission and the biological activity of its vectors, the rapidity with which new variants arise,

and difficulties in the control of the vectors, make TSWV one of the most feared plant viruses by growers of agricultural crops. Preventive and integrated cultural practices such as the eradication of weed hosts able to serve as virus reservoirs, combined with vector management strategies, play a crucial role in the control of the virus.

Tomato black ring virus (TBRV) belong to the Nepoviruses group (nematode-transmitted virus) that infect many plant families: annual, perennial and woody plants, economically important crop species like: grapevine, cherry, apricot, peach, berry-fruits, different ornamental plants and weeds, and solanaceous species like: potato, pepper, tobacco and tomato (Brunt et al., 1997; Edwardson, Christie, 1997). They cause of economic importance diseases in a wide range of cultivated, and concerned as quarantine worldwide (Šneideris et al., 2012). Their wide host range combined with ability to be transmitted by nematodes, seed and/or pollen makes them sevear problem, hard to eradicate and control (Murant, 1981; Card et al., 2007). TBRV is transmitted both through seeds and by free-living nematodes *Longidorus elongatus* and *Longidorus attenuatus* (Harrison et al., 1961; Brown et al., 1989) by feeding on roots. The virus has been reported in Europe, North and South America, India and Japan (Brunt et al., 1997; Harper et al., 2011), Australia, New Zealand (Šneideris et al., 2012). Known until now, Tomato black ring virus (TBRV) was previously detected on potato and grapevine in some parts of Yugoslavia. Isolates from sugarbeet, pepper and tobacco were found in North Bosnia, the second tobacco isolate in Herzegovina, and the potato isolate in West Bosnia (Buturović et al., 1979).

Tomato brown rugose fruit virus (ToBRFV) belong to the genus *Tobamovirus*, and has been identified from tomato plants (Luria et al., 2017; Salem et al., 2016). ToBRFV was discovered in greenhouse tomato plants grown in Jordan and its first outbreak was in Israel (Salem et al., 2016). To date, the virus has been reported in at least 35 countries across four continents in the world. ToBRFV infects tomato as the primary host and considered the most serious threat to tomato production in the world. Recently, virus has caused devastating disease outbreaks in tomato production areas in many countries, resulting in a severe reduction in yield (Avni et al., 2021; EPPO, 2020; Jones, 2021; Oladokun et al., 2019). ToBRV is transmitted by mehanical contact, propagation material, plant debris, contaminated soil, growing media, circular water, workers farming activities and tools (Oladokun et al., 22019). Italy is the nearest country to Bosnia and Herzegovina that is identified the presence of this virus (Panno et al., 2019a). EPPO Working Party on Phytosanitary Regulations and Council agreed that ToBRFV should be added to the A2 List of pest recommended for regulation as quarantine pests in 2020.

MATERIALS and METHODS

Plant material

The research was conducted on 30 tomato accessions from the Gene Bank of Republic of Srpska: GB00415, GB00498, GB00545, GB00548, GB00874, GB00875, GB01092, GB01106, GB01107, GB01108, GB01109, GB01110, GB01122, GB01123, GB01124, GB01125, GB01126, GB01128, GB01129, GB01132, GB01238, GB01239, GB01240, GB01323, GB01324, GB01325, GB01345, GB01353, GB01414 and GB01421.

Containerized tomato seedlings were produced according to standard agricultural technology in the unheated glass greenhouse at the Faculty of Agriculture, University of Banja Luka. Total of 60 plants (2 plants per accession) were planted in pots in a tunnel-type polypropylene greenhouse at Institute of Genetic Resources (158 m altitude, 44.774971 latitude and 17.211463 longitude) with total area of 115 m^2. Fertilization was applied before planting and during vegetation. Plants were maintained using standard horticultural practices such as trellising and pinching. Insecticide was sprayed twice on the plants after transplanting in greenhouse to exterminate any thrips vector.

Leaf samples were collected on 105th day of vegetation when fruits on the 1st truss were ripe. All samples were collected in duplicate. All plants were tested for the presence of all 3 viruses, no matter if leaf simpthoms were present.

Sample preparation

Fresh leaf samples were homogenized using Bioreba extraction bags. Prepared samples were stored at 4°C over night.

Serological analysis

Prepared samples were analyzed by double-antibody sandwich enzyme-linked immunsorbent assay (DAS-ELISA) using commercial diagnostic kits (Bioreba AG, Reinach, Switzerland) against: TSWV (Tomato spotted wilt virus, Tospovirus), TBRV (Tomato black ring virus, Nepovirus) and ToBRFV (Tomato brown rugose fruit virus, Tobamoviruses) according to manufacturer's instructions. Commercial positive and negative controls were included in each assay. ELISA reactions were read for absorbance at 405 nm using a HiPo MPP-96 Microplate Reader (BioSan, Lithuania). Also, yellow colour development was assessed visually after 30 and 60 minutes.

Statistical analysis

All obtained results were analyzed by standard descriptive statistical methods. Samples with absorbance values twice higher than in healthy uninfected negative controls were considered positive for virus infection.

RESULTS and DISCUSSION

A total of 30 domesticated germplasm of tomato samples were collected in greenhouse. The main aim of this work is to check the presence of those 3 viruses in tomato accessions that are multiplicated for seed collection in the Gene Bank. Several leaf samples of each plant were collected from all accessions, symptomatic and asymptomatic plants. Mild symptoms like leaf chlorosis and leaf nerve yellowing were visible during sample collection and other symptoms were not noticed.

DAS-ELISA positive tests resulted in 46.67% (14/30) of TSWV infected plants, most of them asymptomatic plants. These results showed a high infection on TSWV which presence is already detected in Bosnia and Herzegovina. Also, the presence of *F. occidentalis*, vector of TSWV, raises possibilities for rapid dissemination of this virus in greenhouse.

Other two viruses TBRV and ToBRFV were negative in DAS-ELISA test for selected 30 tomato accession. This paper represents preliminary work and first results of virus presence in domesticated germplasm in Gene Bank.

Conclusion

During multiplication for seeds collection, plants must be checked in quality and helth status before storage in the Gene Bank. However, this pilot study represents background for a wider survey of TSWV, TBRV and ToBRFV presence and thrips species as potential insect-vectors in BiH.

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APPLICATION OF LIPOSOMAL ENCAPSULATED ANTIMICROBIAL BIOACTIVE COMPONENTS IN FOOD PRODUCTS AS NATURAL PRESERVATION

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ABSTRACT

Encapsulation technology is needed to make more durable and effective of alternative natural preservatives and nutritional components. In recent years, liposomal structures have attracted attention and liposomes ensure the preservation of the encapsulated material until the appropriate place and time thanks to controlled or delayed release capability. Liposomal structures prevent the conversion into harmful components during storage and increase the bioavailability. The liposomal encapsulation process provides to be more stable and more durable bioactive compounds in the food and the digestive system. The slowly release of antimicrobial components during storage against microbiological contaminations can be realized without allowing mold contamination and mycelium formation in food products. In addition, which will be carried out on non-chemical "hurdle" technologies in order to control the development of food-borne microorganisms and increase antioxidant activity in order to respond to consumer expectations, aims to produce product formulations suitable for the concept of 'Clean Label'. In addition, in order to respond to consumer expectations, it is possible to control the development of food-borne microorganisms and to produce product formulations in accordance with the concept of 'Clean Label' with liposomal systems suitable for "hurdle" technologies without chemical content.

Keywords: Liposom, natural preservation, bioactive compounds, Clean Label

INTRODUCTION

Bioactive components have important antioxidant and antimicrobial effects and also effective on human health. Encapsulation procedures have implemented to increase the mechanism of bioactive components' action. Liposome encapsulation is an important application thanks to provide the development of controlled release of bioactive components in food and increased stability. One of the biggest advantages of liposomes is to made from natural components. Liposomes can be included in food formulations without the need for any legal regulation due to natural structure. Liposomes are no usage limit compared to chemical origin substances, and so excessive limits lead to no health problems. This feature removes the obstacles to the use of liposome structures in foods.

Liposomes are used to improve the water dispersibility of hydrophobic components, to increase bioavailability and to protect the encapsulated components from adverse conditions such as light, heat, pH, oxidation, hydrolysis or chemical reactions, to enable the delivery of an encapsulated agent to a specific location, to reduce negative effects and particle toxicity. Liposome systems provide to control the circulation in the body by modulation of their size and regulating the release profiles with surface modifications of the bioactive components (Alavi et al., 2017; Lila and Ishida, 2017). Unlike other encapsulation methods, liposomal structures have no negative effect on product rheology properties thanks to very low phosphorylcholine-based lecithin concentration. In addition, the encapsulated components are more resistant to processes

such as cooking and pasteurization with the controlled release of bioactive substances in the liposomal structure.

ENCAPSULATION TECHNIQUES

Encapsulation is an excellent method for the preservation of bioactive, volatile and readily degradable compounds and additives in food applications. The purpose of encapsulation is to protected active ingredient from external factors, to ensure stable in the digestive system and to release slowly, to increase bioavailability, to mask the negative taste and odor, and to prevent the active ingredient from reacting with other ingredients (Delshadi et al., 2020). In the encapsulation process consists of the active components as the core material and the appropriate wall material. The coating agent plays a key role and an ideal coating material should have low hygroscopicity, high solubility, low viscosity, low cost, ability to produce a stable emulsion and provide high protection (Gomez et al., 2018). Lipids, proteins and carbohydrates are widely used as coating material in encapsulation systems. The coating materials are desirable to be inexpensive, plentiful, non-toxic, and compatible with the food matrix (Jafari et al., 2008; Delshadi et al., 2020).

Lipid-based coating agents have excellent functionality in emulsification, film formation and encapsulation of active compounds. These coating materials are less toxic and have many potential uses in industrial applications (Fathi et al., 2012). The lipid-based coating materials are polar lipids (eg monoglycerides, phospholipids) and non-polar lipids (eg triacylglycerol, cholesterol) (Đorđević et al., 2016). Polar lipids such as phospholipids have some properties as biocompatible, suitable for stabilization, preservation and controlled release of active compounds and good surfactants (Đorđević et al., 2016; Shishir et al., 2018). Encapsulation contains microcapsules, submicron capsules, and nanocapsules sizes. Micro and nano encapsulation techniques include high pressure homogenization (HPH), micro fluidization, ultrasonic technique, spray drying, spray cooling/cooling, freeze drying, spray freeze drying, complex coacervation, emulsification (spontaneous, phase inversion, miscellaneous), anti-solvent precipitation, extrusion, electro-spinning and electro-spraying, layer deposition, solid dispersion, fluid bed coating, molecular inclusion in cyclodextrins. Different forms of micro and nano encapsulation systems are reservoir and matrix, emulsions (multilayer emulsions, nano emulsions), lipid nanoparticles (solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), lipid vesicular carriers (liposomes, niosomes, phytosomes, bilosomes), hydrogel particles, molecular inclusion complexes, nanofibers, nanotubes, micelles.

LIPOSOMAL ENCAPSULATION
LIPOSOMES

Liposomes are basically amphipathic vesicles in phospholipid structure, similar in structure to the cell membrane, with polar and nonpolar heads and double lipid layer structure. Liposomes are versatile, biocompatible and biodegradable structures that can be used as carrier systems for unstable components due to their amphipathic properties (Subramani and Ganapathyswamy, 2020). Liposomes were first described in 1965 by Bangham et al. (1965) are small intracellular shaped structures consisting of a closed membrane storing or transporting lipid-based substances. Phospholipids are one of the main groups providing liposome formation. Phospholipids are mainly composed of three-carbon alcohol, glycerol or sphingosine. Common alcohol components of glycerol-derived phospholipids are called phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylinositol (PI). Phospholipids are formed by esterification of the primary hydroxyl group of glycerol with phosphoric acid. The remaining

two hydroxyl groups of the glycerol backbone are esterified to fatty acids (saturated or unsaturated) and form the nonpolar tails of the lipid (Segota and Tezak, 2006). Liposomes consist of two layers of molecules with nonpolar groups. In the liposomal structure, polar head groups are directed outward, while non-polar parts are directed inward. Hydrophobic interactions and Vander Walls bonds that hold long hydrocarbon tails together play an important role in bilayer formation (Bozzuto and Molinari, 2015).

Liposomes are divided into different categories based on their structural properties and composition. Liposomes differ from each other in size and physical morphology, depending on lipid composition and preparation method, and may consist of one or more lipid bilayers. The phospholipid type influences the dimensions and physicochemical properties of the liposomes (Singh et al., 2012). Liposomes according to the composition and mechanism of intracellular delivery as follows: pH sensitive liposomes, conventional liposomes immuno-liposomes, cationic liposomes and long-circulating liposomes (Sharma and Sharma, 1997). Generally, the size change from 20 nm to 5000 nm and consist of one or more lipid bilayers. Liposomes according to lipid composition; preparation method and diameter as follows: multilamellar vesicles-MLV (>500 nm), small unilamellar vesicles-SUV (<50 nm), large unilamellar vesicles-LUV (100-1000 nm), giant unilamellar vesicles-GUV (>1000 nm), Multiple vesicles-MLV (>5000 nm), Oligomellar vesicles-OLV (100–1000 nm) and intermediate unilamellar vesicles-IUV (40-100 nm) (Lasic, 1998; Storm and Crommelin, 1998).

LIPOSOME PRODUCTION METHODS

Bangham method (thin film hydration method); One of the simplest methods for liposome formation in multilamellar vesicles is the thin-film hydration procedure. Thin-film hydration method is the most widely used technique to prepare liposomes (Bangham et al., 1965). The thin-film hydration method consists of sequentially dissolving phospholipids in an organic solvent (mostly chloroform), evaporating the solvent to form a thin film, and then dispersing the dry lipid film in an aqueous phase. In this method, sonication is used to reduce the size of large-sized liposomes (Maja et al., 2020). Apart from this, the methods applied are as follows, solvent (ether or ethanol) injection method, reverse phase evaporation (REV), dialysis, extrusion, spray drying, heating, freeze drying, cross flow injection, microfluidization, membrane contactor, supercritical reverse phase evaporation (SCRPE), improved SCRPE method (ISCRPE), supercritical antisolvent (SAS), depressurization of an expanded liquid organic solution-suspension (DELOS) and ultrasonication method. Each of these methods has different advantages and disadvantages.

LIPOSOMAL ENCAPSULATION METHOD PROPERTIES

Liposomes are preferred in the encapsulation process thanks to biocompatible, biodegradable, no show toxic effects, and high ratio protect of coated material (Laye et al., 2008; Gibis et al., 2012; Chun et al., 2013). One of the most important features of liposomes is that can be obtained from nature components. The natural structure of liposomes enables the usage in food systems without the need for any legal regulation (Taylor et al., 2005). In food science, the liposomal encapsulation method is used to encapsulate antioxidant components, antimicrobial components, enzymes and additives. The liposomal system is used in the encapsulation of many bioactive components, including fatty acids such as gambogenic acid (Tang et al., 2018), resveratrol (Caddeo et al., 2008), tea catechins (Zou et al., 2014) and linolenic acid (Vélez et al., 2019), omega-3 and protein hydrolysates (Li et al., 2015). Liposomal encapsulation offers a versatile approach in terms of preservation and controlled release of sensitive bioactive ingredients, delaying food spoilage, protecting bioactive

ingredients from degradation after consumption, and increasing the bioavailability of ingredients during adsorption (Liu et al., 2020).

Liposome structures improve the solubility of lipophilic compounds in aqueous solutions or hydrophilic compounds in hydrophobic systems. Thanks to high dispersion in water, liposomes can be used to produce low-calorie and fat-reduced products. In addition, liposomes have an important effect in preventing oxidation, removing negative flavors and reducing the energy density of food products (Farrokh et al., 2017). The structural similarity to the cell membrane provides distribution and release some bioactive components to specific areas in the body (Gabizon et al., 2004; Laye et al., 2008). This unique structure allows liposomal nanoparticles to enter the intercellular space in the body. Liposomes have no adverse effects on health and also many health benefits such as liver protection, memory enhancement and inhibition of cholesterol absorption is revealed in studies.

STUDIES ON LIPOSOMAL ENCAPSULATED INGREDIENTS IN FOOD PRODUCTS AS ANTIMICROBIAL AGENTS

The antibacterial activities of clove oil and liposome-encapsulated clove oil were investigated by Cui et al. (2015) and stated that liposome-encapsulated clove oil can be use efficiently as an antimicrobial agent for S. aureus in tofu. In a study by Pinilla and Brandelli (2016) determined the antimicrobial activity efficiency of liposome lysine and garlic extract encapsulated with phosphatidylcholine. Nanoliposome-encapsulated nisin-GE has potential as an antimicrobial formulation for food use. According to results, the use of natural antimicrobial nanoliposomes in dairy products is an important alternative way to improve food quality and shelf life. Lopes et al. (2017) carried out the encapsulation of nisin by nanoliposomes obtained using soybean phosphatidylcholine (PC), pectin or polygalacturonic acid. Antimicrobial activities of liposomes were observed against five different strains of Listeria, and showed the highest activity against L. innocua. In-vitro release studies have indicated that the nisin release rate of PC-pectin and PC-polygalacturonic acid liposomes is lower than that of PC liposomes.

Ghorbanzade et al. (2017) stated that fish oil has important benefits in the daily diet, but applications in food formulations are limited due to strong odor and rapid deterioration. So, fish oil encapsulated with nano-liposomal process and usage in the yogurt formulation. It has been stated that nano-liposome fish oil capsules provide a significant reduction in acidity, syneresis and peroxide values of yogurt. In terms of sensory properties, the addition of nano-encapsulated fish oil in yogurt shows similar properties with the control sample enriched with free fish oil. Pabast et al. (2018) investigated the effects of lamb meat in capsules containing free or chitosan-nano-liposomal encapsulated Satureja khuzestanica essential oil on chemical, microbial and sensory properties of lamb at 4°C for 20 days. As a result of the study, the chitosan-liposom encapsulated essential oil of Satureja khuzestanica could be a promising active packaging material to extend the shelf life of lamb. Lopes et al. (2019), lysozyme and nisin were lipozomal encapsulated with phosphatidylcholine (PC) and pectin or polygalacturonic acid. The co-encapsulation of lysozyme and nisin with liposome has a synergistic antimicrobial effect on L. *monocytogenes* and *S. enteritidis*, but provides greater inhibition against *L. monocytogenes*. The PC-pectin liposomes used in full-fat and skim milk medium reduced the *L. monocytogenes* population by 2 log cfu/ml in whole milk and 5 log cfu/ml in skim milk at 37°C. The L. monocytogenes population remained below the detection limit in milk stored for 25 days under refrigeration temperature. This shows that liposomes can be a promising technology to provide controlled release and stability in complex food systems.

Pinilla et al. (2019) used garlic extract encapsulated with liposome process with phosphatidylcholine and oleic acid as an antifungal agent in bread formulation. They reported that bread samples containing encapsulated garlic extract and free garlic extract (0.65ml/100g

dough) were more microbiologically stable and showed mold inhibition for five days compared to control samples. As a result of the study oleic acid and liposomal garlic extract can be used as natural antifungal agents to improve the microbiological stability of cooked food products due to their thermal properties. In a study made by Lin et al. (2022), a bio-responsive composite liposome with silk fibroin, L-fucose and *Litsea cubeba* essential oil were designed for chicken preservation as antibacterial agent and as results indicated that 20% (v/v) of the composite liposomes could inactivate 99% *Campylobacter jejuni* (C. jejuni).

CONCLUSION

Food safety is an important issue for people in the food production process and consumption. In this respect, food production is faced with many technological challenges due to the increasing demand for naturally additive foods. The natural preservative components are significantly affected by environmental conditions and therefore components must be protected by encapsulation. In the food industry, liposomes have been investigated to deliver proteins, enzymes, vitamins, antioxidants and flavors. Many studies indicate that the efficacy of antimicrobial components is increased with liposome encapsulation. The great advantage of liposomes over other encapsulation technologies is their high stability. As a result, it has been demonstrated that there is a significant potential for use of liposome-encapsulated antimicrobials to improve the quality and healthiness of a wide variety of food products.

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EFFECTS OF AQUAFABA AS ALTERNATIVE PLANT ADDITIVE ON PHYSICAL, TEXTURAL AND SENSORY CHARACTERISTICS OF EGGLESS TURKISH PASTA (ERIŞTE)

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ABSTRACT

Consumers experience health problems with egg consumption and also preference of vegan and vegetarian nutrition leads to the search for alternative egg substitutes in new product development. This research determined the quality and acceptability of Turkish noodle (Erişte) substituted with 25%, 50%, 75% and 100% chickpea aquafaba instead of egg. Erişte were analyzed for physical, textural, and sensory properties compared with sample containing egg. An increase in substitution led to a rise of 12.76% in water uptake, 12.94% in volume increase and 40.19% in the cooking loss. The addition of up to 75% aquafaba increased the firmness of .erişte significantly compared to the control sample. Erişte sample containing 100% aquafaba showed significantly $p < 0.05$ higher values in L* (75.11) and hue angle (94.85), while lower values in a* (-2.13), b* (24.30) and saturation index (24.39). The odor (7.00), taste (7.00), appearance (7.00), chewiness (7.00) and overall acceptability (6.88) of samples containing aquafaba were found more acceptable than control sample (4.00, 5.50, 5.90, 5.95 and 5.47, respectively). Based on our results, possible to produce erişte with acceptable sensory properties, and good physical quality product by adding up to 50% aquafaba.

Keywords: Aquafaba, egg-less noodle, physical properties, textural properties

INTRODUCTION

Turkish pasta (Erişte) is one of the traditional Turkish foods was generally produced from wheat flour, salt and egg. Milk, whey powder and some other additives can also be added in different regions of Turkey (Özkaya et al., 2004). The high rate of egg component used in pasta products can reduce production and consumption due to both the cost of the product and various reasons. So, many issues such as changing consumer preferences, increasing allergens, improving food safety, improving nutritional balance, reducing price variability and supporting environmental sustainability have increased the interest in researching egg substitutes and alternatives (Grizio and Specht, 2018).

Plant-based proteins are one of the most important food components to use as egg substitution in product development. Recently, the viscous liquid obtained from cooked chickpeas or other legumes and pulses called 'aquafaba' according to the Latin origin of water (aqua) and beans (faba) has been recently used as eggs replacement in many foods (Erem et al., 2021). Some studies in the literature revealed that aquafaba has many functional properties such as water and oil holding capacity, emulsion stabilizer, foaming, gelling and thickening in various formulations (Mustafa and Reaney 2020) and can be used as an egg substitute in vegan products (Raikos et al., 2020).

Aquafaba has used in meringue (Stantiall et al. 2018), sponge cake (Mustafa et al. 2018) and vegan mayonnaise (Raikos et al., 2020) as an emulsifier instead of egg. To our knowledge, no studies have been conducted on the inclusion of aquafaba in the formulation of egg-less

Turkish pasta (Erişte). The objective of this study was to investigate egg substitutes for Turkish pasta using aquafaba. The effects of aquafaba on physical, textural and sensory properties of eggless pasta were evaluated.

MATERIAL AND METHOD

Materials

Commercial chickpea, wheat flour, whole egg and salt were purchased from local markets in Konya, Turkey.

Methods

Production of Aquafaba

Aquafaba was prepared according to the method described by Baik and Han (2012) with some modifications. Firstly, chickpeas were washed and were cleared from dirt, dust and foreign matter. Aquafaba was obtained by cooking 100 g chickpea in 500 mL water (1:5 chickpea/water ratio) for 30 min in boiling water at 98 °C. After the cooking, water and cooked chickpeas waited for 12 hr in a refrigerator at 4 °C. Finally, aquafaba was obtained by removing cooked chickpeas.

Production of Turkish noodle (Erişte)

For production of control Turkish pasta sample, firstly wheat flour (100 g), whole egg (40 g), salt (1 g) and water were mixed and kneaded in a laboratory-type mixer (Hobart N50, Offenburg, Germany) for 8 min. The kneaded dough rested for 20 min and 2 mm thickness/5 mm wide long strips were obtained (1 time in section 6 and 7) by a pasta machine (Shule Pasta Machine; Jiangsu, China). Then, strip-shaped dough were cut to a length of 4 cm. The drying of samples took place in a laboratory-type oven (Nüve KD 200, Ankara, Turkey) at 50 °C for 18 h. In the other samples, aquafaba were replaced at levels of 25, 50, 75 and 100% on the basis of whole egg. The samples were preserved in sealed polyethylene bags at 4 °C.

Cooking properties

Volume increase, weight increase and cooking loss values of pasta were determined according to Bilgiçli et. al. (2011). The weight increase and volume increase were found by differences of dry and cooked erişte samples weights and by the volume difference of water overflow, respectively. For cooking loss determination, cooking water was evaporated to constant weight in an oven and the weight of total solids expressed as a percentage (AACC, 2000).

Texture analysis

The firmness values of erişte samples were measured by TAXT Plus Texture Analyser (Stable Microsystems, Surrey, UK) and A/LKB-F was used as a probe (AACC, 1990). Firstly, 10 g samples were cooked in 200 mL distilled water for optimum cooking time and drained. Then, three strips of erişte sample were placed onto the stand and analyzed.

Color analysis

Color measurement was made by measuring L* value [(0) black- (100) white], a* value [(+) red- (−) green] and b* value [(+) yellow - (−) blue] using the Hunter Lab Color Quest II Minolta CR-400 (Konica Minolta Sensing, Inc., Osaka, Japan) (Francis, 1998). Three measurements were taken on each sample. The hue angle and the saturation index value were determined with $\arctan(b^*/a^*)$ and $(a^{*2}+b^{*2})^{1/2}$ equations, respectively.

Sensory analysis

Sensory analysis was carried out for cooked Turkish pasta samples. Turkish pasta samples were evaluated in terms of color, odor, taste, appearance, chewiness and general acceptability properties by 12 panelists (aged 20–50).Sensory properties of samples were scored using a 7-point hedonic scale in which a score of 7 = like extremely, 1 = dislike extremely.

Statistical analysis

The results were expressed as mean ± standard deviation and were analyzed using the Statistical software JMP 5.0.1 (SAS Institute). The averages of the main variation sources were compared at $p < .05$ level. All measurements were performed in duplicate for each sample.

RESULTS AND DISCUSSION

The data on the effect of different levels of aquafaba on the cooking quality and firmness properties are shown in Table 1. The water uptake values of Turkish pasta samples increased with the use of more than 50%. Compared to control, addition of aquafaba increased volume increase value of Turkish pasta samples from 219.69 to 248.11%. Similar behavior in terms of cooking loss properties was observed Turkish pasta samples produced with different aquafaba. While Turkish pasta prepared with 50-100% legume flour revealed the highest cooking loss, the addition of 25% aquafaba showed similar cooking loss with control sample. The findings of this study demonstrated that the addition of aquafaba increased 1.82-fold with 100% aquafaba the firmness values of pasta samples compared to the control sample (Table 1).

Table 1. Physical properties of Turkish pasta[1]

	Water uptake (%)	Volume increase (%)	Cooking loss (%)	Firmness (g)
Control	215.59±1.89b	219.69±6.03b	4.23±0.11b	642.12±23.35c
25% Aquafaba	220.06±3.44b	229.72±4.81ab	4.75±0.25b	698.98±61.62c
50% Aquafaba	236.70±3.03a	244.86±6.52a	5.43±0.10a	809.39±90.10bc
75% Aquafaba	238.14±1.25a	245.15±5.82a	5.74±0.16a	988.47±35.67ab
100% Aquafaba	243.12±3.16a	248.11±3.95a	5.93±0.13a	1166.62±92.43a

[1]Means followed by the different letters within a column are significantly ($P < 0.05$) different.

Color values of Turkish pasta samples are demonstrated in Table 2. Color L*, a*, and b* results of Turkish pasta samples varied in the range of 65.24-75.11, 0.26-(-2.13), and 24.30-30.56, respectively. Compared to the control sample, the addition of aquafaba into the formulation of Turkish pasta significantly ($p < .05$) increased L*, but decreased a* and b*values. The findings are associated with the natural color properties of eggs. The reason of this result was lower lightness and higher yellowness values of egg compared with aquafaba. The SI value of Turkish pasta sample incorporated with 100% aquafaba were lower than the control and other samples. The hue angle values demonstrated an increase with high aquafaba usage ratio.

Table 2. Color properties of Turkish pasta[1]

	L*	a*	b*	Saturation Index	Hue angle
Control	65.24±1.58c	0.26±0.07a	30.56±0.83a	30.56±0.77a	89.51±0.58b
25% Aquafaba	67.72±1.98bc	-0.37±0.10b	29.94±0.69a	29.94±0.10a	90.71±0.99b
50% Aquafaba	70.52±1.37abc	-0.92±0.14c	29.73±0.59a	29.74±0.84a	91.77±1.02ab
75% Aquafaba	73.12±1.87ab	-2.06±0.03d	28.50±1.92ab	28.58±0.68a	94.27±0.35a
100% Aquafaba	75.11±0.66a	-2.13±0.04d	24.30±1.00b	24.39±0.52b	94.85±0.91a

[1]Means followed by the different letters within a column are significantly ($P < 0.05$) different

Sensory properties of Turkish pasta samples are presented in Figure 1. According to the results; color score shown no any change with incorporation of aquafaba in pasta formulation. When compared with the control sample, the odor, taste, appearance, chewiness and overall acceptability scores of the Turkish pasta samples were found higher with high aquafaba addition

levels, statistically. As a result, 50% and more aquafaba usage improved the sensory properties and overall acceptability of Turkish pasta. Mustafa et al. (2018) prepared sponge cake with egg white and aquafaba. The color and texture of sponge cake made with egg white or aquafaba was similar and acceptable, but cakes made with aquafaba were less pliable and less sticky than cakes made with egg whites.

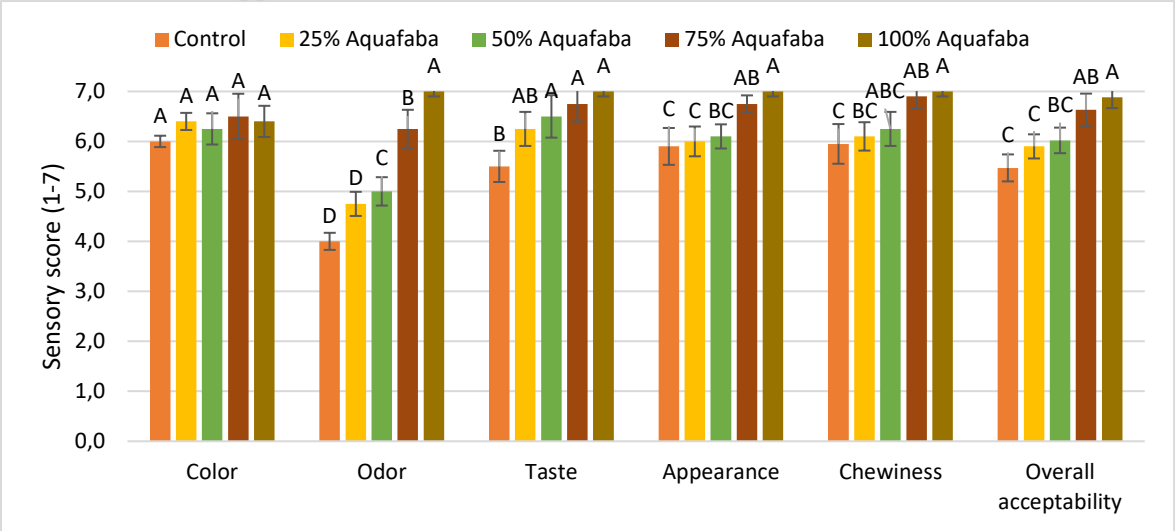

Figure 1. Sensory properties of Turkish pasta

Aslan and Ertaş (2020) used aquafaba as an egg substitute in the cake formulation. According to the results of sensory evaluation, they reported that samples containing 25% aquafaba were preferred more by the panelists.

CONCLUSIONS

In this study, the usability of aquafaba as egg substitute in Turkish pasta (Erişte) production was investigated. According to results, the use of aquafaba as an egg substitute was concluded with an increase in the water uptake, volume increase and cooking loss of pasta samples and so, in terms of cooking quality properties considerably not caused a negative effect up to 50% ratio. Also, the use of aquafaba increased the firmness values compared to the control samples. The incorporation of aquafaba in the pasta samples positively affected the sensory profile in terms of all sensory scores except to color scores.

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BIBLIOMETRIC ANALYSIS OF POLLEN CONTAMINATION DETERMINATION IN SEED ORCHARDS WITH MOLECULAR MARKERS

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ABSTRACT

Seed orchards are special plantations established to produce genetically superior seeds/seedlings from genetically superior candidate parents. Pollen contamination is one of the most important factors affecting the yield, adaptation, and genetic quality of seeds produced from seed orchards in forest tree breeding programs. Potential pollen from the forests surrounding the seed orchard is a concern in tree breeding studies, as it causes the loss of genetic gains expected from seed orchards. It has been determined that different molecular marker techniques are used in the determination of pollen contamination. These molecular markers have advantages and disadvantages over each other. In this study, bibliometric analysis was performed to quantitatively and qualitatively evaluate the published articles on the determination of seed orchards and pollen contamination with molecular markers. Searching the Web of Science (WOS) by "seed orchard", "pollen contamination", and "marker" criteria revealed that 67 articles were published. Japan, Canada, Sweden, China, and France were the countries that contributed the most to pollen contamination studies in the seed orchards of forest trees, respectively. According to the data obtained, it has been shown that the pollen contamination level of forest tree species in seed orchards is generally between 5% and 90%. In Turkey, three studies on this subject were found. It was concluded that studies on pollen contamination were carried out in only two *Pinus brutia* orchards in Turkey, which has 189 seed orchards, most of which are coniferous, and that similar studies should be planned in other seed orchards.

Keywords: Bibliometric analysis, Isoenzymes, Pollen contamination, RAPD, Seed Orchards, SSRs

INTRODUCTION

Biodiversity is necessary and very important for the continuity of all life on Earth. In order to have a healthy ecosystem, we need various animals, plants and microorganisms. Plant biodiversity of a country has very significant role agriculture, forestry, medicine, pharmacy, and industry, both economically and in terms of use. However, the biological diversity is rapidly depleting due to many reasons like rapid population growth, urbanization, industrialization, forest fires, air pollution, agricultural land acquisition, global warming, erosion, and misuse of our natural resources. For conservation programs, the protection of forest ecosystems with rich biodiversity has primary importance in terms of ecological, aesthetic, and economic aspects, and also preventing the extinction of endangered species. In Türkiye, forest ecosystems exist in the mountainous regions and the climate, soil, and biological environmental factors change over short distances and more frequently in these areas. Gene pools and gene combinations are different due to different environmental factors and selection pressures in neighboring populations of the same species. Because of this, races having different fitness values may occur

in short distances. The existence of different races or sub-races has been demonstrated by the studies (Bradshaw, 1972; Hamann et al., 1998; Işık, 1999a, b; Ohsawa & Ide 2008).

The estimation of genetic diversity is vital for sustainability. Sustainable management of forests is possible with studies from the gene level to the ecosystem level. To increase productivity in forest trees, it is necessary to determine and improve the genotypes that are fast-growing and resistant to biotic and abiotic factors. Genetic diversity is the main resource for establishing genetic breeding programs (Sütcü et al., 2022). Natural forest populations, seed stands, seed plantations, or seed orchards, whose genetic diversity has been determined, based on morphological data or at the molecular level, are used for the establishment of new forests. The main purpose of this study is bibliometric analysis of pollen contamination determination in seed orchards with molecular markers from Web of Science (WOS) and to compare them with the studies conducted in our country.

CONIFER SEED ORCHARDS AND POLLEN CONTAMINATION

Conifer seed orchards are specialized forest plantations to obtain genetically superior seeds and seedlings from selected genetically superior candidate parents for use in forestry studies (Buiteveld et al., 2001; Zhuowen, 2002; Funda & El-Kassaby, 2012; Bilgen & Kaya 2014, 2016). The pollen flow from outside the orchard is reduced or destroyed in conifer seed orchards and they are specially operated to produce easy and abundant forest tree seeds (Kang et al., 2001a, 2004). Seed source is very significant for afforestation. The genetic gain expected from the forests to be established should be high by ensuring the superior genetic characteristics of the seeds. To obtain the superior species and breeds required for use in forestry and afforestation studies, selecting and bringing together genetically superior individuals for the establishment of seed orchards is major target. The seed orchards are isolated from other pollen sources, and vital for obtaining frequent, abundant, easy, high genetic and physiological value seeds, and are subjected to special care and management (El-Kassaby et al., 1989; Di-Giovanni & Kevan, 1991; Kang et al., 2004). There are mainly two types of seed orchards, i.e. the vegetative or clonal seed orchard, and the seedling seed orchard (Tunçtaner, 2007).

The first clonal seed orchard was established on the island of Java/Netherlands in 1880 for the Cinchona ledgeriana species (Feilberg & Soegaard, 1975; Ertekin, 2012). In Türkiye, the first seed orchards were established in 1964 in Istanbul-Belgrad Forest with *Pinus nigra* and *P. sylvestris* species by the Istanbul University Faculty of Forestry Department of Silviculture and Afforestation (Tunçtaner, 2007). Until 2023, 189 seed orchards (*P. brutia, P. nigra, P. sylvestris, P. pinea, P. halepensis, P. pinaster, Picea orientalis, Cedrus libani, Juniperus phoenicea, Sorbus torminalis, Liquidambar orientalis,* and *Ziziphus jujuba*) were established in different regions of Türkiye by the Republic of Türkiye, Ministry of Agriculture and Forestry (OATIAM, 2023).

There are potentially two important problems with pollination in seed orchards. First one is the pollen contamination from individuals outside the seed orchard, and second is self-pollination (Adams & Birkes, 1989). Correct determination of pollen contamination is of great importance for determining genetic gain in the orchard, developing seed orchard management strategies to reduce pollen contamination, and evaluating the effectiveness of seed orchards (Torimaru et al., 2009).

BIBLIOMETRIC ANALYSIS OF POLLEN CONTAMINATION STUDIES

In this study, bibliometric analysis was performed to assess the published articles related to the determination of pollen contamination with molecular markers in seed orchards. Searching the Web of Science Core Collection (WOS) by "seed orchard", "pollen contamination", and "marker" criteria revealed that 67 articles were published (Figure 1). It has been observed that these articles are belongs to 12 different WOS categories and the article can

be indexed in more than one category (Figure 2). The WOS results of which academic journals published the publications on pollen contamination in seed orchards are shown in Table 1. When the journals in which 67 publications were published from 1986 to 2022 were examined, it was observed that the majority of them were published in specific journals on forestry (Table 1). Tree Genetics Genomes, Scandinavian Journal of Forest Research, and Silvae Genetica take the first three ranks in these journals.

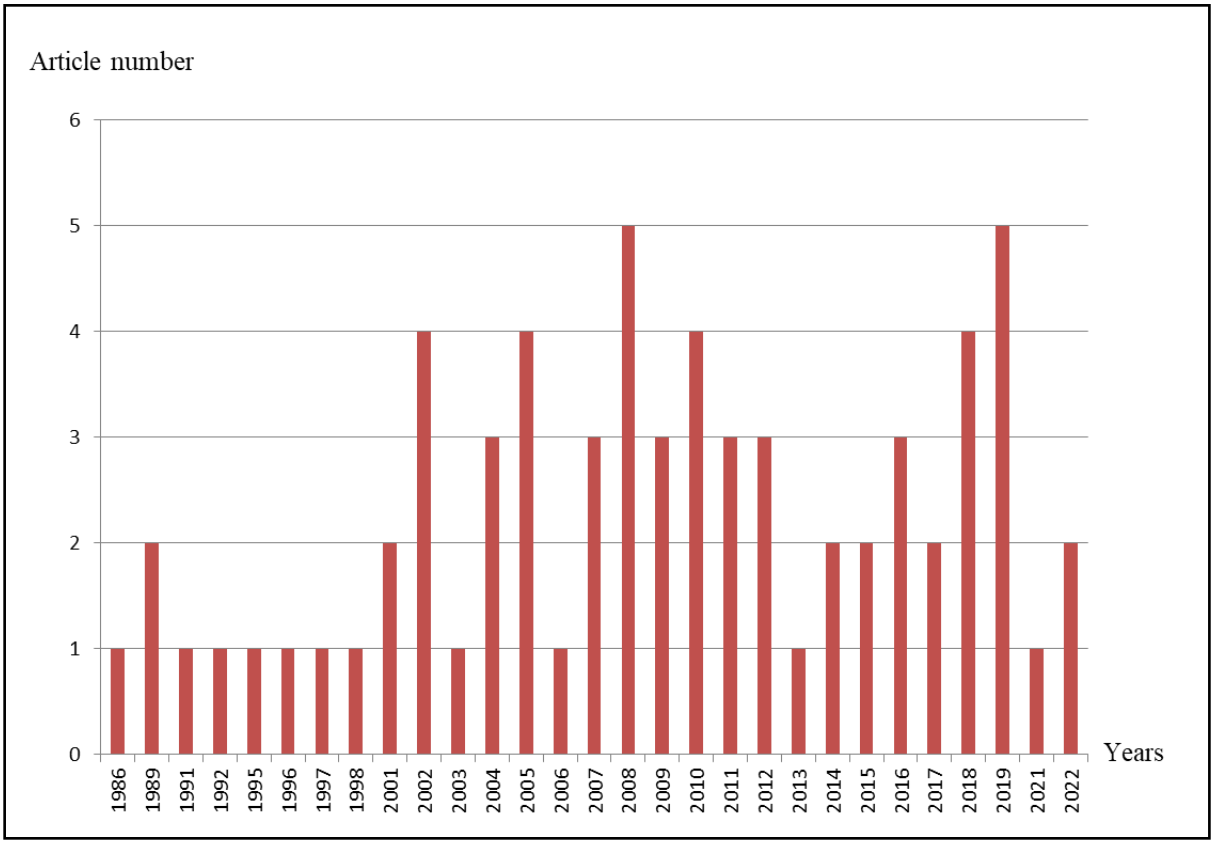

Figure 1. Search results of Web of Science Core Collection (WOS) by "seed orchard", "pollen contamination", and "marker" criteria

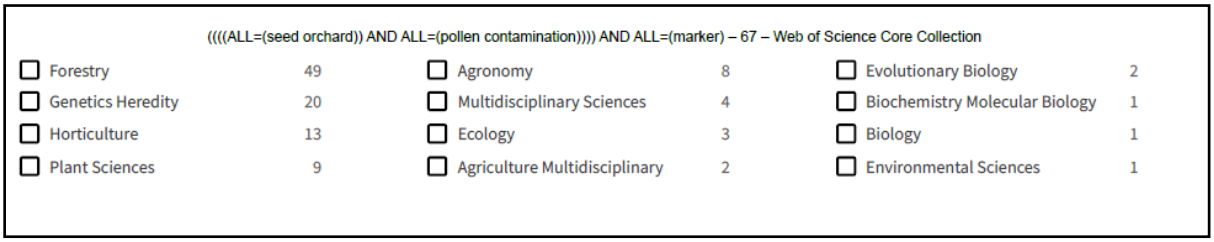

Figure 2. Web of Science Categories of 67 published articles during 1986-2022

Table 1. Search results of WOS Publication Titles of published 67 articles

Publication Titles	Number of Articles
Tree Genetics Genomes	7
Scandinavian Journal of Forest Research	6
Silvae Genetica	6
Annals Of Forest Science	4
Canadian Journal of Forest Research	4
Canadian Journal of Forest Research Revue Canadienne De Recherche Forestiere	4
Theoretical and Applied Genetics	4
Forest Ecology and Management	3
Plos One	3
Allgemeine Forst Und Jagdzeitung	2
Heredity	2
Journal of Tropical Forest Science	2
New Forests	2
Turkish Journal of Agriculture and Forestry	2
Acta Botanica Sinica	1
Breeding Science	1
Dendrobiology	1
Ecological Modelling	1
European Journal of Forest Research	1
Forestry Chronicle	1
Forestry Sciences	1
Fresenius Environmental Bulletin	1
Frontiers In Plant Science	1
Iforest Biogeosciences and Forestry	1
Journal of Horticultural Science Biotechnology	1
Molecular Breeding	1
Population Genetics of Forest Trees	1
Science China Life Sciences	1
Scientific Reports	1
Silva Fennica	1
Tree Physiology	1
Total	67

Japan, Canada, Sweden, China, and France were the countries that contributed the most to pollen contamination studies in the seed orchards of forest trees during 1986-2022, respectively (Figure 3).

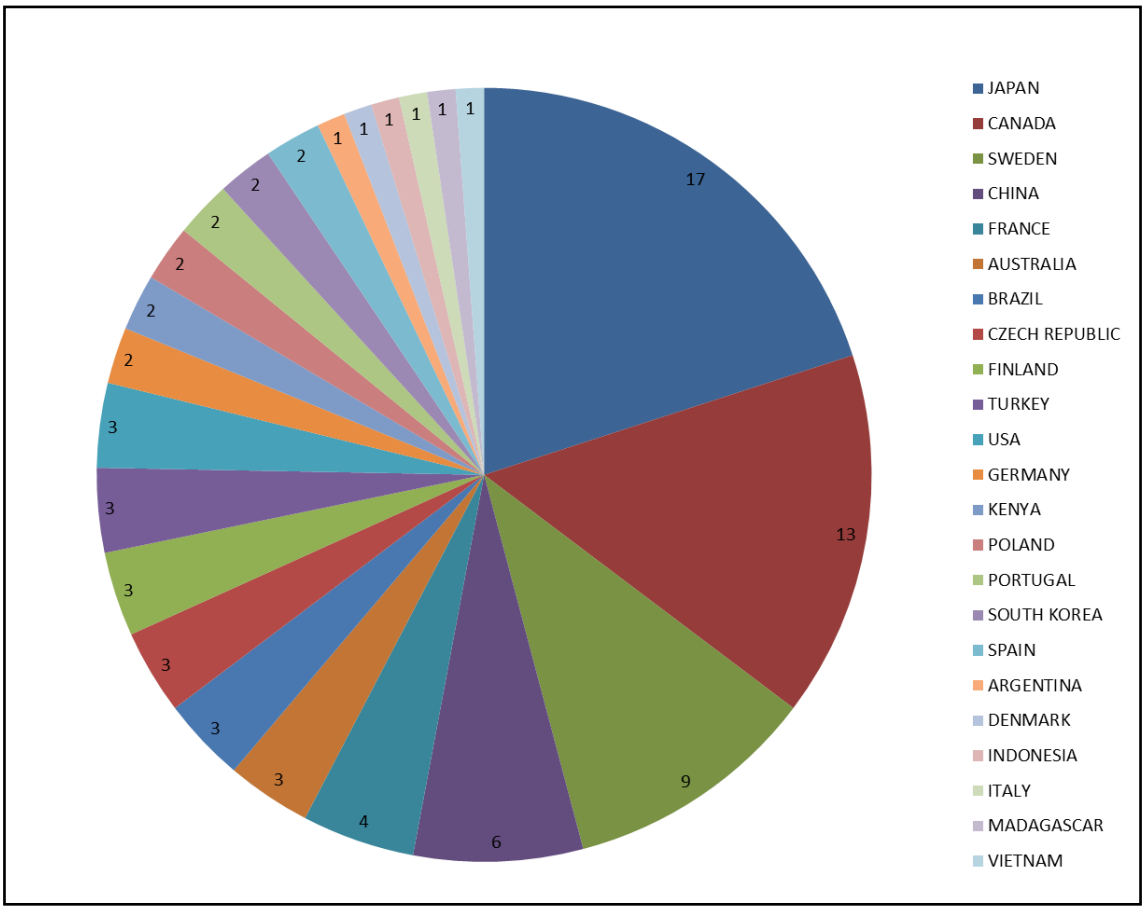

Figure 3. Countries/regions that contributed to publications on pollen contamination in seed orchards

According to the data obtained, it has been shown that the pollen contamination level of forest tree species in seed orchards is approximately between 5% and 90% (Table 2). When we look at pollen contamination studies in the past years, the use of isoenzyme markers is observed. El-Kassaby et al., (1989), reported 36% pollen contaminateion rate by 21 isoenzyme loci in *P. sylvestris* seed orchard. Kaya et al., (2006) estimated pollen contamination rate as 85% with isozyme analysis (14 loci) in Antalya-Asar *P. brutia* seed orchard and this was the first study in Türkiye. Over time, biochemical markers have been replaced by DNA markers. In *P. thunbergii* seed orchard, pollen contamination rate was estimated by 28 RAPD loci (Goto et al., 2002). In two *P. pinaster* seed orchards, pollen contamination rates were determined as 36% and 52.4% with 6 and 3 SSR loci, respectively (Plomion et al., 2001; Fernandes et al., 2008). Sonstebo et al., 2018 determined pollen contamination rate 20% in *Picea abies* by 11 SSR loci. The second study in Türkiye is performed by Bilgen and Kaya (2014) with the use of cpSSR markers in Antalya *P. brutia* seed orchard and the pollen contamination rate was calculated as %39.3. Bilgen and Kaya (2016) studied clonal identity and genetic structure of *P. brutia* clonal seed orchard via nSSR markers (Table 2).

Table 2. Estimates of pollen contamination rate (m) in different tree seed orchards by molecular markers

Species name	Molecular marker used (locus number)	m (%)	Reference
Pinus sylvestris	Isoenzyme (21)	36; 21	El-Kassaby et al., 1989
Pinus sylvestris	Isoenzyme (21)	24-40	Yazdani and Lindgren 1991
Pseudotsuga menziesii	Isoenzyme (11)	49	Adams et al., 1997
Picea abies	Isoenzyme (11)	70	Pakkanen et al., 2000
Pinus brutia	Isoenzyme (14)	85.7	Kaya et al., 2006
Pinus thunbergii	RAPD (28)	2.4	Goto et al., 2002
Pinus pinaster	SSR (6)	36	Plomion et al., 2001
Quercus robur	SSR (6)	70	Buiteveld et al., 2001
Pinus contorta	SSR (6)	5.5	Stoehr & Newton, 2002
Pseudotsuga menziesii	SSR (9)	35.3	Slavov et al., 2005
Pinus pinaster	SSR (3)	52.4	Fernandes et al., 2008
Pinus sylvestris	SSR (9)	52	Torimaru et al., 2009
Pinus koraiensis	SSR (13)	25	Feng et al., 2010
Pinus brutia	SSR (6)	39.3	Bilgen & Kaya, 2014
Pseudotsuga menziesii	SSR (6)	18.4	Kess & El-Kassaby, 2015
Eucalyptus camaldulensis	SSR (11)	14.7	Gonzaga et al., 2016
Schima superba	SSR (13)	7.01	Yang et al., 2017
Picea abies	SSR (11)	20	Sonstebo et al., 2018
Larix kaempferi	SSR (17)	6.3	Chen et al., 2018
Prosopis alba	SSR (10)	28-37	D'Amico et al., 2019
Eucalyptus urophylla	SSR (12)	11.9	Pupin et al., 2019

CONCLUSION

The size of the seed orchard, the distance between the seed orchard and populations with genetically undesirable traits, the amount of pollen produced by ramets in the seed orchard, and the overlapping of flowering times in the surrounding populations are some of the factors that influence pollen contamination rate. Different techniques (such as pollen traps and emasculation, biochemical markers) used to determine the rate of pollen contamination in the seed orchards have now been replaced by the use of DNA markers. DNA markers have been used more widely in recent years to determine the degree of pollen migration and genetic pollution due to their advantages (Fernandes et al., 2008, Torimaru et al., 2009, Feng et al., 2010; Bilgen and Kaya, 2014; Kess and El-Kassaby, 2015; Gonzaga et al., 2016; Yang et al., 2017; Chen et al., 2018; Sonstebo et al., 2018; D'Amico et al., 2019; Pupin et al., 2019).

In the WOS analysis, 67 publications were determined when the studies on pollen contamination from 1986 to the present were examined. Only three of these publications were made in two seed orchards of our country. It was concluded that studies on pollen contamination were carried out in only two *Pinus brutia* orchards in Turkey, which has 189 seed orchards, most of which are coniferous, and that similar studies should be planned in other seed orchards.

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HARD SEED FRUIT GENE RESOURCES OF TÜRKİYE AND MOLECULAR CHARACTERIZATION STUDIES

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ABSTRACT

Türkiye has rich soils that offer suitable habitat for many plants. Our country, which is rich in gene resources, is the gene center of many plants. Fruits, which can be classified according to their different characteristics, are examined in seven groups when classified according to fruit characteristics. The most important stone fruits grown in our country are peach (*Prunus persica* L.), nectarine (*Prunus persica* var. *nectarina*), apricot (*Prunus armeniaca* L.), cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.), and plum (*Prunus domestica* L.). Many varieties of these fruits, which are widely consumed in the world, have been obtained by using both traditional and modern breeding methods. While applying modern breeding methods, it is very significant to use and protect natural populations in factors such as expanding the gene pools of this group, which have a lot of wild ones in our country, and resistance to biotic and abiotic stress. Molecular markers are the most efficient and reliable methods used in the genetic characterization and identification of wild varieties. RAPD, SSR, ISSR, AFLP, and SRAP markers, which are used for many purposes, such as the characterization of wild and cultivated fruits and advanced breeding programs, are just some of them. This review examines the molecular characterization studies of stone fruits carried out in Turkey via molecular markers.

Keywords: Breeding, Drupe fruits, Gene resources, Molecular characterization

INTRODUCTION

More than one fruit variety can be grown in Turkey due to the favorable climate condition and soil types. There are different types of classifications of fruits. When classified according to fruit characteristics (pomologically); Mediterranean fruits are divided into seven groups as hard-skinned, soft-core, drupe, berry, citrus, and pleasure fruits. Cherry, sour cherry, apricot, peach, nectarine, and plum fruits are the leading stone fruits produced in our country. Apart from these, even though the production amount is less, the fruits of buckthorn, cranberry, and jujube, which are included in the production of our country, are also included in the class of stone fruits. Our country is the leader in all of these fruits, each of which grows in different conditions (Duru et al., 2022). Stone fruits are biologically classified as Rosales team, Rosaceae family. It is in the Prunoideae subfamily and comes from the genus *Prunus*. Traded *Prunus* species were first seen between Eastern Europe and Western China, and are suitable for cultivation between 30°-40° latitudes, in climates with long and dry seasons (Duru et al., 2022).

Although wild varieties are plants that can adapt to various conditions, they are endangered day by day due to some reasons such as pollution and the destruction of their habitats. Plants can be lost due to such factors, and gene resources that will be significant to use

in important studies such as breeding in the future are lost. Studies are carried out to ensure that gene resources are not lost. Chemical and *in vitro* techniques, cryo-storage, slow-growth techniques, artificial seed storage, and DNA storage methods are used to preserve gene resources. These protected wild varieties are important to use in molecular studies in the development of new varieties in breeding studies (Bilir, 2016; Balkaya and Yanmaz, 2001).

Identification of plant genetic resources is as important as diversity and protection. Thanks to the development of biotechnology and plant genetics in recent years, many DNA-based techniques have been developed for breeding and variety development. Some of these are RAPD, PCR-RFLP, AFLP, ISSR, and SSR. With these techniques, progress has been made in many varieties of development and molecular studies (Kose, 2013). This review aimed to examine the molecular characterization studies of stone fruits carried out in Turkey via molecular markers.

HARD SEEDS AND GENETIC RESOURCES

Turkey has significant and very rich plant biodiversity. The most obvious example of this is that approximately 3500 of 9500 plant species are endemic to our country. These endemic species can be found in most parts of the country. Moreover, it is mostly seen in the mountainous parts of Southern and Southeastern Anatolia. These plants are also found in the Thrace region. The Iran-Turan Region and Mediterranean Region of our country contain the largest number of endemic species (Tan, 2010). Gene resources and rich genetic diversity are important for plant breeding studies. Breeders use local varieties, especially in the development of new varieties. Most of our proprietary varieties originate from Turkey's plant genetic resources collections (Tan, 2010).

The genetic diversity of fruits is quite high. They are sources of various antioxidants, rich in vitamins and minerals. Therefore, fruit gene resources should be protected at least as much as other plants. With the selection and characterization studies pioneered by Ministries, Research Institutions, and universities, it has been ensured that the fruit gene source in most of the regions was determined and defined, protected, and used in breeding studies. In addition to various gene banks established as a result of these research and development studies, name-specific Research Institutes were established in fruit species such as Hazelnut, Pistachio, Viticulture, Fig, Apricot, Nuts (Almond), and Olive, which have an important place in the Turkish economy (Kuden and Dasgan, 2021; Bozhuyuk, 2020). When the TUIK data are examined, it has been seen that stone fruit types have an important place in Turkey's fresh fruit production and export. According to TUIK data, peach and nectarine are the most produced stone fruits in Turkey whereas the least produced were jujube in 2022 (Table 1).

MOLECULAR MARKERS AND THEIR USES IN CHARACTERIZATION

In breeding studies, classical breeding methods are used for most species for the development of new varieties, but in the classical breeding method, the process is long, it requires a lot of effort, and it is not sufficient alone for the food needed with increased resistance to disease and pests. Morphological and phenotypic measurements are not considered sufficient in today's studies to determine the kinship relations between selected species and resistant varieties. As a result of developments in the field of biotechnology, molecular markers have been developed (Ehliz et al., 2021). Today, using molecular markers to define the correctness of the names of the varieties in a short time has an important place in fruit growing (Aksu, 2015). Molecular markers are not affected by environmental conditions, they are used in every period of plant development without waiting for maturation, they show wide variation and provide advantages compared to other markers and are used more widely (Sönmezoğlu et al., 2010).

Table 1. Production amount of hard seed fruits in Turkey in 2022 (tonnes) (TUIK, 2022)

Hard Seed Fruits	Production Amount in Turkey Tonnes
Peaches and Nectarine	1.008.185
Plums	348.750
Apricots	803.000
Wild apricots	20.832
Cherries	656.041
Sour Cherries	176.770
Cranberry	13.750
Silverberry	3.903
Jujube	2.248

Molecular markers have many advantages over morphological and biochemical markers with features such as high polymorphism, frequent and uniform distribution in the genome, being dominant and codominant, identifying more than one region, being easy, fast, and reproducible, but not all of these features are present in all molecular markers. While selecting the marker that is suitable for our purpose in the study, it is desired that some of these features be together (Shidfar, 2014). Molecular markers are categorized as dominant markers and codominant markers according to their scoreability and heredity in terms of heterozygosity and homozygosity (Devran, 2003). Molecular markers can also be used in analysis such as selection with the help of markers (MAS), characterization studies of gene sources, phylogenetic analyses, determination of varieties, and genetic relatedness (Shidfar, 2014). Markers such as SSR, AFLP, and ISSR which can give more precise results due to the low reproducibility level of the DNA-based RAPD technique, have been started to be used by many researchers (Ehliz et al., 2021).

GENETIC CHARACTERIZATION OF PEACH AND NECTARINE WITH MOLECULAR MARKERS

The latin name is *Prunus persica* L., was thought to be native to Iran and the Caucasus. In 1883, however, De'candolle proved that East Asia and China were the origin of the peach. The name peach has been found in Chinese literature dating back to 2000 BC. Due to its adaptability to different climates, peach has spread throughout the world (Ercan and Özkarakaş, 2003). The most important fruit type in Europe after apple is the peach, and it is also the most important species of the genus *Prunus*. Peach is a diploid species (2n = 16) (Dirlewanger et al., 2006). Contrary to popular belief, nectarine is not different from peach. It is in the sub-variety of peach, *Prunus persica* var *nectarina,* named as nectarina (Erbil and Erenoğlu, 2006).

If we examine the molecular studies on peach, Gür and Şeker (2012) examined the genetic relationships of white nectarine cultivars with other *Prunus* (peach, nectarine, cherry, almond, apricot, and plum) in their study. The *Prunus* species included in their study were taken from the Çanakkale Onsekiz Mart University fruit plots and producer gardens in 2011. The AFLP marker was chosen to determine the genetic relationship between fruits. As a result of the analysis, 182 of the 282 AFLP fragments from 6 primer pairs were observed as polymorphic. As a result of the study, it was seen that the white nectarine has different genetic characteristics

from other *Prunus*. White nectarines were defined as closest to each other, and the most distant related ratio was observed in cherry cultivars.

Demirel et al., (2023), using 32 ISSR markers, the genetic characterization study of a total of 54 peach genotypes, 52 local and 2 commercial varieties, obtained from the province of Iğdır, was carried out. A total of 213 alleles, 154 of which were polymorphic, were obtained in the study and 54 genotypes were grouped into four groups according to the UPGMA dendrogram. According to the results they obtained, they reported that 54 peach genotypes were different in terms of genetic similarity; ISSR markers used in the study could benefit breeding programs in selecting individuals as parents. In another study conducted by Demirel (2018), it was desired to determine the genetic differences between two local varieties of Iğdır province, Zeferan and Ağşeftali. ISSR marker was chosen for the study. 54 peach varieties were studied. Of the 57 primers, 42 were optimized and 32 were selected as polymorphic. 7 ISSR primers with the highest rate of polymorphism (100%) used in the study were determined. It was decided that the characterization of peach genotypes could be made with these 7 primers. As a result of the study, Zaferan6 and Ağşeftali18 genotypes were determined to be far from each other and other genotypes in the study. Ağşefali6 and Ağşeftali16 genotypes were almost very similar to each other, so it was decided that Ağşefali6 and Ağşeftali16 genotypes could not be selected as parents in the breeding study. Distant genotypes were found suitable for selection as parents in breeding.

In a study conducted in Spain, Bouhadida et al., (2007) worked with local varieties unique to Spain. In their study, by using the SSR marker, the similarity to the known local varieties was evaluated by having information about the genetic diversity of 19 varieties of Miraflores, whose pedigree is unknown. High polymorphic 20 SSR markers developed for peach were used. As a result of the analysis, 46 scoreable alleles were obtained. While 14 of the SSRs used in the study were found to be polymorphic, 16 of the 30 cultivars studied were clearly distinguishable.

GENETIC CHARACTERIZATION OF APRICOT AND WITH MOLECULAR MARKERS

The origin of the cultivated apricot (*Prunus armeniaca* L.) was stated by Vavilov's China and Central Asia (Vavilov, 1951). Near Eastern centers extending from Northeast Iran to the Caucasus and Central Anatolia are also defined as the second origin. The *P. armeniaca* species was divided into 4 major eco-geographical groups and 13 regional subgroups by Kostina and added our country to the Iran-Caucasus eco-geographic group (Bakır et al., 2018). All apricot species contain 8 pairs of chromosomes (2n = 16) (Asma and Ozturk 2005).

Phylogenetic analysis of genotypes collected from Malatya, RAPD-PCR, ISSR-PCR, and DNA sequence analysis methods were used in the study conducted by Sevindik et al., (2020) examining some Turkish *P. armeniaca* L. cultivars. 11 RAPD primers and 15 ISSR primers were used to determine the molecular characterization of apricot genotypes. As a result of the analysis, RAPD gave 46 bands, while ISSR gave 95 bands. Sequence analysis results vary between 398-403 nucleotides. As a result, they observed that the use of markers is more polymorphic than DNA sequencing. Sheikh et al., (2021), using 4 ISSR markers, performed the genetic characterization study of the differentiation of 50 varieties of apricots collected from various geographical regions of Jammu and Kashmir from native germplasm from exotic germplasm. While the similarity ratio between 0.48 and 0.94 was obtained by using Jaccard's similarity coefficient of the 50 genotypes used in the study, it was observed that two main groups were formed in the UPGMA clustering analysis. They concluded that the four ISSR markers were able to distinctly distinguish native germplasm from exotic germplasm. However, more ISSR markers should be scanned in order to better understand the distribution of diversity

in the region. The genetic difference between native genotypes and exotic genotypes has been reported to be useful in apricot breeding studies. Another study was carried out in our country, Bakır et al. (2018) with 44 wild apricots collected from Cappadocia. These wild varieties were compared with 5 locally known varieties suitable for trade and market in terms of their characteristics. Thirteen of the 16 SSR primers worked successfully and a total of 107 alleles were detected. It has been reported that the similarity rates of wild apricots vary from 12% to 96%, and high genetic diversity was estimated.

In another study, Ehliz et al., (2021) investigated the differences between 7 apricot cultivars collected from farmers' orchards in Mersin Mut Collecta Village, using the SSR marker. As a result of the analysis, apricot cultivars were divided into two main groups. The similarity coefficients of these groups varied between 0.677 and 0.938. While the lowest similarity value was seen between A1 and Italian buckle, the highest similarity value was seen in Septik and Iğdır Şalâğı varieties. SSR findings of apricot cultivars grown in Turkey can be an important guide for the selection of parents for breeding studies carried out in the country, and for determining factors such as differences in fruit quality parameters or resistance to some specific diseases. It can also be used to determine the distribution areas of apricot cultivars, to compare genetic collections, and to characterize apricot cultivars.

GENETIC CHARACTERIZATION OF CHERRY AND SOUR CHERRY WITH MOLECULAR MARKERS

Cherry (*Prunus avium* L.) is seen to be included in the Rosaceae family, Prunoideae subfamily, *Prunus* genus when the taxonomy is examined. The region between the South Caucasus, the Caspian Sea, and Northeast Anatolia is the region of cherry (*P. avium* L.) is known as the origin. From the area of origin, it spread to the east and west of the world and gained a large production area. As with most fruits, one of the ancient cultural areas of cherries is Anatolia. Therefore, Anatolia is one of the origin centers of cherries. There are about 1500 varieties in the world (Çelik and Sarıaltın, 2019). Especially the species produced are sweet cherry and sour cherry. Sweet cherry (*P. avium* L.) is diploid (2n = 16), and sour cherry (*P. cerasus* L.) is a tetraploid species (2n = 32). It is thought that sour cherry emerged as a result of the natural hybridization of *P. avium* and *P. fruticosa* L. species (Khadivi et al., 2019). Gülen et al., (2010) used 6 SSR and 4 AFLP molecular markers to detect genetic diversity in 78 local sweet cherry cultivars. The similarity rate was found to be more than 42%. In the study, it was concluded that these two marker systems are unique in all 78 genotypes, genetic diversity is high among genotypes and this genetic variation can be used in breeding programs in the future. Patzak et al., (2019), 20 SSR markers and 5 EST-SSR markers were used for the molecular characterization of 123 old and local varieties obtained from the genetic resources of the Pomology Research and Breeding Institute in Holovousy. 115 polymorphic bands were obtained. In the dendrogram, 3 main clusters and 16 subcluster were observed. As a result of the study, they concluded that the SSR marker would be beneficial in maintaining genetic diversity and providing information while creating genetic resource collections.

In a study by Pınar et al., (2018) in cherry, it is aimed to determine genetic relatedness by using the RAPD marker. 16 RAPD primers were used for 20 different cherry genotypes and 109 bands were obtained, 92 of which were polymorphic. The average polymorphism rate was determined as 84.40%. In another study, Uzan Eken et al., (2022) in order to prevented the loss of diversity in the wild cherry (*P. avium* L, syn. *Cerasus avium* L. Moench.) tree, which is ecologically widespread but whose genetic diversity was endangered and shed light on breeding studies and gene resources, molecular studies was carried out to protect. 440 genotypes from 22 different populations in our country were analyzed with 10 SSR markers. While the intra-population genetic diversity rate was found to be 88.5%, the inter-population genetic diversity rate was found to be 9.8%. Veliköy and Kemerköprü populations grown at high altitudes,

Macara population closest to European varieties, and Tota sample taken from the Mediterranean Region population showed genetic differences compared to other populations. For this reason, as a result of the study, Kemerköprü, Macara, Veliköy, and Tota populations have suggested *in situ* protection. The data they obtained for the protection of wild cherry gene resources of our country were interpreted as useful.

GENETIC CHARACTERIZATION OF PLUM WITH MOLECULAR MARKERS

Prunus genus and *Prunophora* subgenus is an important stone fruit species that has spread over a wide area in the world. It is reported that there are about 200 plum species belonging to the genus *Prunus* in the world (Yaşar et al., 2022). The European plum (*Prunus domestica* L.) is a hexaploid (2n = 48) species and its biological origin remains controversial and unclear. According to archaeological findings, the use of plums by humans dates back to the 6,000s. It is also known that it was grown a lot in Roman times. A long history of adaptation has resulted in diversity. Çakal plum (*P. spinosa* L.) belonging to the *Prunus* genus in the Prunoideae subfamily of Rosaceae is a tetraploid (2n = 32) species thought to be native to Southern Europe, Turkey, and Armenia, and the cherry plum is known as diploid (2n = 16) (Erturk et al., 2009).

Erturk et al., (2009) with *P. spinosa*, Çakal plum, wild populations were collected from the Coruh Valley in Northeast Turkey. 16 distinct genotypes were analyzed using RAPD. Fifteen of the 51 primers yielded reproducible patterns. These 15 primers produced 226 bands, 65% of which were polymorphic. As a result of the study, they decided that RAPD could be used to measure the genetic difference of the Çakal plum. In another study, Çakır et al., (2021) investigated 66 genotypes in the Turkish National *P. cerasifera* collection in their study with *P. cerasifera* Ehrh, a highly preferred variety in our country. These 66 varieties were taken from Denizli, Balıkesir, İzmir, Aydın, Manisa, and Muğla, and our important local varieties, Can and Papaz plum, were used. Analyzes of the samples were performed with the SRAP marker. Of the 495 bands showing polymorphism, 98 percent were identified as polymorphic. The dice coefficient was used to determine the mean of diversity. After the Dice coefficient was determined as 0.39, it was decided that this study had similar results to the studies conducted in Belarus, France, and Iran, but higher results than the studies conducted in China. In addition, in this study, it was determined that although Can and Papaz plums were different from each other morphologically, they were molecularly the same. Qiao et al., (2006) studied a total of 56 genotypes using 54 Japanese plums and 2 European plums. 10 ISSR, 21 SSR, and 24 RAPD markers were used in the study. As a result of the study, 86 ISSR bands, 102 SSR alleles, and 21 RAPD bands were obtained. When the similarities of the Japanese plums to the European plums are examined, it has been observed that the two varieties have different distributions. Within-group similarity rates of Japanese plums varied between 0.286 and 0.730.

In another study, Antanyniene et al., (2023) evaluated genetic diversity with SSR markers using European plum (*P. domestica*) and its hybrids from Lithuania. A total of 107 *P. domestica* L. genotype with SSR markers, 68 European and 39 hybrids, were studied from the genetic resource collection of the Horticulture Institute of the Lithuanian Agriculture and Forestry Research Center. The number of alleles in each primer ranged from 18 to 30 with an average of 24.33. The result of the study was the characterization and identification of Lithuanian plum genotypes. The uniqueness of the analyzed varieties is emphasized. European plum varieties originating in Lithuania have been interpreted as having several unique alleles required for plant breeding under exceptional northern climatic conditions.

CONCLUSION

Our country is important habitat for many plant varieties with its wide genetic diversity and endemic species. There will always be a need for our wild endemic varieties for the breeding studies of our country and the development of new varieties. Fruit genetic resources

should also be protected and a lot of work should be done on our unstudied gene resources. Stone fruits have a very important place, especially in trade and market. It is very important to make more varieties known by conducting molecular studies on stone fruits such as peach, nectarine, apricot, cherry, sour cherry, and plum. Many marker systems such as RAPD, SSR, ISSR, and AFLP can be used in such studies. This information sheds light on breeders in selecting varieties to be used in breeding. Our wild varieties are plants that are of great value in order to deliver new, more durable plants that are of great importance in the protection of gene resources for future generations. It is very important to protect the genetic resources of these varieties. Wild varieties, which are resistant to most diseases compared to local varieties, may be the only solution for us to overcome a possible plant disease that we may encounter in the future. We need to increase our knowledge of these plants and identify their genetic variation so that we can use these more resistant wild varieties in the future. For this reason, wild varieties of stone fruits, which have an important place in the market, should be researched and determined, multiplied, studies should be done by making collection gardens and these studies should be supported by molecular analysis in order to be used in further breeding studies in the future.

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HARD SEED FRUIT GENE RESOURCES OF TÜRKİYE AND MOLECULAR CHARACTERIZATION STUDIES

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ABSTRACT

Türkiye has rich soils that offer suitable habitat for many plants. Our country, which is rich in gene resources, is the gene center of many plants. Fruits, which can be classified according to their different characteristics, are examined in seven groups when classified according to fruit characteristics. The most important stone fruits grown in our country are peach (*Prunus persica* L.), nectarine (*Prunus persica* var. *nectarina*), apricot (*Prunus armeniaca* L.), cherry (*Prunus avium* L.), sour cherry (*Prunus cerasus* L.), and plum (*Prunus domestica* L.). Many varieties of these fruits, which are widely consumed in the world, have been obtained by using both traditional and modern breeding methods. While applying modern breeding methods, it is very significant to use and protect natural populations in factors such as expanding the gene pools of this group, which have a lot of wild ones in our country, and resistance to biotic and abiotic stress. Molecular markers are the most efficient and reliable methods used in the genetic characterization and identification of wild varieties. RAPD, SSR, ISSR, AFLP, and SRAP markers, which are used for many purposes, such as the characterization of wild and cultivated fruits and advanced breeding programs, are just some of them. This review examines the molecular characterization studies of stone fruits carried out in Turkey via molecular markers.

Keywords: Breeding, Drupe fruits, Gene resources, Molecular characterization

INTRODUCTION

More than one fruit variety can be grown in Turkey due to the favorable climate condition and soil types. There are different types of classifications of fruits. When classified according to fruit characteristics (pomologically); Mediterranean fruits are divided into seven groups as hard-skinned, soft-core, drupe, berry, citrus, and pleasure fruits. Cherry, sour cherry, apricot, peach, nectarine, and plum fruits are the leading stone fruits produced in our country. Apart from these, even though the production amount is less, the fruits of buckthorn, cranberry, and jujube, which are included in the production of our country, are also included in the class of stone fruits. Our country is the leader in all of these fruits, each of which grows in different conditions (Duru et al., 2022). Stone fruits are biologically classified as Rosales team, Rosaceae family. It is in the Prunoideae subfamily and comes from the genus *Prunus*. Traded *Prunus* species were first seen between Eastern Europe and Western China, and are suitable for cultivation between 30°-40° latitudes, in climates with long and dry seasons (Duru et al., 2022).

Although wild varieties are plants that can adapt to various conditions, they are endangered day by day due to some reasons such as pollution and the destruction of their habitats. Plants can be lost due to such factors, and gene resources that will be significant to use

in important studies such as breeding in the future are lost. Studies are carried out to ensure that gene resources are not lost. Chemical and *in vitro* techniques, cryo-storage, slow-growth techniques, artificial seed storage, and DNA storage methods are used to preserve gene resources. These protected wild varieties are important to use in molecular studies in the development of new varieties in breeding studies (Bilir, 2016; Balkaya and Yanmaz, 2001).

Identification of plant genetic resources is as important as diversity and protection. Thanks to the development of biotechnology and plant genetics in recent years, many DNA-based techniques have been developed for breeding and variety development. Some of these are RAPD, PCR-RFLP, AFLP, ISSR, and SSR. With these techniques, progress has been made in many varieties of development and molecular studies (Kose, 2013). This review aimed to examine the molecular characterization studies of stone fruits carried out in Turkey via molecular markers.

HARD SEEDS AND GENETIC RESOURCES

Turkey has significant and very rich plant biodiversity. The most obvious example of this is that approximately 3500 of 9500 plant species are endemic to our country. These endemic species can be found in most parts of the country. Moreover, it is mostly seen in the mountainous parts of Southern and Southeastern Anatolia. These plants are also found in the Thrace region. The Iran-Turan Region and Mediterranean Region of our country contain the largest number of endemic species (Tan, 2010). Gene resources and rich genetic diversity are important for plant breeding studies. Breeders use local varieties, especially in the development of new varieties. Most of our proprietary varieties originate from Turkey's plant genetic resources collections (Tan, 2010).

The genetic diversity of fruits is quite high. They are sources of various antioxidants, rich in vitamins and minerals. Therefore, fruit gene resources should be protected at least as much as other plants. With the selection and characterization studies pioneered by Ministries, Research Institutions, and universities, it has been ensured that the fruit gene source in most of the regions was determined and defined, protected, and used in breeding studies. In addition to various gene banks established as a result of these research and development studies, name-specific Research Institutes were established in fruit species such as Hazelnut, Pistachio, Viticulture, Fig, Apricot, Nuts (Almond), and Olive, which have an important place in the Turkish economy (Kuden and Dasgan, 2021; Bozhuyuk, 2020). When the TUIK data are examined, it has been seen that stone fruit types have an important place in Turkey's fresh fruit production and export. According to TUIK data, peach and nectarine are the most produced stone fruits in Turkey whereas the least produced were jujube in 2022 (Table 1).

MOLECULAR MARKERS AND THEIR USES IN CHARACTERIZATION

In breeding studies, classical breeding methods are used for most species for the development of new varieties, but in the classical breeding method, the process is long, it requires a lot of effort, and it is not sufficient alone for the food needed with increased resistance to disease and pests. Morphological and phenotypic measurements are not considered sufficient in today's studies to determine the kinship relations between selected species and resistant varieties. As a result of developments in the field of biotechnology, molecular markers have been developed (Ehliz et al., 2021). Today, using molecular markers to define the correctness of the names of the varieties in a short time has an important place in fruit growing (Aksu, 2015). Molecular markers are not affected by environmental conditions, they are used in every period of plant development without waiting for maturation, they show wide variation and provide advantages compared to other markers and are used more widely (Sönmezoğlu et al., 2010).

Table 1. Production amount of hard seed fruits in Turkey in 2022 (tonnes) (TUIK, 2022)

Hard Seed Fruits	Production Amount in Turkey Tonnes
Peaches and Nectarine	1.008.185
Plums	348.750
Apricots	803.000
Wild apricots	20.832
Cherries	656.041
Sour Cherries	176.770
Cranberry	13.750
Silverberry	3.903
Jujube	2.248

Molecular markers have many advantages over morphological and biochemical markers with features such as high polymorphism, frequent and uniform distribution in the genome, being dominant and codominant, identifying more than one region, being easy, fast, and reproducible, but not all of these features are present in all molecular markers. While selecting the marker that is suitable for our purpose in the study, it is desired that some of these features be together (Shidfar, 2014). Molecular markers are categorized as dominant markers and codominant markers according to their scoreability and heredity in terms of heterozygosity and homozygosity (Devran, 2003). Molecular markers can also be used in analysis such as selection with the help of markers (MAS), characterization studies of gene sources, phylogenetic analyses, determination of varieties, and genetic relatedness (Shidfar, 2014). Markers such as SSR, AFLP, and ISSR which can give more precise results due to the low reproducibility level of the DNA-based RAPD technique, have been started to be used by many researchers (Ehliz et al., 2021).

GENETIC CHARACTERIZATION OF PEACH AND NECTARINE WITH MOLECULAR MARKERS

The latin name is *Prunus persica* L., was thought to be native to Iran and the Caucasus. In 1883, however, De'candolle proved that East Asia and China were the origin of the peach. The name peach has been found in Chinese literature dating back to 2000 BC. Due to its adaptability to different climates, peach has spread throughout the world (Ercan and Özkarakaş, 2003). The most important fruit type in Europe after apple is the peach, and it is also the most important species of the genus *Prunus*. Peach is a diploid species (2n = 16) (Dirlewanger et al., 2006). Contrary to popular belief, nectarine is not different from peach. It is in the sub-variety of peach, *Prunus persica* var *nectarina,* named as nectarina (Erbil and Erenoğlu, 2006).

If we examine the molecular studies on peach, Gür and Şeker (2012) examined the genetic relationships of white nectarine cultivars with other *Prunus* (peach, nectarine, cherry, almond, apricot, and plum) in their study. The *Prunus* species included in their study were taken from the Çanakkale Onsekiz Mart University fruit plots and producer gardens in 2011. The AFLP marker was chosen to determine the genetic relationship between fruits. As a result of the analysis, 182 of the 282 AFLP fragments from 6 primer pairs were observed as polymorphic. As a result of the study, it was seen that the white nectarine has different genetic characteristics

from other *Prunus*. White nectarines were defined as closest to each other, and the most distant related ratio was observed in cherry cultivars.

Demirel et al., (2023), using 32 ISSR markers, the genetic characterization study of a total of 54 peach genotypes, 52 local and 2 commercial varieties, obtained from the province of Iğdır, was carried out. A total of 213 alleles, 154 of which were polymorphic, were obtained in the study and 54 genotypes were grouped into four groups according to the UPGMA dendrogram. According to the results they obtained, they reported that 54 peach genotypes were different in terms of genetic similarity; ISSR markers used in the study could benefit breeding programs in selecting individuals as parents. In another study conducted by Demirel (2018), it was desired to determine the genetic differences between two local varieties of Iğdır province, Zeferan and Ağşeftali. ISSR marker was chosen for the study. 54 peach varieties were studied. Of the 57 primers, 42 were optimized and 32 were selected as polymorphic. 7 ISSR primers with the highest rate of polymorphism (100%) used in the study were determined. It was decided that the characterization of peach genotypes could be made with these 7 primers. As a result of the study, Zaferan6 and Ağşeftali18 genotypes were determined to be far from each other and other genotypes in the study. Ağşefali6 and Ağşeftali16 genotypes were almost very similar to each other, so it was decided that Ağşefali6 and Ağşeftali16 genotypes could not be selected as parents in the breeding study. Distant genotypes were found suitable for selection as parents in breeding.

In a study conducted in Spain, Bouhadida et al., (2007) worked with local varieties unique to Spain. In their study, by using the SSR marker, the similarity to the known local varieties was evaluated by having information about the genetic diversity of 19 varieties of Miraflores, whose pedigree is unknown. High polymorphic 20 SSR markers developed for peach were used. As a result of the analysis, 46 scoreable alleles were obtained. While 14 of the SSRs used in the study were found to be polymorphic, 16 of the 30 cultivars studied were clearly distinguishable.

GENETIC CHARACTERIZATION OF APRICOT AND WITH MOLECULAR MARKERS

The origin of the cultivated apricot (*Prunus armeniaca* L.) was stated by Vavilov's China and Central Asia (Vavilov, 1951). Near Eastern centers extending from Northeast Iran to the Caucasus and Central Anatolia are also defined as the second origin. The *P. armeniaca* species was divided into 4 major eco-geographical groups and 13 regional subgroups by Kostina and added our country to the Iran-Caucasus eco-geographic group (Bakır et al., 2018). All apricot species contain 8 pairs of chromosomes (2n = 16) (Asma and Ozturk 2005).

Phylogenetic analysis of genotypes collected from Malatya, RAPD-PCR, ISSR-PCR, and DNA sequence analysis methods were used in the study conducted by Sevindik et al., (2020) examining some Turkish *P. armeniaca* L. cultivars. 11 RAPD primers and 15 ISSR primers were used to determine the molecular characterization of apricot genotypes. As a result of the analysis, RAPD gave 46 bands, while ISSR gave 95 bands. Sequence analysis results vary between 398-403 nucleotides. As a result, they observed that the use of markers is more polymorphic than DNA sequencing. Sheikh et al., (2021), using 4 ISSR markers, performed the genetic characterization study of the differentiation of 50 varieties of apricots collected from various geographical regions of Jammu and Kashmir from native germplasm from exotic germplasm. While the similarity ratio between 0.48 and 0.94 was obtained by using Jaccard's similarity coefficient of the 50 genotypes used in the study, it was observed that two main groups were formed in the UPGMA clustering analysis. They concluded that the four ISSR markers were able to distinctly distinguish native germplasm from exotic germplasm. However, more ISSR markers should be scanned in order to better understand the distribution of diversity

in the region. The genetic difference between native genotypes and exotic genotypes has been reported to be useful in apricot breeding studies. Another study was carried out in our country, Bakır et al. (2018) with 44 wild apricots collected from Cappadocia. These wild varieties were compared with 5 locally known varieties suitable for trade and market in terms of their characteristics. Thirteen of the 16 SSR primers worked successfully and a total of 107 alleles were detected. It has been reported that the similarity rates of wild apricots vary from 12% to 96%, and high genetic diversity was estimated.

In another study, Ehliz et al., (2021) investigated the differences between 7 apricot cultivars collected from farmers' orchards in Mersin Mut Collecta Village, using the SSR marker. As a result of the analysis, apricot cultivars were divided into two main groups. The similarity coefficients of these groups varied between 0.677 and 0.938. While the lowest similarity value was seen between A1 and Italian buckle, the highest similarity value was seen in Septik and Iğdır Şalâğı varieties. SSR findings of apricot cultivars grown in Turkey can be an important guide for the selection of parents for breeding studies carried out in the country, and for determining factors such as differences in fruit quality parameters or resistance to some specific diseases. It can also be used to determine the distribution areas of apricot cultivars, to compare genetic collections, and to characterize apricot cultivars.

GENETIC CHARACTERIZATION OF CHERRY AND SOUR CHERRY WITH MOLECULAR MARKERS

Cherry (*Prunus avium* L.) is seen to be included in the Rosaceae family, Prunoideae subfamily, *Prunus* genus when the taxonomy is examined. The region between the South Caucasus, the Caspian Sea, and Northeast Anatolia is the region of cherry (*P. avium* L.) is known as the origin. From the area of origin, it spread to the east and west of the world and gained a large production area. As with most fruits, one of the ancient cultural areas of cherries is Anatolia. Therefore, Anatolia is one of the origin centers of cherries. There are about 1500 varieties in the world (Çelik and Sarıaltın, 2019). Especially the species produced are sweet cherry and sour cherry. Sweet cherry (*P. avium* L.) is diploid (2n = 16), and sour cherry (*P. cerasus* L.) is a tetraploid species (2n = 32). It is thought that sour cherry emerged as a result of the natural hybridization of *P. avium* and *P. fruticosa* L. species (Khadivi et al., 2019). Gülen et al., (2010) used 6 SSR and 4 AFLP molecular markers to detect genetic diversity in 78 local sweet cherry cultivars. The similarity rate was found to be more than 42%. In the study, it was concluded that these two marker systems are unique in all 78 genotypes, genetic diversity is high among genotypes and this genetic variation can be used in breeding programs in the future. Patzak et al., (2019), 20 SSR markers and 5 EST-SSR markers were used for the molecular characterization of 123 old and local varieties obtained from the genetic resources of the Pomology Research and Breeding Institute in Holovousy. 115 polymorphic bands were obtained. In the dendrogram, 3 main clusters and 16 subcluster were observed. As a result of the study, they concluded that the SSR marker would be beneficial in maintaining genetic diversity and providing information while creating genetic resource collections.

In a study by Pınar et al., (2018) in cherry, it is aimed to determine genetic relatedness by using the RAPD marker. 16 RAPD primers were used for 20 different cherry genotypes and 109 bands were obtained, 92 of which were polymorphic. The average polymorphism rate was determined as 84.40%. In another study, Uzan Eken et al., (2022) in order to prevented the loss of diversity in the wild cherry (*P. avium* L, syn. *Cerasus avium* L. Moench.) tree, which is ecologically widespread but whose genetic diversity was endangered and shed light on breeding studies and gene resources, molecular studies was carried out to protect. 440 genotypes from 22 different populations in our country were analyzed with 10 SSR markers. While the intra-population genetic diversity rate was found to be 88.5%, the inter-population genetic diversity rate was found to be 9.8%. Veliköy and Kemerköprü populations grown at high altitudes,

Macara population closest to European varieties, and Tota sample taken from the Mediterranean Region population showed genetic differences compared to other populations. For this reason, as a result of the study, Kemerköprü, Macara, Veliköy, and Tota populations have suggested *in situ* protection. The data they obtained for the protection of wild cherry gene resources of our country were interpreted as useful.

GENETIC CHARACTERIZATION OF PLUM WITH MOLECULAR MARKERS

Prunus genus and *Prunophora* subgenus is an important stone fruit species that has spread over a wide area in the world. It is reported that there are about 200 plum species belonging to the genus *Prunus* in the world (Yaşar et al., 2022). The European plum (*Prunus domestica* L.) is a hexaploid (2n = 48) species and its biological origin remains controversial and unclear. According to archaeological findings, the use of plums by humans dates back to the 6,000s. It is also known that it was grown a lot in Roman times. A long history of adaptation has resulted in diversity. Çakal plum (*P. spinosa* L.) belonging to the *Prunus* genus in the Prunoideae subfamily of Rosaceae is a tetraploid (2n = 32) species thought to be native to Southern Europe, Turkey, and Armenia, and the cherry plum is known as diploid (2n = 16) (Erturk et al., 2009).

Erturk et al., (2009) with *P. spinosa*, Çakal plum, wild populations were collected from the Coruh Valley in Northeast Turkey. 16 distinct genotypes were analyzed using RAPD. Fifteen of the 51 primers yielded reproducible patterns. These 15 primers produced 226 bands, 65% of which were polymorphic. As a result of the study, they decided that RAPD could be used to measure the genetic difference of the Çakal plum. In another study, Çakır et al., (2021) investigated 66 genotypes in the Turkish National *P. cerasifera* collection in their study with *P. cerasifera* Ehrh, a highly preferred variety in our country. These 66 varieties were taken from Denizli, Balıkesir, İzmir, Aydın, Manisa, and Muğla, and our important local varieties, Can and Papaz plum, were used. Analyzes of the samples were performed with the SRAP marker. Of the 495 bands showing polymorphism, 98 percent were identified as polymorphic. The dice coefficient was used to determine the mean of diversity. After the Dice coefficient was determined as 0.39, it was decided that this study had similar results to the studies conducted in Belarus, France, and Iran, but higher results than the studies conducted in China. In addition, in this study, it was determined that although Can and Papaz plums were different from each other morphologically, they were molecularly the same. Qiao et al., (2006) studied a total of 56 genotypes using 54 Japanese plums and 2 European plums. 10 ISSR, 21 SSR, and 24 RAPD markers were used in the study. As a result of the study, 86 ISSR bands, 102 SSR alleles, and 21 RAPD bands were obtained. When the similarities of the Japanese plums to the European plums are examined, it has been observed that the two varieties have different distributions. Within-group similarity rates of Japanese plums varied between 0.286 and 0.730.

In another study, Antanyniene et al., (2023) evaluated genetic diversity with SSR markers using European plum (*P. domestica*) and its hybrids from Lithuania. A total of 107 *P. domestica* L. genotype with SSR markers, 68 European and 39 hybrids, were studied from the genetic resource collection of the Horticulture Institute of the Lithuanian Agriculture and Forestry Research Center. The number of alleles in each primer ranged from 18 to 30 with an average of 24.33. The result of the study was the characterization and identification of Lithuanian plum genotypes. The uniqueness of the analyzed varieties is emphasized. European plum varieties originating in Lithuania have been interpreted as having several unique alleles required for plant breeding under exceptional northern climatic conditions.

CONCLUSION

Our country is important habitat for many plant varieties with its wide genetic diversity and endemic species. There will always be a need for our wild endemic varieties for the breeding studies of our country and the development of new varieties. Fruit genetic resources

should also be protected and a lot of work should be done on our unstudied gene resources. Stone fruits have a very important place, especially in trade and market. It is very important to make more varieties known by conducting molecular studies on stone fruits such as peach, nectarine, apricot, cherry, sour cherry, and plum. Many marker systems such as RAPD, SSR, ISSR, and AFLP can be used in such studies. This information sheds light on breeders in selecting varieties to be used in breeding. Our wild varieties are plants that are of great value in order to deliver new, more durable plants that are of great importance in the protection of gene resources for future generations. It is very important to protect the genetic resources of these varieties. Wild varieties, which are resistant to most diseases compared to local varieties, may be the only solution for us to overcome a possible plant disease that we may encounter in the future. We need to increase our knowledge of these plants and identify their genetic variation so that we can use these more resistant wild varieties in the future. For this reason, wild varieties of stone fruits, which have an important place in the market, should be researched and determined, multiplied, studies should be done by making collection gardens and these studies should be supported by molecular analysis in order to be used in further breeding studies in the future.

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MORPHOLOGICAL CHARACTERISTICS OF GIANT STINGING NETTLE (*Girardinia Diversifolia*) IN GIRESUN ECOLOGICAL CONDITIONS

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Abstract

Girardinia diversifolia is a perennial plant belonging to the Urticaceae family, reaching a height of 1.5 to 3 meters. *Girardinia diversifolia* is commonly known as the Giant Nettle. It is naturally abundant in forested areas, along riverbanks, and moist habitats in the Himalayas, India, Sri Lanka, and China. The plant grows in clusters, with multiple stems in each cluster. Its stems are upright, pentagonal, and branch out from the base. The stems are also covered with stinging, pointed, and soft hairs. *Girardinia diversifolia* is known for its bast (bark) fibers, which are long, strong, smooth, and shiny. The fibers of the Giant Nettle are used in the production of various textiles, ropes, mats, sacks, and various other household items. It is a crucial fiber source that can be cultivated to generate income in rural areas. Considering its ecological requirements and growth conditions, it has been experimented to evaluate its potential for fiber production in the rural areas of the Black Sea region. This study aims to determine the morphological characteristics of Girardinia diversifolia cultivated under the ecological conditions of Giresun province.

Keywords: Bast fiber, Giant stinging nettle, *Girardinia diversifolia*, Giresun, Morphological characteristics

INTRODUCTION

Fiber plants are the raw materials for the textile industry and a source of cellulose. In our country, cotton production is the main source of fiber. Cotton fiber production in our country cannot meet the consumption demand. The textile sector meets its raw material needs through imports (Mert and Çopur, 2010). In some areas where cotton cultivation is not possible, bast fiber plants (hemp, flax, nettle) are cultivated to supply raw materials to the textile industry. The Giant Nettle (*G. diversifolia*), which naturally grows in the Black Sea region with its ecological adaptability, is thought to have the potential to become an alternative fiber source for the textile industry due to its long, high-quality, and durable fibers.

Figure 1. The general appearance of the *Girardinia diversifolia* plant and flower.

Girardinia diversifolia L. is a perennial plant from the Urticaceae family, which can reach a height of 1.5-3.5 meters. It has more pronounced stinging hairs on its stem compared to common nettles. Its leaves are 5-lobed and serrated. The stem is covered with fine thorns. The width of the leaf averages 24-26 cm. Its flowers bloom from July to September, and the seeds mature around November (Sethmann, 2004*). G. diversifolia* grows in shaded areas at altitudes ranging from sea level to 3000 meters and requires a high moisture content, frost resistance, and a fertile environment (Subedee, 2018). The bark of *Girardinia diversifolia* contains fibers that possess unique qualities, including strength, smoothness, lightness, and a silk-like shine when processed correctly (Lanzilao et al., 2016). These fibers have a wide range of applications, including weaving, medicine, papermaking, biofuel, cosmetics, and the automotive industry. In the Himalayas, its leaves are cooked as a vegetable and consumed as food, as well as used in traditional medicine for treating headaches, fevers, and swollen joints (Gurung et al., 2012; Rokaya et al., 2010). Its flowers are also commonly cooked and consumed as a vegetable along with its leaves. Long, strong, smooth, and shiny high-quality fibers can be obtained from its stems. Woody stem parts can be used in papermaking, biofuel, and biocomposite production (Gurung et al., 2012).

MATERIAL AND METHOD

This study was conducted in the village of Balıklısu, Keşap district, Giresun province, within the scope of the "Dissemination of Nettle Farming and Technology" project supported by DOKAP in 2021. The nettle seedlings required for a total area of 1000 m2 were grown in pots at Ondokuz Mayıs University, Faculty of Agriculture. Planting was carried out on June 11, 2021, with a spacing of 50x50 cm. Observations and measurements for morphological characteristics were made in the next year (2022) to determine the morphological characteristics. The soil structure of the experimental area was medium-textured and had an acidic reaction, with an elevation of 300 meters above sea level. To increase the organic matter content in the soil, manure (450 kg per hectare) was applied for soil correction. The climate data for the Keşap district of Giresun province in 2022, where the trial was conducted,

especially during the flowering and maturation period, provided suitable conditions for the development of Giant Nettle (Table 1).

Table 1. Climate data for the Keşap district of Giresun province (January 2022-December 2022).

Months	*Relative Humidity (%)*	*Temperature (°C)*	*Precipitation (mm)*
January	**68,5**	**3,5**	**224,7**
February	**66**	**6,1**	**96,1**
March	**79,3**	**1,6**	**436**
April	**58,8**	**12,6**	**63,9**
May	**69,4**	**12,9**	**138,1**
June	**86,3**	**17,2**	**69,4**
July	**87,1**	**18**	**119,2**
Auğust	**89,7**	**21,1**	**138,6**
September	**77,8**	**18,2**	**196**
October	**84,8**	**12,7**	**236,4**
November	**76,5**	**11,4**	**107,4**
December	**72,6**	**8,8**	**151**
Average	**76,4**	**12,0**	**164,0**

The research was conducted on 10 randomly selected plants from the Giant Nettle trial established in an area of 1000 m2. Measurements of plant height, technical stem length, stem diameter, and the number of lateral branches were made to determine morphological characteristics. These measurements were made according to UPOV criteria.

Results

As a result of the research, data related to plant height, technical stem length, stem diameter, and the number of lateral branches, etc., are given in Table 2.

Table 2. Average data for some of the examined characteristics of Giant Nettle cultivated under ecological conditions.

Replication	Plant Height(m)	Technical Stem Length (m)	Stem Diameter (mm)	Lateral Branches(max-min) avg
1	2,18	0,53	11,94	(0-4) 2,00
2	1,80	1,25	11,00	(0-5)1,25
3	3,35	1,26	15,22	(0-5)1,71
4	2,41	2,18	11,39	(0-5)0,20
5	2,24	1,98	10,97	(0-2)0,40
6	2,36	2,13	9,24	(0-1)0,20
7	2,43	2,21	9,25	(0-2)0,40
8	2,47	2,47	9,86	(0)0,00
9	2,23	1,91	10,10	(0-1)0,20
10	2,06	2,06	8,40	(0)0,00
Avarage	2,35	1,80	10,74	0,64

Upon examination of Table 2, it is determined that the plant height varies between 1.80 m and 3.35 m, with an average plant height of 2.35 m. Technical stem length (m) ranged from 0.53 m to 2.21 m, with an average technical stem length of 1.80 m. Stem diameter (mm) varied from 8.40 mm to 16.06 mm, with an average stem diameter of 10.82 mm. The average number of lateral branches on the plant ranged from 0 to 2.00, with an average of 0.65.

In addition to genetic factors, breeding techniques and environmental factors play a limiting role in the emergence of yield and yield elements in production. Considering the quality and yield factors, the multifaceted effects of these factors should be considered in determining the varieties or genotypes that can adapt to the ecological conditions of a region.

RESULTS AND DISCUSSION

With the data obtained in this study, the effect of *Girardinia diversifolia* plant on its morphological features on the adaptation quality and yield elements of Giresun province Keşap district Balıklısu village was investigated. Within these compatible parameters, plant heights between 1.80 and 3.35 were obtained. Since the first 2 years of this plant were the establishment years, it was expected that the yield would be low. In this study, although we made the measurements in the 2nd year, the efficiency was higher than our expectations.

The distance (meters) from the root of the plants to the extreme point was measured as plant height. Based on literature studies of giant nettle, it has been stated that the plant height is 1.5 m to 3.5 m. In this study, plant height varied between 1.80 m and 3.35 m. Although it is the second year of this study, these results have emerged. Since it is a perennial plant, its economic harvest life is 8-10 years (Ayan et al., 2020). Giresun's ecological conditions have also been achieved in this second year.

The technical stem is defined as the length of the technical stem up to the point where the generative part or branching begins (Kara, 2013). It is emphasized that technical stem length can be affected by climatic conditions as well as a variety features (Aksoy and Aytaç, 2021). Although our genotypes were different, they showed non-type characteristics. When the general population was looked at, some plants showed different developmental patterns. There were both upright and spreading plants in the same parcel.

In giant nettle, stem diameter was measured in the middle part of the stem with the help of calipers. Stem thickness is affected by environmental conditions and genotypic characteristics. The stem thickness of the nettle (*Urtica diocia L.*), which grows naturally in our country, is thinner than the giant nettle. This difference is because the plant height of the giant nettle plant is longer than the nettle. The advantage of having more stalk thickness provides higher fiber yield.

Generally, parameters such as side branches are not taken into consideration in fiber plants. However, since this plant is an unknown plant in our country, different parameters are included to know the morphological characteristics of the plant by including wider parameters.

CONCLUSION AND RECOMMENDATIONS

Girardinia diversifolia started to bloom in October in Giresun conditions. The plant was harvested in January and morphological observations were taken. As a result of this study, it was determined that the average plant height was 2.35 m and the average technical stem length was 1.80 m. The shank diameter (mm) and the average shank diameter were

determined to be 10.82 mm. It was determined that the number of side branches (max-min) in the plant varied between 0 and 2.00, the average was 0.65.

As a recommendation, it is important to conduct further studies to determine its chemical and physical properties. Although the giant nettle *(G. diversifolia*) is from the same family as the nettle (*Urtica spp.)* that grows naturally in our country, *G. diversifolia* is in a more primitive form, but in terms of plant height and technical stem. is superior. Considering these features, it carries raw material potential to the textile industry. The plant grows fast and has the potential to be an important energy plant in terms of being a perennial. This study was carried out to determine the morphological characteristics of the plant. It is envisaged that studies should be carried out to recognize the plant and understand its content by using wider parameters.

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EFFECT OF BASAL MEDIA ON GROWTH AND EXOPOLYSACCHARIDES PRODUCTION BY ARONIA MELANOCARPA (MICHX.) ELLIOTT CELL SUSPENSION CULTURE

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ABSTRACT

Aronia melanocarpa (Michx.) Elliott (black chokeberry) is well known plant among the consumers and its berries are widely used for production of jam, wines, juices and food colorants. Nowadays, with the stunning advance in cellular agriculture, different in vitro systems from many edible plants are considered as perspective renewable sources of valuable phytochemicals. In this study, the effect of basal medium composition on biomass accumulation and exopolysaccharides production by *Aronia melanocarpa* cell suspension culture was investigated. The results showed that maximal amount of accumulated biomass (ADB=10.37±0.52 g/L; GI=2.13±0.05) and exopolysaccharide content (3.44±0.20 g/L) were achieved when the culture was cultivated on Gamborg B5 medium, whereas, when grown on Murashige and Skoog medium the biomass was significantly lower (ADB=0.21±0.11 g/L; GI=0.06±0.03). It worth noting, that there was no significant difference in total phenolic content between the cells grown on B5, WP and MS media. The reported results are the base for further development of black chokeberry cell suspension culture as alternative platform for sustainable production of valuable food additives.

Keywords: Cellular agriculture, Sustainable production, Black chokeberry, Exopolysaccharides, Nutrient media,

INTRODUCTION

Black chokeberry *(Aronia melanocarpa* [Michx.] Elliot) is a shrub that produces edible black colored berries. The plant belongs to the Rosaceae family and originates from North America and East Canada. Nowadays is widely cultivated in Europe, Russia and China (Kulling and Rawel, 2008). The fruits of *Aronia* are among the richest source of antioxidants and colorants among all berries (Sidor and Gramza-Michałowska, 2019). Moreover, the fruits contains high amount of dietary fibers and polysaccharides with interesting medicinal properties (Kulling and Rawel, 2008, Zhao et al., 2021, Oziembłowski et al., 2022, Zhao et al., 2022, Wen et al., 2023).

Cellular agriculture, an technology for controlled and sustainable manufacture of agricultural products by using single cells and tissues without involving plants or animals, is attracting lot of attention over the past decade (Eibl et al., 2021). Large scale cultivation of plant cells, tissues and organs was proved to be highly efficient eco-friendly technology for production of active ingredients for cosmetics, pharmacy and foods (Georgiev et al., 2018). The fast advance in the field now allows plant cells from different species to be used for commercial production of biomass, phytochemicals and plant-derived technical goods (Krasteva et al., 2021). However, the optimal composition of nutrient medium is one of the key factors,

responsible for productivity and economical effectiveness of the process (Ananga et al., 2013). Among many commercially available formulations, there are three widely used basal media for cultivation of plant cells - Murashige and Skoog (MS), WP (Woody Plant) or Gamborg B5 (B5) media. The basic differences between them is the amount of nitrogen and the ratio between ammonia and nitrate sources, as well as the amount of calcium and vitamin composition (Ananga et al., 2013, Krasteva, 2022). The optimal composition of basal medium varies for different plant species and has to be determined experimentally.

This study was conducted to determine the effect of basal nutrient medium on biomass, exopolysaccharide production and phenolic antioxidants accumulation by cell suspension of *Aronia melanocarpa* (Michx.) Elliott (black chokeberry).

MATERIAL AND METHOD

Plant material

Aronia melanocarpa (Michx.) Elliott cell suspension culture was initiated from callus as described elsewhere (Krasteva et al., 2023). The cell suspension was cultivated on Woody Plant medium, supplemented with 30 g/L sucrose, 0.5 mg/L kinetin and 2.0 mg/L picloram, on orbital shaker at 110 rpm, on darkness. The cells were sub-cultured every 7 days. For the experiments, 7 days old cell suspension was centrifuged at 4000 rpm for 10 min in sterile 50 ml tubes and the cells were separated from the culture liquids. 20 grams of fresh cells were inoculated in 100 ml sterile Murashige and Skoog (MS), WP (Woody Plant) or Gamborg B5 (B5) media and cultivated for 7 days under the described conditions. The cell growth was evaluated on the base on Accumulated Dry Biomass (ADB, g/L) and Growth Index (GI).

Evaluation of exopolysaccharide production

The exopolysaccharides, secreted into the culture media, were determined by weight after precipitation with ethanol. The culture liquids, after the cells removal, were treated with 4 volumes of cooled ethanol (4°C) and kept in the fringe for 12 hours. The precipitated exopolysaccharides were filtrated under vacuum and dried at 50°C until reach constant weight.

Total phenolic content and antioxidant activity assays

Dry Aronia cell biomass was extracted in triplicate with 70% methanol under ultrasound (1:10 w/v). The combined methanol extracts were evaporated at 40°C under vacuum for methanol removal and the volume was adjusted to 10 ml with dH_2O. For purification of phenolic fraction, 5 ml of extracts were subject to solid phase extraction (C18 Strata, Phenomenex) following the manufacturer instructions. The phenolic fraction was eluted with 2 ml HPLC grade methanol and used for future analyses. The evaluation of total phenolic content was done by using Folin–Ciocalteu assay, whereas the evaluation of antioxidant activity was performed by using DPPH radical scavenging, TEAC (Trolox equivalent antioxidant capacity), FRAP (Ferric reducing antioxidant power), and CUPRAC (Cupric ion reducing antioxidant capacity) assays as described elsewhere (Krasteva et al., 2022). The results were expressed as mg gallic acid equivalents (GAE) per gram of dry biomass for total phenolics, and as μM Trolox equivalents (TE) per gram of dry biomass for antioxidant activity assays.

HPLC profiling and quantification of phenolic compounds

Methanol extracts from dry Aronia cell biomass were analyzed by High-performance liquid chromatography (HPLC) as described previously (Krasteva et al., 2022). The HPLC system consisted of a Waters 1525 Binary Pump, equipped with a Waters 2484 dual λ Absorbance Detector and Supelco Discovery HS C18 column (5 μm, 25 cm × 4.6 mm).

Statistical analyses

All data is presented as mean with standard deviations (±SD) of three independent biological experiments (n = 3). All spectrophotometric experiments were carried out in 8 technical repeats. The means were statistically compared using one-way ANOVA, with Tukey post hoc test. The differences between the means were considered significant for values of $p \leq 0.01$.

RESULTS AND DISCUSSION

Plant cell suspension of *A. melanocarpa* is homogenous and grow in high dens culture with maximum of biomass accumulation after 7 days of cultivation (Krasteva et al., 2023). When the basal medium was changed, the cells showed significantly different growth with visible changes in culture density (Figure 1).

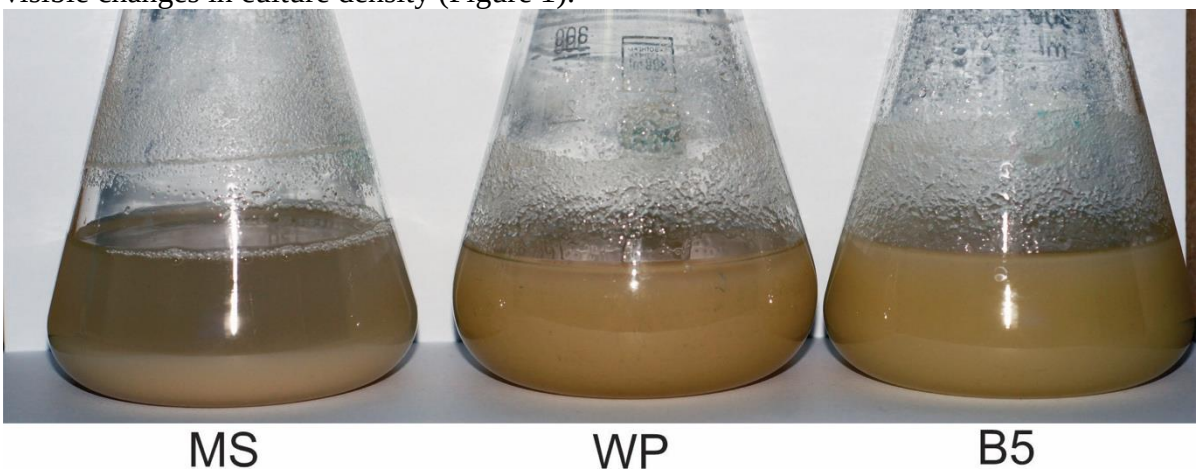

Figure 1. *Aronia* cell suspension culture, grown on Murashige and Skoog (MS), WP (Woody Plant) or Gamborg B5 (B5) media for 7 days

The *Aronia* cells growth was significantly decreased in MS medium (ADB=0.21±0.11 g/L) when compared to WP and B5 media (ADB=9.19±1.43 g/L and ADB=10.37±0.52 g/L, respectively) (Figure 2A).

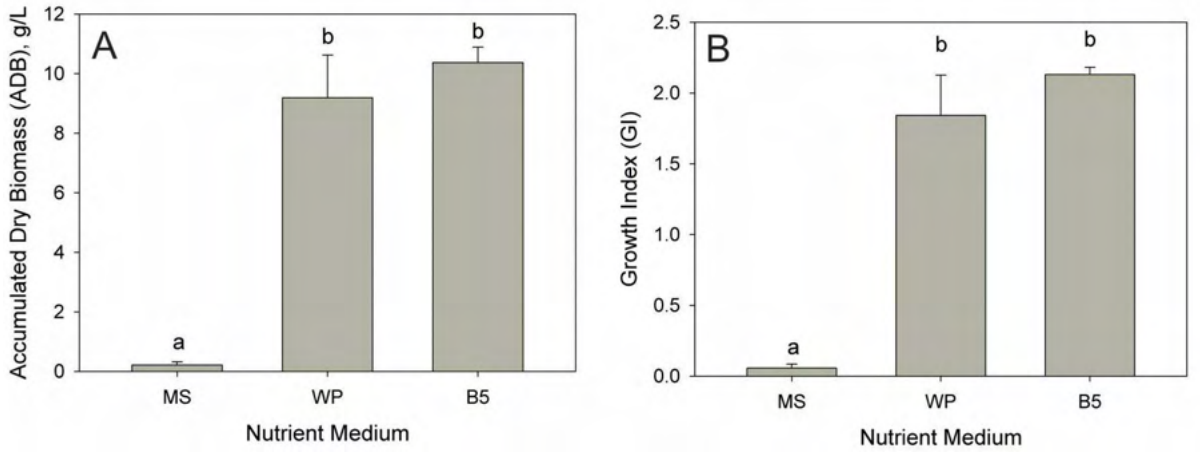

Figure 2. Accumulated Dry Biomass (A) and Growth Index (B) of *Aronia* cell suspension culture, grown on Murashige and Skoog (MS), WP (Woody Plant) or Gamborg B5 (B5) media for 7 days. The mean that do not share a letter were significantly different at $p \leq 0.01$ (one-way ANOVA with Tukey post hoc test, n=3).

The changes in growth index follows the changes in accumulated dry biomass (GI=0.06±0.03, GI=1.84±0.28 and GI=2.13±0.05, for cells cultivated on MS, WP and B5 media) (Figure 2B). Study of cell morphology showed significant changes in cell shape and size, depending on the medium, used for they growth (Figure 3).

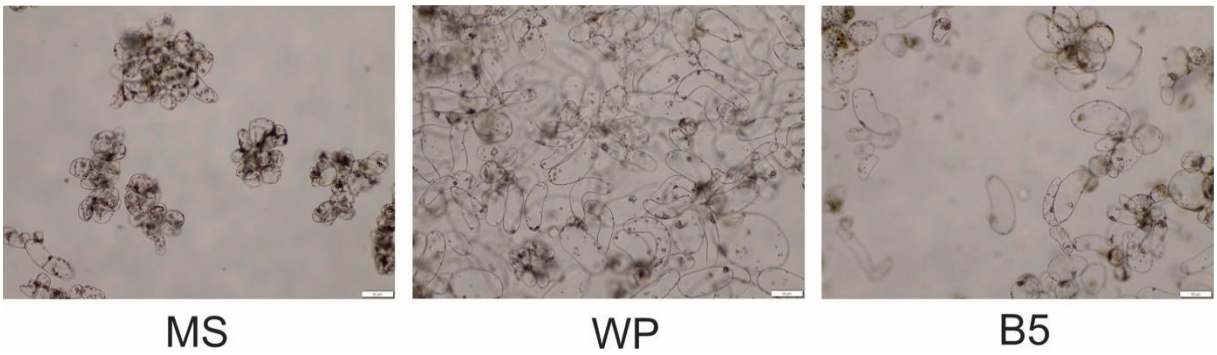

Figure 3. Light microscopy (Olympus CX23, 40x) of *Aronia* cell suspension culture, grown on Murashige and Skoog (MS), WP (Woody Plant) or Gamborg B5 (B5) media for 7 days

It is well known, that the ammonia/nitrate ratio, concentration of phosphates and calcium ions could have dramatic effect on biomass accumulation, cell morphology and secondary metabolite production of plant cells (Ananga et al., 2013). The results confirm that statement for *Aronia* cell suspension culture as well.

The composition of basal medium also affects the production of exopolysaccharides from cultured cells (Figure 4). The maximal amount of secreted polysaccharides was achieved when the cells were grown on B5 (3.44±0.20 g/L) and WP (3.37±0.04 g/L), whereas the amount was significantly lower (1.13±0.03 g/L) when MS medium was used. It worth noting, that there is no significant differences in growth and exopolysaccharides production between *Aronia* cells grown on B5 and WP media in short term cultivation, but our future experiments showed that prolonged cultivation on B5 medium (more than 10 sub-culturing cycles) lead to cell arrest and block biomass accumulation, whereas on WP such negative effect was not observed.

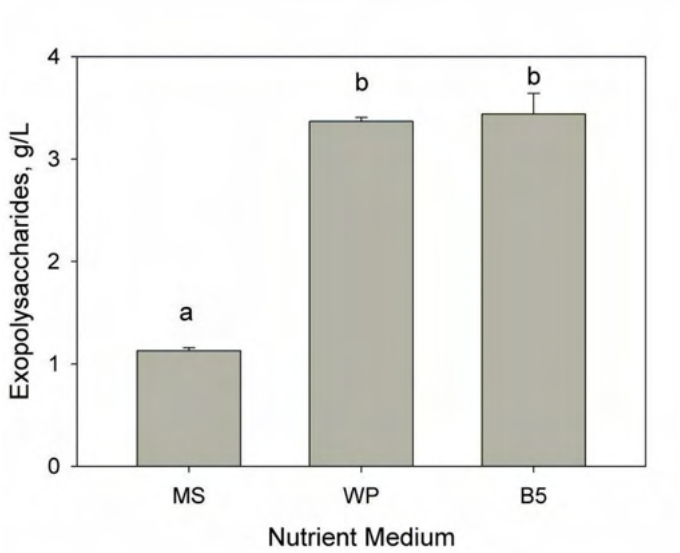

Figure 4. Accumulation of exopolysaccharides in culture medium by *Aronia* cell suspension culture, grown on Murashige and Skoog (MS), WP (Woody Plant) or Gamborg B5 (B5) media for 7 days. The mean that do not share a letter were significantly different at $p \leq 0.01$ (one-way ANOVA with Tukey post hoc test, n=3).

The HPLC fingerprint (Figure 5), showed dramatic differences in HPLC profiles of *Aronia* cells biomasses when cultured on different media.

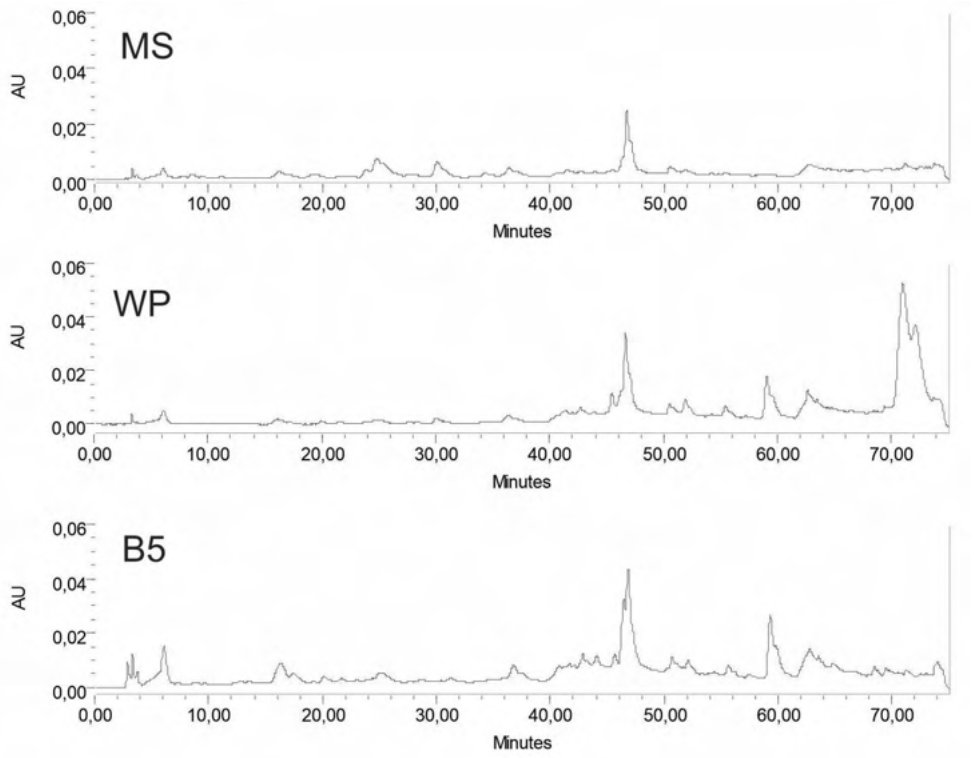

Figure 5. HPLC fingerprint (280 nm) of *Aronia* cell suspension culture, grown on Murashige and Skoog (MS), WP (Woody Plant) or Gamborg B5 (B5) media for 7 days

The quantitative analyses showed that Aronia cells accumulates highest amounts of chlorogenic acid, caffeic acid and (-)-epicatechin when cultivated on MS medium (Table 1). This culd be explained with the fact that secondary metabolite production in plants is stimulated by the stress and the results showed that in MS medium the Aronia cells are exposed to higher stress and showed the slowest growth and highest morphological changes (Figure 1, 2 and 3).

Table 1. HPLC analyses of phenolics found in biomass of *Aronia* cell suspension culture, grown on Murashige and Skoog (MS), WP (Woody Plant) or Gamborg B5 (B5) media for 7 days. The mean that do not share a letter in columns were significantly different at $p \leq 0.01$ (one-way ANOVA with Tukey post hoc test, n=3).

Nutrient Medium	(+)-Catechin, µg/g DW	Chlorogenic acid, µg/g DW	Caffeic acid, µg/g DW	(-)-Epicatechin, µg/g DW	Salicylic acid, µg/g DW
MS	ND	49.41^{a}±1.64	40.51^{a}±5.82	65.26^{a}±0.61	19.17^{a}±0.69
WP	ND	ND	9.63^{b}±1.43	28.27^{b}±0.69	19.59^{a}±10.01
B5	16.08^{a}±0.87	9.87^{b}±1.39	ND	25.83^{b}±1.46	29.76^{a}±3.07

Analyses of total phenolic content and antioxidant activities follow the tendency, observed with HPLC assay (Table 2). However, even some there is no significant differences between total phenolic content and antioxidant activities of extracts from *Aronia* cells when grown in different media. An exception are the CUPRAC and TEAC assays where is significant changes in activities. These methods are based on electron exchange and evaluate the potential of antioxidants to reduce cupric ad ferric ions (Krasteva et al., 2022). The observed differences could be explained with possible differences in phenolic composition of investigated extracts

(supported by chromatograms, presented in Figure 5), rather than by the observed differences in phenolic concentrations.

Table 2. Total phenolic content and antioxidant activities of extracts from biomass of *Aronia* cell suspension culture, grown on Murashige and Skoog (MS), WP (Woody Plant) or Gamborg B5 (B5) media for 7 days. The mean that do not share a letter in columns were significantly different at $p \leq 0.01$ (one-way ANOVA with Tukey post hoc test, n=3).

Nutrient Medium	Total Phenolic, mg GAE/g DW	CUPRAC, µM TE/g DW	FRAP, µM TE/g DW	DPPH, µM TE/g DW	TEAC, µM TE/g DW
MS	0.95^{a}±0.33	9.87^{a}±0.90	2.54^{a}±0.19	2.34^{a}±0.14	0.15^{c}±0.05
WP	0.84^{a}±0.09	9.15^{ab}±0.98	2.21^{a}±0.27	2.12^{a}±0.37	0.30^{a}±0.04
B5	0.75^{a}±0.10	8.24^{b}±0.74	2.25^{a}±0.20	2.17^{a}±0.22	0.22^{b}±0.03

CONCLUSIONS

Composition of basal media has strong effect on biomass accumulation, exopolysaccharide production and accumulation of phenolic and antioxidant compounds in *Aronia* cell suspension culture. The Murashige and Skoog (MS) suppress the culture growth and exopolysaccharide production but stimulate the accumulation of phenolic compounds, probably in response to stress. B5 medium provide maximal biomass production, but there is no significant differences compared to WP medium in short term cultivation. However, prolonged cultivation on B5 medium (more than 10 sub-culturing cycles) inhibited cells growth, whereas such negative effect was not observed on WP medium. The reported results are the base for further development of black chokeberry cell suspension culture as alternative platform for sustainable production of valuable food additives.

ACKNOWLEDGEMENTS

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SOME TECHNOLOGICAL PROPERTIES AND BIOACTIVE COMPONENTS OF LEAVENED AND UNLEAVENED FLATBREADS SUBSTITUTED WITH GERMINATED MILLET FLOUR

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ABSTRACT

In this study, millet (*Panicum miliaceum* L.) was germinated for three days to improve bioactive components. Flour obtained from germinated millet was used at different ratios (0-20%) in the production of leavened and unleavened flatbread with replacing wheat flour. Control leavened and unleavened flatbreads were produced from wheat flour. Color, diameter, thickness, spread ratio, antioxidant activity (DPPH, FRAP and CUPRAC) and phenolic (free, bound and total) contents of all breads were determined. The use of high ratios of germinated millet flour (GMF) increased the diameter, and surface a* and b* color values of both. The bound, free and total phenolic content of leavened flatbread increased up to 2522.22 mg GAE/kg, 5250.83 mg GAE/kg and 7773.04 mg GAE/kg, respectively with 20% GMF usage. As the GMF ratio increased, antioxidant activity values (DPPH, FRAP and CUPRAC) of leavened and unleavened flatbreads were also increased. The highest phenolic contents and antioxidant activity values were achieved especially at the 20% GMF addition ratio.

Keywords: Germination, millet, leavened bread, unleavened bread, flatbread.

INTRODUCTION

Proso millet (*Panicum miliaceum* L.) is one of the oldest cereals. The caryopsis of millet is rich in carbohydrates, protein, mineral substances, and vitamins and its nutritive parameters are comparable or better than common cereals. Millet was richer in essential amino acids (leucine, isoleucine, methionine) than wheat. Functional components found in millet such as phenolic, antioxidant, beta-glucan and dietary fibers have positive effects on health and nutrition. On the other hand, some antinutritional factors such as phytic acid, oxalate, and tannins decrease the nutritional value of millet (Kalinova and Moudry, 2006; Kalinova, 2007). Germination is an economical and effective bioprocessing technique that increases digestibility and bioavailability of nutrients by reducing antinutritional factors. Germination results in the biosynthesis and accumulation of various secondary metabolites such as vitamin C, tocopherols, flavonoids, tocotrienols, γ-aminobutyric acid and phenolic compounds. Germinated grains have beneficial effects on health and free radical scavenging abilities (Koehler et al., 2007; Azeke et al., 2011; Kaur and Gill, 2020; Dhillon et al., 2020; Ceccaroni et al., 2020). There have been conducted many studies in the literature on the use of germinated cereals, legumes and pseudocereals in the preparation of cereal products (Torres et al., 2007; Tok, 2017; Zhu et al., 2017; Demir and Bilgiçli, 2020). In these studies, significant improvement in the nutritional and functional properties of end products has been determined with the use of germinated grains.

Bread is a cereal product that has an important place in meeting daily energy requirements. In recent years, interest in leavened and unleavened traditional flatbreads has increased. Various studies have been carried out to improve the nutritional and functional properties of these breads, which are commonly produced from refined wheat flour (Başman and Köksel, 1999, 2001; Coşkuner and Karababa, 2005; Levent et al., 2012; Levent and Bilgiçli, 2012a; Levent and Bilgiçli, 2012b; Madenci et al., 2012; Yıldız and Bilgiçli, 2012). In this study, the effect of GMF on some technological and functional properties of leavened and unleavened flatbreads was investigated.

MATERIAL AND METHOD

Materials

Millet was purchased from Taşan Ticaret, Konya, Turkey. Wheat flour, salt, baker's yeast and sugar were procured from a local market in Konya.

Germination of millet

The millet germination process was performed according to Parameswaran and Sadasivam (1994) and Li et al., (2017) with some modifications. The surfaces of the samples were sterilized by soaking in a solution of 1.0% aqueous sodium hypochlorite for 15 minutes at room temperature and then rinsed with distilled water. Before germination, the grains were soaked overnight in distilled water (at room temperature) and germinated in the dark for 4 days at room temperature. The germinated grain was dried in an oven at 45 °C for 12 h and milled (< 500 μm) on a laboratory grinder with a 100% extraction ratio.

Leavened and unleavened breadmaking

Leavened flatbread samples were prepared according to the method given by Akbaş (2000). Wheat flour (200 g), salt (3 g), sugar (2 g), fresh yeast (5 g) and water were used to control leavened flatbread production. Unleavened flatbread was prepared according to Başman and Köksel (2001). To control unleavened flatbread production, wheat flour (200 g), salt (3 g) and water were used. In other leavened and unleavened flatbread formulations, wheat flour was replaced with GMF at 5, 10, 15 and 20% ratios.

Color measurement

The color measurements of flatbreads were performed using a chromometer Minolta CR-400 (Minolta Camera, Co., Ltd., Osaka, Japan). Parameters L*, a* and b* determine a three-dimensional color space, in which L* indicates lightness (100 = white; 0 = black), a* values determine the redness (+) and greenness (–), and b* values determine yellowness (+) and blueness (–). Hue angle (arctan (b*/a*)) and SI value ($(a^{*2}+b^{*2})^{1/2}$) was calculated.

Technologic properties

The diameter and thickness of leavened flatbread and unleavened flatbread samples were determined according to Yıldız and Bilgiçli (2012). The spread ratio values of samples were found by dividing the diameter to the thickness value of flatbreads.

Antioxidant activity and phenolic content

The antioxidant activity of leavened flatbread and unleavened flatbread samples was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Gyamfi et al., 1999; Beta et al., 2005), ferric reducing antioxidant power assay (FRAP) (Yılmaz, 2019) and cupric ion reducing antioxidant activity assay (CUPRAC) (Apak et al., 2008). The free and bound phenolic content was determined based on the Folin-Ciocalteu colorimetric method as described by Naczk and Shahidi (2004). Total phenolic content was calculated as the sum of free and bound phenolic content. Phenolic content was expressed as gallic acid equivalents (mg of GAE/100 kg).

Statistical analysis

Leavened and unleavened flatbread results were analyzed separately. SPSS statistical program version 22.0 (SAS Institute Inc., Cary, NC, USA) was used for statistical data analysis. Mean values were compared with Duncan's multiple range test.

RESULTS AND DISCUSSION

Color values of leavened and unleavened flatbreads

Color values of leavened and unleavened flatbread containing different ratios of GMF are shown in Table 1. Increasing usage ratios of GMF decreased the L* value of both bread types. For leavened flatbread, a* value increased in all GMF usage ratios, but for unleavened flatbreads, 10% or more GMF usage increased a* value. The reason for the decrease in lightness of the flatbread surface is probably due to the Maillard reaction. Increasing amylase and protease enzyme activity with germination causes the Maillard reaction by increasing the amount of free sugar and amino acids (Goesaert et al., 2009).

Table 1. Color values of leavened and unleavened flatbread containing different ratios of GMF

Leavened flatbread					
GMF (%)	**L***	**a***	**b***	**SI**	**Hue**
0	79.01±0.31a	-4.95±0.05e	19.10±0.59c	104.52±0.29a	19.73±0.58c
5	74.19±0.12b	-4.22±0.13d	21.91±0.69b	100.92±0.65b	22.31±0.65b
10	72.99±0.99bc	-3.35±0.00c	22.45±0.38b	98.48±0.13c	22.70±0.37b
15	71.82±0.13c	-3.06±0.10b	24.57±0.80a	97.15±1.41cd	24.76±0.17a
20	69.37±0.43d	-2.77±0.17a	24.97±0.86a	96.31±0.24d	25.13±1.02a
Unleavened flatbread					
GMF (%)	**L***	**a***	**b***	**SI**	**Hue**
0	77.89±0.24a	-4.41±0.20d	21.05±0.12c	101.88±0.51a	21.51±0.17c
5	75.76±0.05b	-3.84±0.28cd	23.84±0.12b	99.14±0.63b	24.15±0.16b
10	70.44±0.75c	-3.25±0.57bc	24.07±0.01b	97.70±1.31bc	24.30±0.07b
15	67.29±0.55d	-2.40±0.63b	24.78±1.37ab	95.51±1.11c	24.90±1.42ab
20	65.86±0.16e	-1.20±0.24a	26.41±1.17a	92.57±0.44d	26.46±1.16a

Means followed by the different letters within a column are significantly ($p < 0.05$) different. GMF: Germinated millet flour.

Marti et al. (2017) reported that the use of germinated wheat flour increased the crumb a*, crust a* and b* values but decreased the bread crumb and crust L* values. The use of GMF in both bread formulations increased the yellowness value and the highest values were reached with the use of 15-20% GMF. The high carotenoid pigment content of millet affected the b* value of flatbreads. While the SI values of the bread decreased with the use of GMF, the Hue values increased.

Technologic properties of leavened and unleavened flatbreads

The diameter, thickness and spread ratio values of leavened and unleavened flatbread containing different ratios of GMF are given in Table 2. The diameter of the leavened flatbreads ranged from 17.32 to 18.28 cm and there was a slight increase in the diameter value with the use of GMF. The diameter value of bread using only 20% GMF was significantly ($p<0.05$) higher than the control bread. In unleavened flatbreads, the diameter value increased with the use of 10% or more GMF. The use of 15-20% GMF in leavened flatbreads decreased the thickness value; on the other hand, all usage ratios of GMF decreased the thickness of unleavened flatbreads compared to control. High GMF usage ratios increased the spread ratio in leavened flatbreads and all GMF usage ratios in unleavened breads. Diluting gluten content with GMF addition may cause a decrease in thickness. There are numerous studies in the literature about decreasing the volume or thickness of breads with the substitution of non-gluten flours or bran (Sidhu et al., 2001; Gómez et al., 2012; Levent and Bilgiçli 2012a; Levent and Bilgiçli 2012b).

Table 2. Technologic properties of leavened and unleavened flatbread containing different ratios of GMF

Leavened flatbread			
GMF (%)	**Diameter (cm)**	**Thickness (cm)**	**Spread ratio**
0	17.32±0.16b	1.63±0.65a	10.63±0.05c
5	17.64±0.34ab	1.47±0.71ab	12.00±0.08bc
10	17.82±0.11ab	1.46±1.24ab	12.21±0.09bc
15	18.00±0.57ab	1.27±1.24bc	14.17±0.10ab
20	18.28±.11a	1.18±1.33c	15.49±0.18a
Unleavened flatbread			
GMF (%)	**Diameter (cm)**	**Thickness (cm)**	**Spread ratio**
0	24.48±0.45c	0.16±0.17a	154.94±2.12e
5	26.34±0.48c	0.12±0.20b	227.07±1.52d
10	30.28±0.68b	0.10±0.03bc	296.86±0.11c
15	31.62±0.31b	0.08±0.03c	405.38±1.94b
20	34.92±2.15a	0.07±0.06c	471.89±0.39a

Means followed by the different letters within a column are significantly ($p < 0.05$) different. GMF: Germinated millet flour.

Antioxidant activities of leavened and unleavened flatbreads

Antioxidant activities leavened and unleavened bread containing different ratios of GMF are presented in Table 5. DPPH, FRAP and CUPRAC antioxidant activity values for leavened bread ranged between 181.77-344.93 mg TE/kg, 0.54-1.39 µmol TE/g and 3.61-8.34 µmol TE/g, and for unleavened bread changed between 152.89-300.91 mg TE/kg, 0.33-1.35 µmol TE/g and 2.91-6.73 µmol TE/g, respectively. With the increasing use of GMF in leavened flatbreads, DPPH, FRAP and CUPRAC antioxidant activity values increased. In unleavened flatbreads, DPPH and CURAC values increased with increasing GMF ratio, while FRAP value increased with 10% or more GMF use. The highest antioxidant activity values were achieved with the use of 20% GMF in both bread types.

Table 5. Antioxidant activities leavened and unleavened flatbread containing different ratios of GMF

Leavened flatbread			
GMF (%)	**DPHH (mg TE/kg)**	**FRAP (µmol TE/g)**	**CUPRAC (µmol TE/g)**
0	181.77±1.39e	0.54±0.05e	3.61±0.05e
5	204.21±1.29d	0.67±0.02d	4.10±0.04d
10	232.13±0.00c	0.88±0.01c	5.20±0.14c
15	292.33±2.97b	1.02±0.02b	6.56±0.002b
20	344.93±1.40a	1.39±0.04a	8.34±0.27a
Unleavened flatbread			
GMF (%)	**DPHH (mg TE/kg)**	**FRAP (µmol TE/g)**	**CUPRAC (µmol TE/g)**
0	152.89±0.87e	0.33±0.08d	2.91±0.05e
5	187.20±1.45d	0.42±0.01d	3.74±0.05d
10	224.87±1.52c	0.84±0.05c	4.15±±0.06c
15	240.18±1.38b	0.96±0.02b	5.03±0.02b
20	300.91±0.48a	1.35±0.01a	6.73±0.12a

Means followed by the different letters within a column are significantly ($p < 0.05$) different. DPPH: 2,2-diphenyl-1picrylhydrazyl radical scavenging (TE: Trolox equivalent). FRAP: Ferric reducing antioxidant power CUPRAC: Cupric ion reducing antioxidant capacity.

Phenolic contents of leavened and unleavened flatbreads

Phenolic contents of leavened and unleavened bread containing different ratios of GMF are given in Table 4. Bound, free and total phenolic content of leavened flatbread increased up to 2522.22 mg GAE/kg, 5250.83 mg GAE/kg and 7773.04 mg GAE/kg with GMF usage. All

addition levels of GMF increased the bound, free and total phenolic content of leavened flatbread. Bound, free and total phenolic content of unleavened flatbreads increased with the use of GMF as in leavened flatbreads, and the highest values were reached with the use of 20% GMF.

There are many studies reporting an increase in antioxidant activity and phenolic content in cereals and legumes with germination (Demir and Bilgiçli, 2021; Alvarez-Jubete et al., 2010; Žilić et al., 2014; Swieca and Dziki, 2015; Cankurtaran-Kömürcü, 2021). Sharma et al. (2015) determined that the total phenolic content (free/bound) increased significantly with the germination of foxtail millet. Cankurtaran-Kömürcü, (2021) used germinated modern and primitive wheat in different ratios to produce bread (0, 5, 10, 15 and 20%) and noodles (0, 15, 30, 45 and 60%). It has been reported that germinated wheat significantly increased the antioxidant activity and phenolic content of bread and noodles. Demir and Bilgiçli (2021) used germinated quinoa flour at the rates of 0, 10, 20 and 30% in gluten-free pasta production and found that the antioxidant activity and phenolic content increased with increasing germinated quinoa flour.

Table 5. Phenolic contents of leavened and unleavened flatbread containing different ratios of GMF

Leavened flatbread			
GMF (%)	**BPC (mg GAE/kg)**	**FPC (mg GAE/kg)**	**TPC (mg GAE/kg)**
0	2095.60±20.86d	4167.68±16.22d	6263.28±37.08e
5	2264.83±12.87c	4523.44±26.27c	6788.27±13.40d
10	2336.61±6.52bc	4683.43±83.63c	7020.04±77.16c
15	2404.78±31.23b	4912.21±29.15b	7316.48±2.08b
20	2522.22±13.99a	5250.83±16.33a	7773.04±30.32a
Unleavened bread			
GMF (%)	**BPC (mg GAE/kg)**	**FPC (mg GAE/kg)**	**TPC (mg GAE/kg)**
0	2042.01±6.85e	4078.20±35.99d	6120.20±42.84e
5	2194.54±10.59d	4486.62±12.35c	6681.15±1.76d
10	2280.36±7.15c	4698.82±37.56b	6979.17±30.40c
15	2357.65±13.49b	4758.87±7.87b	7116.52±5.62b
20	2425.90±10.99a	5159.43±4.27a	7585.33±15.26a

Means followed by the different letters within a column are significantly ($p < 0.05$) different. BFC: Bound phenolic content, FPC: Free phenolic content TPC: Total phenolic content (GAE, gallic acid equivalent).

CONCLUSIONS

In this study, the effects of GMF on the technological properties and bioactive components of leavened and unleavened flatbreads were examined. The findings were evaluated separately for leavened and unleavened flat breads. While the increase in the use of GMF decreased the L* and SI values of the bread, it increased the a*, b* and Hue values. While higher a* and b* color values were determined in unleavened bread samples compared to leavened breads, lower L* values were generally measured. Especially high utilization ratios of GMF increased the spread ratio values of the both breads. As predicted, unleavened flatbreads were determined as breads with higher diameter and spread ratio than leavened flatbreads. The use of GMF in flatbread production significantly ($p<0.05$) increased DPPH, FRAP and CUPRAC antioxidant activity values. The highest antioxidant activity values were obtained with the use of 20% GMF. In addition, it was determined that the amount of free, bound and total phenolic content increased in both bread types with the use of GMF, and these values reached the highest amounts at the highest use of GMF. Utilization of 20% GMF resulted in the greatest redness, yellowness, diameter, spread ratio value and bioactive components of the breads.

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INVESTIGATION OF MORPHOMETRIC VARIATIONS *ON PTEROCHLOROIDES PERSICAE* (CHOLODKOVSKY, 1898) (HEMIPTERA: APHIDIDAE) DEPENDS ON HOST PLANT PREFERENCES

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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).

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

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).

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).

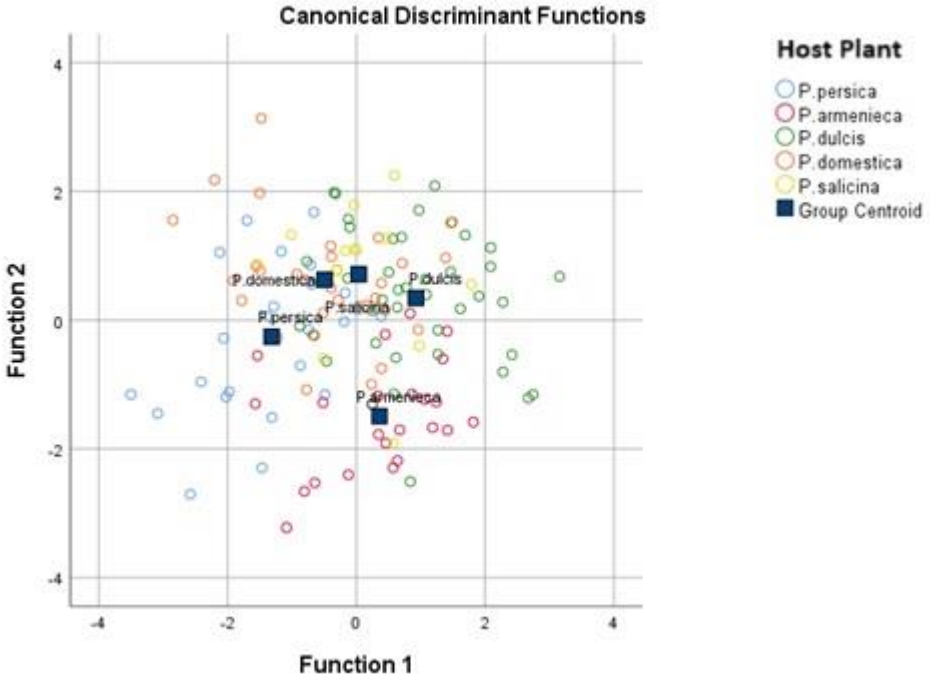

Figure 1. Classification of *Pterochloroides persicae* populations according to canonical discriminant function depending on host plant

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.

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EFFECT OF CAULIFLOWER POWDER ON THE CHEMICAL AND FUNCTIONAL PROPERTIES OF GLUTEN-FREE SNACK PRODUCT

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ABSTRACT
Cauliflower (Brassica oleracea) is one of the most important winter vegetables grown throughout the world and is widely used in food formulations due to its high nutritional quality. In this study, cauliflower powder (0-15%) was used in gluten-free gluten-free cracker formulations. Moisture, ash, protein, fat, antioxidant activity (DPPH, FRAP and CUPRAC) and phenolic (free, bound and total) contents of the cracker samples were determined. The use of cauliflower powder in the gluten-free cracker formulation increased the ash, fat, protein, antioxidant activity and phenolic content compared to the control gluten-free crackers. DPPH, FRAP and CUPRAC antioxidant activity values of control gluten-free crackers were found as 79.87 mg TE/kg, 1.47 µmol TE/kg and 251.62 µmol TE/kg, respectively. Those antioxidant activity values were 477.21 mg/kg, 7.38 µmol TE/kg and 478.48 µmol TE/kg for gluten-free crackers containing 20% cauliflower powder. The total phenolic content of the control was determined as 2889.58 mg GAE/kg. In comparison, the same value was 5175.15 mg GAE/kg in containing 20% cauliflower powder gluten-free cracker samples. It was concluded that the use of cauliflower powder contributed to the improvement of the nutritional and functional properties of gluten-free crackers.

Keywords: Gluten-free cracker, vegetable powder, cauliflower, antioxidant activity, bioactive component.

INTRODUCTION
Vegetables have an important place in our daily diet due to their high dietary fiber, mineral matter, phenolic substances and antioxidant contents. The bioactive components of vegetables have an important role in the prevention of many diseases. Cauliflower (Brassica oleracea var. botrytis L.) is a vegetable of the cabbage group. Antioxidants (E, C and β-carotene), flavonoids, flavones and phenolic compounds of cauliflower have a protective effect against cancer (Sadik, 1962; Lin and Chang, 2005). Vegetables can be used for a long time by drying in seasons when their production is high. The obtained vegetable powders can be added to meals or evaluated in functional food formulations.

Cracker is a snack product with low nutritional and functional properties that is produced from refined flour. There are various studies in the literature on fortifying crackers and cookies with vegetables. In these studies, cabbage, broccoli, carrot, and tomatoes have been added into cracker or cookie formulation (Gül et al., 2013; Ahmad et al., 2016; Lafarga et al., 2019). There are limited studies on the use of cauliflower powder in cereal products. Cauliflower powder and other parts of cauliflower (stalk and leaves) were used in the production of wheat/rice crackers, cookies, bread and bakery products (El Sheikh et al., 2021; Ribeiro et al., 2015; Saleh, 2022) In this study, the effects of cauliflower powder on nutritional and functional properties of gluten-free crackers were investigated.

MATERIAL AND METHOD
Materials

Gluten-free cracker ingredients (rice flour, corn flour, shortening, salt, sugar, baking powder baker's yeast) and fresh cauliflower were obtained from local markets in Konya. Protease enzyme was procured from Vatan Enzyme (İstanbul, Turkey).

Preparation cauliflower powder

Fresh cauliflower was washed under running tap water, then cut into small pieces and dried in a dryer at 60 ± 2 °C for 12 hours. After drying, cauliflower samples were ground and passed through a 500 μm sieve to obtain cauliflower powder.

Preparation of gluten-free crackers

The formulation of control gluten-free control cracker was 50 g rice flour, 50 g corn flour, 20 g shortening, 1.6 g salt, 1.5 g sugar, 1.5 g baking powder and 0.2 g baker's yeast. In other gluten-free crackers, formulations replace rice flour: corn flour (50:50) with 5, 10, 15 and 20% levels of cauliflower powders. Gluten-free cracker samples were prepared according to Davidson (2016) with small modifications. After baking, the gluten-free crackers were cooled and ground and then stored in polyethylene packaging until laboratory analysis.

Proximate composition, antioxidant activity and phenolic content

Moisture, ash, protein and fat content of the gluten-free cracker samples were determined according to AACC methods (AACC, 2000). The antioxidant activity of gluten-free cracker samples was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Gyamfi, et al.,1999; Beta et al., 2005), ferric reducing antioxidant power assay (FRAP) (Yılmaz, 2019) and cupric ion reducing antioxidant activity assay (CUPRAC) (Apak et al., 2008). The free and bound phenolic content was determined based on Folin-Ciocalteu colorimetric method as described by Naczk and Shahidi (2004). Total phenolic content was calculated as the sum of free and bound phenolic content. Phenolic content was expressed as gallic acid equivalents (mg of GAE/100 kg).

Statistical analysis

All analyses were performed in duplicate. For statistical analysis, the JMP statistical program, version 10.0 (SAS Institute Inc., Cary, NC, USA) was used.

RESULTS AND DISCUSSION

Proximate compositions of gluten-free cracker samples are given in Table 1. The moisture content of the gluten-free cracker samples prepared in different amounts of cauliflower powder ranged between 4.02 and 6.48%. Cauliflower powder addition increased the moisture content of the gluten–free crackers and the highest value was obtained with 20% cauliflower powder addition. Increasing the amount of cauliflower powder ratio in gluten-free crackers significantly ($p<0.05$) increased the ash content. Gluten-free crackers containing 20% cauliflower powder had 2.2 times higher ash content than control gluten-free crackers. The use of 10% or more cauliflower powder increased the fat content of gluten-free crackers. Increasing amount of cauliflower powder ratio also increased the protein content of gluten-free crackers up to 8.19% from 6.23%. El Sheikh et al. (2021) found ash protein and fat content of wheat crackers containing 25% cauliflower powder as 2.68, 14.82 and 2.94%, respectively. The same values were 0.79, 12.49 and 2.63% in the control cracker respectively. It has been reported that crackers containing 25% cauliflower powder provide a significant increase in the amount of ash and protein content compared to the control. Gül et al. (2013) used different ratios (%0-7.5) of white cabbage powder in cookie production and found that ash, fat and protein content between 1.06-1.50%, 19.68-20.30% and 4.35-4.93, respectively. In the present study, the chemical properties of cauliflower powder, which is used as a raw material, are reflected in the final product.

Table 1. Proximate composition of gluten-free cracker samples

Cauliflower powder ratio (%)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)
0	4.02±0.15c	1.14±0.02e	13.99±0.17c	6.23±0.14e
5	5.16±0.22b	1.44±0.02d	14.23±0.16c	6.70±0.03d
10	5.74±0.19ab	1.85±0.04c	14.74±0.11b	7.20±0.03c
15	6.13±0.18ab	2.11±0.02b	14.82±0.12ab	7.62±0.08b
20	6.48±0.71a	2.51±0.04a	15.09±0.15a	8.19±0.11a

Means with the same letter within a column are not significantly different ($p > 0.05$). Results are dry matter basis.

Antioxidant activities and phenolic contents of gluten-free cracker samples are presented in Table 2 and Table 3. The antioxidant activity values measured by all methods increased with the addition of cauliflower powder. DPPH, FRAP and CUPRAC antioxidant activity values of control gluten-free crackers were found as 79.87 mg TE/kg, 1.47 µmol TE/kg and 251.62 µmol TE/kg, respectively. Those antioxidant activity values were 477.21 mg/TEkg, 7.38 µmol TE/kg and 478.48 µmol TE/kg for gluten-free crackers containing 20% cauliflower powder. The amount of antioxidant activity determined by the DPPH method of the cracker with 20% cauliflower powder increased 6 times compared to the control gluten-free cracker. DPPH, FRAP and CUPRAC antioxidant activity values of wheat flour and cauliflower powder which were used as raw material in cracker formulation were 115.81 mg TE/kg, 0.22 µmol TE/kg and 4.71 µmol TE/kg; 2009.35 mg TE/kg, 14.53 µmol TE/kg and 603.17 µmol TE/kg, respectively (data not shown). The higher antioxidant activity of cauliflower powder compared to wheat flour may have been effective in increasing antioxidant activity in cracker samples. Similarly, El Sheikh et al., (2021) reported that the amount of antioxidant activity in crackers increased with increasing use of cauliflower powder. Lafarga et al. (2019) found that the incorporation of broccoli co-products (12.5 and 15%) into crackers significantly increased the total phenolic content and antioxidant capacity.

Free, bound and total phenolic content of gluten-free crackers changed between 1722.46-2690.71 mg GAE/kg, 1425.06-2484.44 mg GAE/kg and 2889.58-5175.15 mg GAE/kg, respectively. Cauliflower powder addition was found significant ($p<0.05$) on free, bound and total phenolic content of gluten-free crackers. As expected, the highest free, bound, and total phenolic content was found in the samples with 20% cauliflower powder. In a study, the total phenolic content of crackers produced with 0, 25, 50 and 75% cauliflower powder were reported as 0.75, 2.68, 3.62 and 5.75 Gallic acid g^{-1}, respectively (El Sheikh et al., 2021). With increasing cauliflower powder amount in the cracker, the amount of total phenolic content also increased significantly ($p<0.05$). Free, bound and total phenolic content of wheat flour and cauliflower powder were found as 2393.86, 3351.41 and 5745.27 mg GAE/kg; 5759.30, 3164.83 and 8924.13 mg GAE/kg, respectively (data not shown). It is known that vegetables and fruits have high antioxidant activity and phenolic content. Since the cauliflower powder used in this study has high antioxidant activity and phenolic content, high increases were observed in the antioxidant activities and total phenolic content of crackers, especially with the high use ratios of cauliflower powder.

Table 2. Antioxidant activities of gluten-free cracker samples

Cauliflower powder ratio (%)	DPPH (mg TE/kg)	FRAP (µmol TE/g)	CUPRAC (µmol TE/g)
0	79.87±1.53d	1.47±0.10d	251.62±1.10e
5	117.13±9.41cd	5.16±0.14c	301.05±0.00d
10	153.83±21.82bc	4.83±0.47bc	345.30±1.49c
15	189.37±26.23b	6.32±0.86ab	400.66±2.59b
20	477.21±15.64a	7.38±0.11a	478.48±3.00a

Means with the same letter within a column are not significantly different ($p > 0.05$). DPPH: 2,2-diphenyl-1picrylhydrazyl radical scavenging (TE: Trolox equivalent). FRAP: Ferric reducing antioxidant power CUPRAC: Cupric ion reducing antioxidant capacity.

Table 3. Phenolic contents of gluten-free cracker samples

Cauliflower powder ratio (%)	FPC (mg GAE/kg)	BPC (mg GAE/kg)	TPC (mg GAE/kg)
0	1722.46±27.72d	1425.06±c13.48e	2889.58±23.58d
5	2138.40±14.69c	1543.05±25.70c	3681.45±11.02c
10	2274.16±15.77b	1981.02±21.47b	4359.14±26.78b
15	2366.00±62.81b	2055.62±16.63b	4421.63±16.96b
20	2690.71±14.68a	2484.44±8.57a	5175.15±23.25a

Means with the same letter within a column are not significantly different ($p > 0.05$). BFC: Bound phenolic content, FPC: Free phenolic content TPC: Total phenolic content (GAE, gallic acid equivalent). Results are dry matter basis.

CONCLUSIONS

In this study, the effects of cauliflower powder on some chemical and functional properties of gluten-free crackers were investigated. It was determined that the addition of cauliflower powder increased the amount of ash, fat and protein in gluten-free crackers, and the increase in ash content according to control was quite high. Antioxidant activity values of gluten-free cracker samples measured by different methods were significantly ($p<0.05$) affected by the addition of cauliflower powder. The highest antioxidant activity values were obtained with the use of 20% cauliflower powder. As the cauliflower powder ratio increased in the gluten-free cracker formulation, there were significant ($p<0.05$) increases in the amounts of free, bound and total phenolic contents. It has been determined that all usage ratios of cauliflower powder are effective in improving the nutritional and functional properties of gluten-free crackers, and there is a much greater increase in functional properties, especially at high usage ratios.

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DETERMINATION OF MOLECULAR MARKER FOR SEEDLING EMERGENCE IN WATERMELON

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ABSTRACT

DNA markers associated with phenotype can be determined by establishing a connection between phenotype and genotype with the association mapping technique that emerged with the development of molecular techniques. In this study, DNA markers related to seedling emergence rate and time in watermelon were determined by using association mapping technique using 96 watermelon genotypes. According to the Q-Q plot graph, the best agreement between the expected and observed values was determined by the GLM (Q) method. In the GLM (Q) analyzes, 11 markers were found to be correlated at the $p<0.01$ level. The model formed as a result of the back regression analysis included 3 markers (iPBS-2392.460, iPBS-2243.420 and iPBS-2081.500). The rate of explaining the seedling emergence of the model depending on these markers is 70.9%. Obtained marker information can be used in marker assisted selection studies.

Keywords: Watermelon, *Citrullus lanatus*, association mapping, seedling emergence

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] is one of the most important members of the Cucurbitaceae family. *C. lanatus* has spread around the world thanks to its delicious fruit flesh and has become one of the most consumed vegetable products. In terms of fresh vegetable production, watermelon production is among the top 5 in the world (Guo et al., 2013). For this reason, improvement studies are carried out continuously with the breeding method in watermelon.

One of the basic conditions to achieve early and high yield in vegetable production is to provide a uniform and fast seedling emergence. Generally, the period from planting to seedling emergence is critical for vegetable species grown from seed. Seedling emergence rate and seedling emergence time are important parameters in watermelon cultivation. Delays in seedling emergence can cause yield losses and significant disruptions in production. Even under optimum conditions, seedling emergence problems may occur in some genotypes and cultivars. Therefore, determining the genetic mechanism of seedling emergence is important for breeding.

Marker-assisted selection method shortens the breeding period and enables this process to be completed with less labor. Genetic markers are the most important factor that helps breeding studies in plants. Molecular markers are the most widely used techniques for genetic

characterization because of their advantages. Preliminary information has been obtained for breeding studies by using many marker techniques on plant genotypes and varieties (Karaman et al., 2018; Kıraç et al., 2022; Coşkun, 2022; Coşkun, 2023).

Marker development studies on important fruit and plant characters in watermelon are insufficient. In molecular breeding studies, it is aimed to identify genomic regions associated with phenotypic traits (Zhu et al., 2008). Quantitative trait locus (QTL) can be determined by detecting DNA markers associated with genes controlling important characters. Genetic mapping studies are carried out in plants to determine these regions. With the increase in genetic techniques, the association mapping method has been developed as an alternative to genetic link maps. With this method, phenotype-related markers can be determined using natural populations without the need for a long-time mapping population (Rafalski, 2010). In addition, with this method, more alleles can be obtained and map resolution increases (Yu and Buckler, 2006). As a result of evolutionary recombination and natural genetic diversity in relational mapping, high resolution maps can be obtained (Zhu et al., 2008). The aim of this study is to determine molecular markers associated to seedling emergence rate and time in watermelon, which is an important vegetable species.

MATERIAL AND METHODS

In the study, 96 genotypes were used. 94 of the genotypes have *C. lanatus* var. *lanatus*, 1 of them is *C. lanatus* var. *citroides* and 1 of them belong to the *P. fistulosus* species. The study was carried out in the greenhouse, molecular biology laboratories and trial field of Erciyes University, Faculty of Agriculture, Department of Horticulture. Morphological measurements were made in 3 replications and 7 times in each replication. For seedling emergence rates and time: Seed emergence rates and the number of days with the highest emergence rate were recorded in viols under controlled greenhouse conditions.

Seeds were sown in 2:1 peat perlite medium, and leaf samples were collected when the seedlings reached the stage with 2 true leaves. CTAP method was used for DNA isolation. PCR study was performed using ISSR, SSR and iPBS primers giving reliable bands. SPSS.22, Structure Harvester, Structure and Tassel 5.2 programs were used for the bioinformatic analysis of the obtained locus data. The LD level between loci, the combined marker data were obtained in the Tassel 5.2 program. Analyzes were performed after removing loci with low number of alleles ($f<0.10$). In the association mapping study, the results obtained using the model containing three different statistical approaches were compared. By using different models, false positive results were eliminated and probability (P) probability values were determined for each marker associated with the feature of interest. The significance level between the marker and phenotypic traits was determined by the Tassel 5.2 program (Bradbury et al., 2007) based on the P values and the F test. The model with the best results was determined by obtaining the QQ (quantile quantile plot) graphs. Quantile Quantile Plot and Manhattan plots were obtained using Tassel 5.2. Regression analyzes were performed with the associated markers obtained by 3 different statistical methods with the Tassel 5.2 program. For this purpose, backward and forward regression models were used in SPSS.22 program.

RESULTS AND DISCUSSION

As a vegetative observation, seedling emergence rate and seedling emergence time parameters were examined. When all genotypes were examined, the average seedling emergence rate was determined as 92.98±1.42%. The smallest value was measured in genotype 224 (23%). When all genotypes were examined, the average number of days when seedling emergence was completed was determined as 9.19±0.3. The lowest value was measured in genotypes 68, 77, 161, 171 and 213 (6 days), while the highest value was measured in genotypes 59, 62, 234 and 354 (18 days). On the 6th day, seedling emergence was observed in viols in 85 genotypes except 11 genotypes (13, 58, 59, 62, 168, 192, 224, 225, 331, 342 and 354). In addition, on the 6th day, the emergence rate reached 100% in 5 genotypes (68, 77, 161, 171 and 213). On the 6th day, 5 genotypes reached the highest emergence rate. At the end of 22 days, the emergence rate of 50 genotypes was determined as 100%. Seedling emergence rates were determined between 90-100% in 30 genotypes, between 70-90% in 11 genotypes, between 50-70% in 2 genotypes and between 0-40% in 3 genotypes. The lowest seedling emergence was recorded in genotypes 224 (23%), 342 (33%), 62 (40%), 23 (57%) and 206 (57%). Except for genotype 58, seedling emergence could not reach 100% in genotypes that started to germinate in more than 6 days. In the correlation analysis, the seedling emergence rate shows a correlation of -45% with the number of seedling emergence days.

Table 1. Seedling emergence rate (SER) information of genotypes

No	SER (%)	No	SER (%)	No	SER (%)	No	SER (%)
3	100	**53**	93	**122**	100	**203**	100
5	80	**56**	93	**125**	100	**206**	57
6	93	**58**	100	**136**	100	**213**	100
9	86	**59**	73	**137**	100	**223**	100
11	100	**62**	40	**138**	97	**224**	23
13	77	**63**	100	**141**	90	**225**	93
18	100	**68**	100	**147**	100	**229**	97
22	100	**70**	93	**149**	100	**234**	100
23	57	**71**	93	**151**	100	**241**	97
28	100	**75**	100	**152**	93	**244**	100
35	100	**77**	100	**161**	100	**247**	100
36	90	**78**	100	**165**	100	**252**	100
37	100	**80**	90	**168**	93	**260**	100
38	97	**85**	100	**171**	100	**285**	100
40	97	**86**	80	**174**	97	**298**	87
41	100	**89**	87	**183**	97	**303**	100
42	100	**90**	93	**184**	93	**305**	100
44	83	**91**	100	**187**	93	**331**	97
45	100	**96**	100	**190**	77	**341**	100
46	97	**111**	100	**192**	80	**342**	33
47	100	**112**	97	**194**	93	**347**	100
48	97	**114**	100	**195**	100	**350**	90
50	93	**117**	97	**199**	90	**354**	83
52	100	**119**	100	**200**	100	**356**	100

Table 2. Seedling emergence time (SET) information of genotypes

No	SET	No	SET	No	SET	No	SET
3	7	**53**	7	**122**	8	**203**	7
5	8	**56**	7	**125**	7	**206**	9
6	8	**58**	11	**136**	11	**213**	6
9	8	**59**	18	**137**	7	**223**	8
11	9	**62**	18	**138**	8	**224**	15
13	9	**63**	10	**141**	10	**225**	9
18	10	**68**	6	**147**	7	**229**	10
22	7	**70**	15	**149**	7	**234**	18
23	9	**71**	8	**151**	7	**241**	7
28	7	**75**	9.	**152**	7	**244**	10
35	8	**77**	6	**161**	6	**247**	11
36	8	**78**	7	**165**	8	**252**	9
37	7	**80**	15	**168**	15	**260**	8
38	9	**85**	7	**171**	6	**285**	7
40	7	**86**	7	**174**	10	**298**	10
41	7	**89**	10	**183**	11	**303**	7
42	9	**90**	9	**184**	9	**305**	10
44	12	**91**	7	**187**	8	**331**	15
45	12	**96**	8	**190**	12	**341**	7
46	12	**111**	7	**192**	15	**342**	11
47	7	**112**	8	**194**	10	**347**	7
48	7	**114**	10	**195**	7	**350**	12
50	7	**117**	7	**199**	12	**354**	18
52	8	**119**	7	**200**	10	**356**	7

The relationship between seedling emergence rate and 583 markers was investigated. In the GLM (Q) analyzes, it was determined that 29 markers were related at the $p<0.05$ level and 11 markers at the $p<0.01$ level. In MLM (K) analyzes, it was determined that 54 markers were correlated at $p<0.05$ level and 12 markers at $p<0.01$ level. In MLM (K+Q) analyzes, it was determined that 28 markers were related at the $p<0.05$ level and 9 markers at the $p<0.01$ level. When the Q-Q plot graphics are examined, it is understood that the agreement between the expected and observed values is best determined by the GLM (Q) method. According to this model, 14 of the related markers belong to iPBS, 3 to ISSR and 12 to SSR primers. The level of correlation (R) ranged from 4.91% to 18.67%. The most highly correlated markers are ISSRGACA4400, SSRCMTp174450, and ISSRCAC6200.

When the seedling emergence values were examined, the average seedling emergence rate was determined as 92.98±1.42% in all genotypes. On the sixth day, seedling emergence from viols was observed in 85 genotypes except 11 genotypes (13, 58, 59, 62, 168, 192, 224, 225, 331, 342 and 354). The lowest seedling emergence was recorded in genotypes 224 (23%), 342 (33%), 62 (40%), 23 (57%) and 206 (57%). Seedling emergence rates showed a 22% correlation with ovary height. The number of seedling emergence days showed a negative

correlation (-45%) with the seedling emergence rate. As the number of seedling emergence days increased, seedling emergence rates decreased moderately. In some studies, germination percentage was determined in watermelon genotypes. Maggs-Kölling et al. (2000) determined the germination percentage as 61.76% in some watermelon genotypes. In this study, seedling emergence rates in watermelon were determined at high rates.

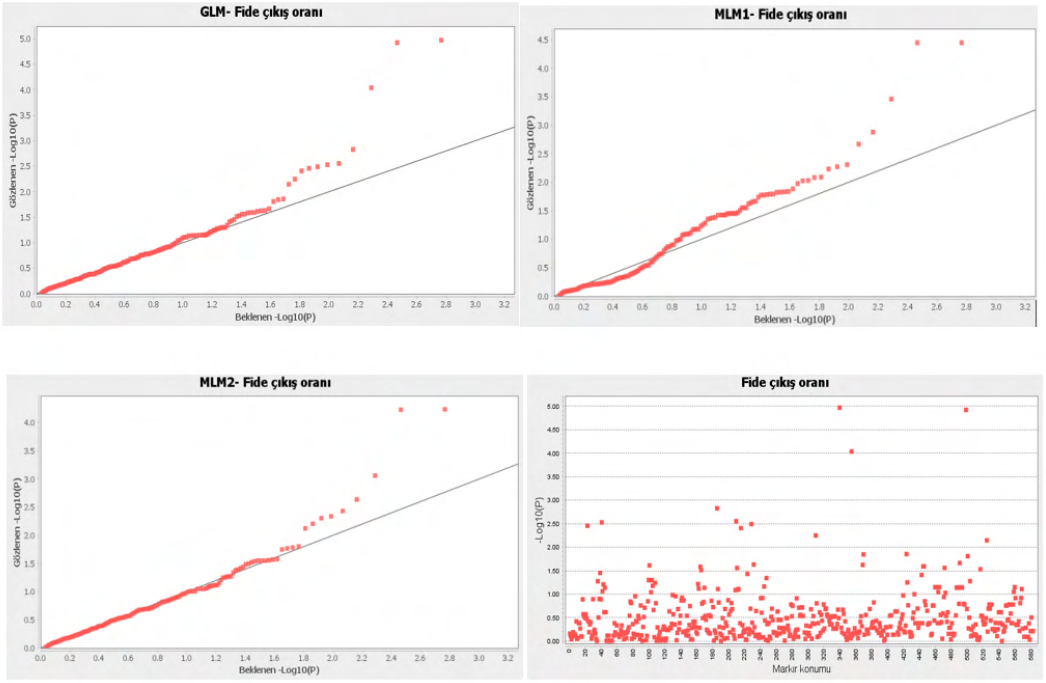

Figure 1. Q-Q and Manhattan graph for seedling emergence rate.

Table 3. Association mapping results obtained for seedling emergence rate using the GLM model ($p<0.05$ and $p<0.01$)

GLM (Q)					
Marker	**p**	**R**	**Marker**	**p**	**R**
ISSR.GACA4.400	0.0001	0.1867	iPBS-2081.500	0.0234	0.0542
SSR.CMTp174.450	0.0001	0.1866	SSR.CSTCC813.200	0.0238	0.0531
ISSR.CAC6.200	0.0001	0.157	iPBS-2392.460	0.0244	0.0526
iPBS-2243.420	0.0015	0.1053	SSR.CI.2-23.330	0.0256	0.0523
iPBS-2249.420	0.0028	0.0939	SSR.CI.2-23.1300	0.0256	0.0523
iPBS-2376.650	0.003	0.0989	iPBS-2230.590	0.0261	0.0526
iPBS-2080.660	0.0032	0.0889	SSR.CMTp201.750	0.0276	0.0576
iPBS-2077.590	0.0035	0.0871	iPBS-2249.590	0.0277	0.0521
iPBS-2249.1600	0.0039	0.0894	SSR.CMTmC14.250	0.0296	0.0551
ISSR.HVHTCC7.400	0.0057	0.0785	iPBS-2230.620	0.0307	0.0497
SSR.CMTp182.150	0.0072	0.0756	iPBS-2376.550	0.0353	0.051
SSR.CSJCT 720.450	0.014	0.0732	iPBS-2252.270	0.037	0.0454
SSR.CSTCC813.600	0.0143	0.0627	SSR.ASUW13.800	0.0391	0.0449
SSR.CMTp174.1500	0.0155	0.0612	iPBS-2095.1200	0.0453	0.0491
SSR.CMTp46.850	0.0217	0.0606			

Regression analysis was performed between the dependent variable of the seedling emergence rate and 29 independent variables (markers) by using the important markers that emerged as a result of the GLM (Q) analysis. In the back regression analysis, 12 significant models were formed. In the 12th model, there were 3 independent variables at the $p<0.05$ level (iPBS-2392.460, iPBS-2243.420 and iPBS-2081.500). There is no high level of correlation between these markers. In the model, the 'Intercept' value was 22.121 and the 'B' value was 23.939, 10.939 and 39.939, respectively. The rate of explaining the seedling emergence of the model depending on these markers is 70.9%.

The relationship between seedling emergence days and 583 markers was examined. In GLM (Q) analyzes, it was determined that 146 markers were correlated at $p<0.05$ level and 75 markers were correlated at $p<0.01$ level. In MLM (K) analyzes, it was determined that 95 markers were correlated at $p<0.05$ level and 26 markers were correlated at $p<0.01$ level. In MLM (K+Q) analysis, it was determined that 54 markers were related at the $p<0.05$ level and 21 markers at the $p<0.01$ level. When the Q-Q plot graphs are examined, it is understood that the agreement between the expected and observed values is best determined by the MLM (K) and MLM (K+Q) methods. According to the MLM (K) model, 20 of the relevant markers belong to iPBS, 17 to ISSR and 58 to SSR primers. The level of correlation (R) varied between 4.21% and 18.14%. The highest associated markers were iPBS-2226.1450, iPBS-2272.680 and SSR.CI.2-23.330. According to the MLM (K+Q) model, 8 of the related markers belong to iPBS, 5 to ISSR and 41 to SSR primers. The level of correlation (R) ranged from 5.77% to 14.3%. The most highly correlated markers are SSR.CI.2-23.330, SSR.CI.2-23.1300 and iPBS-2272.680.

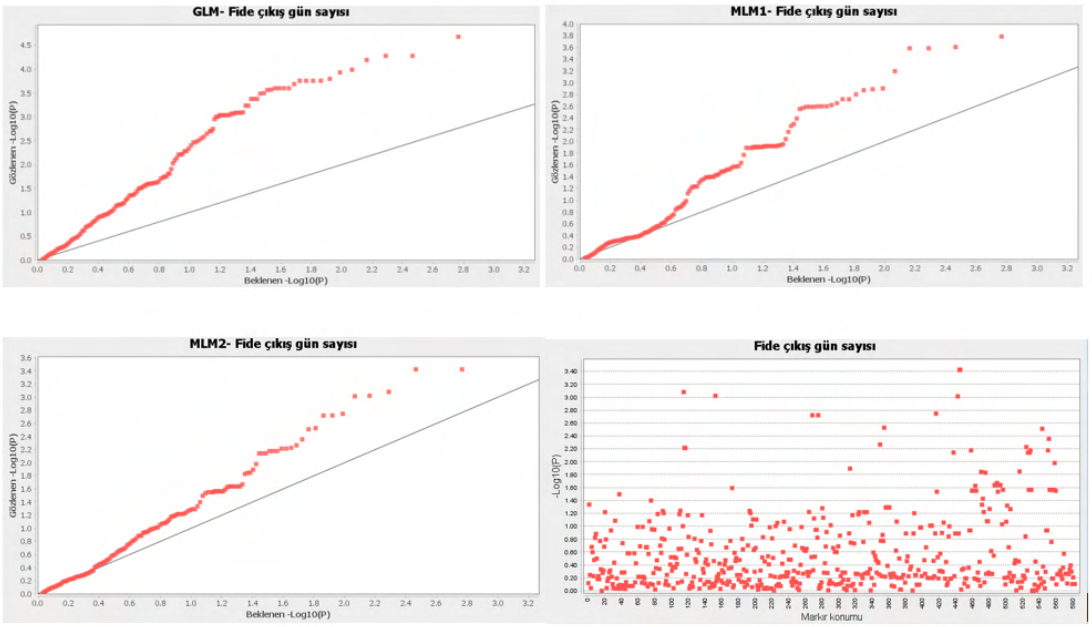

Figure 2. Q-Q and Manhattan graph for number of seedling emergence time.

Table 4. Association mapping results obtained for seedling emergence time using MLM (K) and MLM (K+Q) models

MLM (K)			**MLM (K+Q)**		
Marker	**p**	**R**	**Marker**	**p**	**R**
iPBS-2226.1450	0.0002	0.1814	SSR.CI.2-23.330	0.0004	0.143
iPBS-2272.680	0.0002	0.1675	SSR.CI.2-23.1300	0.0004	0.143
SSR.CI.2-23.330	0.0003	0.1522	iPBS-2272.680	0.0008	0.1366
SSR.CI.2-23.1300	0.0003	0.1522	iPBS-2226.1450	0.001	0.1366
SSR.ASUW13.800	0.0006	0.1318	SSR.ASUW13.800	0.001	0.1219
SSR.CSJCT 315.100	0.0013	0.1165	SSR.CSJCT 315.100	0.0018	0.1082
ISSR.CT8TG.720	0.0013	0.1176	ISSR.CT8TG.720	0.0019	0.1086
ISSR.TAA8.880	0.0013	0.1157	ISSR.TAA8.880	0.0019	0.1075
SSR.CMTm111.380	0.0016	0.1257	ISSR.CAC6.200	0.003	0.1124
iPBS-2272.870	0.0019	0.1173	SSR.CMTm111.380	0.0031	0.109
iPBS-2272.950	0.0019	0.1173	SSR.CMTmC34.700	0.0044	0.1006
ISSR.CAC6.200	0.0022	0.1196	ISSR.VHVGTG7.900	0.0054	0.0854
SSR.CMTmC34.700	0.0024	0.1155	SSR.CMTp182.150	0.0059	0.0936
SSR.CSJCT746.255	0.0025	0.1143	iPBS-2272.870	0.0061	0.09
SSR.CMTp182.300	0.0025	0.1143	iPBS-2272.950	0.0061	0.09
SSR.CMTp182.490	0.0025	0.1143	SSR.CMTp193.380	0.0067	0.091
SSR.CMTp193.380	0.0026	0.1138	SSR.CMTp182.830	0.0067	0.091
SSR.CMTp182.830	0.0026	0.1138	SSR.CMTmC34.580	0.0067	0.091
SSR.CMTmC34.580	0.0026	0.1138	SSR.CSJCT746.255	0.0072	0.0891
ISSR.VHVGTG7.900	0.0027	0.1005	SSR.CMTp182.300	0.0072	0.0891
SSR.CMTp182.150	0.0028	0.1117	SSR.CMTp182.490	0.0072	0.0891
SSR.CMTp125.405	0.0041	0.1029			
SSR.CMTp201.600	0.005	0.1065			
SSR.CMTp201.1200	0.0055	0.1044			
iPBS-2376.530	0.0069	0.0939			
ISSR.HVHTCC7.1090	0.0091	0.0746			

Regression analysis was performed between the dependent variable of the number of days of seedling emergence and 26 independent variables (markers) by using the important markers that emerged as a result of MLM (K) analyzes. In the back regression analysis, 8 significant models were formed. In the 8th model, there were 3 independent variables at the $p<0.05$ level (iPBS-2226.1450, ISSR.CAC6.200 and 552). There is no high level of correlation between these markers. In the model, the 'Intercept' value was 21,688 and the 'B' value was -2.854, -6.833 and -3.688, respectively. The rate of explaining the number of seedling emergence days of the model depending on these markers is 35.2%. Regression analysis was performed between the dependent variable of the number of seedling emergence days and 21 independent variables (markers) by using the important markers that emerged as a result of MLM (K+Q) analyzes. In the back regression analysis, 7 significant models were formed. In the 7th model,

there were 3 independent variables at the $p<0.05$ level (iPBS-2226.1450, ISSR.CAC6.200 and SSR.CMTmC34.700). In the model, the 'Intercept' value was 20.725 and the 'B' value was -2.17, -7.389 and -2.725. The rate of explaining the number of seedling emergence days of the model depending on this marker is 28.8%.

High yield and high fruit quality are the main goals of today's watermelon growers. Within the scope of this study, DNA markers related to seedling emergence characteristics were tried to be determined by association mapping analysis by using different marker techniques in watermelon. Association mapping method is a powerful method that reveals gene-marker relationships. This study brings together different mapping models and provides information on the suitability of watermelon genotypes for association mapping analyses. The data obtained as a result of the study will contribute to future genetic and breeding studies.

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NUCLEAR DNA CONTENT ANALYSES IN *ALYSSUM CARICUM* T.R.DUDLEY & HUB.-MOR. (BRASSICACEAE)

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ABSTRACT

Alyssum caricum is an endemic in the flora of Türkiye and classified as endangered (EN) categories by the Red Data Book of Turkish Plants. The species distributed in Denizli and Muğla, Türkiye. Nuclear DNA content is one of the most important and fundamental biological character of the genome. However, this essential information is missing for *A. caricum*. The objective of this study was to determine the nuclear DNA content of *A. caricum* species using 23-week-old *in vitro* germinated and grown plants. Also, the DNA content *in vitro* propagated plantlets of *A. caricum* were analysed and compared to determine the genetic stability based on flow cytometry. For this, the plantlets that propagated in medium A (2 mg/L BAP+ 0.1 mg/L NAA) and Medium B (2 mg/L KIN + 0.1 mg/L IAA) were used for analyses. Flow cytometric analysis revealed that 23-week-old *in vitro* grown plants had 1.75 pg/2C ± 0.01, propagated plantlets ranged from 1.65 pg/2C± 0.01 to 1.67 pg/2C ± 0.1 mean nuclear DNA content. The results proved that propagated plants had similar DNA content to the seed-derived plants which may suggest that analysed plants were genetically stable.

Keywords: Nuclear DNA Content, Tissue culture, *A. caricum*

INTRODUCTION

The genus *Alyssum* (Brassicaceae) is distributed in America, Asia, Europe, and North Africa, and the centre of diversity is the Eastern Mediterranean region (Dudley, 1964, Li et al., 2014). The distribution of Alyssum species is 70 in Europe, 38 species in Greece, 9 species on the island of Cyprus, and 21 species in Iraq (Ball and Dudley, 1964; Dudley, 1965; Meikle, 1977; Townsend, 1980; Davis et al., 1988; Hartvig, 2002). The *Alyssum* genus is among the largest with consisting of 107 species and subspecies for the flora of Türkiye (Babaoğlu et al., 2006). The genus is known by the public as 'Rabid weed' or 'Kevke'. The rate of endemism is approximately 55% (Adıgüzel and Reeves, 2002, Aytaç and Duman, 2000; Orcan and Mısırdalı, 2000; Orcan 2002; Orcan, 2006; Orcan and Binzet, 2006; Yılmaz, 2012). *Alyssum* has been reported as a represent of Ni-hyperaccumulator genus with many of its taxa (Babaoğlu et al., 2006; Reeves and Adıgüzel 2008) e.g. *A. cassium* Boiss, *A. dubertretii* Gomb., *A. callichroum* Boiss. & Bal. (Reeves and Adıgüzel 2008). Several *Alyssum* has been reported not only accumulation of important amount of nickel but also drought tolerance feature. It has been mentioned that since *Alyssum has drought tolerance and low soil selectivity, it is important preventing disasters such as erosion* (Tozyılmaz et al., 2021). *Alyssum caricum* is an endemic plant in the flora of Türkiye and classified as an endangered (EN) categories by the Red Data Book of Turkish Plants (Ekim et al., 2000; Yeşilyurt and Akaydın 2012). The species is perennial which distributed in Denizli and Muğla, Türkiye and show semi blush type (Çördük et al., 2023). *A. caricum* is Ni accumulator and a serpentine endemic (Adıgüzel and Reeves, 2012). Plant tissue culture methods have been used for the propagating different plant species e.g endangered, endemic, threatened, or commercial plants. The effective *in vitro* propagation

protocol has already optimized for *Alyssum caricum* (Çördük et al., 2023). Phylogenetic analyses (Li 2014), pollen morphology, antibacterial and anti-biofim activities (Arslan 2019) of different *Alyssum* species have been reported but, there is limited research exist about *A. caricum*. Nuclear DNA content is one of the most important and fundamental biological character of the genome. However, this essential information is missing for *A. caricum*. The objective of this study was to determine the nuclear DNA content of *A. caricum* species using 23-week-old *in vitro* germinated and propagated plants for the first time by flow cytometry.

MATERIAL AND METHOD

Plant Materials

Seeds of *A. caricum*, collected in previous studies (Çördük et al., 2023), were used as plant materials. All seeds were initially treated with 70% ethanol for 1 min and then disinfected by stirring in 5% (v/v) sodium hypochlorite with 0.1% Tween 20 (2 drops) for 20 min. Then, the seeds were rinsed with sterile water at least five times. The sterilized seeds were aseptically transferred onto Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) with 3% (w/v) sucrose and 0.7% (w/v) phytoagar for germination. The seedlings were immediately cultured in a culture room with controlled environment conditions (25±2°C, 72 µmol $m^{-2}s^{-1}$ light intensity, and 16/8 h photoperiod, 60% humidity) for their developments.

In vitro propagation cultures of *A. caricum* were established according to the protocol optimized in a previous study (Çördük et. al., 2023). The nodal segments (ca 5mm long) of *in vitro* seedlings were aseptically cultured on MS medium containing 2 mg/L BAP+ 0.1 mg/L NAA (Medium A), 2 mg/L KIN + 0.1 mg/L IAA (Medium B) with 3% (w/v) sucrose and 0.7% (w/v) phytoagar. The cultures were maintained in the plant culture room (25±2°C, 16/8 h photoperiod with a light intensity of 72 µmol m^{-2} s^{-1}). The 2–3 cm in length propagated adventitious shoots were transferred onto MS with 3% sucrose and 0.7% phytoagar without plant growth regulators for root induction. Plantlets were grown during 24 weeks of culture.

Nuclear DNA Content Estimation

The nuclear DNA content of *A. caricum* samples was determined by the flow cytometer (Partec, CyFlow® Space Münster, Germany). *Vicia sativa* (2C = 3.95 2C/pg) was used as an internal standard. The intact nuclei suspension was prepared from the young and healthy leaves of propagated plants and *in vitro* grown plants. The intact nuclei suspensions were prepared using commercial kits manufactured by Sysmex Partec GmbH (Münster, Germany). The fresh leaf of sample (20 mg) and standard leaf tissue (50 mg) was co-chopped into small pieces for approximately 40–60 s using a razor blade in a petri dish with 500 µl nuclei extraction buffer. The homogenized solution was transferred into a glass tube through a 30 µm filter. A 2 µl of staining buffer (CyStain PI Absolute P) was added to each tube. The samples were incubated at room temperature in the dark for approximately 1 h before analysis. 2C nuclear DNA contents of samples were calculated based on the ratios of the G1 peak means of sample and internal standard with three replicates for per sample using the following equation: Nuclear DNA content of sample = (Fluorescence intensity of sample (mean of G1 peak) / Fluorescence intensity of standard (mean of G1 peak) × Known DNA content of standard (pg)

RESULTS AND DISCUSSION

The seedlings were healthy grown *in vitro* conditions for 23 weeks (Figure 1). The previously optimized protocol followed (Çördük et al., 2023) and the obtained materials used to estimate mean nuclear DNA contents of the materials using flow cytometry.

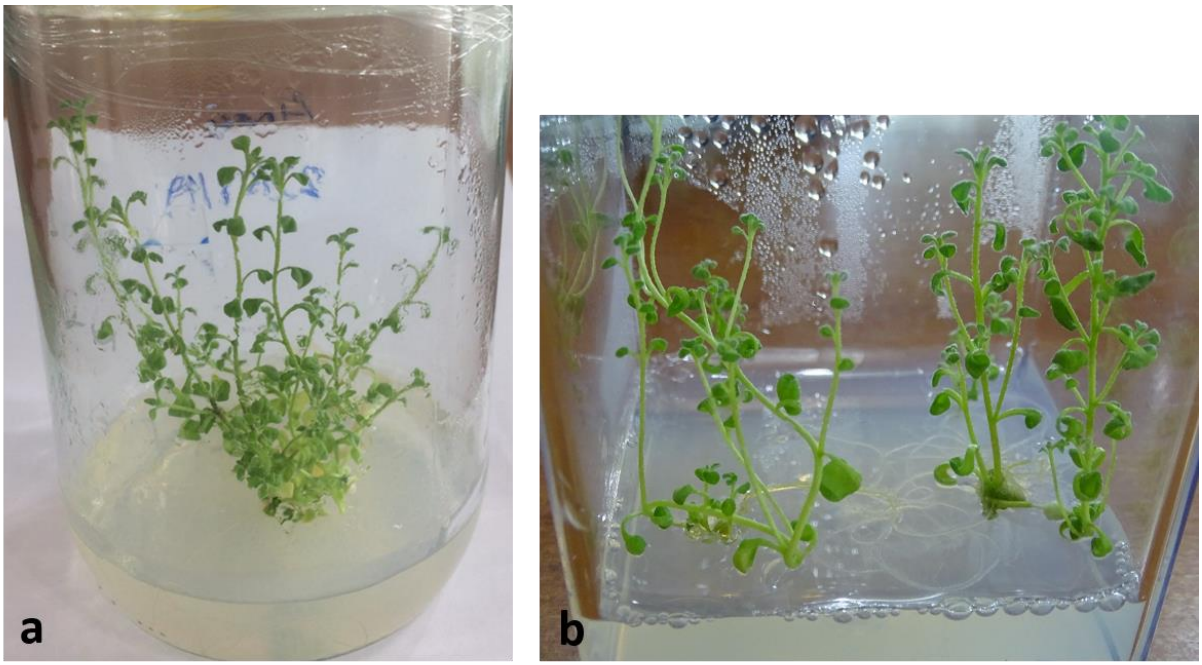

Figure 1. 23-week-old *in vitro* seedling (a), propagated plantlet of *A. caricum* (b)

Technically, the analyses of the nuclear DNA content were efficient *in vitro* grown and propagated individuals of *A. caricum.* Flow cytometric analysis resulted with high resolution histograms for the each analysed individual plants (Figure 2.). The mean nuclear DNA content of *A. carricum* was detected using flow cytometry with *V. sativa* (3.65 pg/2C) as an internal standard plant. *V. sativa* was excellent as an internal standard for *A. caricum* nuclear DNA content analyses since analysed plant G1 peak was clearly distinguishable from the standard plant G1 peak (Figure 2). Based on the flow cytometric analysis of nuclear DNA content, the *in vitro* grown plants and propagated plantlets have similar amount of nuclear DNA. Flow cytometric analysis revealed that 23-week-old *in vitro* grown plants had 1.75 pg/2C ± 0.01, propagated plantlets ranged from 1.65 pg/2C± 0.01 (Medium A) to 1.67 pg/2C ± 0.1 (Medium B) mean nuclear DNA content (Figure 2).

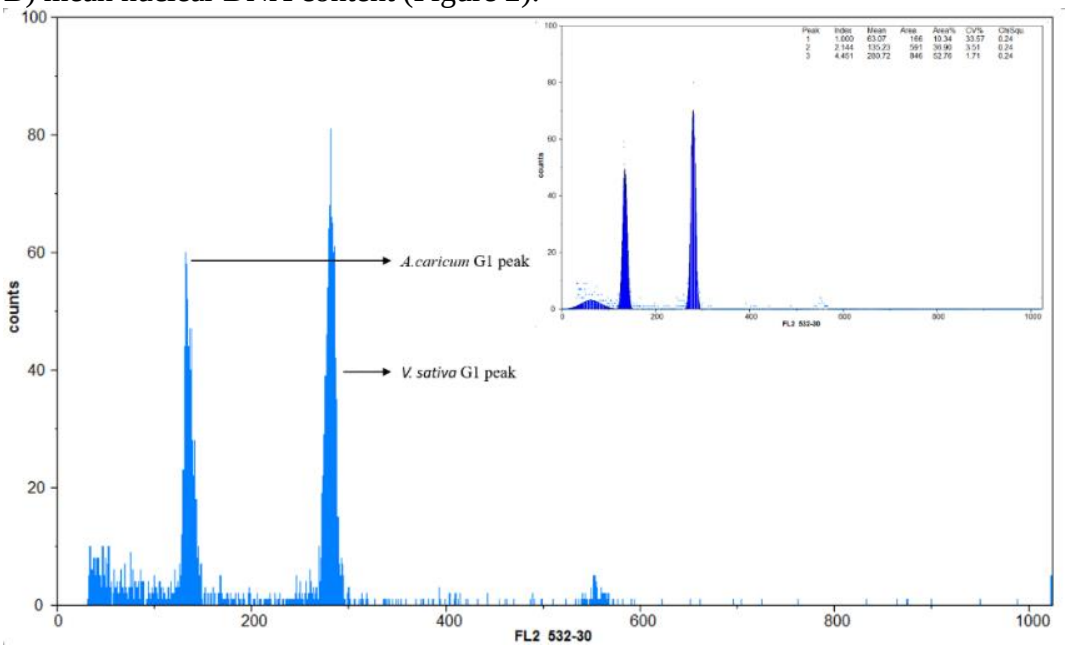

Figure 2. Relative positions of the G1 peaks of *A. caricum* L. and internal standard (*Vicia sativa*).

Previously analysed species, *in vitro* grown plants and propagated plants had similar nuclear DNA content e.g. *Digitalis trojana* (about 2.80 pg/2C; Çördük et al., 2017), *Verbascum scamandri* (from 0.73 pg/2C to 0.79 pg/2C; Yücel et al., 2023). The *Plantago asiatica* and *Silene vulgaris* (Çördük et al., 2018) *in vitro* cultures reported that produce genetically stable material since the 2C DNA content of *in vitro* cultures were similar to the source of the material (Makowczynska et al., 2008). Since there is no variation based on nuclear DNA content of *in vitro* grown and propagated plants which may suggest that the plants were genetically stable.

CONCLUSIONS

In conclusion, mean nuclear DNA content remained stable during the successive subcultures which may suggest that the culture conditions were suitable for *in vitro* propagation of this species since no variation based on flow cytometric analyses occurred during the culture. According to the flow cytometry results, regenerated plants showed similar nuclear DNA content to the source of the material which help us to control the nuclear DNA content stability during *in vitro* culture. However, more comprehensive studies e.g. chromosome counting, different tissue culture conditions are necessary to have comprehensive information about *A. caricum.*

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HERBAL TEA PRODUCTION TECHNOLOGY AND CURRENT APPROACHES

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ABSTRACT

Herbal tea is produced as fresh or dried mixtures of botanical elements other than Camellia sinensis species. It has different advantages such as ease of use, consumer preferences, nutraceutical ingredients blended in small bags, and being profitable for both consumers and producers. The type of raw material used in the preparation of herbal teas, the diffusion rate of the tea bags, the extraction efficiency, the phytochemical potential, the loading size of the bag and the safety aspects are the most important factors affecting the success of the tea bags in the market. In this study, the information in the literature has been compiled from a current perspective regarding the production, preparation methods and packaging materials of herbal tea. In addition, the case of adulteration and the plants used in the treatment of some diseases are included. The production, risks, technological features and abaca fiber (Manila hemp), which is a completely natural source used in production, are mentioned. The prior consideration includes important parameters such as the type of materials used, its pore size, shape, loading capacity, infusion rate, holding time, temperature and so forth. As a result, herbal teas are products that are frequently consumed, which have distinctive sensory qualities as they contain various volatile fractions.

Keywords: Herbal tea, Production, Packaging materials

INTRODUCTION

There is a lot of archaeological evidence that people used plants for medicinal purposes in prehistoric times (Varlı et al., 2020). Before black tea and coffee, which we frequently consume today, people used plants as tea in every region of the world. They benefited from the therapeutic properties of plants and preferred herbal teas because of their pleasant taste. Reasons such as the fact that herbs support healthy life, consumers' awareness and caution against artificial substances, and the increasing interest in ethnic products have led to the re-popularization of herbal teas and enabled them to capture a large market share all over the world (Akgül and Ünver, 2001).

Türkiye has very diverse and endemic plant species in terms of its geographical location. There are approximately 11000 plant taxa and about 500 of them are used for alternative medicine. Lavender, poppy, St. John's wort, mint, cassia, cumin, fennel, chamomile, thyme, sage, black cumin are among the commercially cultivated plants. Exported plants are mainly laurel, thyme, poppy, aniseed, while imported plants are mainly black pepper. Plants are consumed as tea as well as spices and are frequently used in kitchens, medicine, pharmacy and cosmetics (Göktaş and Gıdık, 2019; Varlı et al., 2020).

People have benefited from medicinal and aromatic plants for therapeutic purposes for ages. Phytotherapy, the method of treatment with plants, is as old as human history. Medicinal and aromatic plants have played a role in the use of plants in diseases since the past. Today, these plants are utilized in many sectors including food, cosmetics, pharmacy, medicine, chemistry and pesticides. Herbal teas, one of the areas of use of medicinal and aromatic plants,

are gaining importance day by day and have become a frequently consumed product due to their benefits proven by studies (Toker et al., 2012).

Herbal medicines are of great importance for human health, mostly in developing countries. Approximately 80% of the world's population uses medicinal plant products in the first stage of preventive and post-illness treatment. Plants contain bioactive secondary metabolites such as flavonoids, phenolic compounds, steroids, tannins, saponins and alkaloids. These metabolites have many properties such as inhibiting fungal and yeast growth, lowering blood sugar, preventing allergies, antioxidant, preventing cancer formation, lowering high blood pressure, reducing inflammation, reducing intestinal parasites, protecting cardiovascular health, pain relief and antispasmodic (Varlı et al., 2020). Although herbal teas are beneficial for health, they also have some disadvantages. These can be listed as containing heavy metals, pesticide residues, being contaminated with pathogenic microorganisms and mycotoxins (Can and Duraklı Velioğlu, 2018).

Among nutraceutical beverages, herbal teas produced from medicinal and aromatic plants and their alternative beverages have an important place (Suna, 2014). Recently, the demand for herbal tea bags is increasing day by day due to the increasing interest in healthy living, the fact that herbs have been consumed as tea since time immemorial and their practicality. These tea bags, which look like filter paper, contain 1-2 grams of product and are commonly offered to the market in boxes containing 20 bags.

Parts of plants such as leaves, roots, bark, seeds, fruits, stems and flowers can be consumed as tea (Sarwar and Lockwood, 2010). Factors affecting the use of a plant as tea are the culture of the region or country and the plant diversity of the region. Herbal teas are an easy way to get bioactive and antioxidant substances into the body without using much sugar and energy. Herbal teas are defined in the European Pharmacopoeia as: "They are orally used aqueous preparations prepared by maceration, decoction and infusion of one or more droplets; they are prepared before use" (Kabakçı, 2016). Herbal teas do not contain caffeine compared to Camellia sinensis species and are easy to drink (Ravikumar, 2014). In our country, sage, linden, thyme, chamomile, chamomile, mint, laurel, rosemary, clove, cinnamon, rosehip, anise, dill, fennel, tarragon, ginger, basil, hibiscus and lavender are some of the herbs used as tea (Akgül and Ünver, 2001; Etheridge and Derbyshire, 2020).

In this study, production of herbal teas, points to be considered in production, microbiological examination of herbal teas, adulteration issue and preparation methods were explained. The effect of harvest time, brewing time and temperature on the amount of active ingredient was explained with the studies conducted, and the plants used in the treatment of some diseases were also included. The raw materials used in the production of tea bags, which have recently increased in popularity, and the properties and risks that they should have are stated.

HERBAL TEA PROCESSING TECHNOLOGY

A wide variety of materials can be used in herbal tea making. Among the factors affecting plant diversity are geographical location and climate diversity (Akgül and Ünver, 2001). The number of medicinal and aromatic plants used only in the food sector is more than 10,000 (Varlı et al., 2020). Although the consumption of wild-growing plants as tea has decreased today, there are still countries that resort to this method. Using these plants, which are abundant in nature, can be preferred because it reduces the cost, but there are some risks it poses. These include inconvenient raw material procurement and quality differences. In addition, the inability to distinguish plants with excessive amounts of some toxic compounds may harm health. Teas that can be dangerous to consume frequently should be prepared and used by experts. As in developed countries, tea production should be realized by cultivating plants with proven health benefits and botanical identification (Akgül and Ünver, 2001).

Harvesting and collection of plants

The first step in herbal tea processing is to harvest and collect the plant at the right time. Because the active ingredient content of the plant depends on its age and stage of development. The cultivation and harvesting of the plant should be carried out by trained people under hygienic conditions. Foreign matter contamination of the material during harvesting should be avoided as much as possible. The main factor affecting the quality is harvesting according to the plant organ to be used. Subsoil organs such as ginger, galangal and licorice should be harvested towards the end of vegetation. The above-ground parts of herbaceous plants, on the other hand, usually contain a large amount of effective compounds at the beginning of flowering. Plants with flowers such as lavender, linden and chamomile should be harvested when the flowers are in full bloom; plants with leaves such as sage, rosemary, rosemary, laurel, mint and senna should be harvested just before flowering; and plants with bark such as cinnamon and buckthorn should be harvested before drying. Plants whose fruits such as rose hips, lemon, bergamot and seeds such as cumin, coriander, anise, fennel should be picked after ripening. The harvested products may be damaged by mechanical action or overfilling. As a result of the activity of enzymes and microorganisms, loss of active ingredients, decay and reduction in the amount of product may occur. Harvested products should be brought to the processing plant as soon as possible under good ventilation conditions, in clean baskets or sacks, avoiding excessive stacking. Harvested plant parts (leaves, flowers, roots, etc.) should not be mixed with other parts and should be transported in separate containers. Harvesting can be done by machine for some plants. The machines used should always be kept clean to prevent contamination, and care should be taken not to contaminate the products with the oils used in the lubrication of machine equipment (Akgül and Ünver, 2001; Douglas et al., 2005; Pandey, 2017).

Separation and cleaning of the material

The second stage in the processing of herbal teas is to clean the collected product from all foreign materials and to separate the plant organ to be used from other parts. The subsoil organs to be used may need to be washed and peeled. In small-scale enterprises, cleaning operations can be done manually. For large-scale enterprises, some methods applied to cereals according to the type of material can be adapted for plants and machines can be used (Akgül and Ünver, 2001). The material should also be made microbiologically safe. Non-thermal decontamination methods for plants are divided into two as physical and chemical. Physical methods are irradiation, pulsed electric field (PEF), cold plasma and pulsed light. Chemical methods include ozonation, ethylene dioxide fumigation and high pressure CO_2 gas application combined with ultrasound.

Gamma rays, X-rays and electron beams are permitted for food irradiation. The smaller a microorganism is, the more resistant it is to irradiation, thus requiring high doses of irradiation. Viruses are more resistant than bacteria, bacteria than molds and yeasts, molds and yeasts than insect larvae and insects. Irradiation prolongs the shelf life of the product and increases its safety. At a certain dose, the nutritional value and sensory quality of the product are at the desired level, but when irradiation is applied at high doses, losses may occur in these criteria. PEF application is a non-thermal decontamination method and is mostly used in the sterilization of liquid foods such as milk, liquid eggs, fruit juice. There are studies on the decontamination of herbs and spices with PEF, but it is a subject that needs research and development.

Plasma is the fourth state of matter and is an ionized gas that contains sufficient energy. Cold plasma is the ionization of gases such as helium, nitrogen, argon, argon, hydrogen, nitrogen and/or a mixture of these gases under ambient conditions without heating. It has been proven by studies that cold plasma applied to plants does not cause loss of volatile oil and content, color and phenolic compounds of the products. It can be successful in preserving ingredients in the use of sensitive products that are not resistant to heat treatment. Pulsed light technology is based on the principle of breaking down and inactivating the DNA and RNA of

microorganisms using short wavelength ultraviolet rays. It is frequently used for disinfection of food contact surfaces and decontamination of liquid foods. With second applications, microorganisms can be inactivated in a short time. On solid surfaces, it can be difficult for the rays to penetrate, so the thickness of the material is important for an effective process.

Although there are few studies on the effectiveness of pulsed light in plant decontamination, effective results have been obtained. Therefore, it is useful to conduct research on its use in plant decontamination. Ozonation, one of the non-thermal chemical methods, is based on the principle of disinfection by utilizing the properties of ozone gas with three oxygen atoms as a strong oxidant and reactive. It has been observed that it significantly reduces the microbiological load when used. It was observed that the microbial load decreased with the application of ozone gas to plants, but high concentration and maximum time (120 min) caused appearance defects and phenolic substance losses. In addition, different microorganisms have different sensitivities to ozone. Its use in plants needs more research.

Fumigation with ethylene dioxide is an inefficient method of destroying microorganisms. In some countries it is used to decontaminate medicinal herbs and spices, but some countries have banned it because it can cause the formation of carcinogens. Ultrasound (US) is used in the food industry for pasteurization, extraction, freezing, drying, enzyme inactivation, filtration, de-foaming, gelling, etc. While high pressure CO_2 (HPCD) is a supercritical fluid, it can diffuse into solids like a gas and dissolve substances like a liquid. Research has shown that HPCD+US is not sufficient for decontamination, although efficient results have been achieved. Non-thermal decontamination processes for crops have advantages and disadvantages, but require less energy and cause less damage to the crop compared to traditional thermal methods. Non-thermal decontamination methods for crops should be further investigated and the appropriate one should be preferred (Perussello, 2020).

Drying process

Depending on the nature of the product to be used, several different drying processes can be applied. For delicate and precious materials, hot air drying in the shade is preferred. Durable products can be dried in the sun. The name of the drying process applied due to the economic structure of the facility, the amount and type of raw material may be drying tunnel, room or cabinet (Akgül and Ünver, 2001).

Studies have shown that drying methods and temperature applied to herbal teas significantly affect the active ingredient content. In a study, different drying methods and temperatures were applied to rosemary, basil, thyme, mint and stevia plants and antioxidant activity and phenolic matter values were investigated. The plants were dried in drying tunnel (30, 40 and 50˚C), microwave oven (450, 600, 700 and 800 W), sun, shade and refrigerator. The highest phenolic content was found in rosemary and basil dried in microwave oven at 800 W power level, in thyme dried in drying tunnel at 30 °C, in mint dried in sun, stevia and shade. The highest antioxidant activity value was observed in rosemary and basil at 700 W and 800 W power levels in microwave oven, in thyme at 30 °C in drying tunnel, in mint at 600 W, 700 W, 800 W power levels in microwave oven and in sun, in stevia in sun and shade dried samples (Güler, 2019).

Aydın et al. (2019) examined the essential oil content and oil components of Anatolian sage (*Salvia fruticosa* Mill. = *Salvia triloba* L.) at different drying temperatures and determined that the applied temperature affected the amount of essential oil and its components.

Grinding

The next stage after drying is grinding. The material supplied to the market in cup/beverage bags or in bulk needs to be ground to a certain size or reduced to particles. It is important that the grinding is not too fine because of the problem of cloudy brew and dust. It is reasonable for products packaged in bulk or in kraft bags in higher quantities to be coarser than bagged products. According to the capacity of the enterprise and the physical structure of the

product, grinder or mill methods can be applied for the grinding process (Akgül and Ünver, 2001).

Blending/additive

If blending and addition are required based on the product type, they are typically carried out post milling. Blending is the mixing of products containing more than one plant. In some herbal teas, additives such as naturally identical flavors, vitamins, minerals and sweeteners can be added. Granulated/powdered products impregnated with essential oils or extracts in carrier solids are not classified as herbal tea (Akgül and Ünver, 2001).

Bagging/packaging

The packaging material used must be clean and dry, and the packaging must be protected from damage by biological pests. The packaged product should be stored in a clean place away from moisture, heat and odor. Regardless of the bagging method, if the plant used contains substances that may cause side effects, it should be reported on the package and the amount should be specified. For example, tea bags containing senna may have an excessive laxative effect when consumed more than two times a day (Akgül & Ünver, 2001; Pandey, 2017).

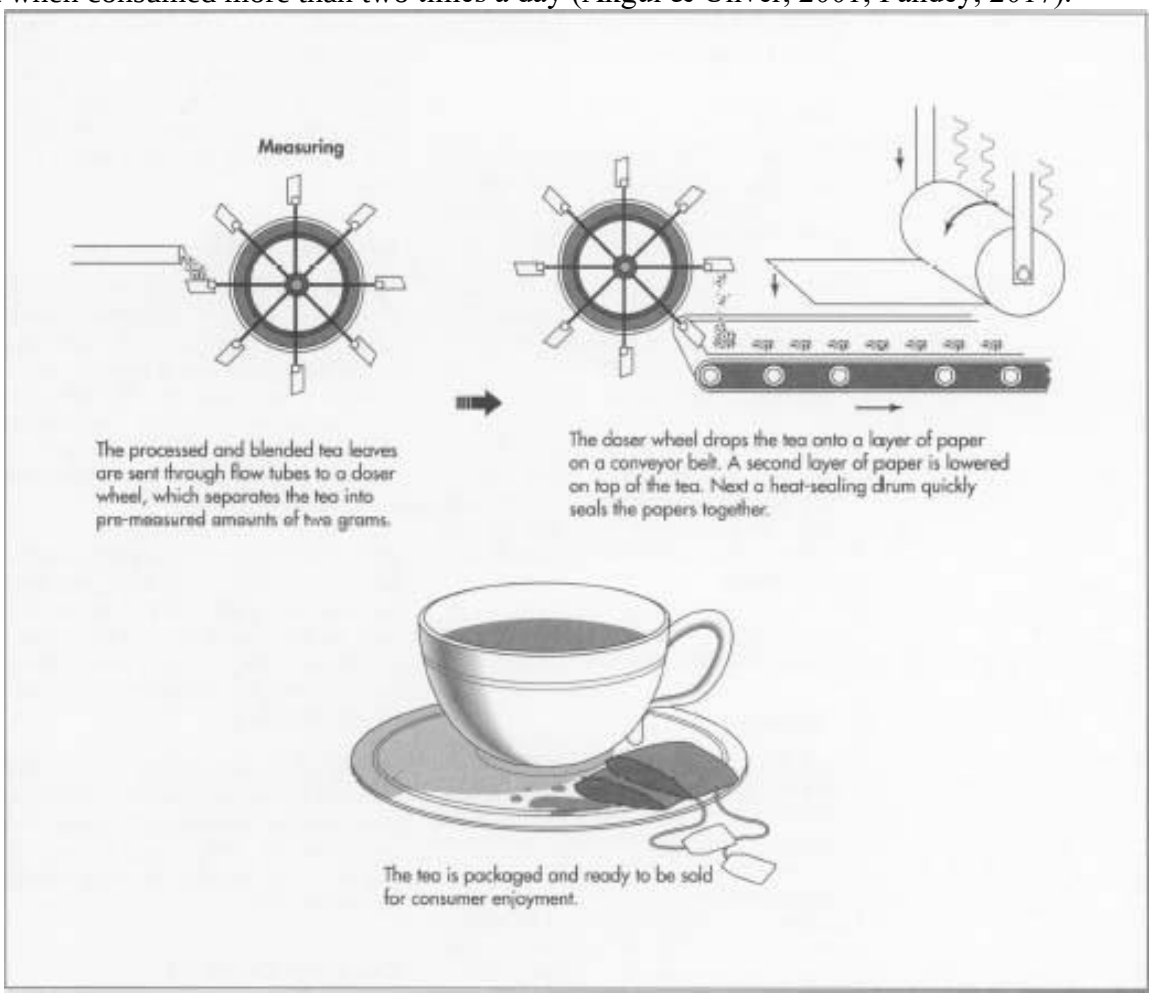

Figure 1. Bagging of blended teas (Anonymous, 2022)

Herbal Teas and Quality Control

There may be a need to determine the quality criteria of herbal teas at more than one stage: raw material, ground product and brew. Chemical analyses such as moisture, ash, extract, volatile oil, fiber and oil content determination, physical analyses such as defective parts and foreign matter, microbiological analyses such as mold contamination, mycotoxins, pathogenic bacteria should be performed. In addition, qualitative and quantitative analysis of effective compounds that vary according to the plant, determination of all contaminants (such as pesticides, microplastics and heavy metal residues) and additives, if used, are other criteria. In

addition, the degree of grinding, the amount of herbs used, and the sensory profile of the material can be determined by experts and panelists (Akgül and Ünver, 2001).

Another issue in quality control criteria is adulteration. Since herbal teas can be sold in bulk, they are open to adulteration. Unfortunately, there are people who add different plants to valuable products, disregarding human health, in order to make more money from valuable and/or expensive plants. Studies have also shown that herbal teas and/or mixtures use products other than the plants specified on the package. In a study conducted by Şaşkara et al. (2010), organic and inorganic pollution elements, foreign plant parts, insect residues, moldy plant parts, organic and inorganic pollution elements, foreign plant parts, insect residues, moldy plant parts were found in open and sacked samples taken from herbalists to examine the lemon balm leaf droplet (used plant part) (*Melissae folium*) obtained from *Melissa officinalis* (Melissa, lemon balm) plant. According to the study, the presence of other plant parts and inorganic contaminants in the herbalist samples is an indication that the herbal was collected and sold by unconscious people. Another sample was taken as a swordwort, but the amount of swordwort in the bag was very small and other drugs and stone fragments were observed. In addition, in the three samples examined, it was determined that the label attached to the box after the purchase stated that it was melissa, but it was a species of mountain tea (*Sideritis* sp.), another genus belonging to the *Lamiaceae* family, and not *M. officinalis*.

In another study, Kabakçı (2016) microscopically and macroscopically examined 153 kinds of packaged herbal teas containing 65 plant species belonging to 5 different brands (A, B, C, D, E) sold only through pharmacies and the internet, and also performed active ingredient controls of 5% infusions with preliminary trials. As a result of his studies, he found that there was a lack of information about the plant names on the packages and that the packaging information of B, C, D, E brands was insufficient. Regarding the samples he examined macroscopically, he found that brand B had lemon balm leaves and *Aloysia triphylla* (L'Hérit.) Britton leaves instead of *Melissa officinalis* L. species stated on the package. In the 5% infusions he made, in line with the literature information, he did not detect the presence of saponin in 14 tea samples, anthracene in 6 tea samples, flavones in 110 tea samples, alkaloids in 17 tea samples and tannins in 28 tea samples.

Microbiological risks in herbal teas

Herbal teas, which are most commonly consumed in diseases requiring mild treatment, carry some risks as well as benefits. These plants growing in nature are of course open to contamination with some microorganisms and their metabolites. However, it is inevitable that plants collected and stored by unconscious people carry microbiological risks. The causes of these microorganism-induced contaminations include pre-harvest, harvesting, drying, grading, grinding, processing, packaging and storage stages through soil, water, fertilizer, sewage, animal wastes and residues (Can and Duraklı Velioğlu, 2018).

Mycotoxins, which enter the body through various routes, can cause impaired liver and kidney function and have neurotoxic effects. Some mycotoxins can interfere with protein synthesis and cause various disorders ranging from skin sensitivity and necrosis to extreme immunodeficiency. Some mycotoxins can be teratogenic (substances that cause permanent deformity and dysfunction in the baby during pregnancy) and/or carcinogenic. Studies show that herbal teas can contain high levels of total bacteria and molds, and are contaminated with coliform group bacteria, *Bacillus cereus*, *Clostridium perfringe*s and their spores, *Salmonella* spp. Although there are not enough studies on the microbiological comparison of plants collected from nature and cultivated plants, it is thought that post-harvest processes cause contamination in cultivated plants (Can and Duraklı Velioğlu, 2018).

Halt (1998) conducted a study with 73 samples to determine the presence of toxigenic molds in herbal teas and found that the most dominant molds were *Penicillium* spp. found in 54.58% of the samples and *Aspergillus* spp. found in 19.80% of the samples, and *Aspergillus*

flavus, one of the most important aflatoxin producers, was found in 16% of the samples. Arslan (2013) investigated the aflatoxin B1 levels and microbiological quality of some organic spices and herbal teas produced in Türkiye and found that 30 (82%) of 37 herbal tea samples had yeast-mold, 33 (89.19%) had *Staphylococcus aureus*, 36 (97.29%) had *Enterobecteriaceae* and 25 (67.56%) had coliform bacteria. Among the herbal teas analyzed, the lowest bacterial contamination was found in fennel. In 32 organic herbal tea samples, aflatoxin B1 was found. The organic herbal tea with the highest aflatoxin B1 (52.50 µg/kg) was rosehip samples.

Studies on herbal products and teas show large differences in contamination rates. The reasons for this situation include the type of plant examined, the number of samples, geographical region, processing, storage and sales conditions and packaging status (Vural et al., 2020). In order to eliminate the microbiological risks of plant material, the processing steps should be applied carefully. In addition, the presence of aflatoxins and other mycotoxins in the final product should be detected. Otherwise, people who use plants for therapeutic purposes may develop different ailments instead of showing signs of improvement (Halt, 1998).

Research results show that it is very important to ensure microbiological stability in plant materials. The most important production steps to be considered for this purpose are drying and storage. The drying stage, which is carried out by taking into account factors such as the protection of the active components of the organ in which the plant will be used and the moisture level, triggers the development of microorganisms in the material when it is carried out at low temperatures, and when it is carried out at high temperatures, the total number of mesophilic aerobic bacteria decreases. Improving storage conditions (ventilation, humidity level, temperature, etc.) will significantly reduce the development of mold and toxins in the material and safe products that do not carry health risks can be produced (Can and Duraklı Velioğlu, 2018).

Herbal Tea Preparation Methods

The nutritional value of herbal teas increases with their antioxidant substances. These substances provide inactivation of free radicals in the body. Natural plant sources of antioxidant substances are phenolic compounds. Flavonoids constitute the largest part of phenolic compounds and play an important role in antioxidant activity. Herbal tea preparation methods are important on the amount of flavonoids in water (Cavlak and Yağmur, 2016). With the high number of plant varieties and organs to be used for herbal tea, different methods are applied to prepare tea. It is the organs of the plant that determine the method to be used. There are three different methods of preparation: brewing (infusion), boiling (decoction) and keeping at room temperature (maceration) (Üstü and Uğurlu, 2018).

Brewing (infusion)

If the soft organs of the plant such as leaves and flowers (for example, sage and mint leaves, chamomile and linden flowers) are to be used, the brewing method should be applied. This technique should also be preferred for valuable plants containing essential oils. It should be prepared fresh each time to prevent bacterial contamination. Generally, 2% of the herb is used. Approximately one tablespoon (approximately 2 grams) of the herb is added to a glass (approximately 150 ml) of boiled water and left to infuse for 5-10 minutes with the lid closed. After straining, it is consumed (Üstü and Uğurlu, 2018). Strained tea bags are added into the cup or teapot, boiled water is added and the brewing time written on the package is applied. At the end of the time, the filter bag is removed and consumed (Akgül & Ünver, 2001).

Boiling (decoction)

This is the method used to use the hard tissues of the plant such as seeds, bark and roots (e.g. turmeric, cinnamon, ginger, anise). The active ingredients in these parts of the plant are difficult to pass into water and therefore need to be boiled. It should be prepared fresh each time to prevent bacterial contamination. Usually 2% of the plant is used. Approximately one tablespoon (approximately 2 grams) of the herb is added to a glass (approximately 150 ml) of

cold water and left to boil. After boiling, lower the heat, boil for 5-10 minutes, then strain and consume (Üstü and Uğurlu, 2018).

Maceration

Room temperature maceration is used for plants containing mucilage (e.g. flaxseed, marshmallow root) and heat-sensitive substances. The plant parts are cut into small pieces, water at room temperature is added and left overnight. It is consumed by straining. It should be prepared in an amount to be drunk daily (Göktaş and Gıdık, 2019; Üstü and Uğurlu, 2018).

Herbal tea can be prepared from a single herb or it is possible to use more than one herb. It should not be forgotten that plants can be harmful as well as beneficial and should be consumed with attention to the toxic compounds they contain. In order to fully utilize the effective compounds contained in plants, attention should be paid to brewing, boiling and maceration (Göktaş and Gıdık, 2019).

Herbal teas can be prepared from both a single plant and a mixture of several plants. When preparing herbal teas, attention should be paid to the preparation times, the effects of the plants, the dose, and care should be taken not to use plants that cannot be adjusted without turning them into medicine.

USE AND EFFECT OF HERBAL TEA

Today, herbal teas, which are used for ailments requiring mild treatment, can be prepared from thousands of different plants. The chemical content of the teas depends on the herbs and/or combinations used. The therapeutic properties of herbal tea are due to the active ingredients of the plants. Flavor is the sum of hot water soluble compounds. The compounds that give herbal teas this characteristic feature are mainly essential oils, resins, heterosides, alkaloids, pigments, tannins, vitamins, minerals, organic acids and polysaccharides. Among the factors affecting the type and amount of these effective compounds of herbal teas are the harvest time of the plant, the organ used, the processes applied to the material and storage (Akgül and Ünver, 2001).

Herbal Teas and Health Benefits

Herbal teas, which have been used in the treatment of various diseases since ancient times, have been proven in today's medicine to cure many diseases with continuous and long-term use. It is necessary to consume the herb and the amount to be used for the disease to be treated in consultation with experts in the field. Otherwise, it may have bad consequences. In addition, the dose used in disease treatment may be higher than normal drinking. Because it may be necessary to take more of the active substance into the body (Akgül & Ünver, 2001; Aslan, 2019). However, poorly produced products may not only be a source of healing but may also cause harm. This is because the products contain harmful contamination elements such as pesticides, heavy metals, aflatoxin, insect larvae. It is beneficial to use products with controlled production and it will be healthier to realize production in high standard facilities to protect against these damages (Poswal et al., 2019).

Other considerations

The fact that disposable tea bags provide convenience in our daily lives, as well as the fact that they are seen quite frequently in the supermarket aisles, clearly shows that teabags are frequently used in our society. In addition to the advantages of tea bags, microplastic (MP) content, which is the subject of studies, is a striking issue. Microplastics have been found to be solid, insoluble, polymeric substances, usually produced directly to a size below 5 mm (primary microplastics) or formed by fragmentation from large plastics (<5 mm, secondary). Microplastics are plastic materials of micro and sub-micro sizes formed from the breakdown of plastics (Kuriş, 2022).

In the study conducted by Kuriş (2022), the release potential was observed in tea bags using polymer materials such as polyethylene, polypropylene and polyester as well as cellulose material used in non-woven fabrics by separation with different methods. In this study on microplastic contamination in food, the release of microplastics from non-woven tea bags used

in the market and their transfer to the beverage through brewing was investigated. The main purpose of the study is to evaluate the release of microplastic fibers from tea bags with different separation methods and to draw attention to microplastic pollution in terms of food safety. Since tea bags produced from nonwoven tissues are disposable and brewed at high temperature, microfibers in the bag tissue have the potential to pass directly into the drinking liquid. Unfortunately, there is no information on the packaging that these bags contain plastic. Studies and patents in the literature confirm that in addition to cellulose (paper), different polymers (polypropylene, polyethylene, polyester, etc.) are used in tea bags to ensure durability and to ensure that the bag is easily adhered with heat in the sealing process. According to the results of the study, a minimum of 4,000 MP and a maximum of 43,000 MP debris can pass into tea from a teabag. It was stated that these materials, which are considered suitable for contact with food, can cause a high release of MP, especially with the effect of temperature, and that this is transferred to the beverage.

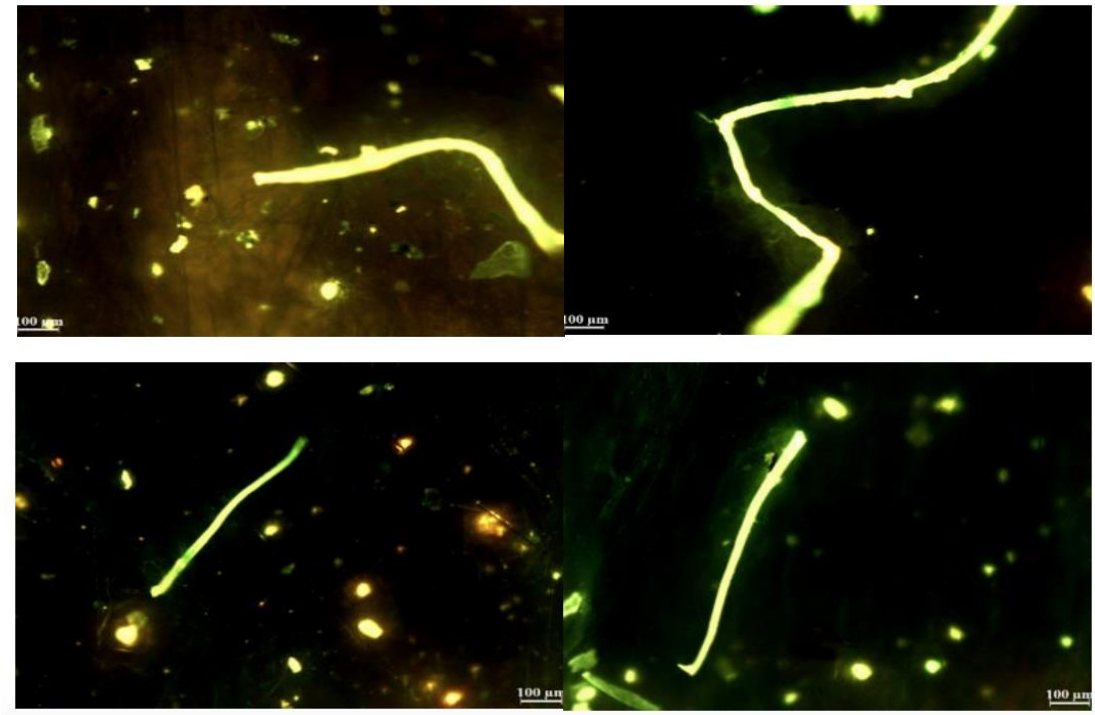

Figure 2. Blue-emitting microplasmacytes released from tea bag samples under BAB fluorescence microscopy (Kuriş, 2022)

Dried herbs (roots, bark, seeds, leaves, flowers, etc.) to be used as herbal tea usually retain their therapeutic properties for 1 year. For this reason, herbs that have been collected more than 1 year after the collection date should not be used for therapeutic purposes. Unless specifically stated, the herbs should not be used for longer than 4-6 weeks (Üstü and Uğurlu, 2018).

TEA BAGS AND PRODUCTION

Tea bags are widely consumed thanks to their advantages such as ease of use, the ability to brew at any time and in any quantity. By weight, 95% of the tea consumed is classic, black bulk tea, while the remaining 5% (900,000,000 tea bags/year) are tea bags, slimming tea and herbal teas such as green tea, rosehip, linden and sage. The tea bag market is increasing by 20-25% every year. Tea bags are mostly consumed by working, studying, educated, middle and upper income, urban consumers, while slimming teas are consumed by women over the age of 25 and green tea is mostly consumed by men.

Tea bags are more preferred than bulk products due to reasons such as easy brewing, practical cleaning from the brewing container, quick preparation in a fast-paced daily life and increased purchasing power (Bassi et al., 2020). There are various rumors about the invention

of the tea bag. The first of these is that in 1908, Thomas Sullivan, a merchant in New York, introduced his teas to his customers by putting small amounts in silk bags that had to be opened before brewing tea, instead of metal boxes. Most of the customers brewed tea by dropping the tea bags into the water as they were. When customers realized that it was easier to brew tea this way and demanded smaller packaged teas from Sullivan, Sullivan took the feedback into account and developed tea bags and used cloth bags. Later, however, paper replaced cloth in commercial tea bag production. In the 1920s, commercial production began for tea bags, which grew in popularity in the USA. With the production of different companies, square, pyramid, glass and teapot tea bags were produced (Anonymous. 2021a; Bajaj, 2016). There are two types of tea bags: stapled and knotted, but stapled tea bags are banned by FSSAI (Food Safety and Standards Authority of India) (Bassi et al., 2020).

Tea bags are usually made from filter paper or food-grade nylon mesh. For traditional tea bags, filter paper made from wood pulp or a blend of wood pulp and vegetable fibers is used. It is designed to be porous enough to allow water to flow through it and extract flavors and compounds from the tea leaves while keeping the leaves inside the bag. The filter paper is heat-sealed or glued to form the bag shape (Aguilar-Cruz et al., 2020).

Tea bags are usually made from the leaves of the abaca tree, a banana tree. The abaca tree, also known as Manila hemp, grows in the Philippines and the Philippines produces 85% of the world's Manila hemp. Cellulose fibers and artificial fibers are also preferred as raw materials for making bags. Artificial bags should not release harmful compounds into the water and the use of bags should be approved for human health (Bajaj, 2016; Bassi et al., 2020).

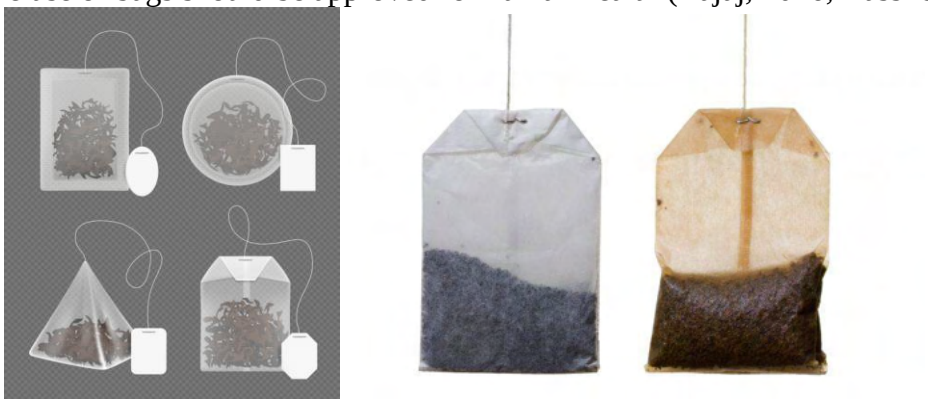

Figure 3. Variety of different shaped and stapled tea bags (Anonymous, 2021b; 2021c)

Tea Bag Production from Abaca Fiber

Abaca fibers, also known as Manila hemp, are obtained from the leaves of the *Musa textilis* tree. Due to their water resistance, abaca fiber is used in many different areas such as rope making in shipping, in many areas in the textile industry, in the paper industry, especially in the production of banknotes and tea bags, and in the production of sausage casings (Hayase, 2018; Vijayalakshmi et al., 2014). Abaca fiber is traditionally obtained by stripping it from the leaf sheath by manual or mechanical processes. In the production of tea bags from abaca fiber, the wet-laid nonwoven fabric method is applied. This method is derived from the paper production method and is also used in the production of long-fiber specialty papers. The extracted fibers are first suspended by swelling in water. In order for the product to become homogeneous, the water is removed by means of special paper machines. The resulting suspension is transferred to a continuously moving sieve. While the filtering process continues, a net is formed on the sieve. The resulting mesh is dried and glued (Bajaj, 2016; Vijayalakshmi et al., 2014).

Figure 4. Abaka (*Musa textilis*) and abaca fibres (Anonymous, 2021d; 2021e)

Figure 5. Abaca fiber roll used for tea bag packaging (Anonymous, 2021f)

Polilaktik Asitin (PLA) Çay Poşeti Üretiminde Kullanımı

PLA, one of the biodegradable polymers, is a biopolyester formed by the polymerization of lactic acid monomers. It is obtained by fermentation of natural resources such as corn starch, starch and sugar cane. Industrially, PLA is used in the production of milk and yogurt, fruit juice, coating of organic fruits and vegetables, mineral water bottles, tea bags, coffee capsules and cups. Strong sealing properties, low temperature adhesion, transparency, thermoplasticity, and easy processing are among the advantages of using PLA in tea bag production (Kılınç et al., 2017; Söbeli et al., 2019).

Apart from cellulose fibers such as abaca and biodegradable polymers, nylon nets are also used to make tea bags. Nylon tea bags have superior flavor compared to cellulosic papers, easy heat sealing and easy infusion. However, when brewed in hot water, there is a possibility of microplastics migrating into the water. Tea bags should have properties such as heat resistance, strong strength, and suitability for use in high-speed machines, and these factors should be considered when selecting raw materials (Bajaj, 2016).

Today, there are various rumors that tea bags contain carcinogenic substances. The chemical epichlorohydrin, which is used to bleach tea bags, converts to the highly carcinogenic compound 3-monochloropropanediol (3-MPCD) at high temperature and this compound passes from packaging materials to food (Bassi et al., 2020). Due to the frequent preference of herbal teas and the harm of epichlorohydrin to the body, research has been conducted on the raw material, design and properties of tea bags.

Negativities such as the fact that paper tea bags cause dust leakage, are not transparent enough and do not show the plant inside, cannot be sealed with heat and/or do not take shape have led to the production of polymer + cellulose tea bags. In this way, both the dust leakage problem of paper bags was solved and the production of non-woven tea bags that can be shaped at a good level was provided (Yurtsever, 2021).

Yaday et al. (2017) in their study on swelling and infusion of tea in tea bags with bulk tea and tea bags, aslo they found that tea bags inhibit swelling and infusion kinetics and increase infusion time compared to bulk tea. According to the study, the tea bag should be kept in water

for 2 minutes for infusion time and should not be removed immediately. In addition, the infusion time is affected by the reduction of the particle size of the tea and the weight filled, and the infusion kinetics, swelling degree and swelling rate of the bags filled with 0.5 grams of tea were higher than the samples studied with 2 grams. According to the study, it was observed that the infusion quality increased as the inflation rate increased.

Jha et al. (2020) characterized tea bags made of three different materials, cellulosic, PLA (polylactic acid) and nylon, in terms of thickness, pore size, porosity, wettability and permeability to understand the effects of tea bags on infusion kinetics. According to the results of the study, they found a relationship between the permeability and porosity of the tea bag papers, and they saw that as the porosity increased, the passage of tea components through the tea bag increased. Woven nylon tea bags exhibited higher permeability than non-woven tea bags (cellulosic, PLA). The reason is the homogeneous distribution of the pore structure in the tea bag paper. The study also showed that although the raw material of the tea bags significantly affects the permeability, different tea bag papers have approximately the same infusion profile.

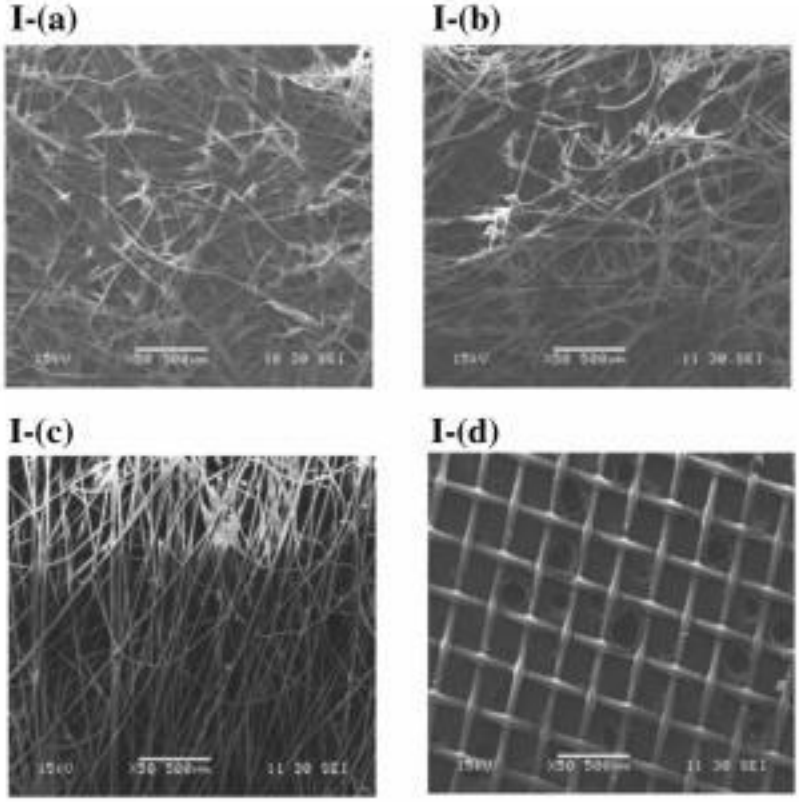

Figure 6. SEM image analysis of different tea bags. I-(a) 100% cellulose, I-(b) cellulose/PP (polypropylene), I-(c) PLA, I-(d) woven nylon (Jha et al., 2020)

In addition to these, there are muslin cloth tea bags that have recently become widespread. These tea bags, which are made of 100% cotton and do not leave nano and microplastic residues in the tea and are completely biodegradable, are seen as a sustainable and environmentally friendly option (Anonymous, 2022).

RESULTS AND DISCUSSION

Improving socio-economic status, consumer preferences, nutraceutical ingredients blended in small sachets are increasing the demand for tea bags with different characteristics such as ease of use and profitability to both consumers and producers. The type of raw material used in the preparation of tea bags (tea, herbs alone or in combination) is the most important factor influencing the success of tea bags in the market; some other factors that can affect the ultimate acceptability of tea bags by consumers include diffusion rate, extraction efficiency, phyto-chemical potential, loading size of the bag and safety considerations. The primary

considerations include important parameters such as the type of paper used, pore size, shape, loading capacity, infusion rate, holding time, temperature and so on.

The use of herbal teas in disease treatment is as old as human history. The ease of preparation and cleaning of tea bags, the chemical content of medicines and the active role of plants in diseases requiring mild treatment increase the demand for herbal teas. In order to benefit from herbal teas at the highest level, it is a priority that the cultivation and production steps are carried out by knowledgeable and experienced people. In addition, plant harvesting and drying methods are among the factors that significantly affect the amount of active ingredients. Harvesting should be done at the right time and the drying method that minimizes the loss of active ingredients should be determined and used.

Light-proof material should be used for packaging and the amount of use, method of preparation, production and expiry date, and any side effects and/or health concerns should be indicated on the packaging. The naming of plants should be regulated by the Ministry of Food, Agriculture and Livestock and the name corresponding to the Latin name should be determined and each brand should use this name. Herbal teas should be produced in a way that eliminates the risks of pesticides, heavy metal residues, microbiological contamination and adulteration.

Brewing (infusion), boiling (decoction) and keeping at room temperature (maceration) are herbal tea preparation methods. In the choice of the preparation method to be used, the parts of the plant used (leaf, flower, root, bark, seed), mucilage of the plant, etc. The application should be carried out taking into account that the plant contains substances and sensitive components. The brewing time and temperature of the herbal tea affect the amount of active substance passing into the water. Studies have generally observed an increase in the active substance as the temperature and time increase. The brewing time and temperature suitable for the plant must be included on the package.

Herbs have been proven to cure diseases. For herbs to be effective, they must be used continuously and for a long time. The herb to be used in treatment and the dose to be taken should not be determined randomly, but in consultation with a specialist. It may be necessary to take a larger dose than usual because more of the active ingredient needs to be absorbed into the body.

In today's conditions, the use of tea bags is increasing day by day. Ease of brewing and practical use are among the advantages of tea bags. However, the raw materials used in its production may cause some health problems. Although the abaca fiber used in tea bag production is a completely natural raw material, it has disadvantages such as inability to heat seal and shape, dust leakage, opacity, difficulty in infusion. PLA, which is used together with cellulose fibers to overcome these disadvantages, is a fully degradable polymer obtained by fermentation of natural resources such as starch, corn and sugar cane. In addition to natural sources, products such as nylon also have advantages such as ease of infusion and not affecting the taste of tea, but microplastics that pose health problems can pass into the water. For tea bags that are frequently used, raw materials that are easy to process and do not cause health problems should be used.

The benefits of herbal teas, which have been used in treatment for thousands of years, are undeniable. In order to fully benefit from their benefits, production steps should be carried out safely and products that will not cause physical, biological and chemical problems in terms of health should be produced.

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THE EFFECT OF DIFFERENT DRYING METHODS ON SOME CHEMICAL AND BIOACTIVE COMPONENTS OF ORANGE AND BLACK CARROT POWDERS

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ABSTRACT

In this study, two different varieties of carrots (orange and black carrots) were dried with three different drying methods (hot air, microwave and freeze-drying) and ground to obtain carrot powders. Color values, ash, protein, fat, antioxidant activity, total phenolic content (TPC), β-carotene, total anthocyanin (TA) and mineral matter of the carrot powders were determined. Orange carrot powders showed higher lightness, redness and yellowness values than black carrot powders. The freeze-drying method provided higher lightness value in both carrot powder varieties. Different drying methods did not cause a significant ($p>0.05$) change on the ash, protein and fat content of carrot powders. The antioxidant activities and TPC of orange and black carrot powders varied between 75.16-76.60% and 87.52-95.75%, 263.50-470.90 mgGAE/100g and 596.10-1353.40 mgGAE/100g, respectively. β-carotene content of orange carrot powders dried with different methods ranged between 39.34 mg/100g and 48.72 mg/100g, and β-carotene content of freeze-dried samples was found to be higher than other drying methods. The TA amount of black carrot powders varied between 259.08 mgCGE/100g and 424.66 mgCGE/100g. The freeze-drying method resulted in the highest TA content, while the hot air drying method revealed the lowest TA content. Black carrot powders for all drying methods had higher Fe, K, Mg and P contents than orange carrot powders. Different drying methods did not change the mineral amounts in both carrot powders.

Keywords: Drying, orange carrot, black carrot, powder, bioactive component

FARKLI KURUTMA YÖNTEMLERİNİN TURUNCU VE SİYAH HAVUÇ TOZLARININ BAZI KİMYASAL VE BİYOAKTIF BİLEŞENLERİ ÜZERİNE ETKİSİ

Bu çalışmada, iki farklı havuç çeşidi (turuncu ve siyah havuç) üç farklı kurutma yöntemiyle (sıcak hava, mikrodalga ve dondurarak kurutma) kurutulmuş ve öğütülerek havuç tozları elde edilmiştir. Havuç tozlarının renk değerleri, kül, protein, yağ, antioksidan aktivite, toplam fenolik madde (TFM), β- karoten, toplam antosiyanin (TA) ve mineral madde miktarları belirlenmiştir. Turuncu havuç tozları, siyah havuç tozlarına göre daha yüksek parlaklık, kırmızılık ve sarılık değerleri göstermiştir. Dondurarak kurutma yöntemi her iki havuç tozu çeşidinde de daha yüksek parlaklık değeri sağlamıştır. Farklı kurutma yöntemleri havuç tozlarının kül, protein ve yağ içeriklerinde önemli ($p>0.05$) bir değişikliğe neden olmamıştır. Turuncu ve siyah havuç tozlarının antioksidan aktiviteleri ve TFM miktarları sırasıyla %75.16-76.60 ve %87.52-95.75, 263.50-470.90 mgGAE/100g ve 596.10-1353.40 mgGAE/100g arasında değişmiştir. Farklı yöntemlerle kurutulan turuncu havuç tozlarının β-karoten içeriği 39.34 mg/100g ile 48.72 mg/100g arasında değişirken, dondurularak kurutulmuş örneklerin β-karoten içeriği diğer kurutma yöntemlerine göre daha yüksek bulunmuştur. Siyah havuç tozlarının TA miktarı 259.08 mgCGE/100g ile 424.66 mgCGE/100g arasında değişmiştir. Dondurarak kurutma yöntemi en yüksek TA içeriği verirken, sıcak havayla kurutma yöntemi en düşük TA içeriğini ortaya çıkarmıştır. Siyah havuç tozları tüm kurutma yöntemlerinde, turuncu havuç tozlarından daha yüksek Fe, K, Mg ve P içeriğine sahip olmuştur. Farklı kurutma yöntemleri her iki havuç tozunda da mineral madde miktarlarını değiştirmemiştir.

Anahtar kelimeler: Kurutma, turuncu havuç, siyah havuç, toz, biyoaktif bileşen

INTRODUCTION

Carrot (*Daucus carota L.*) is one of the most widely produced root vegetables in world agriculture. Daucus, which includes sixty species of several cultivars, has color ranging from white to yellow, orange, light purple, deep red or violet (Rodriguez et al., 1975). Orange carrots are an important source of carotenoids. Approximately 60% of the total carotenoid content of ripe orange carrots is β-carotene, 20% α-carotene, and the remainder is lycopene, γ-carotene, ζ-carotene, and/or β-zeacarotene (Banga & De Bruyn, 1964; Gabelman, 1974; Simon & Wolff, 1987). Although orange color is the dominant color for carrots, black carrot has been attracting attention for its bluish color with high levels of anthocyanins. The origin of black carrot is Turkey, Middle and Far East, and it has been cultivated for at least 3000 years (Kamiloglu et al., 2016). Carrot varieties are also rich sources of vitamins, minerals and dietary fiber. The nutritional properties and bioactive content and also attractive colors of orange and black carrots have led to research on the usage areas of carrots.

There are some studies in the literature on the powdering of carrots using different methods and the use of its powder in various products. Wang & Xi, (2005) used the microwave drying method for drying carrots. The technological and nutritional differences between the dried samples were investigated by changing the amount of carrots placed in the microwave tray (100g, 200g and 300g) and the applied microwave power (120, 160 and 240 W). A decrease in the amount of β-carotene was observed as the microwave power increased. Gong et al. (2015) compared the effects of different drying methods on the color characteristics and β-carotene contents of carrot powders. Carrot powder was obtained by using vacuum drying, hot air drying, microwave drying and freeze-drying methods. Carrot powders dried with hot air had the lowest β-carotene content (114.4 mg/kg), while the highest value (344.8 mg/kg) was obtained by freeze- drying method.

In this study, it was aimed to determine the effects of different drying methods (hot air, microwave and freeze-drying) on the color, some chemical and bioactive components of carrot powders obtained from orange and black carrots.

MATERIAL AND METHODS

Materials

Orange and black carrots were obtained from the cold storages of Kaşınhanı town in Konya, Turkey. All chemicals used in analysis were of analytical grade quality.

Production of carrot powders

Carrots were washed, peeled and cut into thin slices. Carrot slices were first heat treated in boiling water (3 minutes at 90 ºC) and then the following drying methods were applied to the carrots. Drying in hot air flow: carrots were dried in a drying cabinet (Nüve KD 200, Ankara, Turkey) at 60 ºC for 10 hours (Akubor & John Ike, 2012). Microwave drying: Carrot samples were dried in a household microwave oven (LG Solardom, Seoul, South Korea) at 360 W power for 45 minutes according to (Prakash et al., 2004). Freeze-drying: carrots were dried in a freeze-drying device (Scanvac, CoolSafe, Denmark) at -54 ºC for 24 hours (Lee et al., 2003). After all the dried samples were ground, they were sieved through a sieve with a diameter of 500 micrometer and stored under refrigerator conditions for analysis.

Color measurement

The color values of the carrot powders were measured using Hunter Lab Chroma Meter (Minolta CR-400, Osaka, Japan) in terms of the Hunter L*, a* and b* values. Also, according to a* and b* values, chroma ($(a^{*2}+b^{*2})^{1/2}$) and Hue angle (if a*>0 and b*>0 Hue=arctan [b*/a*]; if a*>0 and b*<0 Hue=360+arctan [b*/a*]) were calculated.

Chemical and bioactive components analysis

Ash, protein and fat content of carrot powders were determined according to AACC 08-01.01, 46-12.01 and 30-25.01 standard methods (AACC, 1999). The TPC was determined colorimetrically using the Folin Ciocalteau method. Extraction was conducted according to Gao et al. (2002) and Beta et al. (2005). Absorbance was measured at 760 nm using a

spectrophotometer (Hitachi-U1800, Japan) and results were expressed as mg Gallic acid equivalent (Gámez-Meza et al., 1999; Slinkard & Singleton, 1977). Antioxidant activity was determined by 2-2-Diphenyl-2-picrylhydrazyl (DPPH) method (Beta et al., 2005; Gyamfi et al., 1999). Absorbances were measured at 517 nm and the inhibition percentage was calculated. The β-carotene content of the samples was determined according to Prakash et al. (2004) with some modification of the method. The analysis of TA in the samples was carried out according to the method specified by (Ficco et al., 2014). For the analysis of Ca, Fe, K, Mg, P and Zn elements 1 g dry sample was dissolved by the wet burning method in the microwave combustion system (Mars 5, CEM Corporation, USA) using 10 ml of sulfuric acid + nitric acid. The mineral substance amounts of the obtained filtrates were measured on an ICP-AES (inductively coupled plasma atomic emission spectrophotometer) instrument (Vista Series, Varian International, AG, Switzerland) (Skujins, 1998).

Statistical analysis

Minitab version 16 statistical program was used for statistical analysis. Means were compared at the $p<0.05$ level.

RESULTS AND DISCUSSION

Color values

Color values of carrot powders produced with different drying methods are given in Table 1. L*, a*, b*, C* and hue^{o} values of carrot powders varied between 39.28 and 81.25, 12.19 and 26.78, -4.02 and 44.26, 12.84 and 51.80 and 57.11 and 346.75, respectively. The highest L* value was determined in the orange carrot powder obtained by freeze-drying. The L* value of the powders obtained by freeze-drying method in both carrot cultivars was found to be higher than the L* value of the powders obtained by other drying method. Exposure of samples to heat in hot air and microwave drying methods may have caused this situation. In general, orange carrot powders gave higher a* and b* values than black carrot powders. The yellow and red color of the carrot is attributed to the presence of carotenes (Wagner & Warthesen, 1995). The fact that orange carrots are richer in carotenes than black carrots may have caused these results. When a* value of orange carrot powders was evaluated in terms of drying method; it was seen that a* value of the orange carrot powders obtained by freeze-drying was higher than a* value of the powders obtained by applying hot air. Although the a* value of black carrot powders did not show a statistical difference depending on the drying methods, the powders obtained by freeze-drying method showed the highest a* value numerically. When the orange carrot powders were examined in terms of b* value; as with a* value, the b* value of the powders obtained by freeze-drying method was found to be higher than the b* value of the powders obtained by hot air. The use of different drying methods in the production of black carrot powder did not cause a statistical difference on the b* value. The fact that the drying time is longer in the hot air drying method compared to the microwave drying method, and the temperature is higher than the freeze-drying method may have caused more loss in carotenoid pigments and a decrease in a* and b* values. Freeze-drying method gave higher C* values in orange carrot powders than hot air and microwave drying methods, and no significant difference was observed between hot air and microwave drying methods in terms of C* value. Although the C* values of black carrot powders did not show a statistical difference according to the drying method, the carrot powder obtained by freeze-drying method gave the highest numerical value. Drying methods were not effective on the hue^{o} value of orange and black carrot powder samples, and no statistical difference was determined between the results. Howard et al. (1996) reported that the lightness of the carrot is affected by the processing temperatures, and higher temperatures cause a darker color. It was reported in another study that the L*, a* and b* values of the dried carrot samples decreased with the increase in the temperature applied during drying (Xiao et al., 2010). In a study, freeze-dried, vacuum-microwave and hot air drying methods were used for the drying of carrots; It was determined that freeze-dried carrot slices had the

highest L*, a* and b* values. It has been reported that drying in hot air causes more reduction in lightness value due to greater exposure to oxygen and higher temperature (Cui et al., 2008).

Table 1. Color values of carrot powders produced with different drying methods

Carrot varieties	Drying method	L*	a*	b*	C*	Hue°
Orange	Hot air	70.81±2.07b	19.20±1.34bc	35.75±1.94b	40.58±2.35b	61.78±0.37b
	Microwave	72.26±0.01b	23.88±2.25ab	36.90±0.73ab	43.98±0.60b	57.11±2.98b
	Freeze-drying	81.25±3.78a	26.78±1.72a	44.26±4.22a	51.80±2.71a	58.72±4.06b
Black	Hot air	39.28±2.59d	12.19±0.1d	-4.02±0.15c	12.84±0.05c	341.73±0.77a
	Microwave	41.53±0.82d	12.84±1.02d	-3.92±0.15c	13.43±0.94c	342.94±1.89a
	Freeze-drying	51.11±0.77c	13.36±1.50cd	-3.12±0.17c	13.73±1.40c	346.75±2.10a

Means with the different letter within a column are significantly different ($p < 0.05$).

Chemical and bioactive components

The chemical analysis results of orange and black carrot powders dried with three different methods are given in Table 2. Average ash, protein and fat amounts of carrot powders were determined as 5.77%, 8.232% and 2.34%, respectively. When the orange and black carrot powders were evaluated in terms of drying method, it was seen that the drying methods did not cause any change on the ash, protein and fat contents of the carrot powders. In general, black carrot powders had higher ash content than orange carrot powders.

Antioxidant activity and TPC of carrot powders obtained by different drying methods are presented in Table 2. Although the drying method is statistically insignificant on the amount of antioxidant activity of orange carrot powders, the lowest value was determined numerically in the samples prepared by the hot air drying method, and the highest value in the samples prepared by the freeze-drying method. When the black carrot powders were evaluated in terms of drying method, the carrot powders obtained by freeze-drying method had numerically higher antioxidant activity than the carrot powders dried in hot air and microwave. These changes may be resulted from the applied temperature during drying and long drying times. Similar results were found in other studies comparing the hot air method with other methods in drying carrots, and it was reported that the losses in microcomponents determined as a result of hot air drying were caused by the length of the process time, high temperature and oxidation (as a result of the presence of oxygen) (Chen et al., 2017; Cui et al., 2008; Lin et al., 1998).

The highest amount of TPC in carrot powders was determined in black carrot powder produced by freeze-drying method. Regardless of the drying method, black carrot powders yielded higher TPC than orange carrot powders. When the orange carrot powders were compared in terms of drying method, drying method did not change the TPC statistically, but the powders obtained by the hot air drying method had a lower TPC numerically. When the black carrot powders were evaluated among themselves in terms of drying method, it was determined that the TPC of black carrot powder obtained by freeze-drying method was significantly higher than the TPC of black carrot powder obtained by other methods. Presence of phenolic compounds in carrots contributes to sensory qualities such as color (Zhang et al., 2005), bitterness (Kreutzmann et al., 2008) and aroma (Naczk & Shahidi, 2003). Therefore, the response of phenolic compounds can be used as a good indicator to evaluate the quality of

vegetables during processing and storage (Gonçalves et al., 2010). In general, it was determined that black carrot powders had higher antioxidant activity and TPC amount than orange carrot powders.

For the orange carrot powders, β-carotene content obtained by freeze-drying method (48.72 mg/100g) was found to be higher than that of powders dried in microwave and hot air (39.34 and 40.35 mg/100g) (Table 2). For black carrot powders, the highest amount of TA was determined in the freeze-drying powder (424.66 mgCGE/100g), followed by microwave (344.24 mgCGE/100g) and hot air (259.08 mgCGE/100g) dried samples. The amounts of β-carotene and TA of orange and black carrot powders obtained by freeze-drying method were found to be higher than powders obtained by other methods. By the fact that the application temperature of the freeze-drying process is low and the drying process takes place under a high vacuum, which reduces the oxidation and degradation of β-carotene and similar microcomponents (Cui et al., 2008; Lin et al., 1998).

Mineral contents

The mineral analysis results of orange and black carrot powders prepared with different drying methods are given in Table 3. Ca, Fe, K, Mg, P and Zn content of carrot powders ranged between 396.27-418.13 mg/100g, 1.29-1.92 mg/100g, 224.23-297.13 mg/100g, 288.98-368.74 mg/100g, and 1.42-2.92 mg/100g. The applied drying methods did not cause a statistical change on the mineral substance content of orange and black carrot powders. Fe, K, Mg and P content of black carrot powders had higher than that of orange one.

DISCUSSION

In this study, the color values, some chemical and bioactive components of orange and black carrot powders prepared using different drying methods were compared. When the results are evaluated in terms of carrot variety; black carrot powder had lower L*, a* and b* color values and generally higher ash, antioxidant activity, TPC and mineral substance amounts than orange carrot powder. In the comparison made according to the drying method; higher lightness, TPC, β-carotene and TA values were obtained in the carrot powders obtained by the freeze-drying method compared to other drying methods.

Table 2. Chemical and bioactive component results of carrot powders produced with different drying methods

Carrot varieties		Drying method	Ash (%)	Protein (%)	Fat (%)	Antioxidant activity (%)	TPC (mgGAE/100g)	β-carotene (mg/100g)	TA (mgCGE/100g)
Orange	Oven	Hot air	5.71±0.07b	7.93±1.95a	2.16±0.25a	75.16±5.40b	263.50±5.50d	40.35±1.12b	nd
		Microwave	5.69±0.07b	7.93±0.40a	2.08±0.14a	75.70±5.02b	430.00±17.80cd	39.34±0.85b	nd
		Freeze-drying	5.68±0.03b	7.97±0.21a	2.15±0.18a	76.60±4.74b	470.90±16.80cd	48.72±0.69a	nd
	Black	Hot air	5.88±0.92a	8.49±0.52a	2.55±0.76a	87.52±1.19ab	596.10±16.70bc	nd	259.08±9.76c
		Microwave	5.84±0.03a	8.54±0.28a	2.53±0.20a	87.69±0.89ab	742.80±21.70b	nd	344.24±6.67b
		Freeze-drying	5.83±0.03a	8.55±0.23a	2.59±0.20a	95.75±3.50a	1353.40±134.60a	nd	424.66±9.01a

Means with the different letter within a column are significantly different ($p < 0.05$). Results are dry matter basis. TPC: Total phenolic content. TA: Total anthocyanin.

Table 3. Mineral matters (mg/100g) of carrot powders produced with different drying methods

Carrot varieties	Drying method	Ca	Fe	K	Mg	P	Zn
Orange	Hot air	403.48±3.18a	1.30±0.06b	1630.90±58.8b	224.23±5.47b	293.19±4.16b	2.92±0.14a
	Microwave	396.27±8.40a	1.29±0.10b	1619.20±24.0b	234.23±7.10b	290.88±12.55b	2.84±0.17a
	Freeze-drying	396.27±4.65a	1.31±0.04b	1623.70±30.4b	235.44±8.75b	288.98±12.40b	2.83±0.23a
Black	Hot air	418.13±3.20a	1.89±0.03a	2296.90±101.0a	296.00±14.90a	361.48±8.79a	1.42±0.23b
	Microwave	414.94±7.13a	1.92±0.04a	2295.50±1.60a	287.07±4.31a	368.74±3.52a	1.50±0.03b
	Freeze-drying	417.69±7.25a	1.90±0.06a	2299.70±4.80a	297.13±7.28a	355.64±10.51a	1.47±0.04b

Means with the different letter within a column are significantly different ($p < 0.05$). Results are dry matter basis.

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ANTIMICROBIAL ACTIVITY OF A NEW DEVELOPED CREAM FORMULATION WITH NATURAL ADDITIVES: *Citrus medica* L. var. *sarcodactylis* FRUIT ETHANOL EXTRACT AND PROBIOTIC

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ABSTRACT

Citrus medica L. var. *sarcodactylis* is a morphologically remarkable fruit that grows in subtropical regions. It is accepted as food and nutrient rich in bioactive components, with high antioxidant activity and can be consumed safely. The chemicals used in cosmetic products cause skin irritation and allergic reactions. For this reason, herbal compounds offer natural options that support and protect skin health with their antimicrobial properties and skin care effects. In this study, it was aimed to create a new cream formulation by combining plant extract and probiotic as natural ingredients and to determine its antimicrobial activity. For this purpose, the cream formulation was developed using *C. medica* L. var. *sarcodactylis* ethanol extract and *Limosilactobacillus fermentum* MA-7, a probiotic candidate strain derived from human milk and commercial cream. The antibacterial and antifungal activities of the developed cream formulations against test microorganisms were determined using the well diffusion method. In the commercial cream (control, C) group, the inhibition zone diameter was not determined against *Candida glabrata* RSKK 04019, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* O157:H7 and *Listeria monocytogenes* ATCC 7644. The developed groups of cream and *L. fermentum* MA-7 (CL), cream and the extract (CE) and cream containing extract and *L. fermentum* MA-7 (CEL) showed the highest inhibition zone diameters against *S. epidermis* ATCC 12228 (6.52 mm), *S. aureus* ATCC 25923 (6.06 mm) and *E. coli* O157:H7 (15.75 mm), respectively. The CEL group against all tested microorganisms exhibited higher antimicrobial activity compared to other developed cream groups (CE and CL). The results showed that the developed cream formulation with natural content can be used as an antimicrobial agent in the cosmetic and pharmaceutical industries to develop alternative products alternative to chemical substances.

Keywords: skin, cosmetic, antibacterial, probiotic

INTRODUCTION

Plants are among the sources to be used as antimicrobial agents (Ginovyan et al., 2017). The antimicrobial properties of plants are realized thanks to the bioactive compounds such as flavonoids, phenolic compounds, alkaloids, terpenoids, tannins, steroids they contain (Archana and Bose, 2022). *Citrus medica* L. var. *sarcodactylis* (Rutaceae) is a morphologically diverse fruit that can grow in subtropic (Karp and Hu, 2018). It is a rich source of terpenoids (Xu et al., 2019). Various chronic diseases are treated using it as a raw material in traditional Chinese medicines. *C. medica* L. var. *sarcodactylis*, with its high antioxidant activity, is reliably consumed as food and nutrients (Mahdi et al., 2019).

The skin is an organ that provides the first interaction of the human body with the external environment and serves as the primary line of defense (Byrd et. al., 2018). The skin surface creates a protective barrier against environmental factors, preventing the invasion of sun rays, harmful substances, and harmful microorganisms, and maintaining the moisture balance of the skin (Yousef et al., 2017). Recently, there has been a significant increase in skin problems. For this reason, people show interest in personal care products applied to the skin surface. However,

many products cause skin irritations and allergic reactions due to their chemical content (Adu et al., 2020). For this reason, natural substances that do not show allergic reactions are preferred in cosmetic products. The substances contained in the ingredients of cosmetic products applied to the skin surface can also create suitable environments for the reproduction of harmful microorganisms (Ecer, 2019). At the same time, cosmetic products carry a risk of microbial contamination. These microorganisms can pose a health hazard and cosmetic products need to be protected from contamination (Michalek et al., 2019).

Recently, probiotics are natural ingredients that have attracted great attention in the health and cosmetic industry. Probiotics exhibit antimicrobial and protective properties against skin and gastrointestinal tract reactions (Patil et. al., 2020; Poruhsky et al., 2018). Probiotics added to creams as a solution to skin problems have topical applications (Patil et al., 2020). Topical probiotics have a promising role in wound healing and the treatment of some inflammatory skin diseases (Poruhsky et al., 2018). The topical use of probiotic products creates direct effects on the application area by strengthening the skin's natural defense barrier (Al-Ghazzewi and Tester, 2014).

In the study, the antimicrobial and antifungal activities of the cream formulation developed with ethanol extract obtained from *C. medica* L. var. *sarcodactylis* fruit and probiotic candidate strain *Limosilactobacillus fermentum* MA-7 originating from human milk was determined against test microorganisms. The potential of the developed cream formulation for use in the cosmetic and pharmaceutical industries has been investigated.

MATERIAL AND METHOD

Supply of plant materials

The *C. medica* L. var. *sarcodactylis* fruit (Figure 1) was obtained from the Alata Horticultural Research Institute (Turkey-Mersin) in November 2022.

Figure 1. A, B: *C. medica* L. var. *sarcodactylis* fruits

Preparation of plant extract

The fruits of *C. medica* L. var. *sarcodactylis* were dried in the shade and powdered with a blender (Waring). A homogeneous mixture of fruit (10 g) and ethanol solvent (30 ml) was obtained. The extraction process was completed using the water bath at 70˚C for 2 days (24 hours). The solvent was removed from the extracts by evaporation. The extracts were dissolved with dimethylsulfoxide (DMSO) and sterilized using a 0.45 µm filter. The extract was stored at +4°C and used for in vitro antimicrobial activity study.

Test microorganisms

The antimicrobial activity of the cream formulation developed with plant extracts and/or probiotics was evaluated using six test microorganisms. The strains include Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *S. epidermis* ATCC 12228 (Nutrient Broth, 37˚C), *Listeria monocytogenes* ATCC 7644 (Tryptic Broth, 30˚C), Gram-negative bacteria *Escherichia coli* O157:H7 (Nutrient Broth, 37˚C) and yeasts *Candida glabrata* RSKK 04019, *C. albicans* ATCC 10231 (Yeast Peptone Dextrose, 30˚C). The probiotic candidate lactic acid bacteria *L. fermentum* MA-7 (Man Rogosa and Sharpe, 37˚C) were incubated for 24 hours.

Determination of antimicrobial activity of cream formulations containing *C. medica* L. var. *sarcodactylis* ethanol extract and/or *Limosilactobacillus fermentum* MA-7

The antimicrobial and antifungal activities of the cream formulation were determined using the method of Asan-Ozusaglam and Celik (2023). In the cream formulations developed for antimicrobial purposes, *C. medica* L. var. *sarcodactylis* ethanol extract and/or human milk originated probiotic strain *L. fermentum* MA-7 (Asan-Ozusaglam and Gunyakti, 2019) were used. The antimicrobial activity of the cream formulations was determined using the well diffusion method. The petri dishes were incubated at suitable conditions as mentioned above for the test microorganisms.

Statistical Analysis

The antibacterial and antifungal activity assay results of the cream formulations developed with the *C. medica* L. var. *sarcodactylis* extract were analyzed using GNU-SPSS software. Statistical significance level was determined by one-way analysis (ANOVA) with Tukey's post-hoc test. The difference between the results was considered significant ($p<0.05$).

RESULTS AND DISCUSSION

The antibacterial and antifungal activities of the cream formulation prepared using the ethanol extract obtained from *C. medica* L. var. *sarcodactylis* fruit and/or the probiotic candidate strain *L. fermentum* MA-7 were determined by the well diffusion method. The inhibition zone diameters of the developed cream formulations against test microorganisms are given in Table 1. The biological activity of the control group (C) against *C. glabrata* RSKK 04019, *S. aureus* ATCC 25923, *E. coli* O157:H7 and *L. monocytogenes* ATCC 7644 strains was not determined. The highest inhibition zone diameter was determined against *S. aureus* ATCC 25923 (6.06 mm) for the cream and extract group (CE), while the extract and probiotic containing group (CEL) was observed against *E. coli* O157:H7 (15.75 mm). The cream formulation developed with *C. medica* L. var. *sarcodactylis* ethanol extract and *L. fermentum* MA-7 strain (CEL) shows a significant inhibitory effect on *E. coli* O157:H7 and *S. epidermis* ATCC 12228, indicating that it may have the potential to be used as a natural antimicrobial agent. It was determined that most of CL group had higher inhibitory activity against the tested microorganisms compared to the cream (control, C) group. However, C and CL groups did not show any antibacterial activity against *E. coli* O157:H7 and *L. monocytogenes* ATCC 7644. The inhibitory activity against all tested strains was observed in all CE groups. Especially, CEL group was found to increase the diameters of the inhibition zone against all test microorganisms compared to other cream groups. *C. medica* L. var. *sarcodactylis* ethanol extract and *L. fermentum* MA-7 strain may have increased biological activity by creating a synergetic effect on the test strains.

Table 1. Antimicrobial activity of the developed cream formulations

Microorganisms	**Inhibition Zone Diameters (mm±SD)**				
	C	**CL**	**CE**	**CEL**	**F(Sig)**
***C. glabrata* RSKK 04019**	NA^{a}	2.21±0.44^{b}	3.63±0.04^{b}	5.70±1.11^{c}	48.782(0.000)
***C. albicans* ATCC 10231**	2.08±0.19^{a}	4.01±1.16^{b}	1.55±0.31^{a}	8.59±0.15^{c}	82.256(0.000)
***S. aureus* ATCC 25923**	NA^{a}	1.25±0.10^{a}	6.06±1.40^{b}	9.42±0.96^{c}	79.251(0.000)
***S. epidermis* ATCC 12228**	3.14±0.39^{a}	6.52±0.44^{b}	2.85±0.34^{a}	12.27±0.73^{c}	231.813(0.000)
***E. coli* O157:H7**	NA^{a}	NA^{a}	3.87±0.38^{b}	15.75±0.89^{c}	716.336(0.000)

***L. monocytogenes* ATCC 7644**	NA[a]	NA[a]	2.36±0.26[b]	3.27±0.57[c]	83.814(0.000)

*C: Cream (Control), CL: Cream and *L. fermentum* MA-7, CE: Cream and Extract, CEL: Cream containing *L. fermentum* MA-7 and Extract, NA: No activity

*Different letters indicate significant difference at $p<0.05$.

Dahmani et al. (2022), the antibacterial activity of the extract obtained from *C. reticulata* peel using methanol solvent was determined against *S. aureus* (ATCC 29213) and *E. coli* (ATCC 25922). The wound healing activities of the ointment prepared using two different concentrations of the extract (5% and 10%) were investigated. It has been determined that the bioactive compounds present in *C. reticulata* peel have the potential for wound healing due to their content. According to Valizadeh et al. (2020) the MBC concentration of *C. aurantifolia* oil against *S. aureus* ATCC 25923, commonly found in wounds, were recorded as 47.61. It was determined that the ointment obtained from *C. aurantifolia* oil may be useful in the development of alternative products to provide tissue repair and accelerate the healing process.

CONCLUSIONS

The antibacterial and antifungal activities of the cream formulation developed with *C. medica* L. var. *sarcodactylis* ethanol extract and *L. fermentum* MA-7 against the tested strains was determined in vitro. It is observed that in the cream formulation, the ethanol extract and the *L. fermentum* MA-7 strain obtained from human milk have a synergetic effect against test microorganisms and increase the inhibition zone diameters. It has been determined that the developed cream formulation can be an alternative to synthetic preservatives used in the cosmetic and pharmaceutical industries as an antimicrobial preservative with natural additives.

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INVESTIGATION OF CORNELIAN CHERRY FRUIT AS A NATURAL ADDITIVE IN THE INDUSTRY

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ABSTRACT

In many industries, antimicrobial agents are used as additives against pathogenic microorganisms. Today, these substances, which are used commercially, are replaced by natural antimicrobial agents obtained from plants. Cornelian cherry (*Cornus mas* L.), which has the potential to be an antimicrobial agent, is a fruit grown in Turkey with high antioxidant and anthocyanin content. In this study, the antibacterial and antifungal activities of cornelian cherry extracts prepared with water and chloroform solvents on *Salmonella pullorum*, *Vibrio angillarum* A4, *Aeromonas hydrophila* ATCC 19570, *Candida albicans* ATCC 10231, *Escherichia coli* O157:H7 pathogens were investigated. The antimicrobial activity of the extracts was determined with disc diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC or MFC) methods. The highest zone diameter of cornelian cherry was determined on *S. pullorum* (18.06 mm) for chloroform extract and on *A. hydrophila* ATCC 19570 (16.06 mm) for water extract. MIC values of the extracts ranged from 5 µg/µl to 40 µg/µl. The lowest cidal value was obtained for the chloroform extract as 10 µg/µl (MBC) against *S. pullorum*. The results determined that cornelian cherry fruit extracts have the potential to be alternative natural antimicrobial additives against synthetic agents in various industries such as food, feed and pharmaceutical.

Keywords: Antimicrobial activity, *Cornus mas* L., Extract, Natural additive

INTRODUCTION

Recently, the use of medicinal plants for disease prevention and treatment purposes has been increasing rapidly. Plants exhibit antimicrobial activity due to biophenols, phenolic compounds and antioxidants in their structures (Rahaiee el al, 2015). Cornelian cherry (*Cornus mas* L.), which has a wide distribution area in our country, belongs to the Cornaceae family. Cornelian cherry fruit has a high biological value and is rich in phenolic compounds, ascorbic acid and anthocyanin content (Kazımierski et al.,2019). It has effects such as anti-inflammatory, antioxidant, antimicrobial, antiparasitic, antidiabetic, hepatoprotective, cardioprotective, nephroprotective and anticancer (Hosseinpour-Jaghdani et al., 2017). It is also used in folk medicine for various diseases such as skin diseases, diarrhea, intestinal inflammation, cancer, fever, urinary tract infections *(Uğur et al., 2020).*

Candida species are commensal microorganisms found in bronchial secretions, the oral mucosa, skin folds, urine, feces, digestive and vaginal tracts of humans (Hsu et al., 2020). Although about 20 species cause infection in humans, *Candida albicans* is the most common pathogenic strain, especially in immunocompromised person (Sardi et al., 2013).

Foodborne diseases are an important problem that threatens people's health (Takó et al., 2020). Food contamination occurring at various stages of the production process poses a serious global health issue, leading to foodborne illnesses and severe diseases (Yang et al., 2017). Even animals raised in hygienic conditions can carry many disease-causing bacteria such as *Salmonella* spp., *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Listeria monocytogenes*. These bacteria cause infection by infecting the human body through food and water. Despite significant efforts in the food industry, the presence of these bacteria persists due to contamination and processing procedures in slaughterhouses (Das et al., 2017). This situation can cause economic losses for the food industry and serious damage to public health. In particular, some plant extracts, essential oils and antioxidants inhibit or slow the growth of bacteria and microorganisms in foods (Takó et al., 2020). These natural ingredients help foods last longer and prevent microbial contamination that can threaten human health. In addition, it provides an alternative to the chemicals used and preservatives (Yu et al., 2021).

Aquaculture, a fast-expanding sector, plays a vital role in supplying humans with a crucial source of protein and micronutrients (Carbone and Faggio, 2016). However, diseases caused by pathogens are important problems in this industry. *Vibrio, Aeromonas, Yersinia, Lactococcus, Streptococcus, Acinetobacter, Clostridium* and *Pseudomonas* species are among the pathogens that cause serious financial losses and diseases (Yi et al., 2018). In addition, the excessive use of antibiotics against emerging diseases and the emergence of antibiotic-resistant strains poses a global threat to both humans and animals (Larsson and Flach, 2022). In aquaculture, secondary metabolites contained in plants are used to keep diseases under control. These plant compounds can be added to the feeds used in aquaculture as additives and natural antimicrobial agents and provide an effective solution to combat disease (Ahmadifar et al., 2021).

In study, the antimicrobial activity of water and chloroform extracts of cornelian cherry fruit against various clinical, food-borne and animal origin pathogens and their potential use as natural additives were investigated.

MATERIAL AND METHOD

Preparation of Extracts

The fruits were washed with distilled water and dried in the open air in a sun-free environment. The dried fruit samples were grounded using a Waring blender. The grounded fruit samples were vortexed with chloroform and water solvents (20 grams of fruit powder and 60 ml of solvent) and then sonicated for 20 minutes (for 2 days). After extraction, the solvents were evaporated and then stored (+4°C).

Determination of Antimicrobial Activity

Antimicrobial activity of cornelian cherry water and chloroform extracts was determined using the disc diffusion method. *S. pullorum* (Nutrient Broth (NB)), *V. anguillarum* A4 (2% salt Tryptic Soy Broth (TSB)), *A. hydrophila* ATCC 19570 (Nutrient Broth (NB)), *E. coli* O157:H7 (Nutrient Broth (NB)), *C. albicans* ATCC 10231 (Yeast Extract Peptone Dextrose (YPD)) strains were used as test microorganisms for 24-hours. Test microorganisms were washed twice with saline solution and bacterial concentration (0.5 McFarland) was adjusted. 0.1 ml of the prepared McFarland solution was spread on solid agar. Then, sterile discs (6 mm) were placed in petri dishes in 3 repetitions. 0.02 ml (4 mg/disc) of fruit extracts were dripped onto the discs. The recorded results were obtained by measuring the zone diameters formed around the discs after a 24-hour incubation period, using a caliper.

Determination Minimum Inhibition (MIC) and Minimum Bactericidal and/or Fungicidal Concentration (MBC and/or MFC)

The minimum inhibition and the minimum bactericidal and/or fungicidal concentration of fruit water and chloroform extracts were determined using the micro-dilution method. The fruit extracts were added to the tubes at a final concentration of 40 µg/µl and the mixture was diluted. After the tubes were incubated for 24-hours, MIC values were recorded. After spot dropping the samples from each tube onto solid media, they were incubated for 24 hours. The resulting MBC or MFC values in the solid medium were then recorded.

Statistical Analysis

The antimicrobial activity assay results of cornelian cherry extract were subjected to statistical analysis using GNU-SPSS software. A one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was performed to assess the significance of differences between the experimental groups.

RESULTS AND DISCUSSION

The biological activities of cornelian cherry extracts (water and chloroform) were determined by disc diffusion and microdilution methods. The inhibition zone diameters against the test microorganisms for the extracts are given in Table 1. The highest inhibition zone diameter of the cornelian cherry water and chloroform extract was determined against *A. hydrophila* ATCC 19570 (16.06 mm) and *S. pullorum* (18.06 mm). The lowest inhibition zone diameter was obtained against *V. anguillarum* A4 for the water extract as 12.15 mm and for the chloroform extract as 11.15 mm. In addition, it was determined that the water and chloroform extracts had an inhibition zone diameter of 13.97 mm and 14.33 mm against *C. albicans* ATCC 10231. It has been determined that cornelian cherry extracts have antibacterial and antifungal effects on the tested microorganisms.

Table 1. Inhibition zone diameter of the extracts from cornelian cherry fruit

Microorganisms	**Extracts**	
	CW (mm±SD)	**CC (mm±SD)**
Salmonella pullorum	13.55±0.3	18.06±0.6^{a}
Escherichia coli O157:H7	13.91±1.1	15.35±1.1^{b}
Aeromonas hydrophila ATCC 19570	16.06±1.6	17.37±0.1^{a,b}
Vibrio angillarum A4	12.15±0.2	11.15±0.3^{c}
Candida albicans ATCC 10231	13.97±2.5	14.33±0.9^{d,b}
F(Sig)	2.780(0.086)	37.975(0.000)

*CW: Cornelian cherry Water extract, CC: Cornelian cherry Chloroform extract
*Different letters show significant difference at $p<0.05$ between samples.

In a study, the biological activity of cornelian cherry water and methanol extracts on some clinical isolates was investigated. The water extract showed an inhibition zone diameter of 10 mm against *E. coli*, but no inhibitory activity against *C. albicans*. It was observed that the methanol extract had an inhibition zone diameter of 10 mm against *E. coli* and 8 mm against *C.*

albicans (Yigit, 2018). Milenković-Anđelković et al. (2015) was determined the antimicrobial activity against *E. coli* (ATCC 25922) and *C. albicans* (ATCC 10231) pathogens by disc diffusion method. The Cornelian cherry fruit harvested at different times was extracted with methanol/acetone/water/formic acid (30/42/27.5/0.5) solvents. It was determined that the extracts had an inhibition zone diameter of 13.8/14.2 mm against *E. coli* ATCC 25922 and 14.7 mm against *C. albicans* ATCC 10231.

The MIC and MBC or MFC values of the extracts were determined using the micro-dilution method and are given in Table 2. MIC values of cornelian cherry water and chloroform extracts varied between 5 µg/µl to 40 µg/µl and MBC and/or MFC values between 10 µg/µl to >40 µg/µl. The lowest MIC value was 5 µg/µl against *S. pullorum* in both extracts. The lowest MBC value of the extracts was determined as 10 µg/µl against *S. pullorum* for water extract. The MFC value of the water and chloroform extracts was obtained as >40 µg/µl against *C. albicans* (ATCC 10231).

Table 2. MIC and MBC or MFC values of cornelian cherry water and chloroform extracts.

Microorganisms	**Extracts**			
	MIC(µg/µl)		**MBC and/or MFC(µg/µl)**	
	CW	**CC**	**CW**	**CC**
Salmonella pullorum	5	5	40	10
Escherichia coli O157:H7	20	40	40	40
Aeromonas hydrophila ATCC 19570	10	20	40	20
Vibrio angillarum A4	10	40	>40	>40
Candida albicans ATCC 10231	10	40	>40	>40

* CW: Cornelian cherry Water extract, CC: Cornelian cherry Chloroform extract

Yiğit (2018) was determined the MIC values of cornelian cherry extracts (water and methanol) using the micro-well dilution method. MIC values of the obtained water and methanol extracts varied between 0.312-0.625 mg/ml. The water and methanol extracts have been determined to have MIC values against *E. coli* as 0.312 mg/ml. The MIC value of methanol extract against *C. albicans* was 0.625 mg/ml. As a result, plants are of great importance due to their strong antimicrobial effects, as well as being a food source.

CONCLUSIONS

In this study, the potential of using cornelian cherry water and chloroform extracts as a natural additive and antimicrobial agent in various industries was investigated. The results showed that fruit water and chloroform extracts had antibacterial and antifungal activities. The Cornelian cherry fruit extracts may have the potential to be used as a natural additive and antimicrobial agent instead of chemical ingredients used in the food, feed, and pharmacology industries.

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THE INFLUENCE OF CLIMATIC FACTORS ON THE BIOECOLOGY OF THE HYBRID SPARROW (PASSER DOMESTICUS X P. HISPANIOLENSIS) IN THE BOUIRA (ALGERIA)

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ABSTRACT

The present work is a contribution to the study of sparrows in Algeria by providing more elements concerning the bio-ecology of species and the influence of climatic factors. Monitoring the behavior of sparrows in different localities in Bouira between 2020 and 2021 shows the dominance of global perching (PG) with rates between 56.32 and 63.93%, followed by foraging (RA) with 28.67% and 36.97% . Vol (V.) is in third position (5.19% to 7.88%). For the influence of climatic factors, significant correlations are recorded between the search for drinking water (BRE), grooming (T.) and average temperatures (Tm) with $P<0.001$, as well as cry activity (Cr) and precipitation (Pre). For the other activities, no significant correlation with climatic factors is noted. The study also reveals a significant difference in the behavior of the Hybrid Passer depending on the locality. The results obtained allow us to say that the behavior of the sparrows is variable according to the months of the year or the seasons, thus defining two periods: a period of intense activity in autumn and a second delimited in spring-summer, coinciding with the breeding period. The period of low activity characterizes the winter and summer period. Two effects of climatic factors on the behavior of sparrows are recorded; a perceptible direct effect and an indirect effect affecting either environmental conditions (food abundance) and/or species phenology. To this can be added other factors encountered such as the human presence (anthropic action), the geographical position of the locality (altitude), and the nature of the open, semi-open or closed environment which can in turn influence the behavior of sparrow species by controlling their numbers in the area.

Keywords: Hybrid Sparrow, Bio-ecology, Behaviour, Climatic factors.

Introduction

The bioecology of an organism reflects its entire natural history, includes all the biological characteristics related to its life cycle and provides a functional interpretation of the use of its habitat (Henry, 2001). Generally, species that occupy a large geographic range are subject to different ecological conditions and specific constraints specific to each habitat to which they must adapt in order to survive. (Blondel, 1995; Chabi, 1998). Thus, to understand the behavior of animal populations, birds are good biological models for assessing the quality of habitats. They are present in all natural environments, including artificial environments, in all food webs, distributed over the three dimensions of space and are therefore sufficient to formulate an ecological diagnosis of terrestrial environments (Blondel, 1975) . For the implications of birds in climate change, it seems to have many effects on bird species, by induction of phenological responses (success of reproduction, use of food resources, etc.) or modification of the ranges of

species , therefore influencing the numerical evolution of populations (Laudelout & Paquet, 2014) . Some of these changes are so noticeable that highlighting them has helped raise awareness of the importance of climate change and the associated risks to biodiversity.

The species considered in this work is the hybrid sparrow (*Passer domesticus x P. hispaniolensis*). the best known bird group due to its distribution and dynamics causing serious damage to agricultural crops. Several works have been carried out in Algeria and around the world in the perspective of understanding the bio-ecology of these species and their congeners. We cite as an example the work carried out in Algeria by (Ait Belkacem,2000,2004) on the reproduction of the hybrid sparrow, in particular those of (Guezoul et al., 2006) , and (Behidj-Benyounes et al., 2013). For studies on the trophic diet of (*Passer domesticusX P hispaniolsis*), mention should be made of the work of (Ait Belkacem 2000,2004), (Guezoul et al., 2011), (Saad et al., 2019), and (Abbassi et al., 2022). However, these studies were carried out without taking into account the potential effects of climatic factors on the bio-ecology of the species, and no temporal analysis at the regional level was carried out. The present work contributes to the study of sparrows in particular and birds in general by providing more information on the bio-ecology of species and the impact of climate.

Materials and methods

The hybrid sparrow behavior study was conducted in the communes attached to the agricultural subdivisions of the Bouira region (Oued El Berdi and El Hachimia). The 1st study area is located at an altitude of 591m, resting on varied geological substrates of stratified form. The landscape is semi-open characterized by areas of plains occupied by cereals, arboriculture and market gardening. As for the 2nd zone El-Hachimia is at an altitude of 713m, the landscape is open with relief characterized by a low slope: 70% of plains exploited in cereal growing and olive growing and 30% of more or less rugged terrain. Bouira is positioned in the sub-humid bioclimatic stage with a temperate variant.

The experiment lasted eight months from November 2020 until June 2021, two outings are carried out per week, i.e. eight observations per month. The observation took place during the first hours following sunrise and during the afternoon a few hours before dusk. As for the equipment used in the field, it consists of a pair of binoculars to identify and follow the evolution of the various activities of the sparrow, namely: Global Perching (PG), Food Search (RA), Flight (V), Search drinking water (BRE) and theft hunting (CV). A timer to calculate the time spent for each activity in seconds and a mimeographed sheet or behavior sheet to fill out, hour by hour.

The data collected is organized in the tables: Average daily time expressed in seconds and in percentages devoted to each type of activity of the hybrid sparrow in Oued-El-Berdi and El-Hachimia. It should be noted that the rates recorded for the Global Perching (PG) activity for each month only represent the cumulative time devoted to the three activities: Simple perching (Ps), Cry (Cr), and Grooming (T). In addition, the climatic information collected by the Bouira weather station for the period 2020-2021 is taken into account with an altitude adjustment. In particular the average temperature Tm (°C), the average precipitation Pre (mm) and the wind speed Vt (km/h).

For the treatment of the results we used the Principal Component Analysis (PCA). With the aim of highlighting the connections (similarities and differences) which exist in the behavior of the sparrow according to the months of the year and the considered stations, and to detect a possible action of the climatic factors on the activity of the sparrow hybrid. In addition, a bilateral mean test was applied based on a comparison between the two localities. For the implementation of our analyzes we used the R software.

Results and discussion

Of all the activities monitored during this study, it turns out that overall perching is the most important activity in terms of time spent, with values fluctuating between (63.93% And56.32%) in Oued el berdi, and (65.23% and 51.42%)in El hachimia when looking for food, it comes in second place with values between (36.97% ,28.67%) And (42.43%,24.96%) respectively . Theft comes in 3rd position with lower percentages ranging between (7.88% And4.31%) And (6.55% and 5.53%)The activities which require less time by the sparrow are represented by the two activities looking for drinking water and hunting theft.These results agree with those found by Aıt Belkacem (2013) in the Djelfa region; who report that the most important activity of sparrows is Global Perching, followed by Diet and Flight.

The analysis of the Graph (Biplote of the Activities of the Hybrid Sparrow and climatic factors in Oued el Berdi) and the reading of the tables of the relative contributions of the individuals and the variables made it possible to highlight this: that the activity of the Hybrid Sparrow in November opposes that recorded in (January and February); the months belonging to the Fall season share strong values of Simple Perching, Screaming, Foraging and Flying. As for the months of the cold season, the behavior of the passer shows low rates of Foraging and Grooming, but also low temperatures are noted during the months. For (May and June) the activity or behavior is quite different to that denoted in the Autumn-Winter period; thus revealing the strong values of the activities: (flying hunting, Finding Drinking Water, and Grooming). High temperatures can be felt during this summer period. These observations make it possible to determine two periods of activity a period of intense activity in Autumn and a second period can be delimited in spring-summer coinciding with the breeding season. Indeed during the breeding period which begins in mid-March and ends in early July, the activity of hybrid sparrows in Bouira is oriented in several directions (the construction of nests, courtship displays, mating, brooding of eggs , and raising chicks) unlike the autumn season which was more devoted to food supply and the formation of reserves..

As for the study of correlations, the combinations (Ps -RA), (RA-Cr) and, (V-Cr) are almost perfectly correlated variables, positively. Which leads to say that these activities are of close importance for the biology of the sparrow. As for the climatic variables, positive correlations were recorded between the average temperatures (Tm) and the activities: Grooming (T) and the search for drinking water (BRE), as well as the Precipitation (Pre) and the cry (Cr). It is obvious that during high temperaturesthe water requirement of birds increases; moreover in the dry period the sparrows consume more dry seeds than insects (prey) rich in water. As for the correlation denoted between the (Tm) and the activity (T), it reveals an effect of the temperatures on the ectoparasites of this Passerine. According to (Mennerat et al., 2021) the average annual parasite load increased with minimum spring temperature and decreased with the increase in average temperature of the previous summer. While suggesting a major effect of temperature during the life cycle blowfly, with potential implications for host (Blue Tit) interactions across their geographic range as the climate continues to warm. Elderd & Reilly (2014), Eads & Hoogland (2016) local weather fluctuations can cause ectoparasite intensities to increase or decrease depending on where populations are relative to these optima.

The correlation recorded between monthly rainfall and Call activity in (*Passer domesticusX P. hispaniolsis)* can be explained by the fact that the sparrow increases the intensity of its calls by emitting more alarming signals during meteorological events. According to (Verboom & Heij, 2018) different types of vocalizations are determined in the house sparrow. Indeed the sparrows in groups produce high frequency chirping, while when taking meals somewhat corresponding chirps are recorded. The alarm call is a hoarse pulsating call consisting of six identical semi-wideband components. As for the territory call, it is repeated several times and consists of three up/down sweep combinations.

Similarly, the analysis of the graph (biplot of behavior of the hybrid sparrow according to the annual cycle in the two stations) makes it possible to note that in November the activity of the hybrid sparrow is very important in the two localities with high rates of: Global Perching (PG), Food Search (RA), and Flight (V), as well as in April at Oued el Berdi. Therefore opposing the activity of the Passer over the short months (January, February, and June) to El Hachimia. As for the behavior of (*Passer domesticus X P hispaniolsis)* in Oued el berdi during the months (April, May and June) and (May and June) in El Hachimia; the two localities share strong activity values: flight hunting (CV) and the search for drinking water (BRE).

It should therefore be said that the hybrid sparrow has differences in its behavior depending on the stations. Indeed during the winter and summer season the reduced activity of the Passer is noted in the two localities. However, it marks more the El Hachimia locality. On the other hand, in the spring-summer period, a delay in the reproduction period was recorded in El Hachimia compared to Oued el Berdi. According to (Pearce Higgins & Green, 2014) the positive effect of temperature on the reproduction of landbirds in temperate zones: increased survival and better reproductive success is less evident in passerines, for which there does not seem to be a clear trend clear. Moreover, in temperate zones, global warming will lead to an increase in the diversity of bird species per station (diversity á). Although in common birds in Britain (Davey et al., 2012), it is however accompanied by a greater "homogenization of communities". In addition, rainfall has a crucial role in conditioning the extent of many wetlands but also it promotes the growth of vegetation and, indirectly, the availability of food resources and therefore a better body condition in birds, high survival rate , and more abundant populations the following spring. Newton (2004). It is important to mention that the climate differs almost entirely depending on the altitude. Temperatures decrease as height increases. The number of frost days differs/changes between November and March depending on altitude. Almost in the same way, the annual precipitation increases with proximity to the sea. While the winddepends more on the local topography.

On the other hand, it should also be indicated that the behavior of the hybrid sparrow is important in the month of April at Oued el Berdi. According to (Aitbelkacem et al., 2002) during the reproduction period a strong activity of individuals is observed near agricultural plots coinciding with the milky stage of wheat.

In addition, the average test applied to the different recorded rates of Hybrid Sparrow activities in Bouira reveals the existence of a significant difference in the behavior of the Passer between the two localities, for flight activity (V) and overall perching. (PG) with p= 0.016 and p=0.011 respectively. this could be explained by the strong anthropic activity noted at the level of the El Hachimia zone which influenced the activity of the sparrows, as well as their number in the station. Furthermore, we hypothesize that habitat variables at the landscape scale play a crucial role in the reproduction and recruitment of sparrow species. According to (Zhang & Zheng, 2010) heavily urbanized areas, major roads and high-rise buildings are not suitable for tree sparrow habitation. resulting in sparrow numbers declining along urbanization gradients in Pikin. these revelations are consistent with our observations at El Hachimia. On the other hand, the presence of trees belonging to different strata, low-rise constructions as well as the reduced number of pedestals in the locality of Oued el berdi make this area an adequate environment to shelter the hybrid sparrow (especially in period Autumn-Winter and during the breeding period; Spring-Summer). At the 50 m scale, the area of low buildings, the number of conifers and pedestrians are the main factors contributing to the distribution of the Tree Sparrow, while at the 400 m scale, the percentage of the area of tall buildings and vegetation remain important habitat variables. (Zhang & Zheng, 2010). In addition, the presence of plants and the abundance of food are two key factors that determine the presence of sparrows in the region. In the Faroe Islands (Bengtson et al., 2010) found a positive correlation between Passer patch occupancy

and area and amount of vegetation. Vincent (2005) House Sparrow brood biomass increased with the extent of vegetated areas around nest sites, suggesting that breeding success may be relatively low in habitats with low vegetation cover. In Algeria (Benyounes & Doumandji, 2009) reports that the presence of trees and water resources, but also constructions are favorable places for the installation of nests. Ultimately, it is argued that the interaction between climate and local environment provides a mechanism by which spatial synchronization in population dynamics can be reduced even in strongly spatially autocorrelated environments. (Rıngsby et al., 2002).

Conclusion

The study of the Bio-ecology of the hybrid Sparrow and the influence of climatic factors in the region of Bouira shows that the most important activity requiring more time is Perching followed by Food Search and Flight. For other activities they are less frequent and of less importance. Our observations also make it possible to note: a variation in the evolution of the different activities of the hybrid sparrow according to the months of the year, consequently we can define two periods of activity: a period of intense activity in Autumn and a second period can be delimited in Spring-Summer, coinciding the period of reproduction. The period of low activity characterizes the cold season in Winter and the dry period in Summer. obacco action of the climatic factors, correlations between the activities seeking drinking water (BRE), grooming (T) and the average monthly temperature values are recorded, as well as the precipitation (Pre) and the cry (Cri). obacco other factors, no correlation is noted. The study also reveals a significant difference in sparrow behavior between the two localities.

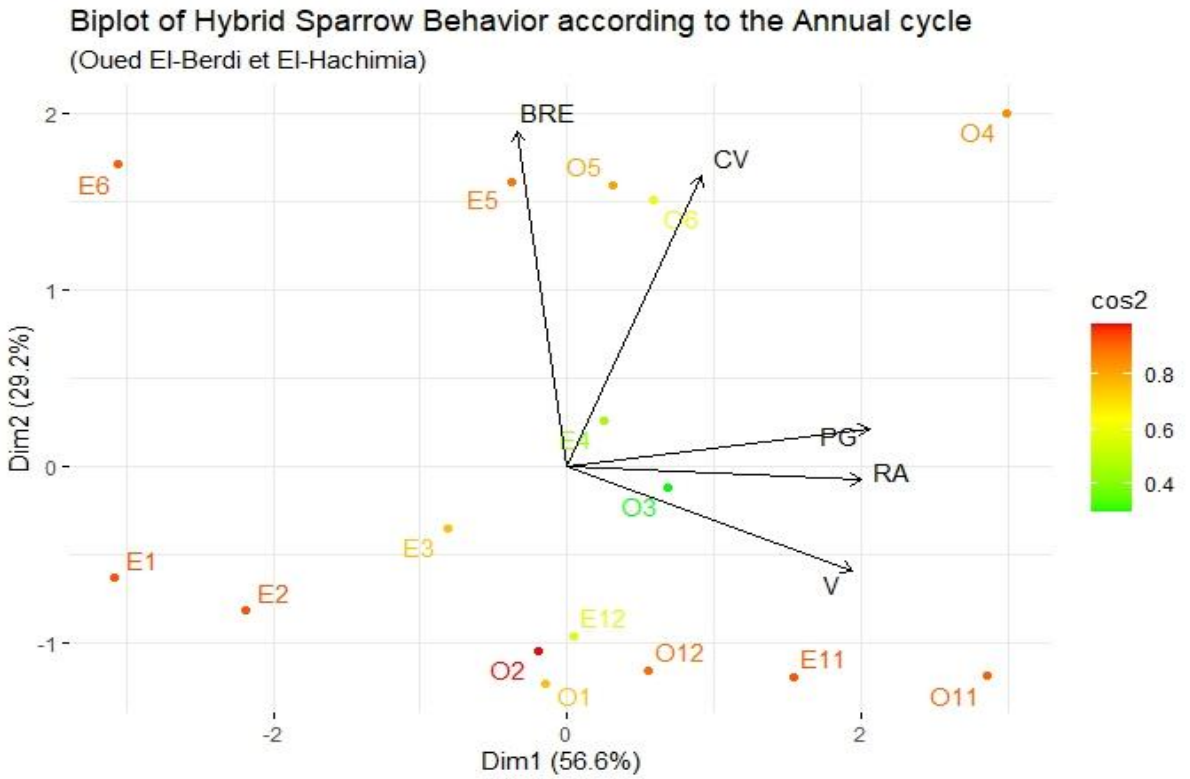
This amounts to saying that climatic factors can have a perceptible direct effect on the behavior of sparrows and indirect either by affecting the conditions of the environment; for example an action on the evolution of plants or even the presence of insects in the area, or even an effect on the phenology of species. To this can be added other factors encountered in the environment such as the human presence, the geographical location of the locality, and the obacc of the open, semi-open or closed environment which can in turn influence the behavior of sparrow species by control of their numbers in the area.

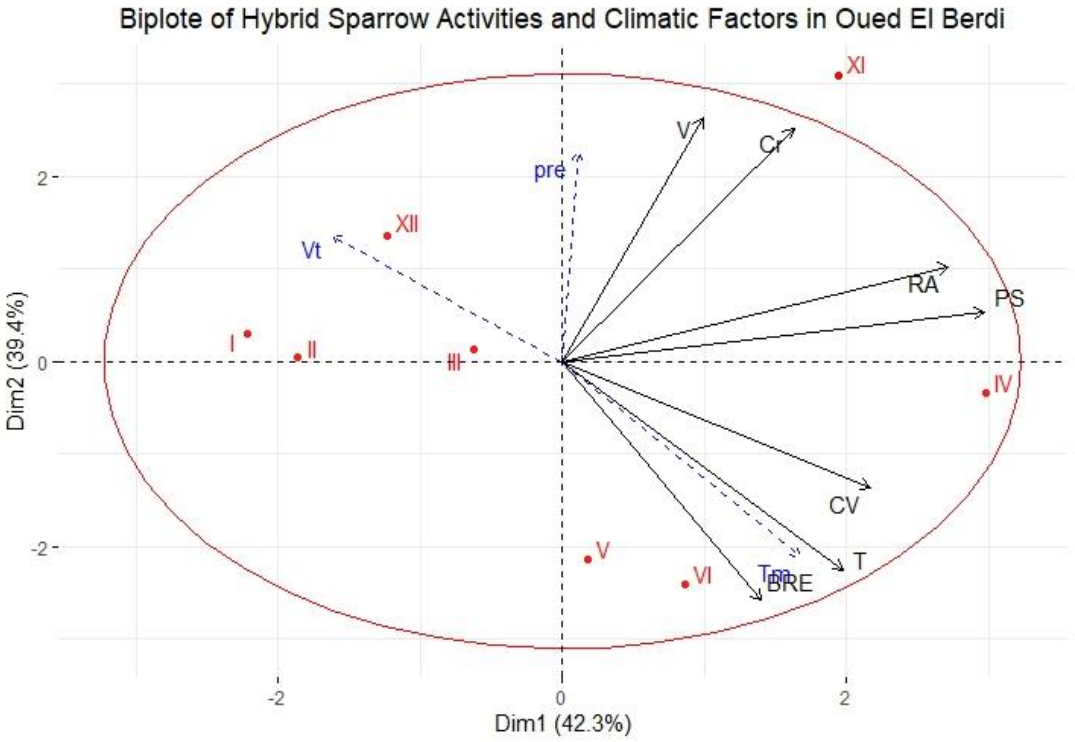
	Months 2020/2021															
	XI		**XII**		**I**		**II**		**III**		**IV**		**V**		**VI**	
	Sec	%	Sec	%	Sec	%	Sec	%	Sec	%	Sec	%	Sec	%	Sec	%
PG	7302	59,18	5065	58,21	4180	56,32	4719	58,99	5603	63,93	6972	59,21	5301	57,01	5929	61,36
RA	4325	35,05	3031	34,83	2656	35,79	2778	34,73	2513	28,67	4012	34,07	3438	36,97	3031	31,37
v	701	5,68	600	6,89	585	7,88	502	6,27	589	6,72	612	5,19	401	4,31	523	5,41
BRE	10	0,08	5	0,05	0	0	0	0	20	0,23	56	0,47	102	1,09	150	1,55
CV	0	0	0	0	0	0	0	0	39	0,44	123	1,04	56	0,6	29	0,30
Totaux	12338	100	8701	100	7421	100	7999	100	8764	100	11775	100	9298	100	9662	100

Tab01. Average obac time expressed in seconds and in percentages devoted to each type of activity of the hybrid sparrow in Oued-el-berdi (Bouira)

	Months2020/2021															
	XI		**XII**		**I**		**II**		**III**		**IV**		**V**		**VI**	
	Sec	%	Sec	%	Sec	%	Sec	%	Sec	%	Sec	%	Sec	%	Sec	%
PG	5985	57,14	4256	51,42	2487	57,59	3349	61,97	4432	59,97	4667	55,94	4252	54,09	3143	65,23
RA	3888	37,12	3512	42,43	1563	36,19	1701	31,47	2513	34	3067	36,76	3001	38,18	1203	24,96
v	601	5,74	499	6,03	263	6,09	354	6,55	412	5,57	522	6,26	435	5,53	294	6,1
BRE	0	0	10	0,12	5	0,11	0	0	18	0,24	51	0,611	123	1,56	166	3,44
CV	0	0	0	0	0	0	0	0	15	0,2	35	0,42	49	0,62	12	0,25
Totaux	10474	100	8277	100	4318	100	5404	100	7390	100	8342	100	7860	100	4818	100

Tab 02. Average obac time expressed in seconds and in percentages devoted to each type of activity of the hybrid sparrow in EL-Hachimia (Bouira).





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T0BACCO BREEDING FOR LEAVES AND YIELD

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ABSTRACT

The aim of this work is to investigated the mode of inheritance for the number of the leaves per stalk, area of the leaves from the middle belt and yield of dry leaf mass per stalk, in four F_1 tobacco hybrids obtained by crossing five varieties, four of which are Oriental in the role of mother and one Broadleaf as a father, in 2020 and 2021. The most common mode of inheritance for the first trait is negative dominance, for the second trait partial dominance and for the third trait intermediate. There is no heterosis. The best results for the size of the leaves from the middle belt and for the yield of dry mass gave P-76/86 x B-1/91. The obtained mode of inheritance is an indicator of good selection of individuals in future generations and quick fixation and stabilization of the traits. The four hybrid combinations represent very interesting starting material for tobacco breeding.

Keywords: *Nicotiana tabacum* L., hybrids, inheritance, F_1 generation, quantitative traits.

INTRODUCTION

The production of oriental tobacco is one of the most important branches in the economy of the Republic of North Macedonia. Most of the tobacco raw material is intended for foreign markets. The participation of our tobacco in the highest quality cigarette brands is proof of top quality and exceptionally pleasant aroma. Because of this, the investigations in genetics and selection of tobacco are of great importance. Using the methods of these sciences, breeders try to create more productive and better quality varieties than the existing ones. By introducing new superior varieties in tobacco production, the economic effect of this culture will increase, thereby improving the standard of producers and increasing the flow of funds in the country.

The aim of this paper is to study the mode of inheritance of the number of leaves per stalk, the area of the leaves of the middle band of the stalk and the yield of dry leaf mass per stalk, in the F_1 generations obtained from different types of tobacco, in order to reveal a possible heterotic effect, as well as to provide material for further successive tobacco breeding activities.

MATERIAL AND METHOD

As material for work, we chose five genotypes from the gene bank of the Scientific Tobacco Institute – Prilep: Prilep P-23, Prilep P 18-50/4, Prilep P 76/86, Basmak MS 8/1 and Burley B-1/91. As a parent-father, we used the broadleaf variety B-1/91, so with its pollen in 2019 and 2020 we made four F_1 hybrids: P-23 x B-1/91 (Figure 6), P 18-50/4 x B-1/91 (Figure 7), P 76/86 x B-1/91 (Figure 8) and MS 8/1 x B-1/91 (Figure 9). The parental varieties and their F_1 hybrids were planted in a randomized block system in four replications, in an experimental field at STI-Prilep, in 2020 and 2021, on a working area of about 291.6 m^2 or a total area of 655.2 m^2 (working surface and paths). The broadleaf variety and F_1 hybrids are planted at a planting distance of 90 cm (between rows) x 50 cm (between plants in a row), while the oriental varieties are planted at a planting distance of 45 cm x 15 cm. The number of leaves per stalk and the dimensions of the leaves from the middle band of the stalk were determined at the full stage of plant development, at the beginning of flowering. The data from the measurements of

the number of leaves per stalk were processed variationally-statistically (the standard deviation-σ and the coefficient of variability-CV). The surface area of the leaves was calculated by multiplying the mean values of the length by the mean values of the width and by the coefficient k=0.6354. Dry leaf mass was calculated after the manipulation of tobacco.

Mode of inheritance of the components was determined on the basis of test-significance of F_1 generation in relation to the average of both parents.

Parental genotypes:

Prilep P-23 – Kosta Nikoloski and Milan Mitreski are authors of thes variety. Belongs to the oriental sun-cured tobaccos (Figure 1). The characteristics of this variety are described by Korubin – Aleksoska (2004).

Prilep P 18-50/4 – Creation by Ana Korubin – Aleksoska. The variety belongs to the group of oriental sun-cured tobaccos (Figure 2).

Prilep P-76/86 – is an oriental sun-cured variety, created by Dimche Chavkaroski and his collaborators (Figure 3). It is distinguished by a long vegetation (from planting to flowering 85-95 days). A description of the variety is given by Korubin – Aleksoska (2004).

Basmak MS 8/1 – created by a group of authors, headed by Dusko Boceski. It belongs to the basmak sun-cured type, which was created from the Jakali type from Greece (Figure 4). The morphological traits of the genotype are described by Korubin – Aleksoska and Ayaz Ahmad (2016).

Burley B-1- 9 – Dimche Cavkaroski and his collaborators are the authors of the variety. Belongs to the group of broadleaf air-cured tobacco (Figure 5). A description of the variety is given by Korubin – Aleksoska (2004).

Figure 1. Prilep P-23

Figure 2. Prilep P 18-50/4

Figure 3. Prilep P-76/86

Figure 4. Basmak MS 8/1

Figure 5. Burley B-1/91

Figure 6. P-23 x B-1/91 (F_1)

Figure 7. P 18-50/4 x B-1/91(F_1)

Figure 8. P-76/86 x B-1/91 (F_1)

Figure 9. MS 8/1 x B-1/91 (F_1)

Climatic and soil conditions in the area of investigations:

During the scientific research of quantitative traits from the aspect of selection and genetics, it is necessary to take into account the environmental conditions in which the studies were conducted.

The climate parameters in 2020 and 2021 are drastically different. So, in 2020 average temperature (May-September) is 22.15°C, minimum tem. Is 15.6°C, maximum tem. Is 28.8°C, humidity 61.2%. The total amount of rains in the given period is 400.6 mm. In 2021 average temperature is 18.9°C, minimum tem. Is 13.1°C, maximum tem. Is 24.1°C, humidity 52.2%. The total amount of rains in the given period is 174 mm (https://en.climate-data.org/ obacc/ obaccos /prilep/prilep-37313/). Basically in 2021 the temperature from May to September is lower, the humidity in the air is lower, and there is about 43% less rainfall.

Our research was conducted in the experimental field in the Scientific Tobacco Institute – Prilep on a deluvial (colluvial) soil type.

RESULTS AND DISCUSSION

Number of leaves per stalk:

One of the most studied quantitative traits by tobacco breeders is the number of leaves per stalk, because it is directly related to yield.

With the smallest number of leaves among the parents is characterized B-1/91 (30.3), and with the biggest P-76/86 (54.4), while in hybrids the least leaves have P-23 x B-1/91 (29.2), and the most P 18-50/4 x B-1/91 (34.3). The standard deviation ranges from 1.2 (P 18-50/4 and P-76/86 x B-1/91) to 2 (B-1/91). The coefficient of variability ranges from 2.2% (P-76/86 x B-1/91) to 4.5 (B-1/91). The coefficient of variability of the variants has a value less than 10, which means that the tested variants are stable and uniform.

The mode of inheritance of this trait is negative dominant (onli in P 18-50/4 x B-1/91 there is partial dominance). There is no heterosis.

Partial dominance in inheritance of leaf number per stalk and absence of heterosis found: Korubin – Aleksoska (2000), in the crosses of three oriental varieties, Korubin – Aleksoska (2001), in ten oriental genotypes, Gixhari and Sulovari (2010), in a semi-diallel of eight oriental genotypes. Different way of inheritance and a weak heterotic effect received Aleksoski (2010), in a one-way diallel of three oriental and one Burley variety. Dyulgerski and Radoukova (2019), in seven hybrids of the Berlay type in the F_1 generation found the dominance of the parents with a larger number of leaves.

Heterosis with a positive heterotic effect on the trait found: Butorac et al. (1999), in F_1 offspring of four Burley varieties, Lalitha et al. (2006), in crosses on six lines and six testers, Dimanov and Dyulgerski (2012), at ten crosses of local and introduced Burley varieties. (high heterotic effect is detected), Aleksoski et al. (2013), in hybrids of four parent genotypes of tobacco of different types (the heterosis had a weak heterotic effect), Ramachandra et al. (2015), in hybrids obtained from six lines of different types of tobacco and eight testers.

Leaf area of the middle belt of the stalk:

The smallest area of the leaves from the middle belt of the stalk in the parental genotypes has the variety P 18-50/4 (173 cm^2), and the biggest B-1/91 (1203.5 cm^2), while in F_1 hybrids with the smallest leaf area is characterized MS 8/1 x B-1/91 (847 cm^2), and with the biggest P-76/86 x B-1/91 (1270 cm^2). The standard deviation and the coefficient of variability are not calculated for this trait, because the values are obtained by applying the formula for area, where the mean values of the length and width of the leaves by repetitions are entered.

The mode of inheritance of this trait is partially dominant (onli in P-76/86 x B-1/91 there is positive dominance). There is no heterosis.

The area of the leaves has been studied by many authors, because the value of this trait correlates with the yield. The most common way of inheritance is the partially dominant and intermediate. Similar results were obtained by: Aleksoski (2010), in a one-way diallel of four parental genotypes of Oriental and Burley origin; Gixhari and Sulovari (2010), in a one-way diallel of eight oriental genotypes; Aleksoski et al. (2013), in a diallel of four parent genotypes of tobacco of different types; Aleksoski (2018), in a diallel of four oriental varieties, etc.

Positive heterosis in inheriting of leaf area received: Korubin – Aleksoska (2000), in diallel of three oriental and one semi-oriental variety (a positive heterotic effect appeared in two crosses where one parent is the introduced variety Pobeda-2); Lalitha et al. (2006), in hybrids of six line and six testers (the resulting heterotic effect was low to moderate in both directions); Aleksoski (2010), in a one-way diallel of four parental genotypes – three oriental

and one Burley (the weak heterotic effect had no economic justification); Gixhari and Sulovari (2010), in a diallel of eight parent oriental genotypes; Aleksoski et al. (2013), in six diallel crosses of four parent tobacco genotypes of different types; Aleksoski (2018), in hybrids of four oriental varieties.

Yield of dry leaf mass per stalk:

The investigations for the yield of dry leaf mass are always present in programs for the creation of new more productive varieties and improving of existing ones.

The lowest yielding variety between the parental genotypes is MS 8/1 (15.5 g/stalk), and the highest yielding B-1/91 (170.5 g/stalk), while in F_1 hybrids with the lowest yield is P-23 x B-1/91 (72 g/stalk), and with the highest yield P-76/86 x B-1/91 (104 g/stalk).

The mode of inheritance of this trait is intermediate (oba in P-23 x B-1/91 there is partial dominance). There is no heterosis.

The dry leaf mass per stalk has been studied by many breeders. The most common way of inheritance is the partially dominant and intermediate.

A partially dominant mode of yield inheritance was obtained by Korubin – Aleksoska (2001), in a diallel of three oriental and one semi-oriental variety, and Gixhari and Sulovari (2010), in a one-way diallel of eight oriental genotypes.

Heterosis with a positive heterotic effect on the trait found: Butorac et al. (1999), in F_1 generation of four Burley varieties; Gixhari and Sulovari (2010), in a diallel of eight parental oriental genotypes; Dyulgerski (2019), on eight Berley newly created hybrid combinations of the first generation, Kinay and Yilmaz (2016), in seven hybrids obtained by one-way diallel crosses between oriental varieties. The heterotic effect for dry mass yield was 4%. Kinay et al.(2020), in 21 F_1 half-diallel hybrids of seven oriental tobaccos mostly from the Black Sea region of Turkey.

Table 1 shows the mean values for the number of leaves per stem, leaf area of the middle belt of the stalk and the yield of dry leaf mass per stalk in parents and F_1 hybrids for 2020 and 2021.

Table 1. Mode of inheritance of quantitative traits in parents and F_1 hybrids of tobacco

No	Parents and F_1 hybrids	Quantitative traits										
		Number of leaves per stalk					Area of the leaves from the middle belt of the stalk			Yield of dry leaf mass per stalk		
		2020	2021	$\overline{x}$	σ (±)	CV (%)	2020	2021	$\overline{x}$ (cm^2)	2020	2021	$\overline{x}$ (g)

1.	P-23	P1-♀	43.5	42.5	43	1.4	3.5	184	172	178	20	19	19.5
2.	P 18-50/4	P1-♀	46.4	44.4	45.4	1.2	3.6	176	170	173	20	21	20.5
3.	P-76/86	P1-♀	53.6	55.2	54.4	1.5	3.6	174	191	182.5	23	25	24
4.	MS 8/1	P1-♀	40.3	42.1	41.2	1.5	4.1	202	203	202.5	15	16	15.5
5.	B-1/91	P2-♂	32.4	28.2	30.3	2.0	4.5	1197	1210	1203.5	169	172	170.5
6.	P-23 x B-1/91	F_1	29.7	28.7	29.2 $^{-d}$	1.5	2.4	1080	1088	1084 ^{pd}	70	74	72 ^{pd}
7.	P18-50/4xB-1/91	F_1	34.8	33.8	34.3 ^{pd}	1.5	2.3	937	917	927 ^{pd}	89	86	87.5 ^{i}
8.	P-76/86 x B-1/91	F_1	30.8	32.2	31.5 $^{-d}$	1.2	2.2	1245	1295	1270 $^{+d}$	98	110	104 ^{i}
9.	MS 8/1 x B-1/91	F_1	32.2	30.4	31.3 $^{-d}$	1.7	3.2	892	802	847 ^{pd}	84	72	78 ^{i}

CONCLUSIONS

From our studies on parental genotypes and their F_1 hybrids, as well as the mode of inheritance on the number of leaves per stalk, leaves sizes from the middle belt and dry leaves yield per stalk, we have got the following conclusions:

- The varieties that are the subject of these studies are characterized by a high degree of stability and uniformity, as a result of their homozygosity. The parents in the role of mothers and the parent in the role of father, differ significantly in the investigated traits.
- Inheritance of the number of leaves per stalk is negative dominant (only in P 18-50/4 x B-1/91 there is partially dominant).
- Inheritance of the leaf area of the middle belt of the stalk is partially dominant (oba in P-76/86 x B-1/91 there is positive dominance).

- Inheritance of the dry leaf mass yield per stalk is intermediate (oba in P-23 x B-1/91 there is partial dominance).
- There is no occurrence of a heterotic effect in the F_1 population in all studied morphological traits, in the two years investigations.
- The best results for leaf area and dry leaf yield per stalk were given by P-76/86 x B-1/91.
- With these investigations we obtained F_1 hybrid offspring, with which we provided material for further breeding activity.
- The results obtained with these studies are useful achievements in the genetics and tobacco breeding, and they have primary importance for science and practice in the process of creating new superior varieties.

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INVESTIGATING PARTICIPATORY LEARNING AS A TOOL TO ENGAGE STUDENTS AND TO RAISE THEIR AWARENESS ABOUT FOOD WASTE ISSUE

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Abstract:
To face today's issues adapting the path of Responsible Research and Innovation (RRI) becomes a must rather than an option. Food waste is an issue gaining concerns worldwide in recent years because of its multidimensional impacts (economical, social, and environmental). Many strategies were suggested to tackle food waste problem such as awareness campaigns, as a way of affecting consumers' behavior. Engaging young consumers in such awareness campaign would be significant as they are the future leaders. This research aimed to using participatory learning as a tool to raise student's awareness toward food waste issue. Collaborative work was initiated through a brainstorming among restricted student groups(n=3) from different departments in the National Institute of Agronomy of Tunisia (University of Carthage, Tunisia). From this brainstorming, students suggested to organize an education and awareness campaign at the university scale. Posters addressing food waste issues were placed in prominent locations around the Campus. In addition, in order to engage their colleagues, students conducted a face-to-face survey (103 respondents) from September 15th to October 1st, 2021. The first part of the survey assessed students' knowledge and attitudes toward food waste. The second part was about solutions they suggest to reduce food waste at university scale and their opinion about the awareness campaign (did they hear about the event, what do they think about, what do they think about posters, would they attend the event…).Survey results showed that 97.8% of student respondents were aware about food waste issue and its impact on environment. About half (49.5%) of respondents declared to throw food moderately. Regarding the education and awareness campaign, 59.8% noticed the posters mainly at the campus cafeteria (62.1%) and 81.2% heard about it, through social media. The posters 'content was appealing for 70.5% of respondents. Students also reported that information clarity, content and graphics were satisfactory. Moreover, 79.6% of students reported that the event and communicated information encouraged them to improve their behavior toward food waste. These results showed that using a participatory learning has engaged students who tried even to engage their colleagues, they felt more responsible as they suggested and implemented a solution. Moreover, our findings have highlighted the importance to take into consideration the specificities of Generation Z and accordingly, to use nontraditional tools (social media, graphical design…).
Key words: Food waste reduction, participatory learning, Responsible Research and Innovation (RRI), Generation Z, awareness campaign.

Introduction:

According to the Food and Agriculture Organization of the United Nations (FAO), food waste refers to the decrease in the quantity or quality of food resulting from decisions and actions by

retailers, food service providers and consumers (FAO, 2019). The financial costs of food wastage are substantial and amount to about USD 1 trillion each year. The impact of food waste exceeds economical dimension to reach social and environmental sustainability ones. Such reflection was already highlighted in the 2030 Agenda for Sustainable Development. In fact, reducing food waste is among ways to meet the second Sustainable Development Goal (SDG 2): Zero hunger. In addition, Target 12.3 (SDG 12: Responsible consumption and production), calls for the halving by 2030 of per capita global food waste at the retail and consumer levels and the reduction of food losses along production and supply chains, including post-harvest losses (FAO, 2019).

To tackle today's issues there is an urgent need to adapt the path of Responsible Research and Innovation (RRI). According to von Schomberg (2013), RRI is defined as "a transparent, interactive process by which societal actors and innovators become mutually responsive to each other with a view to the (ethical) acceptability, sustainability and societal desirability of the innovation process and its marketable products (in order to allow a proper embedding of scientific and technological advances in our society)." In fact, researchers and innovators have a significant role to play in reflecting on and anticipating the future effects of their research and development – both positive and negative social, ethical, and environmental – during their routine decision making practices (Owen et al., 2013). Science education as among "RRI keys". It can play an important role not only in engaging publics but also in preparing experts for more robust relations between science and society (Lukovics et al., 2019). Thus, combining activities that synergistically enhance scientific creativity and societal responsibility (Lukovics et al., 2019).

Generation Z (Gen Z) includes persons who were born on mid-to-late 1990s and the early 2010s. This generation represents the future consumers and leaders. Gen Z is the generation who will become the successor and who will live and experience various environmental issues (Lemy et al., 2020). Moreover, Gen Z had been raised during the technology boom of the millennium and so was comfortable with and reliant on technology for most aspects of their lives. They can thus be seen as early adopters of new technology-mediated (Kymäläinen et al., 2021). Accordingly, using nontraditional tools with Gen Z would be a must rather than an option.

Numerous strategies were suggested to tackle food waste issue (Vizzoto et al., 2021). Awareness campaigns represent a way of affecting consumer behavior as it targets his beliefs (Kuo & Shih, 2016). Such approach at university scale could be considered a low cost solution contributing in sustainability at university campus (Ellison et al., 2019). A review by Reynolds et al. (2019) reported that information campaigns could reduce up to 28% of food waste. This research aimed to use participatory learning as a tool to raise student's awareness toward food waste issue.

Methodology:

This research was conducted in the National Institute of Agronomy of Tunisia (INAT) from September to November 2021. INAT is the first engineering school in North Africa. In fact, it was established in 1898 under the dual supervision of the Ministry of Agriculture and the Ministry of Higher Education and Scientific Research. Currently, 350 students are enrolled in the engineering cycle and 300 students enrolled between Masters and Doctorates. Work focuses on a wide range of topics related to climate change and sustainable development issues, including biodiversity, environment, functioning and engineering of natural and cultivated ecosystems, marine ecosystem, water, animal production, and agri-food sciences and technology.

Collaborative inquiry:
Based on evaluation of previous activities and discussion among group researchers from INAT food waste topic was selected. Inter disciplinary groups of students, from engineering cycle, were formed (10 students). Then, participatory learning approach was adapted.
Participatory learning:
Participatory learning is an approach to teaching and learning which focuses on the learner. It encourages learning by doing, using small groups, concrete materials, open questioning, and peer teaching. Collaborative work was initiated through a brain storming among students. From this brain storming students suggested to organize an education and awareness campaign at the university scale. To involve their colleagues who are not in the group students suggested to conduct a survey.
Survey:
An online survey with 103 students from INAT (82.6% women, 17.4% men, average age 23) was conducted from September 15th to October 1st, 2021. The questionnaire is based on two main parts: the first one assessing students knowledge and attitudes toward food waste. The second part focused on involving students outside the group.
Microsoft Excel software for frequency analysis of data.

Results:

Students and food waste
Students were asked if they believe there is a link between food waste and environment, 97.8% answered yes and 98.9% declared that there is a need to reduce food waste amounts. This reflects the awareness of student. Meanwhile, it is important to take into consideration that respondents spent at least three years at university. Moreover, they belong to an Agricultural Science university where issues like climate change and sustainability are parts of University's lectures. These results reflecting young consumers' awareness were previously reported by Jribi et al. (2022).

Respondents were then asked about their generated quantity of food waste (Figure 1). Almost half respondents (49%) considered that the food quantity of thrown food is moderate and 32% of them think it is reasonable while only 8% believe it is important. These trends were also observed by Lemy et al. (2020) with Gen Z Indonesian students. Such answers would be expected as it was a self-assessment: there is a trend to underestimate the thrown food to avoid guilt. Such findings suggest the importance of communication and introducing real life examples. In other words, showing the impact of thrown food even low quantities. Although only 8% of respondents declared they throw important amounts of food, 98.9% declared that they need to reduce their food waste and 55.4% set a target to do so.

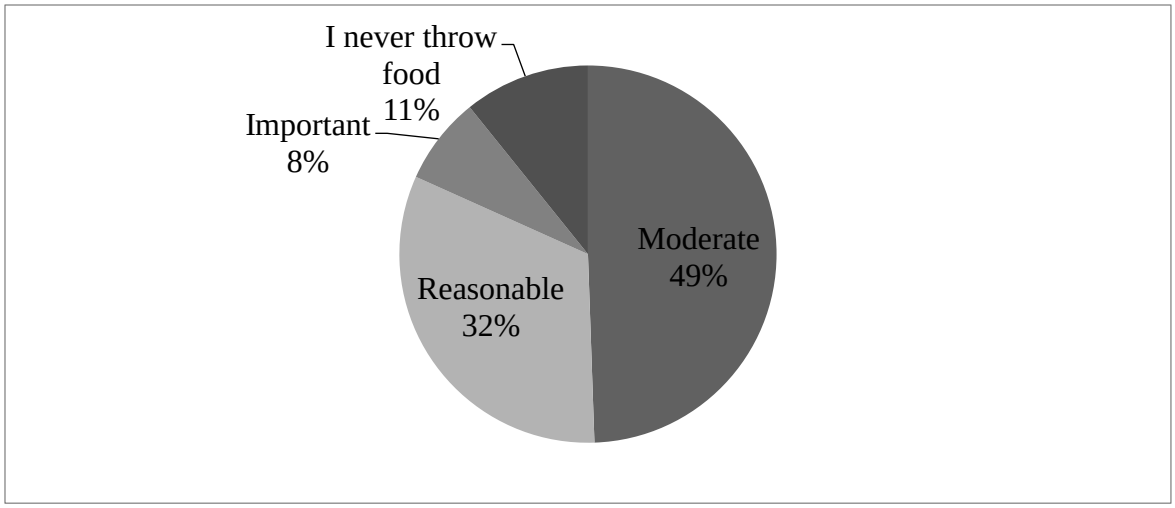
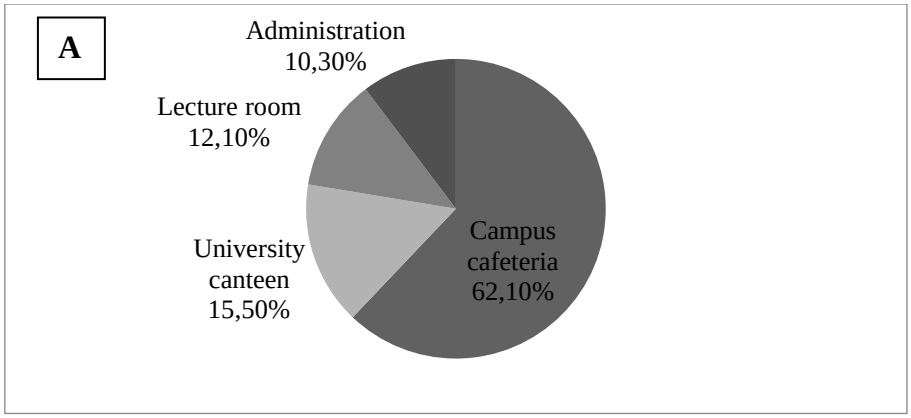


Figure1: Students' self-assessment of their generated food waste

Students were also asked if they know methods to treat waste. Remarquably, 52.7% said yes. The main suggested approaches were: sorting, recycling and composting.

Students and awareness campaign:

The second target of the survey was to raise students' awareness by evolving them in education and awareness campaign. Students were firstly asked if they would be willing to participating in an action to reduce food waste. 91.3% of participants replied positively. An indirect way to inform students about the campaign was used as respondents were asked if they heard about it. It was the case for 59.8%. Campaign posters were developed and placed around the Campus. Since the target of this awareness campaign was students, it was important to assess the main effective places and channels for communication. Results (Figure 2A) revealed that Campus cafeteria, a high-traffic area, was the main place where students noticed campaign posters (62.1%). According to Figure 2B, 81.2% of respondents heard about the campaign through social media. In Tunisia, until January 2022, 72.75% of the population use social media and 25% of them are 18-24 years old. Thus, social media could be considered as a new pedagogical tool that may be used to engage students both inside and outside university. The effectiveness of social media was even reported in engaging students for courses (Al Bahrani et al., 2015; Bal et al., 2015). These results highlight the importance of up-to-date pedagogical tools and approaches with Gen Z.



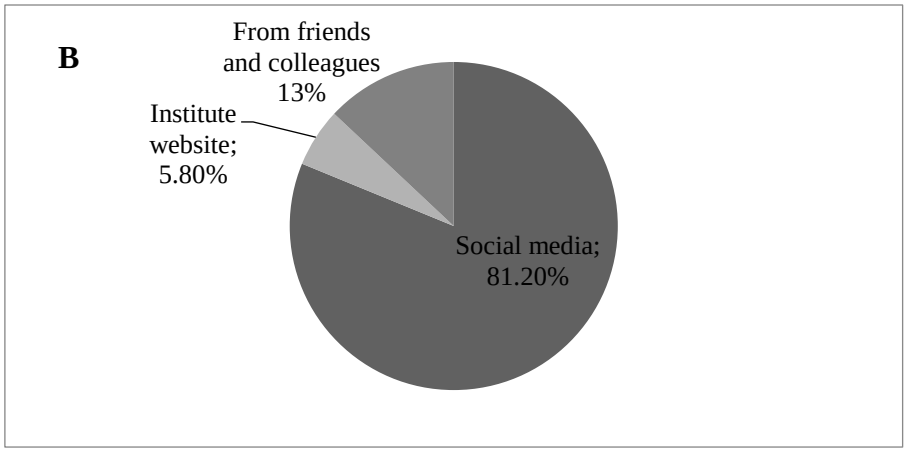


Figure 2: Locations and channels of communication

Regarding evaluation of campaign posters (Figure 3) respondents were asked to rate information clarity, graphical design and to give their global appreciation on a five-point scale. Communicated information were judged clear enough as only 16.2% (8.1 + 8.1%) rated them under the average. Similarly, graphical design and global appreciation were rated up the average for 77.5% and 87.1% of respondents, respectively. Moreover, 70.5% of respondents reported that the posters content was appealing and 67.2% declared that the posters encouraged them to improve their behavior toward food waste.

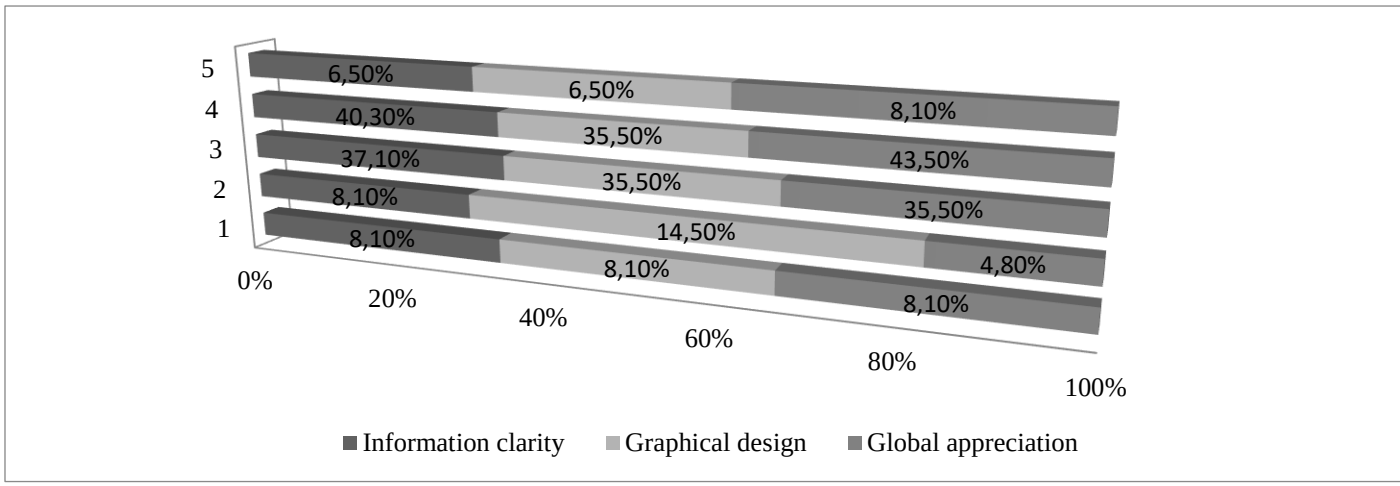


Figure 3: Respondents' campaign posters rating (1:very poor; 5: excellent)

These findings reflect the success of used support of communication but also the self-esteem of students and survey respondents. In fact, students (organizers) communicated in a correct way adapted to their colleagues. Getting good marks would encourage them more. For respondents, rating posters is a way to engage them more by feeling as influencers.

Conclusion:

This exploratory research aimed to use participatory approach learning to engage Gen Z students and raise their awareness about food waste issue. This approach of RRI combined enhancing scientific creativity and societal responsibility simultaneously. Results showed that Gen Z Students, particularly agricultural engineer, were already aware about food waste and environmental issues. The used tools (social media, adapted design…) showed their effectiveness in appealing and engaging students. These finding highlight the importance of considering Gen Z specificities and using nontraditional tools. In this way RRI would encourage responsible citizenship. Our findings may be of special interest for policy makers, researchers,

civil society organizations and other actors for designing and implementing successful food waste reduction management strategies.

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THE INFLUENCE OF GRASS CARP ON THE SPECIES COMPOSITION AND BIOMASS OF PHYTOPLANKTON

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ABSTRACT
The grass carp, brought from China, has been part of the ichthyofauna of the inland waters of Albania since the beginning of the 60s of the last century. The purpose of its introduction has been the cultivation in the polyculture system with other species of the carp family (*Cyprinidae*) for human consumption and the use of this species for the control and management of aquatic *macrophytes*. The tests to prove the influence of grass carp on the composition of phytoplankton were carried out in Experimental Didactic Center of Tapiza in two ponds with a surface area of 1000 m² and a water depth of 1.0-1.2 m, during a period of 12 months (April - March). The taxonomic study of phytoplankton showed that in both ponds, before grass carp introduction, the dominant groups were green algae (*Clorophyta*) and diatoms (*Bacillariophyta*). After the introduction of the grass carps in one of the test ponds, some changes were observed, which are mainly related to the shift of algae dominance. The average value of the concentration of *chlorophyll-a*, according to the tests carried out before grass carp introduction, was 10.92 mg/m³. The measurements performed after the introduction of grass carp in the pond showed that the average value of this indicator was 11.73 mg/m³. The increase of the *chlorophyll-a* concentration was also accompanied by the decrease of water transparency. Before the introduction of grass carp, the average value of water transparency was 43.4 cm, while after the introduction of the fish, the transparence value decreased to 32.8 cm.
Key words: grass carp, phytoplankton abundance, polyculture

INTRODUCTION:

Grass carp (*Ctenopharyngodon idella*), brought from China, is now part of ichthyophauna of Albania inland waters from the beginning of the 60th of the past century. The aim was the cultivation ponds both with other species of *Cyprinidae* family and the exploitation of this species for the control and management of aquatic macrophytes.

There exist many works that had as an object the determination of directions of grass carp influence on aquatic biocoenosis and the mechanisms of this influence (Mitchell C.P. and coauthors 1984; Richard D.I. and coauthors 1985; Leslie A.J. and coauthors 1985; Vranovsky M.1991; Kirkagac M., Demir N.2004; Pipalova I.2002, 2006).

A general opinion is that the primary influence of grass carp on aquatic ecosystems are first caused from the exploitation of the macrophytes during grazing since this species manifests phytophagus feeding regime. Secondary influence manifested on plankton and benthos community is caused as a consequence of habitat structure change that result after alterations that suffer water transparency, integrity of sediments and the concentration of nutrients after depositing the excrements by grass carp (Zweerde W. van der, 1982; Richard D.I. and coauthors.,1985).

The aim of this work has been to value some direct and indirect influence of grass carp on species composition and the biomass of some hydrobionts on the ecosystem of cyprinidae cultivation ponds, in application conditions of standard technical populating.

MATERIAL AND METHOD:

The experiments are done in Tapiza plant (Fushe Kruje), Figure 1 in two ponds with a surface of 1000m² and water depth 1.0-1.2m, during the period of 12 months April – March. The work is focused on the comparison of samples taken in one of the ponds populated with grass carp (experiment pond), with the samples taken in the other pond not stocked with grass carp (control pond). On the other side, during one year alterations have been followed in time, which specific components of biocoenosis suffer in the pond stocked with grass carp.

In the beginning of the experiment, two year old (1+) grass carp individuals were stocked in the experiment pond, with a population density of 30kg/ha (94 individuals with individual average weight 0.320+ 0.098kg), whereas the control pond wasn't stocked with this species.

Figure 1 Satellite view of Tapiza plant

The samples from plankton and benthos are taken every month from the beginning of April to the end of November and March. To take the plankton samples, in every pond without preliminary choice eight points are determined. The sampling is done by using Fieldmaster Advanced Water Sampler, that is a bathometric bottle of transparent polybicarbonat with a capacity 1.2lt (sample of every month has been 9.6lt). The equipment is immersed till near the ground of the pond and then is pulled up in the surface so that the sample included all the depth of the water.

The preparation of microscopic preparates for the determination of alga taxon is done immediately after taking the samples, without being necessary the conservation of the material. The determination of *clorophyll-a* is excecuted every month using Hydrolab D55X Multiparameter Sonde.

The biometric elaboration of data is done by using the variance analysis (ANOVA) with the pond and the year as standard factors. The influence of grass carp on proper studied parameters was considered significant when the relation between two systems comparable had the value $P<0.05$ (Ter Braak C.J.F and Smilauer P.,1998).

RESULTS AND DISCUSSION:

The taxonomic study of phytoplankton done in the period April-November, showed that in both ponds included in the study the dominant groups were green alga (*Clorophyta*) and diatomea (*Bacillariophyta*). The greater biomass, particularly in the period from the middle of April to beginning of June and from the end of August to the end of October were created from the green alga *Scendesmus quadricauda* (Turp.), *Pediastrum tetras* (Ehrenberg), Ralfs., *Hydrodyction reticulatum* (Lin.,Lagerheim), *Cladophora globulina* (Kutz.), *Diatomea Navicula sp., Pinularia viridis* (Nitzsch.), *Gyrosigma sp.* and green-blue alga (*Cyanophyta*), *Oscillatoria tenuis* (C.Agardh), *Microcystis aeruginosa* (Kutzing) and *Anabaena affinis* (Lemm.).

After stocking the experiment pond with grass carp we observed some alterations mainly focused on the displacement of dominances as in the group of green alga species as between the green alga on one side, and the other groups of alga on the other side. In the period from the middle of May till the beginning of September, we have proved the decrease of biomass of filamentous clorophyta (*H.reticulatum, C.globulina*) and *Scendesmus clorophyta* that form a colony with small number of cells. On the other side, two phenomenon were noticed in the experiment pond, particularly from the middle of July till the beginning of November: The rise of green-blue alga biomass, of some green unicellular alga, *Euglenphyta* and the appearance of some species not present in the control pond.

We have information that confirm the fact that filamentous alga of *Cladophora* and *Hydrodiction* are common components of grass carp diet meanwhile some alga that form a colony might have a determinating role in the feeding of this species. It seems that the inclusion of macrophytes and green filamentous alga in the grass carp diet removes from the ecosystem the main nutrient consumators, creating for the phytoplankton better trophic conditions. In such conditions, the abundance values of unicellular clorophyta and others that form small colonies, for the cyanophyta as a searching of high nitrogen concentration rises. A growth is proved for an individual number of some diatomea, particularly in April-June and September-November (Figure 2).

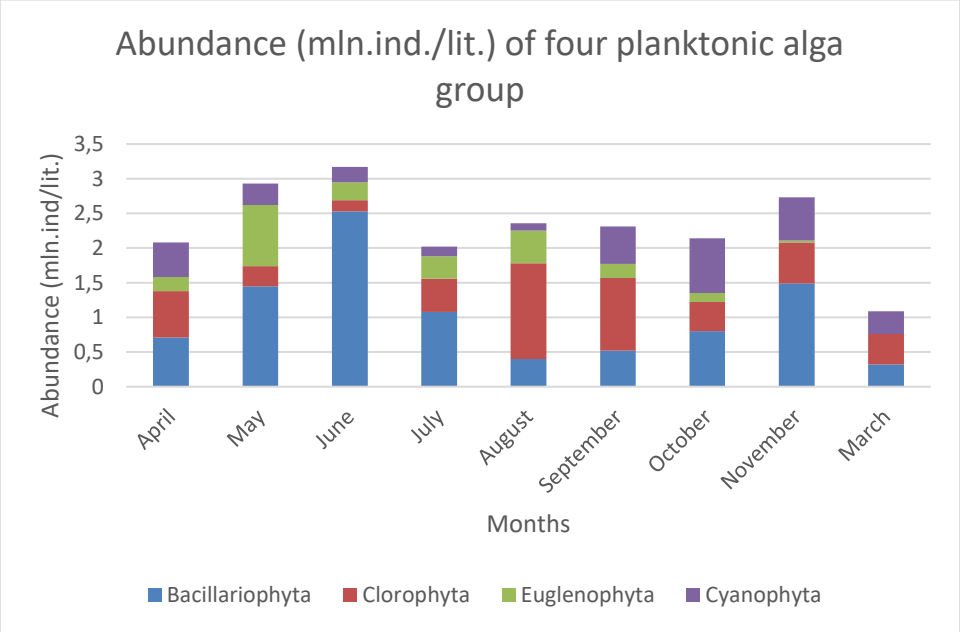

Figure 2 The dynamics of the abundance of four planktonic alga group in experiment pond after stocking with grass carp (*C. idella*) according to sampling done during April-November-March.

With all the alterations that suffer the density of some taxons of filamentous clorophyta alga and those that form a colony after stocking with grass carp, the general abundance of planktonic alga remain almost unchangeable as a consequence of abundance value rise for diatomea, unicellular clorophyta, euglenophyta and cyanophyta.

The fact that the influence of grass carp on *clorophyll-a* concentration wasn't statistically proved (F = 0.38; P = 0.0.57) is an indicative of the situation noticed during the study of general abundance dynamics of planktonic alga. Average value of *clorophyll-a* according to the tests carried out before grass carp introduction, was 10.92 mg/m³. The measurements performed after the introduction of grass carp in the pond showed that the average value of this indicator was 11.73 mg/m³. The rise of *clorophyll-a* concentration is accompanied with lessening of water transparency values. Before stoking with grass carp, the average value of water transparency was 43.4 cm, when after stocking with grass carp the average value of this indicator was 32.8cm.

The reduction of water transparency values is connected with the rise of organic and mineral detritus quantities in water, after the sediment displacement from the eradication of plant that happens during the grazing of grass carp (Mitchell C.P. and coauthors., 1984; Bonar S.A. and coauthors, 2002). Pipalova I. and coauthor (2009) underline that when all the plants are eliminated from the pond or when only uneatable plants are present in the pond then the grass carp begins to search the food near the ground causing sediment displacement and rise of water turbulence. On the other side, the rise of biomass of typical planktonic alga that happens after the reduction of filamentous alga abundance influences in reduction of water transparency values (Maceina M.J and coauthors 1992; Pipalova I. and coauthor, 2009).

Based on the working aim, in our experiment we stocked grass carp as a "monoculture". This solution is done for the fact that in comparison with fish fry with a weight of 10g, used for stocking polycultural systems, the two year old individuals we have stocked the experiment pond are directly consumers of aquatic macrophytes. Applied ichthtyomass guarantees full information in respect to influence of this phytophagus ichthtyc species on aquatic macrophytes, on grass carp impacts over the components of biocoenosis basins and simultaneously permits alimentary preference valuation of grass carp.

The presence of grass carp in experiment pond caused some changes in plankton alga community that were mainly focused on dominance displacement within the groups of green alga species and between the green alga in one side and alga of other groups at the other side. The study proved that the stocking of grass carp caused the lessening of filamentous clorophyta (*Hydrodiction reticulatum, Cladophora globulina*) and *Scendesmus* that make a colony with small number of cells. This manifestation was accompanied with rise of general blue green alga biomass, of some green unicellular Euglenophyta alga and with the appearance of some species not present in control pond.

The lessening of values for *C.globulina* alga abundance because of fact that during grazing, grass carp demonstrates chooser ability for this alga, is accepted in other studies too (Pine R.T. and Anderson L.W.J.,1991; Kirkagac M. and Demir N.,2004).

The rise of abundance indexes for some phytoplankton components as green unicellular alga, blue-green alga and euglenoidines are, it seems that it is cause of nutrients exploitation after lessening of strong competitors, as macrophytes (Pipalova I. and coauthor, 2009). Buck D.H and coauthors (1975) observed that *Ceratophyllum demersum* species completed in succesfull way for the nutrients with phytoplankton.

CONCLUSIONS:

Stocking with grass carp caused the rise of abundance indicator for some components of phytoplankton such as unicellular green alga, green-blue alga and euglenoidines. This alteration is a consequence of nutrients utilization, after the reduction of strong concurents presence, such as macrophyta.

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COMPARATIVE RESISTANCE OF STORED CEREALS AND PULSE TO *Sitophilus zeamais* MOTSCHULSKY (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

We hypothesized the degree of resistance of stored grains and pulse to *Sitophilus zeamais*, a cosmopolitan insect pest of stored foods in the tropics. Three varieties each of maize (TZPB SR, DMR 9943, DMR 9928), sorghum (NGBO1354, NGBO1469, NGBO1582) and wheat (NGBO1123, NGBO1124, NGBO1224) and a commonly grown cowpea variety (Ife brown) were used for the experiment. In a no-choice experiment, 20 g of each food variety was weighed into each of ten 1 L Kilner jar and five pairs of *S. zeamais* were introduced and covered with muslin cloth. Similarly, a free choice experiment was conducted on a white circular cardboard divided into ten equal sectors with each containing the food sample. All experiments were laid out in a CRD, r = 4. Data were collected on F_1 emergence, percentage survival, days to emergence, seed weight loss and susceptibility index and analyzed using ANOVA and means were separated with the NDMRT ($p<0.05$). The highest mortality (90%) of *S. zeamais* was observed on Ife-Brown and wheat variety, NGBO 1123 in all the days of the trials. Significantly ($p<0.05$) higher numbers (10.67, 9.86) of adult *S. zeamais* emerged from NGBO 1582 (sorghum) and NGBO 1124 (wheat) respectively. Susceptibility indices ranged from 0 to 5.8 in both no-choice and free-choice experiments. Cowpea variety (Ife Brown) and the wheat variety (NGBO 1123) were the least suitable host to *Sitophilus zeamais.* Desirable characteristics from these resistant grains could be useful in breeding programs to develop varieties that are resistant to the insect pests.

Keywords: Free choice experiment, Developmental time, Susceptibility index, Breeding programs
Mode of presentation at 2021 AGBIO CONFERENCE: Oral

INTRODUCTION

Cereals are a good source of rich-dietary fibre, vital nutrients like vitamin E, omega 3 fatty acid, phosphorous, magnesium and zinc (Macauley, 2015), constituting the largest source of food for human beings, as well as for animals, especially in Africa. Each cereal, such as maize, rice and sorghum, has its important nutrients that help to boost the body health (Klopcic *et al.*, 2020; Baniwal *et al.*, 2022). Cowpea is one of the most versatile food legumes in the tropics and subtropical regions of the world; the most important seed legume in Africa (Dakora and Belane, 2019), with a particularly high demand in Nigeria. The incessant rise in human population necessitates growing need for human food and animal feed, and consequently, there is a high demand for the maintenance of quality and quantity grain food products (Garcia-Correia, 2002).

Insect pests are among the main biotic agents that disrupt food substances in storage. They do these either by eating grains, contaminate commodities with their faeces, webbing, as well as their body parts (Hodges *et al.*, 2011; Berhe *et al*, 2022). The maize weevil (*Sitophilus zeamais*) is an important cosmopolitan pest of grains, including, maize, wheat, rice and sorghum, in the tropics. Its infestation begins in the field when the grain moisture content is between 50–55%, allowing the weevils to already complete one generation, and lay eggs for the second generation (Adedire, 2001). Earlier reports have also shown that it attacks other plants like *Carya illinoinensis* and *Prunus persica* (Bloem *et al*., 2002).

During harvest of crops, many smallholder farmers in Africa, especially Nigeria allocate sections of their local storage facilities for their produce and do not usually construct separate structures for each produce, leading to cross infestation of the produce by insect pests. Although cereal grains are the main host crops, infestation and damage caused by *Sitophilus zeamais* of cereal crop varieties are not well documented. As well, members of the genus *Sitophilus,* have been reportedly found in some other classes of crops. For example, in Nigeria, a strain of *S. zeamais* was found in some cowpea cultivars and laboratory observation revealed that the strain utilized the cowpea cultivars as its food, evidenced by the leftover powdery materials in the jars containing the cowpea seeds (Babarinde *et al.*, 2008). Gupta *et al*. (1985) reported that *S. rugicollis*, infested the seeds of sal (*Shorea robusta*) and caused damage on the oil crop and Coombs *et al.* (1977) reported that some strains of *S. oryzae* were found to develop on grain legumes such as peas, lentils and black grams. These developments necessitated the conduct of this current study in other to determine and compare the level of resistance of food host crops to *Sitophilus zeamais.*

MATERIALS AND METHODS

Study location

This study was carried out in the Entomology Research Laboratory of Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan under ambient temperature of 27 ± 5^{o} C, relative humidity of 72± 6% and 12 hours photoperiod.

Sources of Crop varieties

Three maize varieties (TZPB SR, DMR 9928, DMR 9943) and one cowpea variety (Ife Brown) were obtained from the Institute of Agriculture Research and Training (IAR&T), MOOR plantation, Ibadan, while three sorghum varieties (NGBO1354, NGBO1469, NGBO1582) and three wheat varieties (NGBO1123, NGBO1124, NGBO 1224) were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB) Moor Plantation, Ibadan, Nigeria. The food hosts were cleaned and kept in a refrigerator for 7 days to kill existing storage insect pest.

Insect colony

Colony of *Sitophilus zeamais* was established in the laboratory with initial stock obtained from Nigerian Stored product Research Institute (NSPRI), Onireke, Ibadan. Fifty (1 male: 1 female) weevils were introduced into 150 g maize grains in each of three 1- Litre Kilner jars with mesh lids. Old weevils were removed after 10 days of mating and oviposition. Teneral adults were used for the experiments.

Survival of *S. zeamais* on food hosts

Twenty (20) grams of each food varieties (Maize, sorghum, wheat and cowpea), were weighed and placed into each of ten 1 L Kilner jars and five pairs (1:1) of one week old *S. zeamais* were introduced into each jar and covered with lids having muslin cloth. Each jar was replicated four times and the experiment was laid out in a Completely Randomized Design (CRD). The set up was left undisturbed in the laboratory for ten days and data on mortality were collected at 6, 8 and 10 days after infestation.

Percentage mortality was calculated as,

$$\frac{\text{Number of dead weevils}}{\text{Total number of weevils}} \times \frac{100}{1}$$

No - choice experiments

Food varieties (20 g each), were weighed and placed in forty jars and ten one week old *S. zeamais* were introduced and the jars were covered with muslin cloth. All insects, both dead and living, were removed after 12 days. The set-up was left undisturbed until the emergence of F_1 progeny. Daily count of the emerged adults was done (until emergence ceased) and every insect was removed to prevent further egg laying on food samples. Adult *S. zeamais* that emerged from each jar were summed up and compared among food hosts. Median developmental time (MDT) was calculated as the time (days) from the middle of the oviposition period to the emergence of 50% of the F_1 progeny (Akinbuluma and Ewete, 2019). Susceptibility index (SI) for each treatment was then calculated using the formula:

$$SI = \frac{Log_{10}F \times 100}{MDT}$$

The grains were later sieved to remove the dust produced from adult feeding and reweighed using a Digital Pocket Weighing balance and percentage weight loss was determined as follows:

$$W\ (\%) = \frac{WI - WF}{WI} \times \frac{100}{1}$$

Where, W (%) = weight loss (%), WF = Final weight, WI = Initial weight

Free choice experiments

The same set up as the no-choice experiment was repeated with some modifications. Briefly, a white cardboard was cut to give a circular shape fitting into the bottom of a bowl and ten equal sectors were traced on the circular cardboard. Twenty (20) g of each grain was randomly placed in each sector. Adult insects (100) were placed at the centre of each bowl and covered with muslin cloth. The set up was replicated 4 times. The bowls were left undisturbed for 7 days for insect to oviposit, after which the grains were carefully transferred into Kilner jars and covered with muslin cloths. As described above, data were collected on emergence of F_1 adults (until completion of emergence), MDT and SI and weight loss and compared among food hosts.

RESULTS

Mortality of *S. zeamais*

Table 1 shows the percentage mortality of *S. zeamais* infested on the different varieties of food maize, sorghum, wheat, and cowpea (Ife brown) varieties. There was no mortality *of S. zeamais* on the infested DMR 9943 (maize) and NGBO 1224 (wheat) up till the 10^{th} day of trial. Mortality was significantly higher ($p < 0.05$) in NGBO 1123 (wheat) and Ife brown (cowpea) than in other food host varieties on all days of trials.

F_1 Emergence in *S. zeamais* and weight loss in food hosts

The number of F_1 emergence of adult *S. zeamais* as well as weight loss in maize, sorghum, wheat and cowpea in a no choice experiment and free choice experiments are presented in Tables 2a and 2b. The highest significant ($p < 0.05$) number of adult *S. zeamais* (10.67, 9.86) emerged on NGBO 1582 (sorghum) and NGBO1124 (wheat), respectively, while the least emergence was observed on NGBO1123 (wheat) and Ife brown (cowpea). Maize variety, TZPB SR-W and sorghum variety, NGBO 1582 had the longest developmental period of 38.63 and 35.75 days, respectively. The highest weight losses in food varieties were recorded on DMR 9943 (maize), NGBO 1582 (sorghum) and NGBO 1124 (wheat), while the least weight losses were found in NGBO 1123 (wheat) and Ife brown (cowpea) (Table 2a). As shown in Table 2b,

the highest significant number of adult *S. zeamais* in a free choice test, emerged from maize varieties, DMR 9928 and DMR 9943. The sorghum variety, NGBO 1354 had the longest median developmental time (34.50), even though not significantly different from developmental time in other food hosts apart from NGBO 1224 (wheat) and DMR 9928 (maize). Significantly higher weight losses were recorded on maize varieties, DMR 9943 and TZPB SR, while Ife brown (cowpea) and NGBO1123 (wheat) recorded the least percentage weight loss (Table 2b).

Fig. 1 shows the susceptibility indices of the food host varieties used in this study. As we had similar susceptibility indices for the no-choice and free choice experiments, we decided to use a representative index for our reports. Ife Brown (cowpea) and NGBO 1123 (wheat) had the lowest (0) susceptibility index, while NGBO 1469 (sorghum) had the highest (5.8) susceptibility value (Fig. 1).

Table 1: Survival of *Sitophilus zeamais* infested on varieties of food hosts in the laboratory

Food host	Varieties	Mortality (± S.E) at days after infestation		
		6	8	10
Maize	DMR 9928	0.00±0.00a	0.00±0.00a	5.00±0.30a
	DMR 9943	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a
	TZBP SR W	4.61± 4.61a	9.22± 5.32a	9.22± 5.32a
Sorghum	NGBO 1354	32.53±4.91b	41.99±5.07b	49.87±5.07b
	NGBO 1469	4.61± 4.61a	9.22±5.32a	16.22±5.32a
	NGBO 1582	0.00±0.00a	0.00±0.00a	7.00±3.40a
Wheat	NGBO 1123	78.75± 6.70c	90.00± 0.00c	90.00± 0.00c
	NGBO 1124	9.22±5.32a	9.22±5.32a	9.22±5.32a
	NGBO 1224	0.00± 0.00a	0.00± 0.00a	0.00± 0.00a
Cowpea	IFE-BROWN	80.78±5.32c	90.00±0.00c	90.00±0.00c

Means within a column with the same letter(s) are not significantly different at $p<0.05$ using New Duncan's Multiple Range Test

Table 2a: Adult Emergence, Median Developmental Time of *Sitophilus zeamais* and weight loss in food hosts in a no-choice test

Food hosts	Varieties	F_1 Emergence	Median Development Time	Loss in weight (%)
Maize	DMR 9928	4.38±0.12bc	34.5±0.83cd	10.57±0.66bc
	DMR 9943	4.41± 0.15bc	33.75± 0.41bc	15.44±1.12d
	TZBP SR	3.67±0.42b	38.63±1.69e	8.51±0.97b

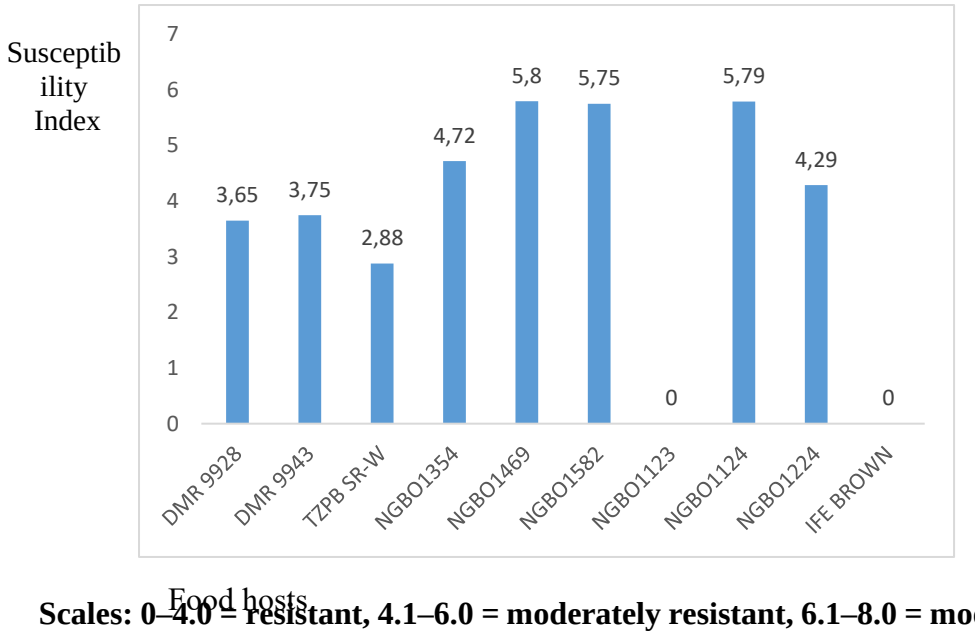
Sorghum	NGBO 1354	5.77±0.55d	32.25±1.11bc	7.37±1.03b
	NGBO 1469	7.95±0.44e	31.00±0.5b	8.06±0.97b
	NGBO 1582	10.67±0.62f	35.75±1.38de	13.89±1.57d
Wheat	NGBO 1123	1.00±0.00a	0.00± 0.00a	1.11±0.21a
	NGBO 1124	9.86±0.18f	34.25±0.95cd	13.39±1.36cd
	NGBO 1224	5.21± 0.45cd	33.25±0.25bcd	7.50±1.03b
Cowpea	IFE-BROWN	1.00±0.00a	0.00±0.00a	0.88±0.09a

Means within a column with the same letter(s) are not significantly different at $p < 0.05$ using New Duncan's Multiple Range Test

Table 2b: Adult Emergence, Median Developmental Time of *Sitophilus zeamais* and weight loss in food hosts in a free-choice test

Food hosts	Varieties	F_1 Emergence	Median Development Time	Loss in weight (%)
Maize	DMR 9928	5.07±0.13de	31.5±0.86bc	36.12±1.75de
	DMR 9943	5.58± 0.31e	32.5± 0.86bcd	41.97±2.48ef
	TZPB SR	4.31±0.22c	32.0±0.91bcd	49.28±1.29f
Sorghum	NGBO1354	4.56±0.42cd	34.5±0.65d	17.73±1.79b
	NGBO1469	4.52±0.16cd	31.75±1.1bcd	28.25±1.34b
	NGBO1582	4.87±0.21cd	31.75±0.94bcd	24.67±7.49cd
Wheat	NGBO1123	1.00±0.00a	0.00± 0.00a	4.18±0.89a
	NGBO1124	3.67±0.09b	33.75±0.95cd	17.82±2.60bc
	NGBO1224	3.55± 0.19b	30.75±0.75b	20.57±1.88b
Cowpea	IFE-BROWN	1.00±0.00a	0.00±0.00a	3.13±1.09a

Means within a column with the same letter(s) are not significantly different at $p < 0.05$ using New Duncan's Multiple Range Test



Scales: 0–4.0 = resistant, 4.1–6.0 = moderately resistant, 6.1–8.0 = moderately susceptible, 8.1–10.0 = susceptible and ≥ 10.1 = highly susceptible (Dobie, 1974)

Fig. 1.: Susceptibility index of food host varieties in a no choice test

DISCUSSION

Significantly high mortality of *S. zeamais* was recorded on Ife-Brown and on wheat variety, NGBO 1123, suggesting that *S. zeamais* must have been starved to death since the food host varieties could not supply the appropriate nutrients to the insect. Conversely, little or no mortality was recorded on all the maize host varieties and other wheat and sorghum hosts, agreeing with earlier reports that *Sitophilus zeamais* preferred some food varieties (especially maize) than others.

There was considerable variation in the F_1 progeny of *S. zeamais* across the food hosts. Significantly higher number of adults emerged from the three sorghum varieties than from the other food varieties ($p<0.05$) could be as a result of the small sizes of sorghum grains, which provided an increase in the number of grains available for oviposition. This finding is in accordance with earlier reports that oviposition and emergence of *S. zeamais* was density dependent (Richards, 1947; Pederson, 1979; Mathias *et al*., 2015). In this study, *S. zeamais* completed its development on the food hosts between 33 - 39 days, on the choice and no-choice experiments. Similar observations have also been made by Ojo and Omoloye, (2016) where they reported that the comparative developmental cycle of *S. zeamais* from egg to adult was 34.7 days (on maize). Akinbuluma and Ewete (2019) also reported a median developmental time of 33.0 days of *S. zeamais* on DMR-ESY maize variety.

Losses in maize hosts were consistently higher than those in other food hosts and this might be due to the high survival rate of *S. zeamais* on the maize grains. Infestation of *S. zeamais* can cause weight loss of above 30% in stored maize (Paneru *et al*., 1996; Sharma *et al.*, 2016). Similarly, a range of between 7 – 29% was observed to be lost to *S. zeamais* feeding on sorghum, agreeing with the reports of Patrick and McClure (2009) that stored sorghum incur losses of up to 20%. Suleiman *et al*. (2013) reported that *S. zeamais* is a serious insect pest of sorghum causing grain damage of up to 65.5% after four months of exposure to *S. zeamais* under laboratory conditions, while Goftishu and Belete (2014) also recorded grain damage of about 30.0% after 2 months of *S. zeamais* infestation. The least percentage weight loss in Ife brown (cowpea) and NGBO 1123 (wheat) varieties might be due to the low survival of the insect pest on these food hosts. The range of susceptibility indices suggests that all the food host varieties used in this study were moderately resistant to *S. zeamais* apart from NGBO1123

(wheat) and Ife brown (cowpea) which were highly resistant (Dobie, 1974; Akinbuluma *et al*., 2019). From this study, maize, sorghum and two wheat varieties NGBO 1124 and NGBO 1224 can serve as suitable hosts, while cowpea and wheat variety (NGBO1123) are the least suitable host of *S. zeamais* in relation to feeding and development.

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SUSTAINABILITY ASSESSMENT OF ANIMAL HUSBANDRY IN TURKEY

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ABSTRACT

This study covers analysis of the sustainability of the animal husbandry activities in Edirne and Kastamonu provinces of Turkey through a thermodynamic sustainability assessment technique, emergy analysis (EA). By classifying energy and material flows as renewable, non-renewable and purchased from economy, EA defines metrics to evaluate the sustainability of a system. This metrics provide insight about evaluated system's renewability, environmental loading and dependence on external inputs. In analyzed systems, 3 breeders raise both cows and sheep; 3 breeders raise cows, only. All animal breeding systems we analyzed are found to have renewability lower than 20%, environmental loading ratio (ELR) of higher than 2 and environmental sustainability index (ESI) of lower than 1. Consequently, they are determined to be unsustainable irrespective of the location of husbandry activities. Purchased animal feed is determined to be the main factor behind the systems' unsustainability. Integration of animal breeding with feed crop cultivation and increasing the ratio of farmer grown food in diets of animals can enhance the sustainability performance of animal husbandry systems.

Keywords: Emergy analysis, system renewability, environmental loading, sustainable animal husbandry, system integration

INTRODUCTION

26% of global greenhouse gas (GHG) emissions and 70% of freshwater use are created by food production. 96% of all mammal biomass excluding humans is livestock and 71% of all bird biomass is poultry livestock (Ritchie and Roser, 2022). This shows the extent of increase in animal-sourced-nutrition production due to human population growth. Hence, performing animal rearing activities in a sustainable manner has the upmost importance in today's world.

Emergy analysis is a thermodynamic sustainability assessment tool that provides insights about a systems renewability, environmental loading and dependence on external resources (Odum, 1996). Biological systems are interconnected through an "energy hierarchy" (Brown and Ulgiati, 2004; Hau and Bakshi, 2004). Hence, the interconnected nature and sustainability of these systems can be analyzed thermodynamically through EA.

EA is widely utilized in sustainability assessment of animal rearing activities as in works of He et al., 2019, Zhang et al., 2007 and Wang et al., 2015. However, studies evaluating sustainability of Turkish husbandry sector through EA are not available. Hence, this work evaluates and compares the sustainability of 6 animal husbandry systems in Edirne and Kastamonu provinces of Turkey.

MATERIAL AND METHOD

Background

Table 1 lists properties of 6 husbandry systems analyzed in this work. Performing research in two different locations enables system comparisons and related recommendations.

Table 1: Location, animal number and type, feeding structure and product characteristics of evaluated animal breeding systems.

System	Location	Animal Number	Feed Type	Products
Husbandry 1	Edirne/ Uzunköprü	100 cows	Purchased + Self-grown	18-20 L milk/animal + 25 male calf/year
Husbandry 2	Edirne/ Merkez	25 cows + 3 sheep	Purchased + Self-grown	15L milk/animal + 5 male calf/year + 60 kg sheep meat/year
Husbandry 3	Edirne/ Merkez	11 cows	Purchased + Self-grown	20 L milk/animal + 5 male calf/year
Husbandry 4	Kastamonu/ Tosya	110 cows + 60 sheep	Purchased	400 kg meat/cow + 30 kg meat/sheep
Husbandry 5	Kastamonu/ Tosya	22 cows + 2 sheep	Purchased + Self-grown	10 L milk/animal + 10 male calf/year.
Husbandry 6	Kastamonu/ Tosya	65 cows	Purchased + Self-grown	15L milk/animal + 15 male calf/year

Emergy Analysis (EA)

Methodologically, EA includes the steps of drawing energy systems diagram (a pictorial model of the system), emergy evaluation table formation (an inventory for inputs and outputs) and the calculation of emergy indicators (Odum, 1996; Brown and Ulgiati, 2004).

Figure 1 shows the energy systems diagram (ESD) for an animal rearing system in Edirne. Here, sun wind and rain are renewable inputs that are provided by the natural environment. Water is the non-renewable local input that is under storage category. It is classified as non-renewable since the groundwater levels are declining in both research locations. Inputs that are exchanged from economy are classified as purchased inputs (Odum,1996).

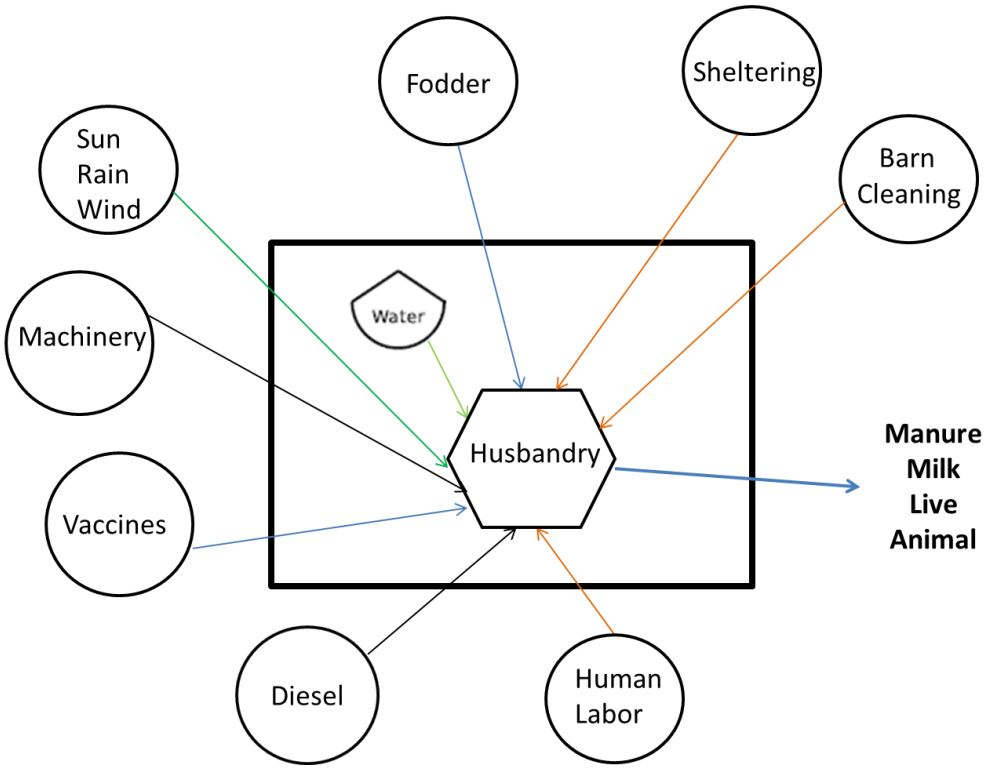

Figure 1: Energy systems diagram for and animal rearing system in Edirne.

Emergy evaluation table (EET) is a listing containing all the energy and material inputs to and the outputs from the system under study. EET is formed based on the determined analysis boundary and the model drawn in the ESD (Odum, 1996).

Calculation of emergy indicators in EA is based on the classification of emergy flows as local renewable (R), non-renewable (N) and purchased (P) (Ulgiati et al, 2010).

The calculation of the emergy indicators is presented in equations 1-6 mathematically.

$$\text{Emergy Yield } (Y) = R + N + P \quad (1)$$

$$\text{Renewability} = \frac{R}{Y} \quad (2)$$

$$\text{EmergyYield Ratio (EYR)} = \frac{Y}{P} \quad (3)$$

$$\text{Environmental Loading Ratio (ELR)} = \frac{(N+P)}{R} \quad (4)$$

$$\text{Environmental Investment Ratio(EIR)} = \frac{P}{(R+N)} \quad (5)$$

$$\text{Environmental Sustainability Index (ESI)} = \frac{EYR}{ELR} \quad (6)$$

Systems having renewability of lower than 20%, EYR of lower than 4, ELR of higher than 2 and ESI of lower than 1 are classified as unsustainable systems. If these systems are improved, they can evolve into being in transition state or sustainable in terms of their sustainability status (Chen et al., 2017). Further information on EA can be found in Kursun and Bakshi (2016).

RESULTS AND DISCUSSION

Figure 2 shows renewability of animal breeding systems evaluated. Out of 6 breeders, 3 have both cow and sheep and 3 breeders solely raise cows. In cow breeding systems renewability changes between 0.42% and 15.9%. In case of sheep, this change is between 1.69 % and 12.1%. The main factor affecting renewability of cow systems is how much of the feed is grown by the farmer. As the integration level of cow rearing and feed crop cultivation systems (feed crops are fertilized with animal manure) increase, renewability of cow rearing increases. The same inclination is also true for sheep rearing, as the portion of farmer grown feed or grazing increases, sheep rearing systems become more renewable.

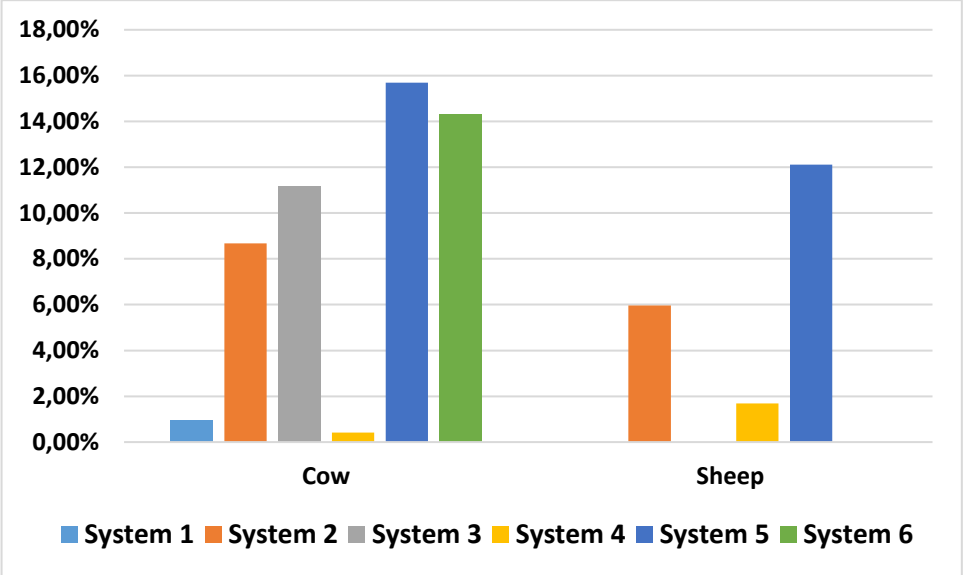

Figure 2: Renewability values for evaluated animal rearing systems.

Figure 3 shows emergy yield ratio (EYR) of animal breeding systems evaluated. In cow breeding systems EYR changes between 1.10 and 1.26. In case of sheep, this change is between 1.02 and 1.17. System renewability and EYR go hand in hand. As renewability increases, system EYR also increases. Here, again how much of the feed is grown by the farmer and level of integration of cow rearing and feed crop cultivation systems are the determining factors that increase EYR value (preferred). For sheep cases as the portion of farmer grown feed or grazing increases, sheep rearing EYR increases.

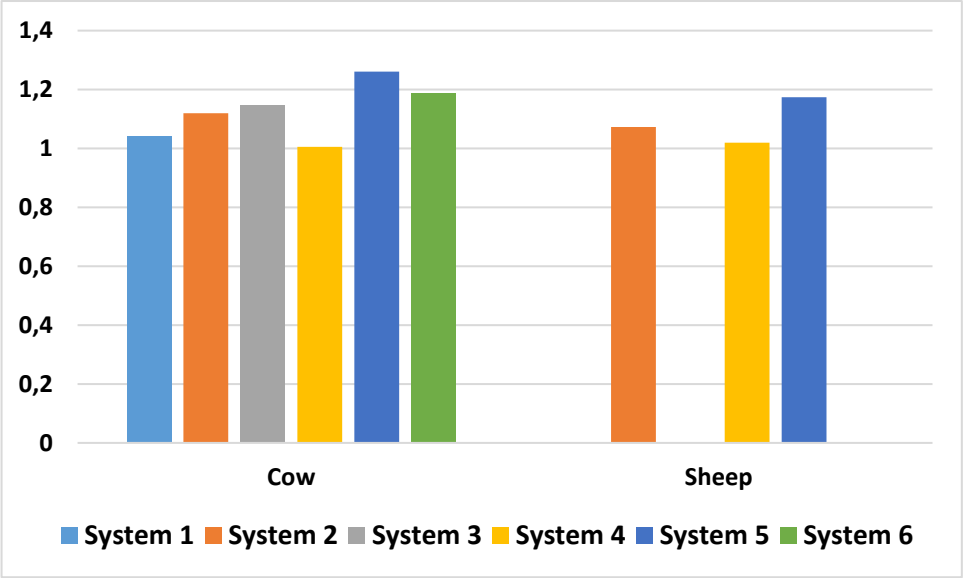

Figure 3: Emergy yield ratio (EYR) values for evaluated animal rearing systems.

Figure 4 shows environmental loading ratio (ELR) of animal breeding systems evaluated. In cow breeding systems ELR changes between 5.33 and 238. In case of sheep, this change is between 7.22 and 58.2. Being purchased feed the largest emergy input to the system followed by non-renewable water create the environmental loading in cow breeding. For sheep, mainly purchased animal feed is responsible from this impact. Due to grazing, sheep breeding generally has lower environmental loading than cow breeding.

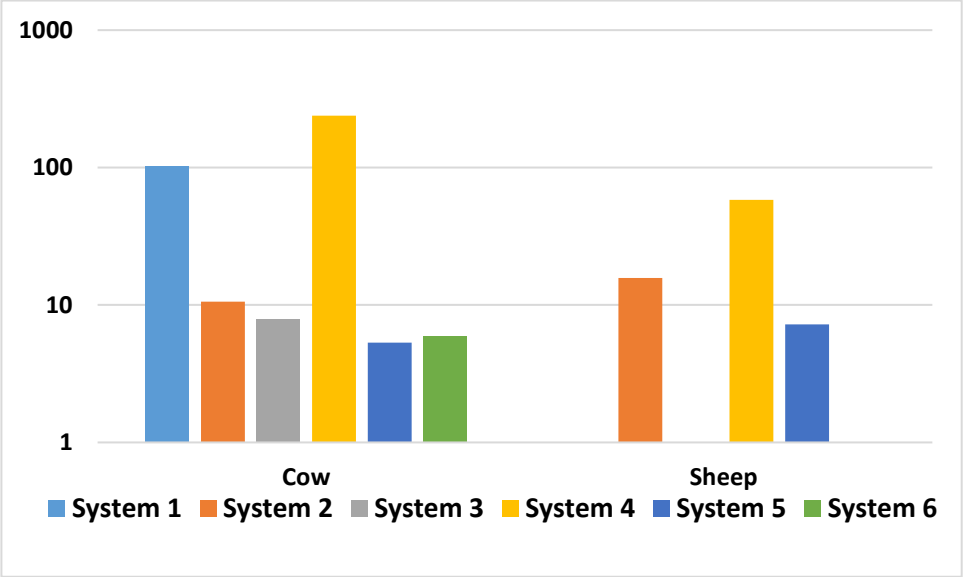

Figure 4: Environmental loading ratio (ELR) values for evaluated animal rearing systems.

Figure 5 shows environmental investment ratio (EIR) of animal breeding systems evaluated. In cow breeding systems EIR changes between 5.36 and 197. In case of sheep, this change is between 5.91 and 13.7. For EIR, dominance of purchased animal feed is the main reason behind high EIR values both for cow and sheep breeding.

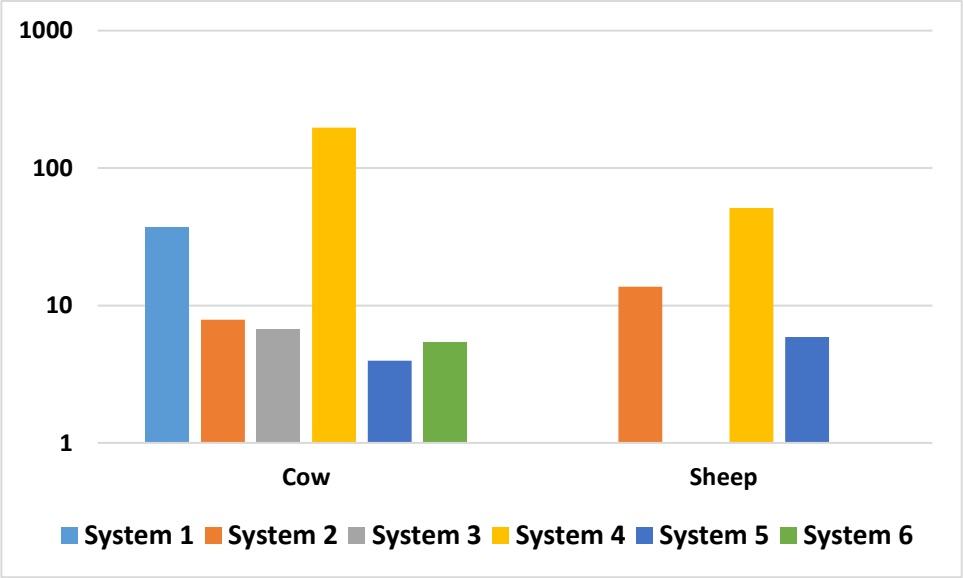

Figure 5: Environmental investment ratio (EIR) values for evaluated animal rearing systems.

Figure 5 shows environmental sustainability index (ESI) of animal breeding systems evaluated. In cow breeding systems ESI changes between 0.01 and 0.24. In case of sheep, this change is between 0.02 and 0.16. ESI is the ratio of EYR to ELR, hence it represents production per environmental loading. Low ESI values obtained both for cow and sheep breeding show that all of the animal breeding systems analyzed are unsustainable.

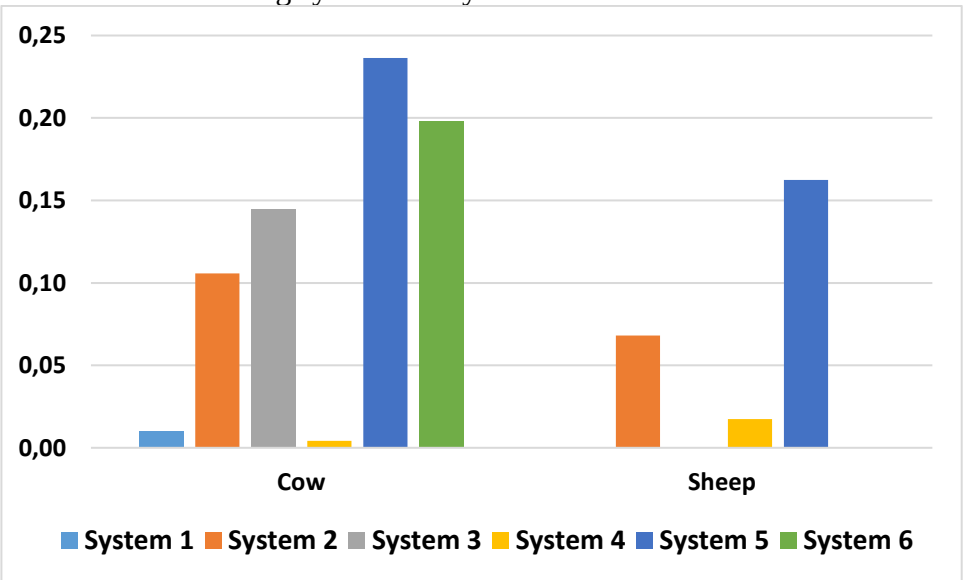

Figure 6: Environmental sustainability index (ESI) values for evaluated animal rearing systems.

CONCLUSIONS

All animal breeding systems studied in this work has renewability lower than 20%, ELR higher than 2 and ESI lower than 1. Consequently, they are found to be unsustainable. Purchased animal feed is determined to be the main factor behind the systems' unsustainability. Integration of animal breeding systems with feed crop cultivation and increasing the ratio of farmer grown food in diets of animals can enhance sustainability performance of animal breeding systems.

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EVALUATION OF ORGANIC LIVESTOCK FARMING EFFICIENCY IN TURKEY WITH DATA ENVELOPMENT ANALYSIS

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ABSTRACT

In recent times, consumers have been placing great importance on the consumption of healthy, high-quality, and safe food. Organic products to protect human health and raise healthier generations has been increasing the significance of organic agriculture and livestock day by day. The primary objective of organic agriculture and livestock is to preserve the environment, plants, animals, and human health without polluting soil, water resources and compromising air quality. Organic livestock farming is a production method where chemical inputs are avoided, and all stages, from production to consumption, are controlled and certified. Residues of feed and additives used in industrial livestock farming leave significant traces in animal-based food products, causing significant health issues for consumers. Hence, an alternative organic livestock farming approach based on pasture and chemical-free feed is proposed, as it is a more environmentally and ethically sound production system. In this study, the efficiencies of organic livestock farming in the twelve regions of Turkey, which we determined it, were evaluated using Data Envelopment Analysis (DEA) with zero data. The regions were considered as Decision Making Units (DMUs), number of poultry, number of small ruminants, number of large ruminants, number of beehives, and number of farmers were determined as inputs. Produced meat (tons), milk (tons), eggs (number), and honey (tons) were determined as outputs.

Keywords: Organic livestock, Organic livestock farming, Data envelopment analysis, Efficiency, Zero data

INTRODUCTION

Until the 1980s, due to the complementary relationship between plant and animal production in enterprises, fertilizer was obtained for animal husbandry and plant production, while plant production also contributed to animal husbandry with feed crops and by-products. Despite the increase in the world population, the number of animals, milk, and especially meat production, which are very important resources for human nutrition, have not increased at the same level, so livestock farming has begun to be carried out industrially in large-scale enterprises. For this reason, the natural relationship between plant agriculture and livestock farming has been broken and small producers have reached the point of extinction, while many environmental, biological and economic damages have occurred. For example, increased susceptibility to diseases as a result of little movement of animals tethered or walking in narrow spaces, and the resulting animal feces and gases disrupt the natural balance, water, soil and air. In addition, factors that negatively affect the consumer have emerged, such as the formation of fatty acid, insulin and antibiotic residues in products obtained from animals. To address these issues, organic livestock farming has emerged as an alternative to industrial practices. It relies on pasture-based feed production and avoids chemical usage, aligning with consumer demands for a more environmentally friendly and ethically sound production system.

Organic livestock farming is a form of production that is controlled and certified at every stage from production to consumption, without using chemical inputs in production. Çelikyürek

& Karakuş (2018) analyze the importance of organic livestock farming in the world and Türkiye. Aygün and Akbulak (2017), evaluate the organic agriculture potential of Ardahan province, which has large and productive pasture areas and no environmental pollution caused by industrial and agricultural activities with SWOT analysis. Hanoğlu (2013) provides a comprehensive discussion of organic cattle farming for both small and large ruminants in Turkey. Furthermore, organic chicken farming has been experiencing a notable increase in popularity. This surge can be attributed to consumer preferences, as chickens are favored for their efficient conversion of feed into high-quality protein within a short time frame. Additionally, both chicken meat and eggs are considered important components of a healthy diet. Moreover, chicken meat is often priced more affordably than red meat (Uruk & Yenilmez, 2018). Furthermore, in addition to its contribution to ecological balance, organic beekeeping is also practiced in rural areas, especially in certain regions of Turkey. Güler (2021) evaluate the efficiency of the provinces in Turkey in organic beekeeping with DEA. In the study, the number of businesses and the number of hives were determined as inputs, and honey production and beeswax were determined as outputs.

MATERIAL AND METHOD

DEA is a non-parametric and linear programming-based method used to measure the relative efficiencies of alternative units that produce similar outputs using similar inputs. The CCR model, which was developed by Charnes, Cooper, and Rhodes as part of a thesis, was introduced first model of DEA in the literature (Charnes, Cooper, & Rhodes, 1978). In the CCR model, efficient and inefficient Decision-Making Units (DMUs) can be distinguished, where efficient DMUs form the efficiency frontier and provide a preference ranking for inefficient DMUs. However, the classical models, known as the CCR under the Constant Returns to Scale (CRS) assumption and BCC model under the Variable Returns to Scale (VRS) assumption, proposed by Banker, Charnes, and Cooper (Banker, Charnes, & Cooper, 1984), cannot provide rankings for efficient DMUs. Due to the inability of classical DEA models to rank efficient DMUs, many methods have been proposed to enhance the discrimination power of DEA, one of which is the super-efficiency method proposed by Andersen and Petersen which developed the Super Efficiency (SE) model (Andersen & Petersen, 1993).

Firstly, the parameters and decision variables for SE model are defined below, followed by the presentation of the input-oriented SE model (Andersen & Petersen, 1993). The model aims to calculate the efficiency score of DMU_0, which is the DMU under evaluation. In this context, θ_k represents the efficiency score, where *k* denotes the number of DMUs.

Parameters:

N	cluster of DMU
M	cluster of input
S	cluster of output
x_{ik}	*i*-th input value of DMU *k*
y_{rk}	*r*-th output value of DMU *k*

Decision Variables:

θ_k	Efficiency score of DMU *k*
λ_k	Matrix containing the weights of inputs and outputs for DMU *k*

(SE Model)

$$\text{Min } \theta_0 \tag{1}$$

$$\sum_{k \in N-\{0\}} \lambda_k x_{ik} \leq \theta_0 \, x_{i0} \qquad \forall \, i \in M \tag{2}$$

$$\sum_{k \in N-\{0\}} \lambda_k y_{rk} \geq y_{r0} \qquad \forall\, r \in S \tag{3}$$

$$\sum_{k \in N-\{0\}} \lambda_k = 1 \tag{4}$$

$$\lambda_k \geq 0 \qquad \forall\, k \in N \tag{5}$$

In the SE model, DMUs is excluded from the dataset, consequently breaking the existing efficient frontier, and establishing a new one. The DMU under evaluation for efficiency is positioned outside this newly formed efficient frontier, resulting in an efficiency score equal or larger than 1. The greater the efficiency score of an efficient DMU, the preferable it. Inefficient DMUs have the same efficiency scores as those obtained from the CCR model which assumes CRS.

In the VRS assumption, super-efficiency model which may be infeasible when some efficient DMUs are under evaluation. Seiford and Zhu (1999) provide the necessary and sufficient conditions for infeasibility of super-efficiency models, and further show that infeasibility must occur in the case of the variable returns to scale (VRS) super-efficiency model. Several studies have tried to solve the problem of VRS super-efficiency model's infeasibility (Lovell & Rouse, 2003; Chen, 2005; Cook, Liang, Zha, & Zhu, 2009). In a recent study by Lee, Chu, and Zhu (2011), the authors develop a two-stage process to overcome the VRS infeasibility issue by yielding a score that characterizes the super-efficiency in both inputs and outputs. Chen and Liang (2011) further prove that the two-stage process can be solved in a single linear program. Lee, Chu, and Zhu (2011), show that infeasibility exists under input-oriented (output-oriented) model when any output surplus (input saving) exists. Under input-oriented (output-oriented) situation, this new approach identifies the radial efficiency and output surplus (input saving) simultaneously and yields a super efficiency score that characterizes both the radial efficiency and output surplus (input saving) if it exists. The proposed model works when data is positive. If the data is nonnegative, i.e., some of the input or output data is zero, these new super-efficiency models can still be infeasible. In an extension of the research conducted by Lee et al. (2011), Lee and Zu (2012) revised the model and feasible when zero data exist in inputs. They state zero output data does not cause infeasibility the output-oriented super-efficiency models developed in prior studies by Lee et al. (2011), Chen and Liang (2011), and Cook et al. (2009). The reason behind this is that the constraints on the output side can always be satisfied. Lee and Zu model is presented below (Lee and Zu, 2012):

(*Lee and Zu model)*

$$Min\ \tau + M\left(\sum_r \beta_r + \sum_i t_i\right) \tag{6}$$

$$\sum_{k \in N-\{0\}} \lambda_k x_{ik} - t_i x_{imax} \leq (1+\tau)\, x_{i0} \qquad \forall\, i \in M \tag{7}$$

$$\sum_{k \in N-\{0\}} \lambda_k y_{rk} \geq (1-\beta_r)\, y_{r0} \qquad \forall\, r \in S \tag{8}$$

$$\sum_{k \in N-\{0\}} \lambda_k = 1 \tag{9}$$

$$\lambda_k \geq 0,\ \beta_r \geq 0,\ t_i \geq 0,\ \tau \text{ is unlimited} \tag{10}$$

where $x_{imax} = max_{k=1}^{1}\{x_{ik}\}$, t_i is input saving and β_r output surplus, Input saving index and output surplus index are calculated as follows with $I = \{i|\ t_i^* > 0\}$ and $R = \{r|\ \beta_r^* > 0\}$.

$$\hat{\imath} = \begin{Bmatrix} 0 & if\ I = \emptyset \\ \frac{\sum_{i \in I}\left(\frac{1+t_i^*}{1}\right)}{|I|} & if\ I \neq \emptyset \end{Bmatrix} \qquad o = \begin{Bmatrix} 0 & if\ R = \emptyset \\ \frac{\sum_{r \in R}\left(\frac{1}{1-\beta_r^*}\right)}{|R|} & if\ R \neq \emptyset \end{Bmatrix}$$

Then, the super-efficiency score can be defined as $\breve{\theta} = 1 + \tau^* + o + \hat{\imath}$.

RESULTS AND DISCUSSION

Worldwide and in Türkiye, organic agriculture, and livestock, also known as ecological agriculture, is becoming a growing market day by day. It can be noted that policymakers, along with providing support, grants, and incentives, have played a significant role in fostering this growth. In addition, concern about food crisis around the world and the need for cleanliness of products and environmental protection have led scientists to search for different production models and encouraged organic agriculture and livestock. In our country, organic livestock farming is constantly growing for the reasons mentioned above. Therefore, in this study, Türkiye's organic livestock farming efficiency was evaluated by DEA with zero data. Türkiye 's provinces are classified 12 regions, and the regions are determined as DMU. Table 1 shows provinces, classified 12 regions, and number of members. In the study, we consider both small ruminants and large ruminants organic livestock farming, and organic chicken farming and beekeeping. In this context, poultry (number), small ruminants (number), large ruminants (number), beehives (number) and farmers (number) are determined as input, and produced meat (tons), milk (tons), eggs (numbers), honey (tons) are determined as output. Table 2 shows inputs and outputs data. Data were taken from the website of the Ministry of Agriculture (Ministry of Agriculture and Forestry, 2023)

Table 1. Provinces and classified 12 regions.

DMU		Cities	Number of class member
1	Marmara Region 1	Bursa, Çanakkale, Balıkesir, Yalova, Bilecik	5
2	Marmara Region 2	Edirne, Kırklareli, Tekirdağ, İstanbul, Kocaeli, Sakarya	6
3	Aegean Region	Kütahya, Denizli, Muğla, İzmir, Aydın, Manisa, Uşak, Afyon	8
4	Central Anatolia Region 1	Eskişehir, Ankara, Çankırı, Kırıkkale, Kırşehir, Konya, Karaman	7
5	Central Anatolia Region 2	Aksaray, Yozgat, Niğde, Nevşehir, Kayseri, Sivas	6
6	the Mediterranean Region	Osmaniye, Adana, Isparta, Antalya, Burdur, Mersin, Hatay, Kahramanmaraş	8
7	Black Sea Region 1	Giresun, Gümüşhane, Trabzon, Bayburt, Rize, Artvin	6
8	Black Sea Region 2	Tokat, Samsun, Sinop, Çorum, Amasya, Ordu	6
9	Black Sea Region 3	Düzce, Bolu, Zonguldak, Karabük, Bartın, Kastamonu	6
10	Southeastern Anatolia Region	Şırnak, Siirt, Batman, Mardin, Diyarbakır, Şanlıurfa, Adıyaman, Gaziantep, Kilis	9
11	Eastern Anatolia Region 1	Malatya, Elazığ, Tunceli, Erzincan, Bingöl, Erzurum, Muş	7
12	Eastern Anatolia Region 2	Iğdır, Ardahan, Kars, Bitlis, Ağrı, Van, Hakkâri	7

Table 2. Inputs and outputs data.

DMUs	Poultry (number)	Small ruminants (number)	Large ruminants (number)	Beehives (number)	Farmers (number)	Meat (tons)	Milk (ton)	Eggs (number)	Honey (ton)
1	20 472	1 172	2 844	1 576	67	27.54	2 572.5	2 743 386	15.809
2	99 700	0	202	35	12	0.991	959.496	24 583 110	0.71
3	352 662	22	2 762	664	28	78	16 054	41 117 289	13.146
4	420	1 651	116	438	6	0	0	54 000	8.706
5	0	0	1 206	4 709	60	0	6 910.8	0	88.19665
6	17 000	2 485	60	12 477	42	0	264	3 517 867	481.527
7	0	0	0	14 429	98	0	0	0	184.863
8	113 500	0	0	1 024	14	0	0	2 000	3.14
9	54 500	0	0	0	3	0	0	4 392 140	0
10	0	0	0	5 033	22	0	0	0	40.85
11	43 968	0	0	13 906	66	0	0	13 190 400	185.923
12	0	0	0	18 646	45	0	0	0	329.306

We take into account zero data to evaluate Turkey's organic livestock farming efficiency and use Lee and Zhu model. Table 3 shows super efficiency score and rank. Looking at the results, the Aegean Region has first rank, Marmara Region 2 has second rank, and Marmara Region 1 ranks 4th. While Central Anatolia Region 1 ranks 3rd, Central Anatolia Region 2 ranks 10th. Black Sea Region 3, which is one of the regions rich natural resources and indigenous races, ranks 5th, while Black Sea Region 1 and 2, located further east, rank 11th and 12th, respectively. Eastern Anatolia Regions 1 and 2, known as the source of large ruminants, are ranked 8th and 6th respectively. Finally, Southeastern Anatolia Region is ranked 7th.

Table 3. Super efficiency score and rank.

DMUs	1+alfa*	t_1*	t_2*	t_3*	t_4*	t_5*	β_1*	β_2*	β_3*	β_4*	Input saving index	Output surplus index	Super efficiency score	Rank
1	6.08	0	0	0	0	0	0	0	0	0	0	0	6.08	4
2	10.43	0	0	0	0	0	0	0	0	0	0	0	10.43	2
3	2.92	0	0	0	0	0	0.99	0.94	0.42	0	0	39.46	42.38	1
4	9.6	0	0	0	0	0	0	0	0	0	0	0	9.60	3
5	0.99	0.43	0	0	0	0	0	0	0	0	1.43	0	2.42	10
6	1.47	0	0	0	0	0	0	0	0	0.33	0	1.49	2.96	9
7	0.82	0	0	0	0	0	0	0	0	0	0	0	0.82	11
8	0.44	0	0	0	0	0	0	0	0	0	0	0	0.44	12
9	4.55	0	0	0	0.05	0	0	0	0	0	1.03	0	5.58	5
10	3.28	0	0	0	0	0	0	0	0	0	0.00	0	3.28	7
11	1.81	0	0	0.2	0	0	0	0	0	0	1.16	0	2.97	8
12	2.18	0	0	0	0	0	0	0	0	0.44	0	1.79	3.97	6

CONCLUSIONS

In recent years, there has been a significant increase in the global demand for products grown using organic farming methods. Consumers are increasingly concerned about food safety and the environment, leading to a shift away from previously used systems. To address these concerns, organizations specializing in organic agriculture and livestock certification have been

established worldwide. They oversee every step of the process, from farming and processing to packaging, labeling, and storage, ensuring the safety and quality of products until they reach consumers.

In this study, Türkiye's organic livestock farming efficiency was evaluated by DEA with zero data. Türkiye 's provinces are classified 12 regions, and the regions are determined as DMU. We consider both small ruminants and large ruminants organic livestock farming, and organic chicken farming and beekeeping. Looking at the results, the Aegean Region ranks first, and the Marmara Region ranks at the top. However, Eastern Anatolia, Southeastern Anatolia and the Black Sea Region, known as regions, where natural resources, local breeds, cattle and sheep breeding are made, ranked lower. As the reason for the low organic livestock efficiency in these regions, one of the important difficulties in the transition to organic livestock farming is that the lands to be used for organic farming are included in the two-year transition period. On the other hand, the expensive control and certification services makes it difficult for small-scale enterprises to transition to organic livestock farming. Finally, policy makers' increase in support and grants to businesses in local and small regions will not only contribute to the development of organic livestock farming but also ensure the economic development of these regions.

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HEAVY METAL RESISTANCE OF MULTIDRUG RESISTANT *Staphylococcus aureus* ISOLATED FROM MEAT

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ABSTRACT

Staphylococcus aureus is widespread in the environment. It is an opportunistic pathogen that causes a wide variety of infections in humans and animals, resulting in severe morbidity and mortality. *S. aureus* found in food, including meat and meat products, is the common cause of foodborne diseases, which are important public health issues. Various virulence factors, antimicrobial and heavy metal resistance play important roles in expressing the pathogenicity of *S. aureus.* The emergence and dissemination of antimicrobial and heavy metal resistance in foodborne pathogens such as *S. aureus* pose a potential threat to human and animal health as well as environmental pollution. Besides, the use and presence of heavy metals in food, agriculture, and animal farming might promote the development and spread of antimicrobial resistance through co-selection. This study aimed to evaluate the resistance of heavy metals among multidrug-resistant (MDR) *S. aureus* isolates from ground beef and chicken meat. The minimum inhibitory concentration (MIC) of six heavy metals was determined by using the broth microdilution method. MDR *S. aureus* isolates were resistant to chromium (Cr), copper (Cu), mercury (Hg), and zinc (Zn) in 81.8%, 72.7%, 54.5%, and 27.3%, respectively. However, resistance to lead (Pb) and cadmium (Cd) was not observed. The findings regarding heavy metal resistance among the MDR *S. aureus* isolates could be useful in assessing consumer health and food safety risks.

Keywords: *Staphylococcus aureus*, heavy metal resistance, multidrug resistance, meat, broth microdilution test

INTRODUCTION

Staphylococcus aureus is the most significant species in the genus *Staphylococcus*. *S. aureus* is a Gram-positive coccus that appears as grape-like clusters under the microscope, is a facultative anaerobe, is non-motile, and produces golden yellow colonies. *S. aureus* is extensively found in the environment and is also present on the skin and in the nasal regions of humans and animals (Bhunia, 2008; Becker and von Eiff, 2011). It can cause a wide range of illnesses, including skin and soft tissue infections, pneumonia, acute endocarditis, meningitis, toxic shock syndrome, osteomyelitis, impetigo, urinary tract infections, mastitis, and food poisoning (Götz et al., 2006).

Staphylococcal food poisoning is one of the most prevalent types of foodborne diseases and is caused by consuming foods contaminated with *S. aureus* toxins (Bhunia, 2008). *S. aureus* has frequently been isolated from a wide range of foods of animal origin, including meat and meat products (Jackson et al. 2013; Wu et al. 2018; Kim et al., 2020), and milk and milk products (Jorgensen et al., 2005; Papadopoulos et al., 2019).

Various virulence factors, antimicrobial and heavy metal resistance play important roles in expressing the pathogenicity of *S. aureus* (Götz et al., 2006; Bhunia, 2008; Becker and von Eiff, 2011). Antimicrobial resistance is an increasingly serious public health and development

threat. The overuse or improper use of antimicrobial agents in veterinary and human medicine, agriculture and farming, and the husbandry of livestock helps the emergence and development of resistance to antimicrobials (Ibrahim et al., 2020; Guo et al., 2020).

Foods of animal origin can be a significant vehicle for the transmission of resistant bacteria to humans. *S. aureus* is infamous for its ability to become resistant to antimicrobial drugs. They have evolved several resistance mechanisms, both chromosomal DNA and plasmid DNA, to almost all antimicrobials used in the treatment of infections (Jensen and Lyon, 2009; Mlynarczyk-Bonikowska et al., 2022). Increasing numbers of investigations have revealed that *S. aureus* has developed drug resistance and evolved from single drug-resistant to multidrug-resistant (MDR), making antibiotic resistance control increasingly difficult (Gomes and Henriques, 2016; Guo et al., 2020; Mlynarczyk-Bonikowska et al., 2022).

In addition to increasing antimicrobial resistance, heavy metal resistance has become one of the most important environmental pollution problems in developing countries with increasing industrialization activities (He et al., 2016; Dahanayake et al., 2019). Bacteria are known to be exposed to heavy metals in the environment because of mine wastes, wastewater, agricultural wastes, and pollutants from industry (Hu et al., 2016; Vats et al., 2022). High prevalence of heavy metal resistance among various pathogenic bacteria, including *S. aureus* isolated from livestock production systems (Dweba et al., 2019), *Escherichia coli* from chicken, cattle, and sheep (Cufaoglu et al., 2022), and *Vibrio parahaemolyticus* from crustaceans and shellfish (Hu et al., 2016), have been reported.

Heavy metals are naturally occurring environmental chemicals that can induce the spread of antimicrobial resistance (Anedda et al., 2023). Metals can usually be classified as essential or non-essential. Essential metals that are implicated in the fundamental metabolic processes of microorganisms include chromium (Cr), calcium (Ca), sodium (Na), potassium (K), iron (Fe), zinc (Zn), copper (Cu), magnesium (Mg), nickel (Ni), cobalt (Co), and manganese (Mn) (Vats et al., 2022; Anedda et al., 2023). Other metals, including mercury (Hg), lead (Pb), cadmium (Cd), silver (Ag), gold (Au), antimony (Sb), arsenic (As), and aluminium (Al) do not play a critical role in biological processes and are thus classified as "nonessential metals." As a result, they are highly hazardous to microorganisms and are used as broad-spectrum antimicrobials. Toxicity is strongly dependent on the environmental conditions of the cells, such as pH, redox potential, and organic matter concentration, because these factors influence the bioavailability and of valency metals (Seiler and Berendonk, 2012; Vats et al., 2022).

Heavy metals such as Zn, Cu, Fe, Mn, and Co are also extensively used in animal production enhancers as feed additives (Yang et al., 2020). Zn and Cu are the most abundant metals in animal feed because they are often utilized as growth promoters (Yazdankhah et al., 2014; Vats et al., 2022). Hg, Pb, As, and Cd can be found as contaminants in animal feed. However, the use of heavy metals in high amounts causes problems in the food chain due to their toxicity, bioaccumulation, and biomagnification (Seiler and Berendonk, 2012).

It is possible for these toxic metals to enter the human body because of heavy metal contamination of foods, including meats. The risk of heavy metals contaminating foods is increasing day by day, and they are taking place in foods more and more every day. Furthermore, heavy metals can induce and sustain antimicrobial resistance in bacteria isolated from food. The prevalence of heavy metal pollutants in the environment poses increasing risks to both food safety and human health (Seiler and Berendonk, 2012; Vats et al., 2022). Therefore, this study aimed to evaluate the resistance of heavy metals among multidrug-resistant (MDR) *S. aureus* isolates from ground beef and chicken meat. The minimum inhibitory concentration (MIC) of six heavy metals was determined by using the broth microdilution method.

MATERIALS AND METHODS

Bacterial isolates

This study used eleven multidrug-resistant (MDR) *S. aureus* isolates from ground beef consisting of cow's meat (n = 7) and chicken meat consisting of breast and leg parts (n = 4). All isolates were recovered from retail meat samples in Bolu (Northwest Turkey) from several public bazaars, supermarkets, and butchers. Biochemical assays and a PCR for the species-specific fragment (Sa442) and thermonuclease gene (*nucA*) were used to identify the isolates previously (Brakstad et al., 1992; Martineau et al., 1998; Götz et al., 2006; Becker and von Eiff, 2011). All *S. aureus* isolates obtained from retail meats were grown overnight in Brain Heart Infusion broth (BHI) (Merck, Germany).

Determination of heavy metal resistance among MDR *S. aureus* isolates

The heavy metal resistance of MDR *S. aureus* isolates was investigated using six heavy metals, including mercury ($HgCl_2$), cadmium ($Cd(NO_3)_2$), lead ($Pb(NO_3)_2$), copper ($CuCl_2$), chromium ($Cr(NO_3)_2$), and zinc ($ZnCl_2$). All heavy metals were purchased from Sigma-Aldrich (Sinopharm Chemical Reagent Co., Shanghai, China). The minimum inhibitory concentrations (MICs) of heavy metals against the isolates were measured quantitatively in 96-well microplates using the broth microdilution method as previously described (CLSI, 2012; He et al., 2016; Dahanayake et al., 2019). Heavy metal concentrations ranged from 3200 to 62.5 µg/mL for Cr, Cu, Cd, Pb, and Zn, whereas Hg concentration ranged from 400 to 0.78 µg/mL. MICs were defined as the lowest concentration of heavy metal that completely inhibited the growth of the organism after 18-20 hours of incubation at 37 ºC. The tests were carried out in triplicates. *Escherichia coli* K-12 strain was used as a quality control in the heavy metal resistance test (Dahanayake et al., 2019).

RESULTS AND DISCUSSION

In this study, multidrug-resistant (MDR) *S. aureus* isolates from ground beef and chicken meat were examined for resistance to heavy metals, including chromium (Cr), copper (Cu), cadmium (Cd), mercury (Hg), lead (Pb), and zinc (Zn). As illustrated in Table 1, a maximum MIC of 3200 µg/mL for cadmium and zinc, 1600 µg/mL for copper, and 12.5 µg/mL for mercury were found when compared to the control strain *E. coli* K12. MDR *S. aureus* isolates were resistant to Cr, Cu, Hg, and Zn in 81.8%, 72.7%, 54.5%, and 27.3%, respectively. However, resistance to Pb and Cd was not observed (Table 1).

Most of the *S. aureus* isolates showed resistance to Cr (81.8%) and Cu (72.7%) in the current study. *Vibrio parahaemolyticus* isolates were resistant to Cr (100%) and Cu (93.3%) in a study published by He et al. (2016). Compared to our Hg findings, Dahanayake et al. (2019) observed no Hg resistance in *Aeromonas* spp. isolated from Manila Clam, the most popular food in Korea.

In contrast to our Pb and Cd results, resistance rates of *S. aureus* from ready-to-slaughter horses in Nigeria against the various heavy metals ranged from moderate to high, with 39.4%, 50.7%, 49.3%, and 60.6% of the isolates resistant to Cd, Cu, Pb, and Zn, respectively (Nwobi et al., 2023). This is consistent with the report of Adekanmbi and Falodun (2015) that *S. aureus* isolated from abattoirs in Nigeria showed high resistance to heavy metals including Cu, Pb, Cd, Zn, Cr, and Ni.

Table 1. Heavy metal resistance of multidrug-resistant *S. aureus* isolates from meat

Heavy metal	*MIC (μg/mL)*													Resistant isolates	
	0.78	1.56	3.125	6.25	12.5	25	50	100	200	400	800	1600	3200	No.	%
Chromium (Cr)												MIC[a] 2	9	9	81.8
Copper (Cu)											MIC[a] 3	8		8	72.7
Mercury (Hg)			1	MIC[a] 4	6									6	54.5
Zinc (Zn)									1		MIC[a] 7		3	3	27.3
Cadmium (Cd)								8	3	MIC[a]				0	0
Lead (Pb)									1	6	MIC[a] 4			0	0

[a] MIC, minimum inhibitory concentration of the quality control strain *E. coli* K-12

In this study, MDR *S. aureus* isolates originated from ground beef and chicken meat samples showed similar resistance to chromium and mercury (Figure 1). Copper resistance was found in 100% of the isolates from ground beef, but only 25% of the isolates from chicken meat. Furthermore, resistance to zinc was 75% in the isolates from chicken meat, while no resistance was detected in the isolates from ground beef.

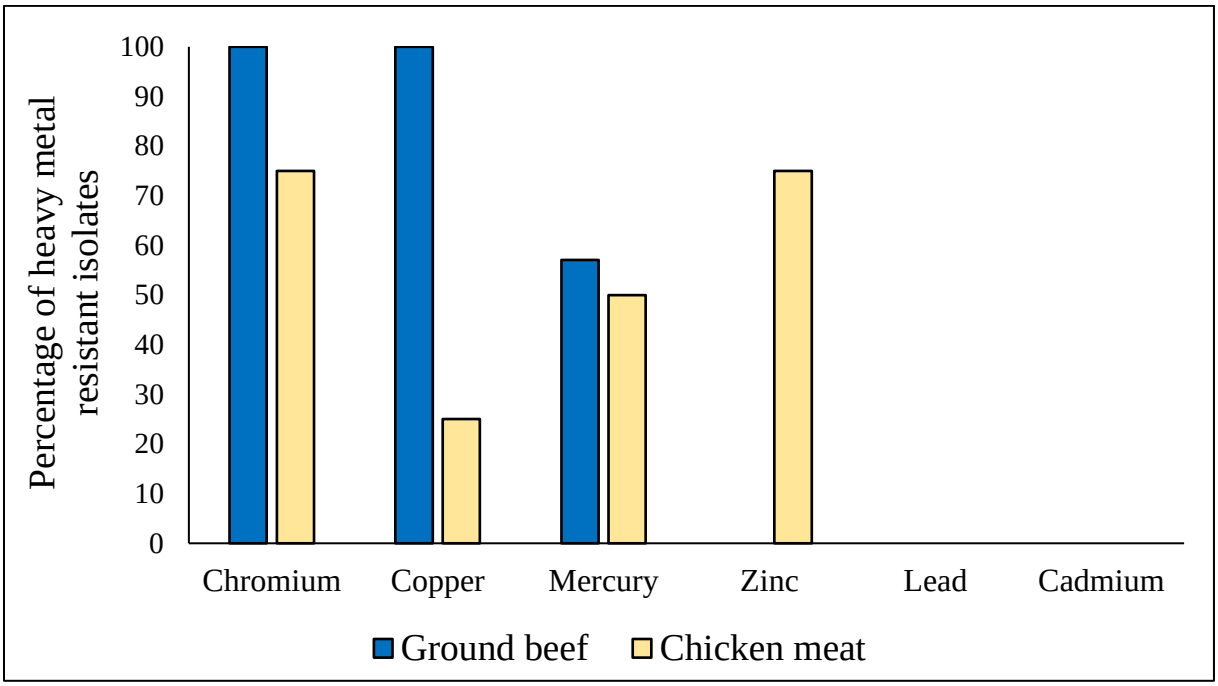

Figure 1. Prevalence of resistance to heavy metals among multi-drug resistant *S. aureus* isolates recovered from ground beef and chicken meat

Zinc has been extensively used in cattle and poultry breeding as a feed additive that helps promote growth and protect animal health (Seiler and Berendonk, 2012). In the present study, the MICs of Zn for the MDR *S. aureus* isolates from ground beef were 800 µg/mL, while the MICs of Zn for the MDR isolates from chicken meat ranged from 200 to 3200 µg/mL. Yang et al. (2020) reported that *E. coli* and *Salmonella* strains from chicken farms and retail meat had the highest MIC was 1600 µg/mL.

None of the MDR *S. aureus* isolates from ground beef and chicken showed resistance to either lead (Pb) or cadmium (Cd) (Figure 1). In agreement with our results, no resistance to lead in *E. coli* isolates from chicken, cattle, and sheep (Cufaoglu et al., 2022). Gufe et al. (2022) documented lead resistance rates among the isolated bacteria, including *Staphylococcus* from Nile tilapia, ranging from 30.8 to 69.2%

Moreover, the results of this study indicated five different heavy metal resistance patterns among the MDR *S. aureus* isolates (Table 2). Four (36.4%) isolates showed Cr, Cu, Hg and four (36.4%) isolates showed Cr, Cu combination patterns. Only one isolate had the Zn pattern. In addition, 45.5% of the isolates were resistant to at least three heavy metals.

Table 2. Resistance patterns of the six heavy metals tested for the MDR *S. aureus* isolated from meat

Resistance pattern	No. of heavy metals	No. (%) of resistant MDR *S. aureus* isolates
Cr, Cu, Hg	3	4 (36.4%)
Cr, Zn, Hg	3	1 (9.1%)
Cr, Cu	2	4 (36.4%)
Hg, Zn	2	1 (9.1%)
Zn	1	1 (9.1%)

CONCLUSIONS

S. aureus is a significant opportunistic human pathogen and can cause difficult-to-treat severe infections because of its great ability to develop antimicrobial resistance and the emergence of multidrug-resistant strains. This study demonstrated that multidrug-resistant (MDR) *S. aureus* isolates recovered from meats were resistant to heavy metals including mercury, chromium, copper, and zinc. As a result, the presence of heavy metal resistance among the MDR *S. aureus* meat isolates may pose potential risks to human health and food safety.

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ROOT ROT DISEASES CAUSED BY FUNGAL PATHOGENS IN PEA AND THEIR CONTROL POSSIBILITIES

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ABSTRACT

Plant diseases that reduce yield and quality cause serious damage to both the producer and the economy of country. Production is constrained for a number of reasons, including annual yield loss from fungal infections and costs of control. The pea (*Pisum sativum* L.), an important food with regard to of nutrients, plays a significant role in our country's agriculture. Some significant fungal pathogens of root rot disease, which were detected in peas, are *Aphanomyces euteiches*, *Fusarium oxysporum* f.sp. pisi, *Fusarium solani* f.sp. *pisi*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. These pathogens infect the pea roots initially, then kill the stem and above-ground tissues by extending through the vascular tissue; as a result of the infection, the pea plants wilt. Significant yield losses ranging from 30% to 57% occur in all pea growing areas, either before or during the flowering stage, as a result of the disease. The use of resistant cultivars combined with cultural control is the most safe, economical and effective method of protecting the pea plant against these disease agents. Within the scope of this review study, the symptoms, short biology, and control strategies of pea root rot pathogens are summarized in light of previous studies carried out by various researchers worldwide.

Keywords: fungal pathogens, root rot, pea, *Pisum sativum*, control

INTRODUCTION

Pea (*Pisum sativum* L.) is a type of vegetable that has a great importance in human nutrition due to its high content of protein and carbohydrate. It is consumed as fresh, canned, or frozen product in our country (Ceyhan et al., 2005). Furthermore, due to nitrogen fixation, which can reduce chemical fertilizer inputs, it is preferred in crop rotations. Türkiye ranks 4th after France, England and Spain among European Union countries, and 12th in the world, with a production amount of 120,455 tons of peas. (Food and Agriculture Organization [FAO], 2021). In our nation, a total of 120,455 tons of fresh peas were cultivated across 124,332 hectares of land in the year 2022, with Bursa leading the production with 42,201 tons (Turkish Statistical Institute [TUIK], 2022).

There are numerous fungal pathogens that contribute to root rot diseases in peas. *Alternaria alternata*, *Aphanomyces euteiches*, *Didymella pinodes*, *Didymella pinodella*, *Fusarium avenaceum*, *Fusarium oxysporum*, *Fusarium redolens*, *Fusarium solani*, *Mycosphaerella pinodes*, *Phytophthora* sp. *Pythium* sp, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and are among them. These pathogens are capable of inducing root rot diseases either individually or collectively (Kraft and Pfleger, 2001; Xue, 2003; Hossain et al., 2012; Taheri et al., 2017). In our country, the presence of *Fusarium* sp., *Rhizoctonia* sp., and *Pythium* sp. has been identified

in the roots of peas cultivated in Samsun province, as well as in diseased plant residues in the soil. Among these, *Fusarium* spp. was reported to be the most prevalent (Erper et al., 2008). In a study conducted in Hatay province, the most prevalent soil-borne fungal pathogen was identified as *Fusarium oxysporum* f.sp. *pisi* (55.6%). The researchers indicated that other fungal agents contributing to root rot included *Pythium* spp. *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Thielaviopsis basicola*, and (Soylu and Derviş, 2011). Within the scope of this review, the symptoms, biology and control of root rot diseases, which are very common in pea production areas and cause great yield losses, are explained in the light of some previous studies.

Pea Root Rot Diseases

Root Rot Disease Caused by *Fusarium solani* f. sp. *pisi* and Control Strategies

As a result of the infection caused by the pathogen, dark black circular lesions with irregular light brown areas are observed on the roots of plants (Figure 1), leading to growth inhibition and eventual plant death (Jung et al., 1999). In susceptible cultivars infected with *Fusarium solani* f.sp. *pisi* (*Fsp*), seeds decay completely (Cook et al., 1968). The pathogen is a soil and seed-borne pathogen that lives in the soil as mycelium and chlamidospores. In seedling or other growing stages, the pathogen enters the plant directly from the epidermal tissue along the epicotyl, hypocotyl, taproot (Kraft and Pfleger 2001), or the root elongation region (Gunawardena et al., 2005). It can also enter through the stoma in the leaves, although this is a less common occurrence compared to direct penetration (Stahl et al., 1994).

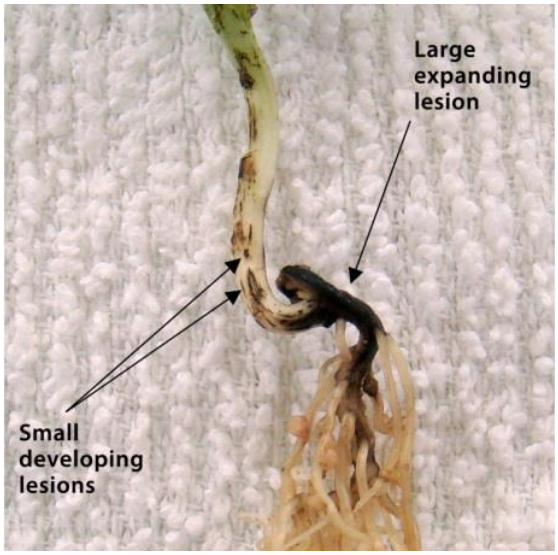

Figure 1. The lesions observed on white epicotyl tissue caused by *Fusarium solani* f. sp. *pisi* of an unknown pea cultivar (Porter et al., 2015).

Cultural practices and biological control agents play a significant role in the management of *Fsp*. In addition to cultural practices such as drainage in the field, rotation, tillage, avoidance of frequent planting, and correct fertilization, weed control before planting are important measures that can be taken to prevent the disease. Cultural practices may decrease the populations of soil-borne pathogens and thus the severity of the root diseases they cause, either directly or indirectly. There have been several important research on the use of specific biological control agents in the management of *Fsp*. Xue (2003), determined that the ACM941

isolate of *Clonostachys rosea* is mycoparasite against *Fsp*. The author reported that seed germination increased by 44%, growth of seedlings increased by 22% and root rot decreased by 76% after treatment of pea seeds with the ACM941 isolate. According to the same study, using a fungicide with Thiram as the active component increased seed germination by 33%, growth of seedlings by 29%, and root rot incidence by 65%. As a result of the study, it was suggested *that Clonostachys rosea* ACM941 isolate is an effective biological control agent in controlling *Fsp* and may be an alternative to chemical seed treatments. Kapoor et al. (2005) determined that *Trichoderma viride* isolates obtained from soil samples reduced disease development by 80% compared to the control group. In a study conducted by Hamid et al. (2012) under *in vitro* conditions using *Trichoderma harzianum, Trichoderma viride, Gliocladium virens* and *Pseudomonas fluorescens*, it was recorded that *T. harzianum* inhibited *Fsp* mycelial growth by 78%. The antagonistic fungi Aspergillus awamori, Aspergillus niger, and Trichoderma harzianum, as well as plant growth-promoting rhizobacteria (PGPR) Bacillus pumilus and Pseudomonas putida, were examined individually or in combination by Akhtar and Azam (2014) in a greenhouse study. They found that *Pseudomonas putida* was more successful in decreasing disease severity compared to *A. niger* and *T. harzianum*.

Root Rot Disease Caused by *Fusarium oxysporum* f. sp. *pisi* and Control Strategies

Fusarium oxysporum f. sp. *pisi* W.C. Snyder and H.N. (*Fop*) is a serious disease that affects peas and causes root rot and wilting in Australia, Algeria, India, Canada, China and the United States (Achari et al., 2020; Merzoug et al., 2014; Deng et al., 2022). Among root rot diseases caused by various fungal pathogens, the disease caused by *Fop* is the most destructive to pea plants. The symptoms observed in the plant generally occur of downward-curling and yellowed leaflets. The plant eventually dies and develops a yellowish-brown color. The pathogen infects pea roots and spreads through vascular tissues, damaging stem and above-ground tissues. When soil temperatures rise beyond 20°C, the disease spread quickly. Under these conditions, wilting symptoms can become severe, and these symptoms may appear during or before pod period. The soil-borne pathogen can remain viable in the soil for more than 10 years as chlamydospore (Kraft, 1994). Under these circumstances, when the pathogen obtains sufficient inoculum and a susceptible variety is planted, serious crop losses occur. Currently, the accepted races of *Fop* include races 1, 2, 5, and 6 (Neumann and AG Xue, 2003). Races 1 and 2 are widespread globally, while races 5 (Figure 2) and 6 have been reported in Algeria, Australia, Canada, China, India and the United States (Achari et al., 2020; Deng et al., 2022).

Figure 2. The response of various pea cultivars to *Fusarium oxysporum* f. sp. *pisi* race 5 isolate PF22b (Deng et al., 2022)

The use of clean, healthy seeds, rotation, and early sowing before the soil temperature is most suitable for fungal growth in are cultural practices that can be taken in terms of disease control. It can be difficult to manage the wilting that *Fop* causes in peas, and no single control strategy is completely successful on its own. Considering the damage and survivability of the fungus, the use of biological control agents and resistant pea varieties is considered an effective control method. The most effective and practical method of disease management worldwide is the use of resistant cultivars (Sakoda et al., 2019). Due to the significant role of using resistant varieties in disease management, pea breeders are making intensive efforts to develop varieties resistant to *Fop* races. Regarding the chemical control of the pathogen, Hannan et al. (2014) conducted a study in Pakistan against *Fop* disease during the years 2012-2013. They applied different active ingredients (Mancozeb, Carbendazim, Copper oxychloride, Metalaxyl + Mancozeb, Trifloxystrobin + Tebuconazole, Thiophanate methyl, Fosetyl-Al, Difenoconazole) at three different concentrations (400, 600, and 800 ppm) under *in vitro* conditions. In the study, it was found that the active ingredients Fosetyl-Al, Trifloxystrobin + Tebuconazole, Thiophanate methyl, and Difenoconazole provided favorable results compared to the control group. As part of alternative control methods, Ali et al. (2014) reported that Aloe vera plant extract inhibited fungal mycelial growth by 69%, while Lantana camera plant extract inhibited it by 50%. The authors observed that essential oil of *Trachyspermum ammi* inhibited fungal mycelial growth by 80%, and *Azadirachta indica* inhibited it by 77%. However, the study suggests that after testing the effectiveness of these plant extracts under field conditions, they could potentially serve as an alternative method of control to fungicides

Root Rot Disease Caused by *Rhizoctonia* sp. and Control Strategies

Rhizoctonia seedling blight caused by *Rhizoctonia* sp. is one of the significant root rot diseases observed in pea production fields. *Rhizoctonia* sp. has several races, which are referred to as anastomosis groups, and they cause disease in numerous plants. Currently, 14 anastomosis groups of *R. solani* have been identified. In studies related to pea root rot, the majority of isolates have been reported to belong to the AG-4 group of strains (Hwang et al., 2007). Regional source, host diversity, morphology and pathogenicity are all different for these groups.

The disease's visible symptoms above ground involve the damping-off of extremely young pea seedlings (Figure 3). After the emergence of seedlings, Rhizoctonia sp. attacks the tip of the hypocotyl (approximately 1-2 cm) before the expansion of leaves (Flentje and Hagedorn, 1964). A dark brown-bronze root rot occurs at the root collar of pea seedlings. The pathogen requires warm weather; when the soil surface temperature exceeds 24-29 °C, significant infection occurs. Rhizoctonia seedling blight is generally more serious in these soils, as sandy soils warm up faster. Heavy soil and high humidity are more conducive to the disease compared to dry conditions (Hagedorn, 1991). Rhizoctonia sp. maintains its viability as a saprophyte in the soil by producing tiny, brown, spherical sclerotia, especially in hot, humid environments. Under favorable conditions, it germinates by producing hyphae that directly invade roots through wounds or natural openings (Anonymous, 2023).

Figure 3. *Rhizoctonia solani* has caused some areas of pea plants to grow poorly. (Sharma-Poudyal et al., 2015)

Effective management against the pathogen includes the use of clean certified seeds, crop rotation, seed treatment with fungicides, and the cultivation of resistant varieties. Crop rotation with crops known to have low susceptibility to the fungus, such as maize, plays a crucial role in disease management. There is currently no commercially available pea variety that is resistant to the disease. It is necessary to avoid dense and deep planting, excessive fertilization, and mechanical damage to roots and stems. Askar and Rashad (2010) reported in their study on the antifungal activity of ethanol-water extracts obtained from cinnamon, anise, black cumin and clove against *Rhizoctonia solani*, which causes root rot in peas, that the highest antifungal activity was observed with clove extract. In this context, it has been reported that while there were no viable plants in the untreated seeds, 40% of peas treated with clove extract and 48% of those treated with Tolclofos methyl (Rhizolex®) active ingredient maintained their viability. Finally, it has been noted that clove plant exctract could have the potential to be used as a seed treatment against *Rhizoctonia solani*. Silva et al. (2012) treated pea seeds with various fungicides containing different active ingredients (Captan, Carbendazim, Carbendazim + Thiram, Iprodione, Iprodione + Thiram, Metalaxyl-M + Fludioxonil, Pencycuron, Procymidone and Tolyfluanid), and then sowed them in soil inoculated with the pathogen. They determined the germination rates. In the control group, the seedling emergence rate was 16%, while applications of fungicides containing Iprodione and Carbendazim + Thiram active ingredients resulted in seedling emergence rates of 82% and 78%, respectively. As a result, the authors have reported that the applications of fungicides containing Iprodione and Carbendazim + Thiram active ingredients would be the most effective seed treatments. Rawat et al. (2014) conducted a study to evaluate the efficacy of *Trichoderma* spp. isolates (Th-14 and Th-21) alone or in combination with the fungicide thiophanate-methyl (Topsin-M®) against Rhizoctonia sp. root rot in peas under both *in vitro* and *in vivo* conditions. The authors found that the control of the disease was more effective by combining *Trichoderma* isolates (Th-14 and Th-21) with Topsin-M® as compared to the applications carried out individually. In the study, the survival rate of seedlings infected with *Rhizoctonia solani* was 33%, while it was determined as 100% in the Th-14+Topsin-M® mixture and 87% in the Th-21+Topsin-M® mixture. In conclusion, it was noted that the combination of *Trichoderma* spp. isolates with thiophanate-methyl active ingredient fungicides exhibited a significant impact on the control of root rot caused by *Rhizoctonia* sp.

Root Rot Disease Caused by *Aphanomyces* sp. and Control Strategies

This disease is significant in Australia, Japan, New Zealand, North America, and Northern Europe. Severe crop losses occur in the production areas (Holub et al., 1991). The pathogen can infect the pea plant at any stage, but the most serious problems occur during the seedling

emergence period (Figure 4). Root cortex tissue turns yellow 7-14 days after infection and gradually turns brown (Kraft and Boge 1996). The cortex softens and eventually decays, leaving only thin veins behind. A similar decay also occurs in the lower parts of the stems. Secondary pathogens contribute to the darkening and decay of the affected tissues. Severely affected plants remain stunted and produce a limited number of pods. The pathogen can remain viable in the soil for up to 10 years with its oospores (Malvick et al., 1994). Hyphae produced by secondary zoospores penetrate host tissues. After a cool, rainy spring season, the damage caused by the disease begins to increase during the hot and dry summer season, and the severity of the disease becomes visually apparent in the field (McMurray et al., 2011).

Figure 4. The fungus Aphanomyces causes field pea root rot. (Anonymous, 2023).

Aphanomyces root rot disease has been recognized as one of the most damaging root diseases in field peas for nearly a century (Jones and Linford, 1925). However, options for controlling this disease are limited. Completely resistant pea varieties against the disease are not available (Pfender 1984; Allmaras et al., 2003), and in some studies, only partial resistance or susceptibility has been reported (Hamon et al., 2013; Conner et al., 2013). Seed fungicide applications, crop rotation, and biological control are effective in controlling the disease. It has been reported that the fungicide containing the active ingredient hymexazol reduces the severity of root rot and increases yield under field conditions (Kotova et al., 1980). Currently, in Canada, the fungicide containing the active ingredient ethaboxam is the only registered fungicide for the control of *Pythium* root rot, *Phytophthora* spp., and *Aphanomyces* root rot (Wu et al., 2018). However, in our country, there is no registered plant protection product available for this disease. Wakelin et al. (2002) conducted a study on the biological control of pea root rot caused by *Aphanomyces euteiches* using bacteria obtained from New Zealand soils under natural conditions. Out of 704 bacterial isolates, 31 of them completely suppressed the pathogen's mycelial growth. They reported that *Bacillus pumilus*, *B. subtilis*, *B. cereus*, *B. mycoides*, and *Paenibacillus polymyxa* reduced root rot symptoms and oospore formation in pea tissues. *B. mycoides* MW27 has been the most effective bacterial isolate, reducing oospore formation in pea roots by 83%. Hossain et al. (2012) reported that the application of isothiocyanate, a compound produced by members of the Brassicaceae family, has the potential for controlling *Aphanomyces* root rot under controlled conditions.

Root Rot Disease Caused by *Sclerotinia sclerotiorum* and Control Strategies

This disease causes root rot during the seedling stage and, root, stem, leaf and fruit rot during the pod formation stage (Figure 5). Symptoms initially appear on the root collar and lower leaves close to the soil surface. As the disease progresses, dense cottony-white mycelial layers develop on the root collar or stem (Jain et al., 2013). Symptoms appearing on the branches, leaves, fruits (Figure 5) and stem initially take on a water-soaked appearance and then the tissues become covered with the fungus's white thread-like mycelia (Fuller et al., 1984).

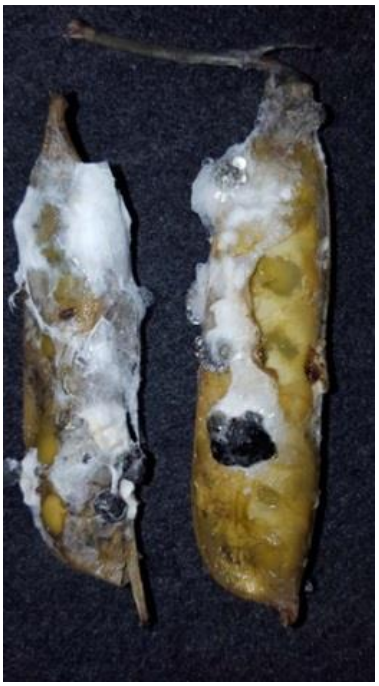

Figure 5. Rotting green peas are characterized by fuzzy mycelium growths and black sclerotia on the pod (Aktaruzzaman et al., 2022).

The pathogen of the disease produces abundant resilient reproductive structures called sclerotia in the infected tissues. If these sclerotia germinate by forming apothecia, infection occurs in the above-ground parts of the plants. The ascospores released from apothecia are transported to the plant by wind. Ascospores can remain viable on leaf surfaces for several weeks and require high humidity. Pea leaves are particularly good sources of nutrients for these pathogens. Secondary infections occur through mycelial growth at points of direct contact between diseased and healthy plant organs (Willets and Wong, 1980).

Currently, there are no varieties resistant to the disease. Firstly, non-infested seeds should be planted. Sensitive varieties should not be planted in fields previously heavily infected or known to have white mold issues. Irrigation should be carried out in the morning to ensure that plant leaves and roots remain dry. Excessive nitrogen fertilization should be avoided, and it should be ensured that the soil has sufficient potassium levels (Tu, 1989). Soil sterilization carried out through chemical, vapor or heat treatments can significantly reduce the presence of sclerotia in the soil. Due to its saprophytic activity and the ability to survive for extended periods as sclerotia under adverse environmental conditions, controlling white mold caused by Sclerotinia is challenging (Nasser et al., 1997). Huang and Erickson (2000) conducted a two-year field

study to determine the effects of soil treatments with four mycoparasites and one antagonist on the apothecium formation of *Sclerotinia sclerotiorum* in dry beans and peas. Among the five evaluated fungal species, *Coniothyrium minitans* and *Talaromyces flavus* were found to be the most effective in reducing the sclerotium formation of *Sclerotinia sclerotiorum*. They noted that treatment with *C. minitans* reduced apothecium formation of *S. sclerotiorum* by 90% in peas. Elsheshtawi et al. (2016) conducted a study on the efficacy of Contans® (*Coniothyrium minitans*) in combination with fungicides against *Sclerotinia sclerotiorum*. The results of this study indicated that, when taken separately in comparison to the control group, Contans® and Topsin® (Thiophanate-methyl) both significantly decreased the disease incidence caused by *S. sclerotiorum* by 90% and 95%, respectively. Additionally, the authors discovered that the combination of Contans® and Sumisclex® 50 WP (Procymidone) completely suppressed the white rot disease.

CONCLUSION

One of the primary causes of yield and quality losses observed in peas is root rot diseases. This group of diseases causes symptoms in many plants, spreading through plant tissues and leading to significant economic damages. As known, root rot diseases can be caused by multiple pathogens and are highly prevalent. Among the fungal agents causing root rot in peas, *Fusarium* spp. is the most common. In areas where the pathogen's inoculum is abundant in the soil, significant yield losses occur. The presence of races of *Fop* among *Fusarium* species creates challenges in the control efforts. The presence of *Fop* races among *Fusarium* species causes difficulties in its control. The development of resistant varieties against different races of the pathogen has become essential for its control. The use of resistant varieties and cultural practices should be combined as part of integrated pest management. In the future, to minimize damage caused by root diseases, it is necessary to develop and register varieties that are effective against various root diseases and make them available to growers. Moreover, it is important to conduct relevant studies in our country to determine the agents and prevalence of root rot diseases in pea cultivation and to develop strategies for their control.

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EVALUATION OF SURFACE WATER AND SEDIMENT MICROPLASTICS OF SULTANSUYU DAM LAKE (MALATYA) IN TURKEY

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ABSTRACT

Synthetic components made from organic polymers are called plastics, and those smaller than five millimeters are classified in the Microplastics group. The amount of microplastics in the aquatic ecosystem has increased significantly in recent years. In this study, the concentration, type, size and color of MPs in surface water and sediment in Sultansuyu Dam Lake were investigated. Fibers, in surface water and sediment were the dominant microplastics type. Microplastics concentrations in surface waters are 153.33 at St.1 and 160 par.m^{-3} at St.2. Microplastic concentrations in the sediments are 960 and 1320 par.m^{-2} in St.1. at St.2. The most common microplastic sizes in surface waters and sediment were 1-2 mm. The dominant color of the detected microplastics was transparent in surface waters and gray in sediment. Of the two stations, St.2 showed a higher microplastic concentration level.

Keywords: Microplastic, Freshwater, Pollution, Sultansuyu Dam Lake

INTRODUCTION

Plastics are materials made of polymers, consisting of many repeating monomer chains. Today, plastic is used in various fields, including chemistry and materials science. Plastic materials that have been included in our lives in various ways, cause irreversible environmental problems in air, water and soil quality (Akçay et al., 2020). Plastics; naturally, and various anthropogenic effects are transformed into microplastics (Yurtsever, 2019). Microplastics are widely dispersed from soil to water and atmosphere. Microplastics can remain in nature for a long time because of their slow degradation rate and cause serious environmental pollution.

Microplastics can be classified using different approaches. They can be divided into primary and secondary microplastics according to their source. Plastics that are discharged directly into the aquatic environment with effects such as human activities are called primary microplastics.Secondary microplastics are plastic parts formed by breaking down larger plastics and reducing their size (Yang et al., 2022).

Studies in marine and freshwater systems have shown that aquatic fauna can ingest microplastics as they mix them with their prey. In laboratory and field observations, microplastics have also been reported to adsorb organic and inorganic contaminants on their surfaces (Egessa et al., 2020). Because of their ubiquity and morphological characteristics, microplastics threaten the life and development of biota through direct and indirect means, including contact, uptake and digestion. Microplastics also pose potential risks to human health as they can be transmitted along the food chain. For this reason, it is very important to determine the environmental conditions and behaviors of MPs. Freshwater systems have many important features such as drinking water and use in fishing. However, these systems are affected by

various pollutants, including microplastics, due to human activities (Su et al., 2016). The aim of this study is to determine the concentrations, color, and size of surface water and sediment microplastic in Sultansuyu Dam Lake, which is a fresh water source.

MATERIAL AND METHOD

Sultansuyu Dam is a dam built between 1986 and 1992 for irrigation purposes on Sultansuyu in Malatya. Microplastics samples were collected in June 2020. 2 sampling stations were determined in Sultansuyu Dam Lake. Station 1 (St.1) (38°31'33.3"N -38°04'54.4"E, Station 2 (St.2) (38°30'44.0"N-38°04'18.1"E).

Surface water sampling from selected stations was taken by filtering 150 L of water with a steel bucket through steel sieves with 5,000 µm, 1,000, 200 and 91 µm pore sizes (Meng et al., 2020). Samples collected from 1000 µm, 200 µm and 91 µm filters were taken into bottles with ultrapure water and preserved in 4% formaldehyde (Aytan et al., 2020).
Sediment samples were taken from the stations by Ekman. Samples taken were stored in jars. (Aytan et al., 2020).

After the water samples were filtered through a filter (10 µm), 30 mL of 30% hydrogen peroxide was added to each sample. Afterwards, the samples were stored in an oven at 50 °C for 3 days. This process is done to digest organic material. After this process, the samples were filtered with a filter (10 µm) (Aytan et al., 2020).

Samples were transferred to beakers and saturated NaCl solution was added for density separation. The supernatant was filtered with 10 µm filters. The residues on the filter were removed. The organic particles were digested with 30% hydrogen peroxide for 168 hours at room temperature, then filtered through 10 µm filters and oven dried.

Microplastic samples were measured with a microscope. Later, microplastics were classified according to their morphological and physical properties.

RESULT AND DISCUSSION

In recent years, microplastic particles have been extensively found in water systems. This can affect aquatic organisms. Therefore, it is becoming increasingly important to observe microplastics in aquatic systems.

Surface water

As a result of this study, total of 47 microplastics particles, 23 particles at St.1 and 24 particles at St.2, were detected in the surface water of Sultansuyu Dam Lake. Microplastics concentrations in surface waters are 153.33 at St.1 and 160 par.m^{-3} at St.2. In the June 2020 sampling, a total of 54 microplastics were detected in the surface water of Sürgü Dam Lake (Turhan, 2022). Compared to Sürgü Dam Lake, the amount of microplastics in Sultansuyu Dam Lake is less.

Microplastic levels of surface water have been determined in some freshwater systems in Turkey. It was determined as 33000 MPs/m^3 in Küçükçekmece Lagoon, 233 MPs/m^3 in Cevdet Dündar Pond and 5.25 MPs/m^3 in Süreyyabey Dam Lake (Çullu et al., 2021; Erdoğan, 2020; Tavşanoğlu et al., 2021).

These determined microplastics were divided into 4 groups as fibers, films, foams and parts. Among these groups, the dominant microplastic group in surface water was determined as fiber at two sampling stations (Figure 1).

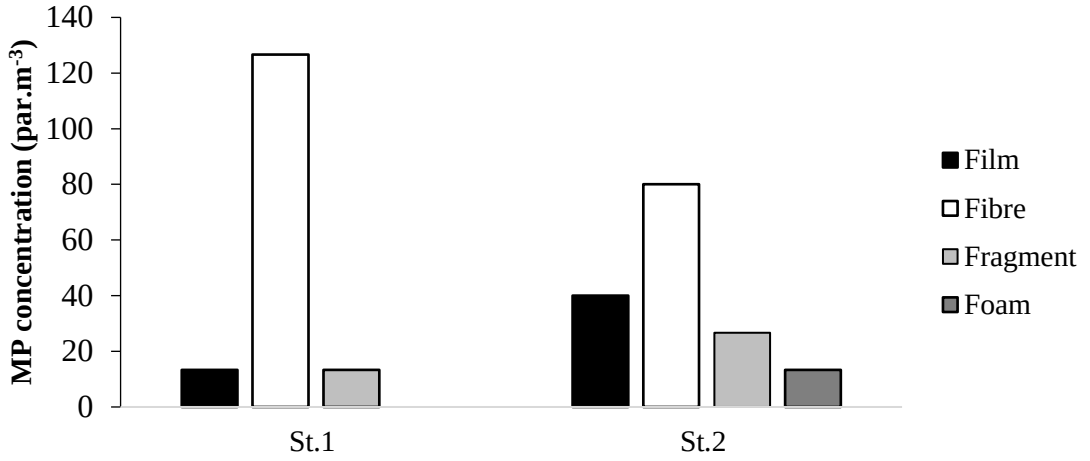

Figure 1. Concentration of microplastics in surface water

It was determined that the microplastics in the surface water consisted of 7 different colors. Among the detected colors, the dominant color was determined as transparent in St.1 and gray in St.2.

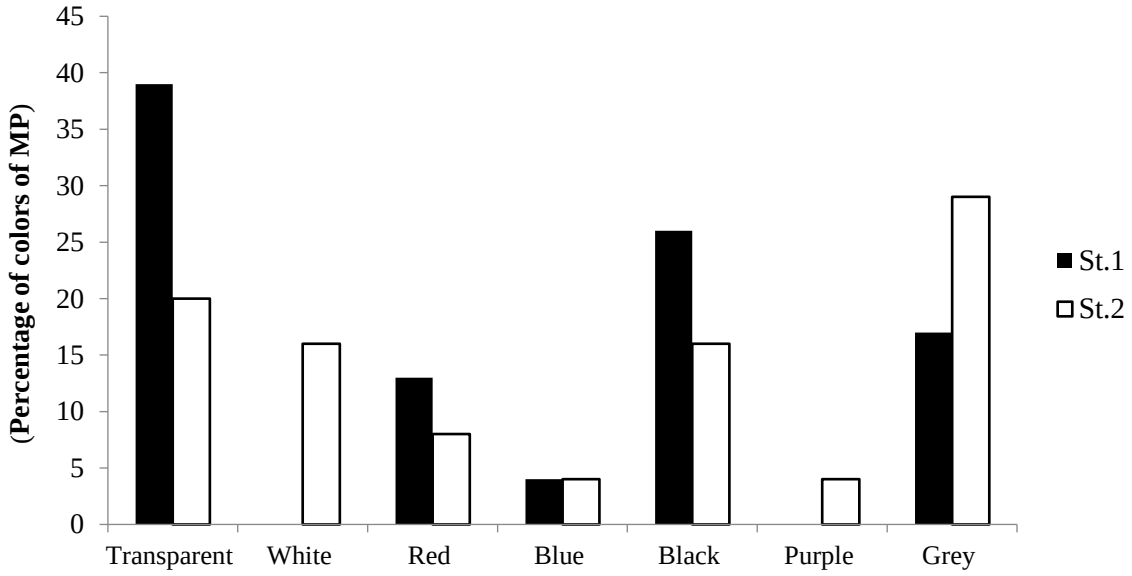

Figure 2. Percentage of colors of MPs in surface water

Microplastics collected from sampling points were classified as <0.2 mm, 0.2-1 mm, 1-2 mm, and 2–5 mm. Microplastics sizes ranged from 0.19 to 4.15 mm. In surface water, microplastics of 1-2 mm size were determined to be more dominant than other sizes at both stations.

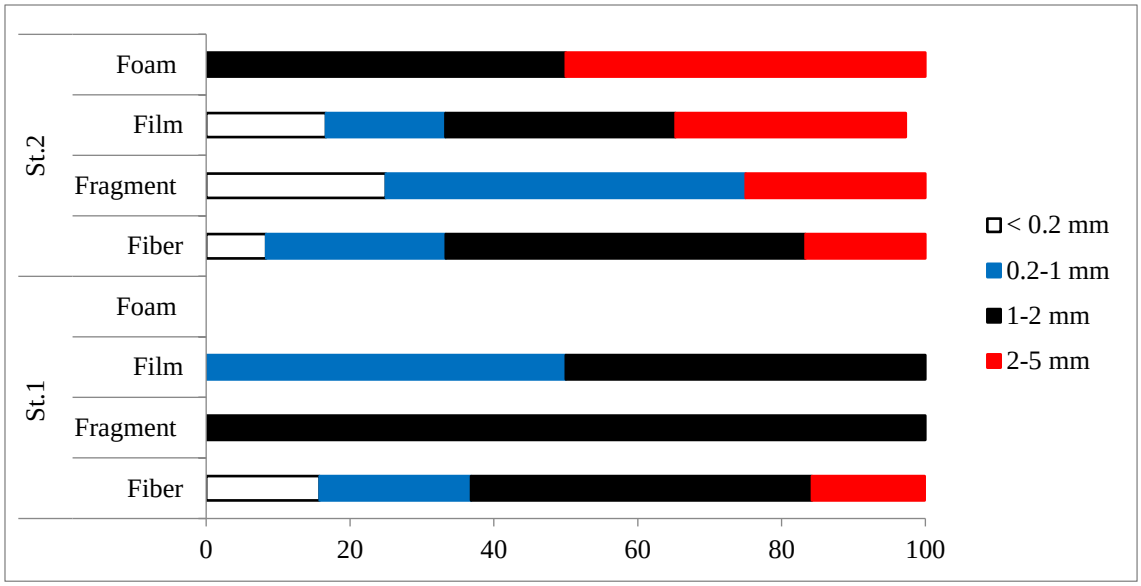

Figure 3. Size composition of microplastics in surface water

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Sediment

In this study, total of 58 microplastic particles, 25 particles at St.1 and 33 particles at St.2, were detected in the sediment of Sultansuyu Dam Lake. MP concentrations in the sediments are 960 and 1320 par.m^{-2} in St.1. at St. 2. In the June 2020 sampling, a total of 41 microplastics were detected in the sediment of Sürgü Dam Lake (Turhan, 2023). The dominant microplastic group in sediment was determined as fiber at two sampling stations (Figure 4).

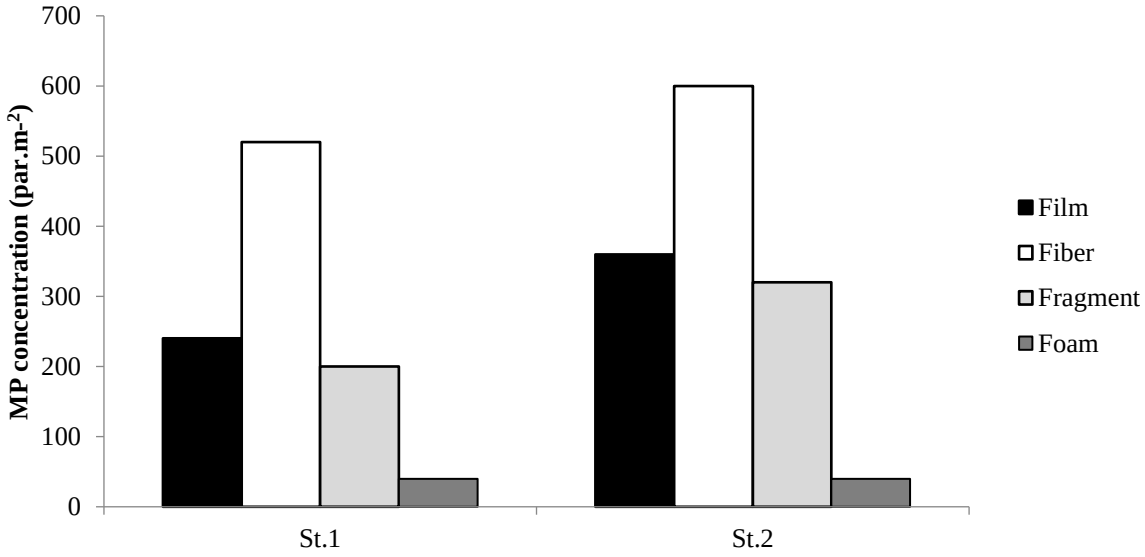

Figure 4. Concentration of microplastics in sediment

It was determined that the microplastics in the sediments consisted of 7 different colors. Among the detected colors, the dominant color was determined as transparent in St.1 and gray in St.2 (Figure 5).

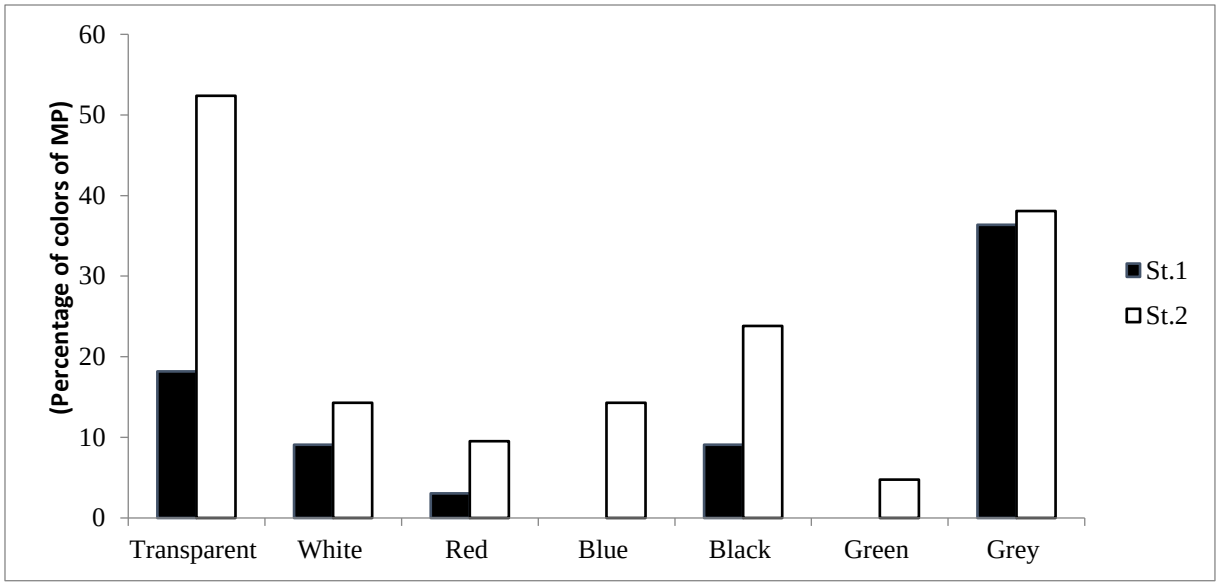

Figure 5. Percentage of colors of microplastics in sediment

Microplastics sizes ranged from 0.1 to 3.98 mm. In sediment, microplastics of 1-2 mm size were determined to be more dominant than other sizes at both stations (Figure 6).

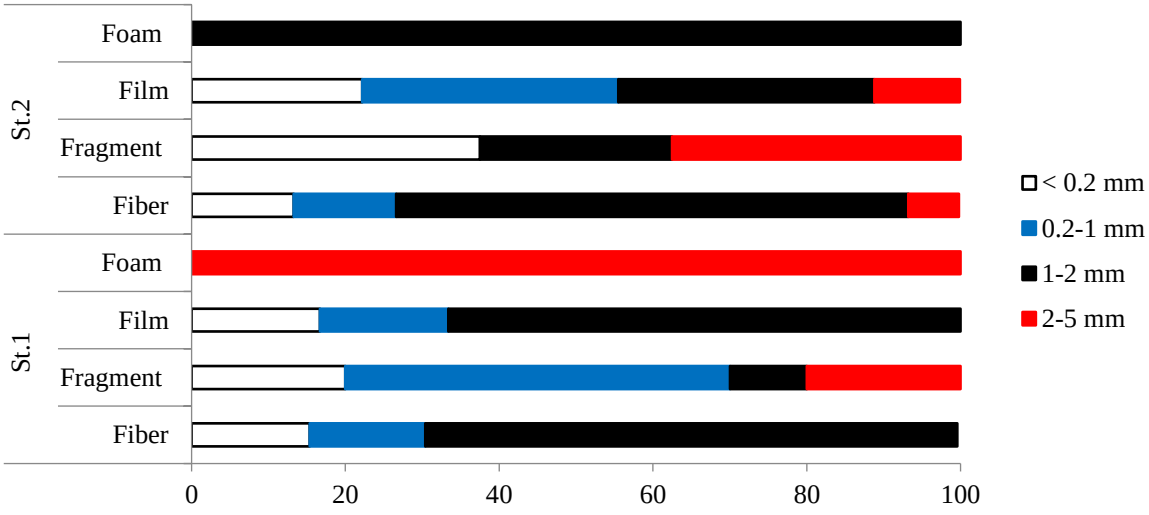

Figure 6. Size composition of microplastics in sediment

Conclusions

In this research, the concentration, color distribution, and size of microplastics in surface water and sediment of the Sultansuyu Dam Lake were determined. Of the two stations, St.2 showed a higher MP concentration level. Fiber was determined as the dominant microplastic type in surface water and sediment. Microplastics of 1-2 mm in size in surface water and sediment are more dominant than other sizes. Transparent and grey were the predominant colour.

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ENRICHMENT OF GRAIN LEGUME GENETIC RESOURCES COLLECTION THROUGH BILATERAL PROJECT BETWEEN BULGARIA AND CHINA

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ABSTRACT

Conservation of plant genetic resources for food and agriculture is an important goal worldwide from strategically and economically points of view. Almost all the relevant documents on the genetic resources, adopted by international bodies, underline the need of crop conservation, not only for this generation, but most of all, for the future of the humanity. Last decades, more and more old and traditional landraces have been replaced by new and modern varieties. Under these circumstances, a holistic approach for biodiversity conservation by using elements of two strategies: *on farm* and *ex situ* preservation, represents a research priority. Through the implementation of the bilateral cooperation between Bulgaria and China a scientific program on inventory and collection of local plant genetic resources from grain legumes is carried out. According to the work plan the activities are focused on collection of accessions and information from different geographical areas with a view to preserve and sustainable use of the diversity and exchange of experience in characterization and evaluation between the two partners. During the period 2021-2023 seven expeditions with the aim of surveying and inventorying rural areas in South Bulgaria according to the methodology of ECPGR were carried out. As a result, 70 local accessions of bean and cowpea species were collected from diverse agricultural conditions. A database with passport information according to the descriptor of FAO/Bioversity has been created. This research work was carried out with the support of Bulgarian National Science Fund by the project "Enrichment diversity of grain legumes between China and Bulgaria – the introduction and evaluation in correspondence with global climate change" (КП-06-Китай/7/20.11.2020) and the obtained inventory results are applying in the project "Bioactive substances from legumes and medicinal species – features and potential for use in changing climatic conditions" (КП-06-Н56/13/19.11.2021).

Keywords: Local varieties, Collection missions, Rural areas, Descriptor, Documentation.

INTRODUCTION

Global climate change and its impact on the environment pose serious challenges. Actions we take today will affect our future ability to respond and adapt to climate change and reduce its impact on the environment (Ortiz et al., 2021). Based on a number of sources and data, the world population is expected to reach 9.6 billion people by 2050. This means that food production must increase by 70 percent to meet the needs of such a huge population. It is therefore increasingly clear that major reforms in the agricultural sector are needed to ensure that our food system is ready to meet the challenges of a growing world population (Alexandratos and Bruinsma, 2012). Specifically, we need to move our agriculture from the conventional agri-food production system to sustainable agriculture (Khan et al., 2022).

Although most of European agricultural production currently relies on formally registered and genetically uniform cultivars, landraces are still grown *on farm*. However, a European inventory of landraces is still lacking and, consequently, there is limited and scattered information on where these materials are grown and to which species they belong to (Raggi et

al., 2021; 2022). Without knowing where landraces are still present in cultivation and cultivation breadth, the elaboration of adequate conservation plan and their implementation is clearly hindered. In term of use, geographical distribution and pedo-climatic characteristics of involved sites are also of great relevance since landraces, cultivated in diverse environments, can hold different traits for local adaptation and are a rich initial material for the breeding programs. The role of home gardens has been evaluated as a repository of agro biodiversity (Galluzzi et al., 2010; Rocha et al., 2017). Local farmers store specific genetic varieties, known as landraces, within their biological, cultural and socio-economical context (Kehlenbeck et al., 2007; Maxted et al., 2009; Negri et al., 2009).

Conservation of plant genetic resources for food and agriculture is an important goal worldwide from strategically and economically points of view. A holistic approach for biodiversity preservation by using elements of two strategies: *on farm* and *ex situ* store, represents a research priority (CDB, 2011; ITPGRFA, 2009). Germplasm management includes activities as collection of genetic resources, study, documentation, conservation, distribution and use. A positive aspect is implementation of new information technologies with a view of successful maintenance of *ex situ* collections. (ECPGR, 2021).

The geographical latitude, climate and agroecological conditions, as well as the food production of Bulgaria are similar to some of the regions of Northern China (Bachev, 2018). In this context, the exchange of plant genetic resources and associated information between the two countries could assist to achieve the sustainable development common goal and respond to global climate challenges. This is the reason for the successful applicability of the achieved scientific results from joint research projects in the area of plant diversity preservation. Bulgaria provides a solution to developing the agro-food sector because of the following specifics and prerequisites: Unique natural conditions for cultivation of wide diversity of traditional crops; Ecologically clean and fertile soils; Very high quality of organic products (ban on GMOs); Established local producers and a strong tradition in the agricultural sector (Liu, 2017; Kandilarov, 2019).

The aim of the study is to evaluate the development of grain legume plant genetic resources' collection in harmony with the two strategies: *on farm* and *ex situ* conservation through implementation of bilateral project between Bulgaria and China.

MATERIAL AND METHOD

Conservation and sustainable preservation of the plant biodiversity from wild and cultivated flora is the main priority of the IPGR-Sadovo as a National Coordinator in the European Programme for Plant Genetic Resources (ECPGR).

During the period 2021-2023 seven expeditions for surveying and inventorying rural areas in South Bulgaria according to the methodology of Guarino et al. (2011) were carried out. GPS system for latitude, longitude and altitude of the collecting site was used. Ethnobotanic data and other information of interest regarding aspects related to the cultivation, utilization and genetic erosion process were also recorded.

According to the international documentation standard Multi-Crop Passport Descriptor (FAO/Bioversity, 2017) the electronic database contains the following data: catalogue number, taxonomy, accession name, acquisition date, country of origin (FAO code), location of collecting site, geographical coordinates, elevation, collecting date, biological status (traditional variety/landrace), acquisition source (cultivated habitat/local market), donor of the accession, organizer of the collecting mission, type of germplasm storage, etc. The taxonomic description of the crops is under the nomenclature of USDA (GRIN, 2015).

The Bulgarian grain legume collection is published and available with open access in the electronic catalogue on Plant Genetic Resources EURISCO (http://eurisco.ecpgr.org).

Collected studies are available based on an electronic database and are a base for creation an *on farm* conservation catalogue of grain legume landraces in Bulgaria according to the Concept of ECPGR (2017).

RESULTS AND DISCUSSION

Status of Phaseolus and Vigna National Inventory in EURISCO

The collection of beans (genus *Phaseolus*) is characterized with one of the largest number of accessions (3,888 acc.), and 47 % from them are with Bulgarian origin (1,837 acc.). The status of Bulgarian *Phaseolus* collection in EURISCO is presented in Table 1.

Table 1. Status of Bulgarian *Phaseolus* collection in EURISCO

Genus	Species	Total number	BGR origin
Phaseolus	*acutifolius*	31	
Phaseolus	*angularis*	3	
Phaseolus	*angulosus*	1	
Phaseolus	*aureus*	39	
Phaseolus	*caffer*	3	
Phaseolus	*calcaratus*	1	
Phaseolus	*coccineus*	241	139
Phaseolus	*calcaratus*	1	
Phaseolus	*gonospermus*	1	
Phaseolus	*hysterinus*	2	
Phaseolus	*lunatus*	34	
Phaseolus	*multiflorum*	5	
Phaseolus	*multiflorus*	8	
Phaseolus	*mungo*	7	
Phaseolus	*radiatus*	8	
Phaseolus	*ricciardinus*	3	
Phaseolus	*semierectus*	2	
Phaseolus	*trilobus*	1	
Phaseolus	*vulgaris*	3488	1698
Phaseolus	*zebra*	1	
Phaseolus	*sp.*	8	
Total number		3888	1837

The collection of cowpea (genus *Vigna*) contains 397 acc., and 40 acc. from them are characterized by Bulgarian origin. The status of Bulgarian *Vigna* collection in EURISCO is presented in Table 2.

Table 2. Status of Bulgarian *Vigna* collection in EURISCO

Genus	Species	Total number	BGR origin
Vigna	*catjang*	3	
Vigna	*mungo*	1	
Vigna	*radiata*	3	
Vigna	*sinensis*	116	18
Vigna	*unguiculata*	192	22
Vigna	*sp.*	82	
Total number		397	40

Enrichment of grain legume collection through the project Bulgaria – China

By the expeditions in Bulgaria through the project between Bulgaria and China the collections are enriched with seed accessions from 51 acc. *Phaseolus vulgaris*, 17 acc. *Phaseolus coccineus* and 2 acc. from *Vigna sp.* Routes for inventory of agricultural areas in South Bulgaria were established using previous results and data in the National Catalogue of Plant Genetic Resources. Accessions from seven areas: Smolyan, Devin, Velingrad, Pazardzhik, Plovdiv, Samokov and Blagoevgrad, including 13 villages were collected.

The bean is one of the most significant crops in the traditional Bulgarian cuisine. With a great value is creation of local beans collection from the mountainous areas in Rhodopes (regions of Smolyan, Devin, Velingrad), which are with the best conditions for growing and the grain legumes are traditional crops with typical dishes. The local populations are local selection and only in the region of collecting these accessions produce seeds with unique traits. This germplasm is one of the best examples where the *on farm* conservation is the most successful practice for its sustainable preservation. In this connection, the information about the specific agro-climatic characteristics of the growing region for further testing and use is very important.

The traditional varieties meet family needs because of the excellent taste quality and biological content. Also, they have economical influence in the mountain areas because they are presented at the local markets as originated food with a very high price.

The analysis of the passport database (Tables 1, 2 and 3) from collecting missions in 2021, 2022 and 2023 shows that expeditions for local grain legumes were carried out in the regions of South Bulgaria, flat, semi-mountainous and mountainous regions with an altitude of 147 to 1185 m. All accessions are collected from small rural and suburban farms or home gardens.

Table 3. Passport data of collected accessions according to the project activities in 2021

N	CAT. N	TAXONOMY	COLLECTING SITE	LATITUDE	LONGITUDE	ELEVATION (m)	SEED DESCRIPTION
1	C1E0014	*Phaseolus vulgaris L.*	Pavelsko, Smolyan	415200N	244200E	730	medium large, white
2	C1E0015	*Phaseolus vulgaris L.*	Pavelsko, Smolyan	415200N	244200E	730	medium large, patterned – white and red
3	C1E0016	*Phaseolus vulgaris L.*	Pavelsko, Smolyan	415200N	244200E	730	medium large, black
4	C1E0017	*Phaseolus vulgaris L.*	Pavelsko, Smolyan	415200N	244200E	730	medium large, white and black
5	C1E0018	*Phaseolus vulgaris L.*	Pavelsko, Smolyan	415200N	244200E	730	medium large, brown-black
6	C1E0019	*Vigna sp. Savi*	Mihalkovo, Smolyan	415200N	244200E	717	small, black
7	C1E0022	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415200N	244200E	717	patterned, white and red
8	C1E0023	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415200N	244200E	717	white
9	C1E0024	*Phaseolus coccineus L.*	Mihalkovo, Smolyan	415200N	244200E	717	white
10	C1E0025	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415200N	244200E	717	patterned
11	C1E0026	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415200N	244200E	717	beige
12	C1E0027	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415200N	244200E	717	patterned
13	C1E0028	*Phaseolus coccineus L.*	Mihalkovo, Smolyan	415200N	244200E	717	white

14	C1E0029	*Phaseolus coccineus L.*	Mihalkovo, Smolyan	415200N	244200E	717	patterned
15	C1E0030	*Phaseolus coccineus L.*	Mihalkovo, Smolyan	415200N	244200E	717	patterned
16	C1E0031	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415200N	244200E	717	patterned, white and purple
17	C1E0032	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415200N	244200E	717	patterned, brown-black
18	C1E0033	*Phaseolus coccineus L.*	Mihalkovo, Smolyan	415200N	244200E	717	white
19	C1E0034	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415200N	244200E	717	patterned
20	C1E0035	*Phaseolus vulgaris L.*	Momchilovtsi, Smolyan	413929N	244633E	1185	white
21	C1E0036	*Phaseolus vulgaris L.*	Momchilovtsi, Smolyan	413929N	244633E	1185	patterned
22	C1E0037	*Phaseolus vulgaris L.*	Momchilovtsi, Smolyan	413929N	244633E	1185	patterned
23	C1E0038	*Phaseolus vulgaris L.*	Momchilovtsi, Smolyan	413929N	244633E	1185	patterned
24	C1E0039	*Phaseolus vulgaris L.*	Novo selo, Plovdiv	420615N	242906E	196	patterned
25	C1E0040	*Phaseolus vulgaris L.*	Grohotno, Devin	414121N	242242E	813	white
26	C1E0041	*Phaseolus vulgaris L.*	Grohotno, Devin	414121N	242242E	813	beige
27	C1E0042	*Phaseolus vulgaris L.*	Grohotno, Devin	414121N	242242E	813	patterned
28	C1E0043	*Phaseolus coccineus L.*	Grohotno, Devin	414121N	242242E	813	white, Smilyanski type
29	C1E0044	*Phaseolus coccineus L.*	Grohotno, Devin	414121N	242242E	813	patterned, Smilyanski type
30	C1E0045	*Phaseolus coccineus L.*	Grohotno, Devin	414121N	242242E	813	patterned, Smilyanski type
31	C1E0072	*Phaseolus vulgaris L.*	Raduil, Samokov	421709N	234118E	900	white
32	C1E0073	*Phaseolus vulgaris L.*	Raduil, Samokov	421709N	234118E	900	patterned
33	C1E0074	*Phaseolus coccineus L.*	Raduil, Samokov	421709N	234118E	900	white, Smilyanski type
34	C1E0085	*Phaseolus vulgaris L.*	Raduil, Samokov	421709N	234118E	900	white, flat, Raduilski type
* Data source – National Catalogue of Plant Genetic Resources / IPGR-Sadovo							

Table 4. Passport data of collected accessions according to the project activities in 2022

N	CAT. N	TAXONOMY	COLLECTING SITE	LATITUDE	LONGITUDE	ELEVATION (m)	SEED DESCRIPTION
1	C2E0011	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	beige
2	C2E0012	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	patterned
3	C2E0013	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	brown
4	C2E0014	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	patterned
5	C2E0015	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	light brown
6	C2E0016	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	black
7	C2E0017	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	dark brown
8	C2E0018	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	white with brown

9	C2E0019	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	white
10	C2E0020	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	patterned
11	C2E0021	*Phaseolus coccineus L.*	Velingrad, Chepino	420139N	235928E	777	white
12	C2E0022	*Phaseolus coccineus L.*	Velingrad, Chepino	420139N	235928E	777	white
13	C2E0023	*Phaseolus coccineus L.*	Velingrad, Chepino	420139N	235928E	777	patterned
14	C2E0024	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	light beige
15	C2E0025	*Phaseolus vulgaris L.*	Velingrad, Chepino	420139N	235928E	777	light beige
16	C2E0026	*Phaseolus vulgaris L.*	Velingrad, St. Petka	420216N	235228E	1114	medicinal, beige-green
17	C2E0027	*Phaseolus vulgaris L.*	Fotinovo, Pazardzhik	415257N	242108E	1124	beige
18	C2E0028	*Phaseolus vulgaris L.*	Fotinovo, Pazardzhik	415257N	242108E	1124	patterned
19	C2E0029	*Phaseolus vulgaris L.*	Fotinovo, Pazardzhik	415257N	242108E	1124	patterned
20	C2E0030	*Phaseolus vulgaris L.*	Fotinovo, Pazardzhik	415257N	242108E	1124	patterned
21	C2E0031	*Phaseolus vulgaris L.*	Fotinovo, Pazardzhik	415257N	242108E	1124	beige with brown
22	C2E0032	*Phaseolus vulgaris L.*	Fotinovo, Pazardzhik	415257N	242108E	1124	patterned
23	C2E0033	*Phaseolus coccineus L.*	Fotinovo, Pazardzhik	415257N	242108E	1124	white
24	C2E0034	*Phaseolus vulgaris L.*	Krichim, Plovdiv	420233N	242752E	253	white
25	C2E0035	*Vigna sp. Savi*	Rupite, Blagoevgrad	412630N	231430E	147	beige
26	C2E0037	*Phaseolus vulgaris L.*	Sadovo, Plovdiv	420752N	245557E	156	white
*	Data source – National Catalogue of Plant Genetic Resources / IPGR-Sadovo						

Table 5. Passport data of collected accessions according to the project activities in 2023

N	CAT. N	TAXONOMY	COLLECTING SITE	LATITUDE	LONGITUDE	ELEVATION (m)	SEED DESCRIPTION
1	C3E0043	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415100N	242600E	717	white
2	C3E0044	*Phaseolus vulgaris L.*	Mihalkovo, Smolyan	415100N	242600E	717	patterned, beige with brown
3	C3E0045	*Phaseolus vulgaris L.*	Grohotno, Devin	414121N	242242E	813	brown
4	C3E0046	*Phaseolus vulgaris L.*	Grohotno, Devin	414121N	242242E	813	white, flat
5	C3E0047	*Phaseolus coccineus L.*	Grohotno, Devin	414121N	242242E	813	Local name: kitkin
6	C3E0048	*Phaseolus coccineus L.*	Mogilitza, Smolyan	412944N	243803E	1041	white
7	C3E0049	*Phaseolus coccineus L.*	Mogilitza, Smolyan	412944N	243803E	1041	white
8	C3E0050	*Phaseolus vulgaris L.*	Mogilitza, Smolyan	412944N	243803E	1041	white
9	C3E0051	*Phaseolus coccineus L.*	Arda, Smolyan	412744N	243818E	1003	white
10	C3E0052	*Phaseolus vulgaris L.*	Arda, Smolyan	412744N	243818E	1003	beige with purple
*	Data source – National Catalogue of Plant Genetic Resources / IPGR-Sadovo						

Documentation, dissemination and implementation of the achieved results

Bulgaria and China are countries where there are favorable conditions for the cultivation of a rich variety of species and varieties. Taking into consideration the aging of the population and the depopulation of rural areas, combined with natural disasters such as floods, drought, etc. of an unpredictable nature, the danger of losing the wealth of local varieties and crop wild relatives is substantially high. In 2023 two scientists from the Chinese Academy of Agricultural Sciences visited Institute in Sadovo and took part in the expedition in South Bulgaria. Exchange of descriptors and experience in characterization and evaluation between the two partners was conducted.

In relation with the ECPGR (2017) objective to promote *on farm* conservation and management of European plant diversity, the realization of this project increases the knowledge of grain legume landraces still present in rural regions in Bulgaria, allows also *ex situ* storage and elaboration of data that is of paramount importance for the implementation the preservation plan in Europe. The inclusion of new data in the National Catalogue of Plant Genetic Resources of IPGR-Sadovo increases the value of datasets related to local gene fund. According to already collected data, the collection is describing successful experiences of conservation and sustainable use of plant genetic resources facilitating the definition of good practices for *on farm* management and preservation for increasing the product added value of the crop. Evidences and lessons that are learnt from the development of the project are close relevant for setting-up similar studies on other crops in Bulgaria (cereals, vegetables, etc.). Vegetables and grain legumes are grown in home gardens, and the surplus is exported to the market, in addition to being a subsidiary farm for the household. Old varieties of cereals are maintained for traditional production of area-specific foods related to local customs.

In Bulgaria there are conditions for environmentally sustainable agriculture and production of unique food and beverages by authentic methods. The added value of the activities of home gardens using local resources are socio-economic and environmental benefits for the regions. Organic production is still weak in the country, but the market for organic products is developing rapidly. The prerequisites for the development of this type of production and the factors motivating farmers in this direction are the rich diversity of natural resources, ecologically preserved areas, the perceived benefits for rural development, the growing demand for healthy food from consumers and the existence of a legal framework. Considering all these positive conditions in Bulgaria and the high economic development of China the partnership is extremely important not only in the field of agricultural science, but also in education and specialization of researchers.

CONCLUSIONS

Based on bilateral partnership IPGR-Sadovo is implementing joint research project with China, focused on conservation of grain legumes. These species are crucial to solve key agriculture-related societal challenges, such as agrobiodiversity conservation, sustainable agriculture, food security and human health.

Expeditions in rural areas of Bulgaria were conducted and 70 local accessions has been collected. Information gathered in the project is useful for *ex situ* back-up of the identified resources for protection and sustainable use.

The study is focused on Inventory of the Bulgarian *on farm* diversity, monitoring and promoting good practices adding value to the crop farm system. Restoration of old varieties in

the agricultural practice as well as preservation of traditional knowledge and good practices has attitude to mitigating the effects of the climate change.

Although Bulgaria has gained achievements in collection of local varieties, there are still a large number of unexplored territories rich with landraces which are in danger the plant diversity to be lost.

Exchange of passport descriptor and database with the Chinese partners was carried out. Regarding the access and sustainable use of this valuable source for crop breeding and sustainable agriculture IPGR-Sadovo is open for collaboration in new projects with Chinese research organizations and trainings, focused on documentation and digitalization of plant biodiversity.

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EFFECT OF PH-SHIFT TREATMENT AND ULTRASONICATION ON THE PHYSICAL STABILITY AND PROPERTIES OF HEMP SEED MILK

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Abstract

Hemp seed milk is a growing beverage with excellent nutritional content and minimal allergenicity, which offers a tasty substitute for other plant-based milk types. During this research, we investigated the individual and combined impact of pH shift and ultrasound (US) on the stability characteristics of hemp seed milk. The effect of pH shift and US were investigated on the physico-chemical properties of hemp, milk, sedimentation index, rheological properties, color, Brix, physical stability, titratable acidity, and emulsion stability index (ESI) measurements. According to the obtained data, applying individual US techniques showed the best results, with the highest stability characteristics and better rheological properties, the highest L* (lightness) and Brix values, and the lowest titratable acidity values. Interestingly, the individual application of the pH-shift technique showed the lowest physical stability results. In comparison, pH shift treatment combined with the US demonstrated moderate stability. Thus, the pH shift and the US are remarkable non-thermal processing methods for producing stable hempseed milk.

Keywords: hemp seed milk, pH-shift, ultrasonication, viscosity, rheological properties.

Introduction

The acknowledged nutritional benefits and minimal allergenic potential of hemp seeds have contributed to a surge in the consumption of hemp products in recent years. Hemp seed market size was assessed at $5.1 billion in 2022 and is expected to reach $11.7 billion by 2032, rising at a CAGR of 8.9% between 2023 and 2032, according to a recent research by Allied Market Research titled, "Hemp Seed Market" (Hemp Seed Market).

Since both hemp and marijuana are produced from the same plant, useful or industrial hemp is referred to by the Latin term Cannabis Sativa. The primary psychoactive ingredient, delta-9 tetrahydrocannabinol (THC), is present in approximately 0.3% to 1.5% of industrial hemp, but it is present in 5% to 10% or more of marijuana (Besir et al., 2022). About 25% of hemp seeds are protein, and 30% are oil. The oil has a high concentration of polyunsaturated fatty acids (PUFAs), particularly linoleic (-6) and -linolenic (-3) acids (Wang et al., 2018). To provide nutritional advantages, hemp seed milk is made from hemp seeds. Most of the original nutrients remain in hemp seed milk, making it a highly nutritious beverage. Hemp seed milk is regarded as a pleasant substitute for dairy, soy, and nut milk, lactose-free, and low in allergens (Besir et al., 2022).

Hemp seed milk is an oil-in-water (O/W) emulsion system that is unstable and tends to flocculate like other milk substitutes created from plant seeds, decreasing quality and shortening

shelf life (Wang et al., 2018). Plant-based milk is a colloidal system that contains large-sized dispersed particles such as fat globules, solid raw material particles, proteins, and starch granules. Because of solid particle sedimentation, it is challenging to produce a stable product that can be kept in stock for an extended period. Therefore, many methods have been applied to increase hemp seed milk's stability, like emulsifiers or stabilizers. However, this method is not recommended due to economic causes and health issues. For instance, several research have indicated that long-term intake of artificial emulsifiers may result in chronic inflammatory diseases linked to obesity and metabolic syndrome. As a result, there is an increasing need for affordable alternative technologies and food items devoid of additives (Cani & Everard, 2015). Such techniques as enzymatic hydrolysis (Yin et al., 2008) and acylation (Yin et al., 2009) have been applied. Wang et al. (2018), in their published research, studied the application of pH-shift and high-pressure homogenization processes to produce additive-free hemp seed milk with a focus on its physical and oxidative stability (Wang et al., 2018). In recent years, high-pressure homogenization (HPH) has also been developed to assist in creating stable O/W food emulsions. Oil is mechanically divided into tiny droplets by HPH, increasing the overall surface area, uniformizing the size distribution, and enhancing stability.

However, a molten globular or fibrous conformation is produced when a protein solution is brought to a high alkaline or acidic pH and maintained briefly to promote structural unfolding, followed by a brief incubation at the neutral pH to allow partial refolding. It has been demonstrated that this procedure is known as pH shift. The resulting structure exhibits molten globule characteristics, which significantly improves proteins' solubility and emulsifying and film-forming capabilities (Jiang et al., 2018). Hence, pH shift has been effectively used to treat soy and pea proteins to increase their emulsifying capabilities (Wang et al., 2018).

Moreover, another promising technique for increasing the stability of plant-based milk is ultrasonication. Ultrasonication is applying high-power ultrasound to a specific product; it may cause cavitation. Cavitation is a process wherein the propagation of high-power sound waves causes the emergence of small gas or vapor bubbles that develop inside the sample. Extreme temperatures and pressures are created as a result of this process. These extreme conditions have the potential to deactivate enzymes and change the secondary structure of proteins, altering both their nutritional value and functional capabilities (Vanga et al., 2020). However, ultrasound is divided into two types: low-intensity ultrasound (with a power intensity of less than one wcm-2 and a frequency of five to ten MHz) and high-intensity ultrasound (with a power intensity of ten to one hundred wcm-2 and a frequency of twenty to one hundred kHz), the latter of which is used in food processing technologies (Sarangapany et al., 2022). Hence, the effect of US on plant proteins has been evaluated in many previous studies on soy, pea, black bean, almond, and wheat proteins (Vanga et al., 2020) and peanut (Salve et al., 2019). However, no study has evaluated the effect of ultrasonication on the structural properties, organoleptic, and functional properties of hemp seed milk. The objective of the current study was to make additive-free hemp seed milk using a pH shift and US procedure while examining its physical, organoleptic, and functional characteristics.

Materials and Methods

Seed material

During this research, defatted hemp seeds have been utilized for milk production. The oil has been previously extracted by cold pressing technique.

Oil extraction by cold pressing

According to the literature, the high lipid content of seeds and nuts may result in undesirable phase separation and decreased product stability; thus these components are eliminated during processing (Tangyu et al., 2019). The current research used cold pressing to extract oil from the hemp seeds. In a technical procedure known as pressing, oil is mainly extracted (drained) from the hemp seeds using mechanical pressure. Cold pressing is performed by directly pressing raw/dried seeds on a continuous screw press at low temperature (Rabrenović et al., 2014).

Hemp Seed Milk Preparation

Each experimental run began with fresh hemp seed milk preparation, according to the method described by Wang et al. (2018), with slight modifications. Briefly, defatted seeds were ground in a 1:8 w/v ratio of deionized water with an ultra-turrax homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 12000 rpm for 10 min, then filtered using Stainless Steel Sieve, to ensure the consistency of milk and to remove particles. The produced milk was immediately kept at 4°C in airtight containers.

pH Shifting and Ultrasonication Treatments

The prepared hemp seed milk underwent three treatments: pH shifting alone, ultrasonication alone, and a combination of both. The control sample had not been modified, only stirred. For pH shift, the pH of hemp seed milk samples was adjusted to 12 using 1 M NaOH at room temperature and then brought back to pH 7 using 1 M HCl. US treatment was applied using a VibraCell VC750 ultrasonic processor (Sonics & Materials, Inc., Newtown, CT, USA) at 20 kHz and 750 W. Using a 13 mm diameter probe, the sonic energy was transferred into hemp seed milk. An ice bath was used to prevent overheating from ultrasonication, which might result in protein denaturation (Kahraman et al., 2022).

The sonication pulse duty cycle (5 s on, 5 s off) was set to 100% amplitude. The hemp seed milk samples were sonicated for 5 or 10 minutes during the treatment with ultrasonication alone or with pH shift. For the samples of pH shifting and ultrasonication combined treatments, the hemp seed milk samples were exposed to pH shift and then immediately sonicated for 5 or 10 min.

Physical stability/ Creaming index

Two 20 ml of processed hemp seed milk were immediately transferred to graduated tubes, sealed, and kept in a refrigerator (4 ± 2°C) to calculate the sedimentation index. According to the Indu et al. (2019) approach, measurements were taken every 24 hours until the samples indicated total separation, at which point the separation index (SI) was determined using the equation given below (Indu et al., 2019).

$$\text{Creaming index (\%)} = \frac{\text{Height of the aqueous layer HA}}{\text{Total height of emulsion HE}} \times 100$$

Color

A colorimeter (CR-400, Konica Minolta, Inc., Japan) was used to measure color properties. The study used the CIE-L* a* b* color coordinate system. L* value is a lightness measuring factor, ranging from blackness (0) to whiteness (100). A* value varies from greenness (-60) to redness (+60), whereas b* value goes from blueness (-60) to yellowness (+60) (Zaaboul et al., 2019).

Total soluble solids (Brix)

On a Brix scale of 0-100, the total soluble solids of the samples were calculated using a refractometer at room temperature (Salve et al., 2019).

Solid particle sedimentation (SPS)

Solid particle sedimentation was conducted according to the method described by (Gul et al., 2017). Each sample was centrifuged at 2500 g for 20 min using 10 mL of the solution. The amount of solid deposition at the bottom of the tube was given as a percentage (w/w)(Gul et al., 2017).

Titratable acidity

The samples (10 ml) were mixed with 10 ml of deionized water after that, titrated with 0.1 N sodium hydroxide (with indicator phenolphthalein) in order to assess the effect of treatment on the titratable acidity of hemp seed milk (Salve et al., 2019).

Rheological properties

Rheological characterization of hemp seed milk was carried out by using Haake Mars III rheometer (Thermo Scientific, Germany) with a cone and plate system (35 mm diameter, 0.105 mm gap, 2_ angle). The temperature was maintained constant at 25ºC by a Peltier plate system. The steady-state shear experiments were measured by shearing the samples at linearly increasing shear rates from 1 to 100 s^{-1} through 120 s. Product flow behavior was modeled using the Ostwaldde-Waele model.

$$\eta_{app} = K\dot{\gamma}^{n-1}$$

where η_{app} is apparent viscosity (Pa s), $\dot{\gamma}$ is the shear rate (s^{-1}), K the consistency index (Pa sn), and n the flow behavior index (dimensionless) (Zaaboul et al., 2019 ; Atalar et al., 2019).

Temperature effects on viscosity

For evaluating the effect of increasing temperature on the viscosity characteristics of hemp seed milk, the temperature of the samples was increased with a heating rate of 0.5°C/min from 10 to 80°C as described perviously by Iskakova & Smanalieva, (2021).

Statistical analysis

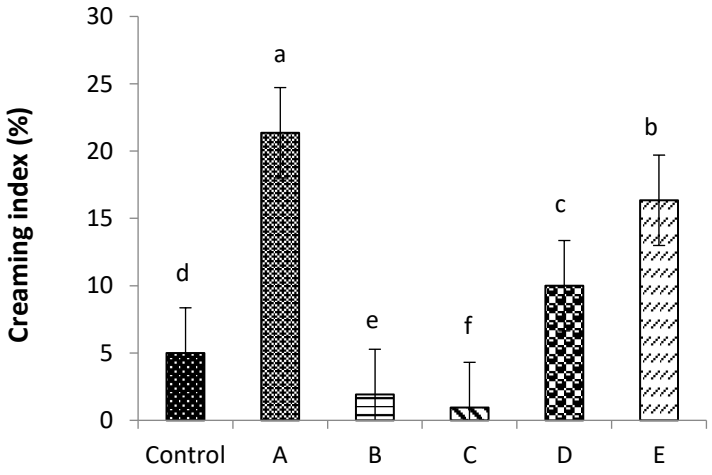
Statistical analysis of data was performed by one-way ANOVA analysis of variance using Minitab software. Thedata was expressed as the mean of triplicate estimation ± standard deviation and at $p<0.05$ level, the differences were considered statistically significant.

Results and discussion

Creaming index

The creaming index is crucial to assess the stability of hemp and milk. The stability of hemp seed milk increases with a decrease in the creaming index %. As shown in **Fig.** (**1A, 1B**), the creaming index of hemp seed milk decreased when the duration of ultrasonication was increased. This could be caused by the cavitation effect, which reduces the size of fat or protein globules and prevents flocculation (Indu et al., 2019). However, the pH-shift treatment showed a noticeable increase in the creaming index. The literature has reported that the pH-shift method, instead of directly changing the pH value to 7-8, could decline the protein solubility and emulsion stability due to the formation of insoluble aggregates (Jiang et al., 2018).

(A)



(B)

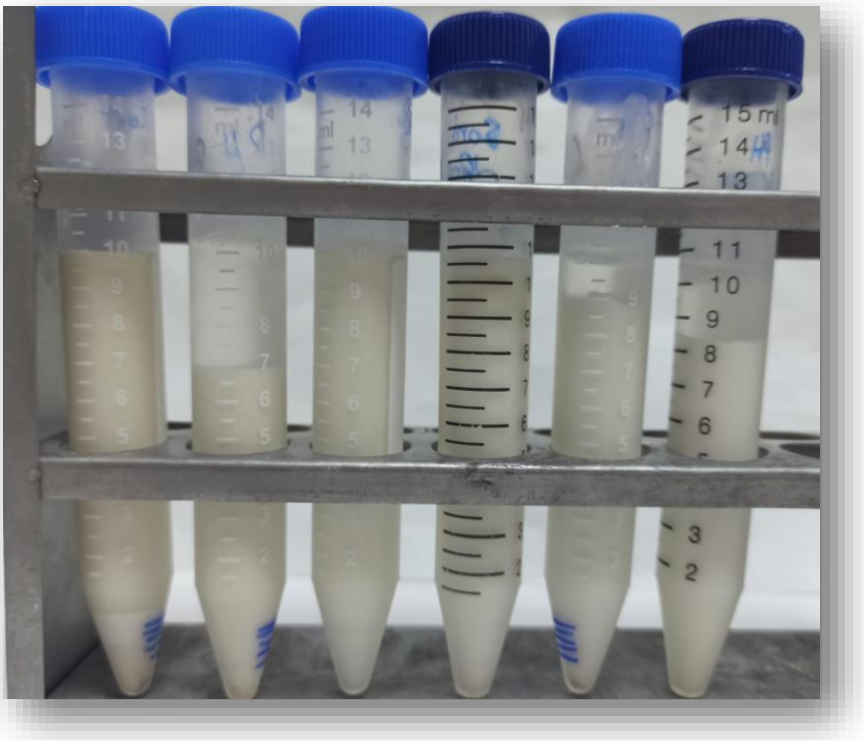


Fig. 1. Creaming index of hemp seed milk after 48 h of low temperature storage (4 ± 2 °C): **(A)** Creaming index of hempmilk, where a', b', c' , d', e',and f' represent statistical differences and **(B)** in sequence photos representing the separation of phases of all treatments. Where Control: without any treatment, A: pH-shift, B: 5 min of US, C: 10 min of US, D: pH-shift & 5 min US, E: pH- shift & 10 min US.

Color properties

The **Table 1.** shows the effect of pH shift and US processing on the colour parameters of hemp seed milk. On analyzing *L** value (lightness), samples treated with with the US statistically showed the highest L* value. Samples treated with pH-shift and US demonstrated high L* values with no statistical differences. As well as Control (untreated) and only pH-shift treated samples showed the lowest values with no significant differences. The increase in light scattering and greater lightness values of the samples can be attributed to the tendency of US treatment to cause the dispersed particles to increase by minimizing their size (Sarangapany et al., 2022). The same outcomes have also been found for peanut milk; ultrasonication increased the color characteristics of the milk, particularly the lightness or L* value (Salve et al., 2019). The* (redness) value showed no statistical differences among all samples except for the control sample, which demonstrated the highest value. For b* (yellowness) value, control and pH-shift treated samples showed the highest values.

Table 1. Effect of pH shift and US treatment on the colour of hemp seed milk.

Treatments	*L**	*a**	*b**
Control	85.960 ± 0.18^{c}	-0.5333 ± 0.12^{a}	10.7133 ± 0.14^{a}
pH-shift	86.497 ± 0.18^{c}	-0.7600 ± 0.05^{b}	9.9167 ± 0.10^{b}
US-5 min	88.137 ± 0.26^{b}	-0.7033 ± 0.06^{ab}	8.8667 ± 0.05^{c}
US-10 min	89.163 ± 0.22^{a}	-0.8467 ± 0.04^{b}	8.5233 ± 0.03^{d}
pH-shift & US-5 min	88.313 ± 0.17^{b}	-0.7633 ± 0.03^{b}	8.27 ± 0.09^{d}
pH-shift & US-10 min	88.117 ± 0.17^{b}	-0.7400 ± 0.02^{b}	8.4233 ± 0.11^{d}

Each value is represented by its mean and standard deviation (n=3). Values in the same column that are vertically present the same superscript letters do not differ statistically ($p > 0.05$).

Soluble solids (Brix) and titratable acidity

The effects of treating hemp seed milk with pH- shift and/or US on total soluble solids (TSS) and titratable acidity of hemp seed milk are shown in **Table 2.** It has been observed that the sonicated samples have shown to have the highest TSS value 4.8 Brix. Increasing the US time significantly affected the Brix value of treated samples ($p<0.05$). However, similar results have been reported in previously published studies (Salve et al., 2019)(Maghsoudlou et al., 2016). Employing the power of sonication can enhance the breaking down of cell walls to speed up the release of their contents. The control, pH-shift, and pH and US-treated samples presented no significant difference. Additionally, it was shown that the titratable acidity of hemp seed milk decreased significantly as the US treatment duration increased. This may be caused by a change in the charge of particles by the sonication process (Salve et al., 2019). However, pH-shift treatment alone or combined with US showed a statistically increased titratable acidity value.

Table.2 Effects of pH shift and US processing on physic chemical properties of hemp seed milk

Treatments	Brix (%)	Titratable acidity	Sedimentation index
Control	1.95 ± 0.212^{c}	0.043 ± 0.002^{d}	37.16 ± 0.1^{e}
pH-shift	1.95 ± 0.283^{c}	0.166 ± 0.007^{b}	42.47 ± 0.2^{c}
US-5 min	3.95 ± 0.141^{b}	0.058 ± 0.004^{C}	37.38 ± 0.1^{d}
US-10 min	4.8 ± 0.07^{a}	0.048 ± 0.006^{cd}	34.04 ± 0.2^{f}
pH-shift & US-5 min	1.95 ± 0.141^{c}	0.207 ± 0.002^{a}	46.95 ± 0.08^{a}
pH-shift & US-10 min	2.0 ± 0.07^{c}	0.154 ± 0.005^{b}	45.97 ± 0.2^{b}

Each value is represented by its mean and standard deviation (n=3). Values in the same column that are vertically present the same superscript letters do not differ statistically ($p > 0.05$).

Sedimentation index

The higher sedimentation value is associated with lower product stability. The results showed that ultrasonication significantly ($p< 0.05$) lower sedimentation values, Fig.2 In another word, treated hemp seed milk without pH- shift resulted in a more stable emulsion. The same

result has been reported by Wang et al. when they studied the effect of pH shift and homogenization pressure on hemp seed milk stability (Wang et al., 2018). Whereas the application of pH-shift or the combined application of pH-shift and the US unexpectedly statistically ($p<0.05$) increased the sedimentation values. The improvement in the sedimentation index in the US-treated samples is driven by some factors, including the reduction in particle size that facilitated intermolecular interactions. Additionally, denaturing hemp proteins aided in their unfolding, revealing their active sites and raising the hydrophobicity of their surfaces (Salve et al., 2019). On another hand, it has been discussed in the literature that the pH-shift process could have resulted in undesirable molten globule conformation in some cases. It is known that the pH-shift process increases the solubility of proteins, but after an excessive pH treatment, solubilized proteins must immediately go through refolding; otherwise, the pH-shift process results in a decreased protein solubility due to the formation of insoluble aggregates; this was observed in the literature on soy globulins (Jiang et al., 2018), a similar result was found out during our study.

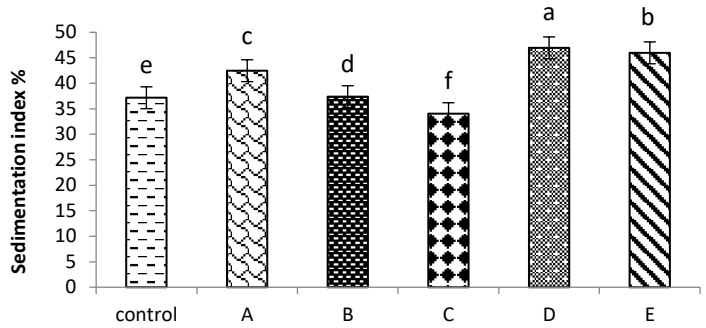


Fig. 2. Sedimentation index of hemp seed milk, where a', b', c' , d', e'and f' represent statistical differences. Control: without any treatment, A: pH-shift, B: 5 min of US, C: 10 min of US, D: pH-shift & 5 min US, E: pH- shift & 10 min US.

Rheological properties

Effect of pH shift and US on rheological parameters

The pH shift and US treatments considerably impacted the viscosity of the hemp seed milk sample **Table. 3** Compared to the untreated (control) milk sample (42.4 mPa.s). For all samples, hemp seed milk had non-Newtonian fluid flow characteristics - have varying relationships with shear stress and non-constant viscosity-. Viscosity was estimated with a Herschel–Bulkley equation. According to the results, only pH-shift treated samples showed significantly reduced viscosity values (12,68 mPa.s) compared to the control samples. As previously discussed, the pH-shift process causes the protein structure to unfold, and when the pH is returned to neutral, where intramolecular charge repulsions are significantly reduced, some degree of refolding occurs. During this study, we noticed phase separation and aggregation, which could be associated with the formation of undesired molten structures during the pH-shift process. However, in their previous published research, Nicoud et al. (2015) claimed that the aggregate distribution's polydispersity is why an aggregated protein solution has a lower viscosity than a monomeric protein solution (Nicoud et al., 2015). Likewise, the viscosity of pH-shift treated samples was statistically lower than those treated with pH shift and US (5 and 10 min). It is worth mentioning that according to the literature, US treatment (400, 600, and 200 W) alone has decreased the viscosity of peanut milk (Salve et al., 2019), and this is consistent with our results for only US-treated samples (5 and 10 min) which had similar

results with no satistical differences. The US impacts the milk by changing the balance and reducing the larger particles into smaller ones. The cavitation treatment caused rheological changes in milk samples that reduced their viscosity, increased their fluidity, and decreased their tendency to behave in a pseudoplastic manner (Salve et al., 2019) **Table.3.**

Table.3 Rheological parameters of pH-shift and US treated hemp seed milk.

	K (Pa sn)	n (-)	R^2	η_{50} (mPa s)
Control	0.0016 ± 0.000^{b}	0.885 ± 0.044^{a}	0.757 ± 0.069^{b}	42.48 ± 1.92^{a}
pH-shift	0.053 ± 0.002^{b}	0.636 ± 0.008^{b}	0.994 ± 0.000^{a}	12.68 ± 1.39^{c}
US-5 min	0.006 ± 0.004^{b}	0.643 ± 0069^{b}	0.732 ± 0.053^{b}	1.332 ± 0.57^{d}
US-10 min	0.002 ± 0.008^{b}	0.881 ± 0.105^{a}	0.964 ± 0.008^{a}	1.368 ± 0.04^{d}
pH-shift & US-5 min	0.475 ± 0.002^{a}	0.253 ± 0.035^{c}	0.992 ± 0.003^{a}	24.88 ± 2.72^{b}
pH-shift & US-10 min	0.369 ± 0.05^{a}	0.298 ± 0.02^{c}	0.994 ± 0.003^{a}	23.46 ± 1.53^{b}

Each value is represented by its mean and standard deviation (n=3). Values in the same column that are vertically present the same superscript letters do not differ statistically ($p>0.05$).

Temperature effects on viscosity

Food flow behavior can be affected by temperature variations (Forster & Ferrier, 1979). Likewise, Simuang et al., (2004), in their study, showed that the viscosity of coconut milk was significantly affected by heat treatment. Thus, the flow behavior of hemp seed milk at various temperatures was investigated. The temperature influence on rheological parameters is shown **Fig. 3.**

Data showed that after the viscosity of all samples demonstrated reduction over continuous heating until it reached a certain extent, the apparent viscosity changed slightly and started to increase at higher temperatures. The literature has reported that increasing temperatures cause liquid viscosity to decrease. The result of increasing a liquid's temperature is a decrease in cohesive forces and an increase in the rate of molecular interchange. In other words, the increase in temperature causes kinetic or thermal energy and the molecules become more mobile (Baily, 2013)(Iskakova & Smanalieva, 2021). On the other hand, increasing viscosity with continuous heating is suggested to be due to protein denaturation and subsequent association/polymerization. However, Liu & Chang, (2007) reported the same result: soy milk's viscosity increased as the heating time increased. They claimed that heating caused both 11S and 7S proteins to dissociate. The dissociated polypeptides and subunits of the 7S and 11S proteins then interacted (Liu & Chang, 2007). Hence, the maximum denaturation of 7S protein, which caused a sharp increase of soymilk viscosity, was observed at 70oC. Likewise, according to the literature, hemp proteins are sensitive and have a lower denaturation temperature, therefore, it is suggested to keep heat treatment below 80°C in order to retain their heat stability and solubility (Besir et al., 2022). The maximum denaturation temperature of hemp protein was not observed during the current study, but it has been noted that the maximum decline and the start of an increase in hemp seed milk viscosity of all samples occurred between 50 and 70^{o}C. We propose that an increase in viscosity at these levels indicates the beginning of protein structural changes, however it may not be a complete denaturation or coagulation. For investigating the effect of pH-shift and US treatment on hemp seed milk viscosity during the application of various temperatures, after a steady and moderate drop in the viscosity of samples treated with pH-shift alone or with US (5 and 10 min), we observed a sharp increase in the viscosity, which indicates the highly sensitivity and heat instability of those samples Fig.3.

These results are highly compatible with the results we discussed previously for measuring sedimentation and creaming indexes for these samples. Whereas, control and only US (5 and 10 min) treated hemp seed milk samples showed a less severe increase in the viscosity, which indicates a more cohesive structure and consequently more heat stability.

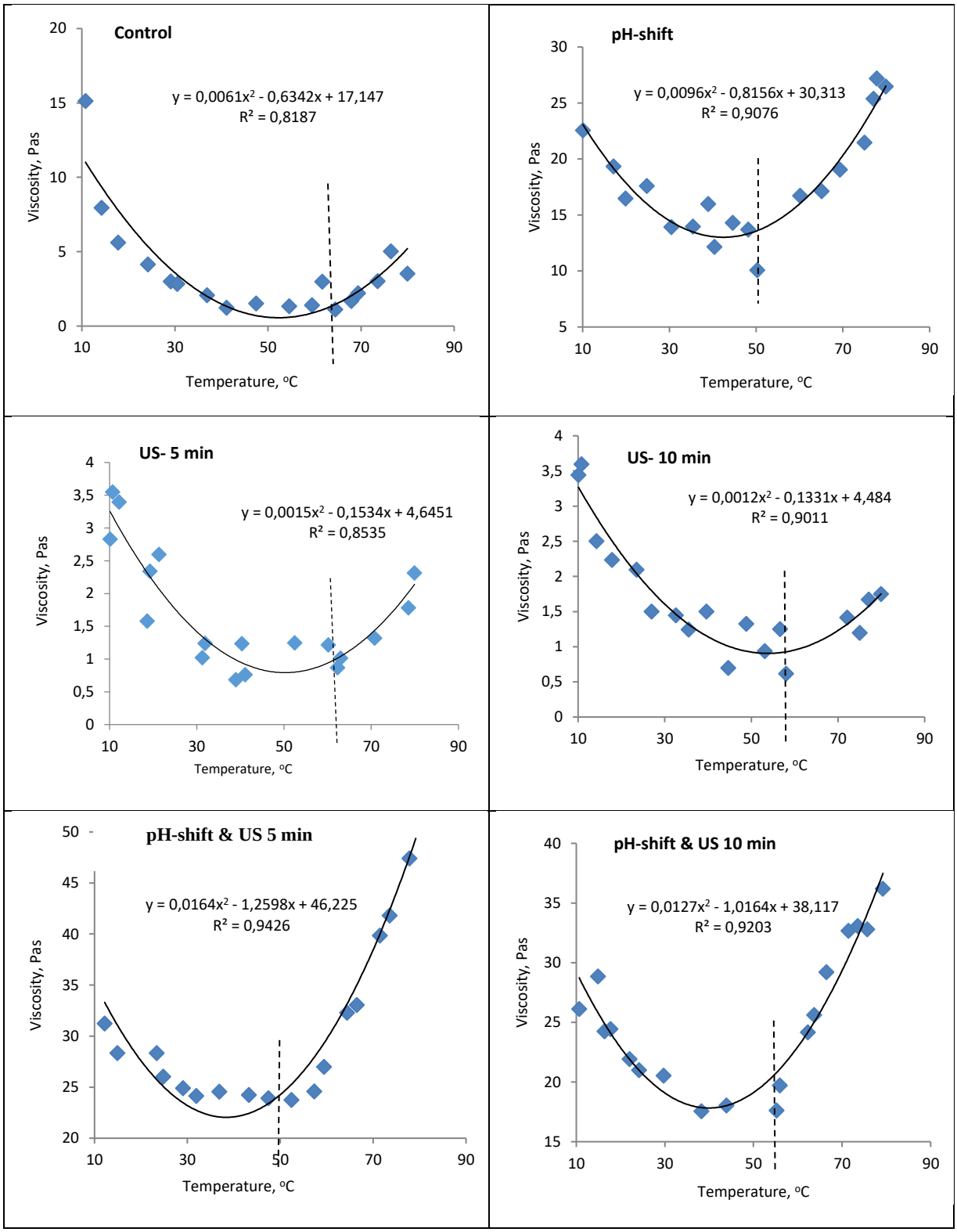


Fig. 3. Temperature effect on the viscosity characteristics of hemp seed milk samples.

Conclusion

Even though each of the pH shifts and US processes had a different stabilizing impact on the hemp seed milk emulsion, their combination did not yield the best results. The pH shift treatment encouraged protein interactions that eventually resulted in large clusters and aggregates, which negatively impact the stabilization of the emulsion. Hemp seed milk treated only with US showed such interactive structures that were stable against coalescence coagulation and showed better temperature-related viscosity characteristics. Since the pH-shift process showed unstable emulsion, it is suggested for the future to apply the direct change of pH to 7-8 (without incubation period), which is expected to be more effective. Finally, last but not least, using US or combining pH shift with US with various modifications may present new potential for producing non-thermally hemp seed milk without stabilizers or emulsifiers.

Data Availability

The datasets of their study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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THE EFFECT OF DIFFERENT DRYING METHODS ON SOME CHEMICAL AND BIOACTIVE COMPONENTS OF ORANGE AND BLACK CARROT POWDERS

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ABSTRACT

In this study, two different varieties of carrots (orange and black carrots) were dried with three different drying methods (hot air, microwave and freeze-drying) and ground to obtain carrot powders. Color values, ash, protein, fat, antioxidant activity, total phenolic content (TPC), β-carotene, total anthocyanin (TA) and mineral matter of the carrot powders were determined. Orange carrot powders showed higher lightness, redness and yellowness values than black carrot powders. The freeze-drying method provided higher lightness value in both carrot powder varieties. Different drying methods did not cause a significant ($p>0.05$) change on the ash, protein and fat content of carrot powders. The antioxidant activities and TPC of orange and black carrot powders varied between 75.16-76.60% and 87.52-95.75%, 263.50-470.90 mgGAE/100g and 596.10-1353.40 mgGAE/100g, respectively. β-carotene content of orange carrot powders dried with different methods ranged between 39.34 mg/100g and 48.72 mg/100g, and β-carotene content of freeze-dried samples was found to be higher than other drying methods. The TA amount of black carrot powders varied between 259.08 mgCGE/100g and 424.66 mgCGE/100g. The freeze-drying method resulted in the highest TA content, while the hot air drying method revealed the lowest TA content. Black carrot powders for all drying methods had higher Fe, K, Mg and P contents than orange carrot powders. Different drying methods did not change the mineral amounts in both carrot powders.

Keywords: Drying, orange carrot, black carrot, powder, bioactive component

INTRODUCTION

Carrot (*Daucus carota L.*) is one of the most widely produced root vegetables in world agriculture. Daucus, which includes sixty species of several cultivars, has color ranging from white to yellow, orange, light purple, deep red or violet (Rodriguez et al., 1975). Orange carrots are an important source of carotenoids. Approximately 60% of the total carotenoid content of ripe orange carrots is β-carotene, 20% α-carotene, and the remainder is lycopene, γ-carotene, ζ-carotene, and/or β-zeacarotene (Banga & De Bruyn, 1964; Gabelman, 1974; Simon & Wolff, 1987). Although orange color is the dominant color for carrots, black carrot has been attracting attention for its bluish color with high levels of anthocyanins. The origin of black carrot is Turkey, Middle and Far East, and it has been cultivated for at least 3000 years (Kamiloglu et al., 2016). Carrot varieties are also rich sources of vitamins, minerals and dietary fiber. The nutritional properties and bioactive content and also attractive colors of orange and black carrots have led to research on the usage areas of carrots.

There are some studies in the literature on the powdering of carrots using different methods and the use of its powder in various products. Wang & Xi, (2005) used the microwave drying method for drying carrots. The technological and nutritional differences between the dried samples were investigated by changing the amount of carrots placed in the microwave tray (100g, 200g and 300g) and the applied microwave power (120, 160 and 240 W). A decrease in the amount of β-carotene was observed as the microwave power increased. Gong et al. (2015) compared the effects of different drying methods on the color characteristics and β-carotene contents of carrot powders. Carrot powder was obtained by using vacuum drying, hot air drying, microwave drying and freeze-drying methods. Carrot powders dried with hot air had the lowest

β-carotene content (114.4 mg/kg), while the highest value (344.8 mg/kg) was obtained by freeze- drying method.

In this study, it was aimed to determine the effects of different drying methods (hot air, microwave and freeze-drying) on the color, some chemical and bioactive components of carrot powders obtained from orange and black carrots.

MATERIAL AND METHODS

Materials

Orange and black carrots were obtained from the cold storages of Kaşınhanı town in Konya, Turkey. All chemicals used in analysis were of analytical grade quality.

Production of carrot powders

Carrots were washed, peeled and cut into thin slices. Carrot slices were first heat treated in boiling water (3 minutes at 90 ºC) and then the following drying methods were applied to the carrots. Drying in hot air flow: carrots were dried in a drying cabinet (Nüve KD 200, Ankara, Turkey) at 60 ºC for 10 hours (Akubor & John Ike, 2012). Microwave drying: Carrot samples were dried in a household microwave oven (LG Solardom, Seoul, South Korea) at 360 W power for 45 minutes according to (Prakash et al., 2004). Freeze-drying: carrots were dried in a freeze-drying device (Scanvac, CoolSafe, Denmark) at -54 ºC for 24 hours (Lee et al., 2003). After all the dried samples were ground, they were sieved through a sieve with a diameter of 500 micrometer and stored under refrigerator conditions for analysis.

Color measurement

The color values of the carrot powders were measured using Hunter Lab Chroma Meter (Minolta CR-400, Osaka, Japan) in terms of the Hunter L*, a* and b* values. Also, according to a* and b* values, chroma ($(a^{*2}+b^{*2})^{1/2}$) and Hue angle (if a*>0 and b*>0 Hue=arctan [b*/a*]; if a*>0 and b*<0 Hue=360+arctan [b*/a*]) were calculated.

Chemical and bioactive components analysis

Ash, protein and fat content of carrot powders were determined according to AACC 08-01.01, 46-12.01 and 30-25.01 standard methods (AACC, 1999). The TPC was determined colorimetrically using the Folin Ciocalteau method. Extraction was conducted according to Gao et al. (2002) and Beta et al. (2005). Absorbance was measured at 760 nm using a spectrophotometer (Hitachi-U1800, Japan) and results were expressed as mg Gallic acid equivalent (Gámez-Meza et al., 1999; Slinkard & Singleton, 1977). Antioxidant activity was determined by 2-2-Diphenyl-2-picrylhydrazyl (DPPH) method (Beta et al., 2005; Gyamfi et al., 1999). Absorbances were measured at 517 nm and the inhibition percentage was calculated. The β-carotene content of the samples was determined according to Prakash et al. (2004) with some modification of the method. The analysis of TA in the samples was carried out according to the method specified by (Ficco et al., 2014). For the analysis of Ca, Fe, K, Mg, P and Zn elements 1 g dry sample was dissolved by the wet burning method in the microwave combustion system (Mars 5, CEM Corporation, USA) using 10 ml of sulfuric acid + nitric acid. The mineral substance amounts of the obtained filtrates were measured on an ICP-AES (inductively coupled plasma atomic emission spectrophotometer) instrument (Vista Series, Varian International, AG, Switzerland) (Skujins, 1998).

Statistical analysis

Minitab version 16 statistical program was used for statistical analysis. Means were compared at the $p<0.05$ level.

RESULTS AND DISCUSSION

Color values

Color values of carrot powders produced with different drying methods are given in Table 1. L*, a*, b*, C* and hueº values of carrot powders varied between 39.28 and 81.25, 12.19 and 26.78, -4.02 and 44.26, 12.84 and 51.80 and 57.11 and 346.75, respectively. The highest L* value was determined in the orange carrot powder obtained by freeze-drying. The

L* value of the powders obtained by freeze-drying method in both carrot cultivars was found to be higher than the L* value of the powders obtained by other drying method. Exposure of samples to heat in hot air and microwave drying methods may have caused this situation. In general, orange carrot powders gave higher a* and b* values than black carrot powders. The yellow and red color of the carrot is attributed to the presence of carotenes (Wagner & Warthesen, 1995). The fact that orange carrots are richer in carotenes than black carrots may have caused these results. When a* value of orange carrot powders was evaluated in terms of drying method; it was seen that a* value of the orange carrot powders obtained by freeze-drying was higher than a* value of the powders obtained by applying hot air. Although the a* value of black carrot powders did not show a statistical difference depending on the drying methods, the powders obtained by freeze-drying method showed the highest a* value numerically. When the orange carrot powders were examined in terms of b* value; as with a* value, the b* value of the powders obtained by freeze-drying method was found to be higher than the b* value of the powders obtained by hot air. The use of different drying methods in the production of black carrot powder did not cause a statistical difference on the b* value. The fact that the drying time is longer in the hot air drying method compared to the microwave drying method, and the temperature is higher than the freeze-drying method may have caused more loss in carotenoid pigments and a decrease in a* and b* values. Freeze-drying method gave higher C* values in orange carrot powders than hot air and microwave drying methods, and no significant difference was observed between hot air and microwave drying methods in terms of C* value. Although the C* values of black carrot powders did not show a statistical difference according to the drying method, the carrot powder obtained by freeze-drying method gave the highest numerical value. Drying methods were not effective on the hue^{o} value of orange and black carrot powder samples, and no statistical difference was determined between the results. Howard et al. (1996) reported that the lightness of the carrot is affected by the processing temperatures, and higher temperatures cause a darker color. It was reported in another study that the L*, a* and b* values of the dried carrot samples decreased with the increase in the temperature applied during drying (Xiao et al., 2010). In a study, freeze-dried, vacuum-microwave and hot air drying methods were used for the drying of carrots; It was determined that freeze-dried carrot slices had the highest L*, a* and b* values. It has been reported that drying in hot air causes more reduction in lightness value due to greater exposure to oxygen and higher temperature (Cui et al., 2008).

Table 1. Color values of carrot powders produced with different drying methods

Carrot varieties	Drying method	L*	a*	b*	C*	Hue^{o}
Orange	Hot air	70.81±2.07b	19.20±1.34bc	35.75±1.94b	40.58±2.35b	61.78±0.37b
	Microwave	72.26±0.01b	23.88±2.25ab	36.90±0.73ab	43.98±0.60b	57.11±2.98b
	Freeze-drying	81.25±3.78a	26.78±1.72a	44.26±4.22a	51.80±2.71a	58.72±4.06b
Black	Hot air	39.28±2.59d	12.19±0.1d	-4.02±0.15c	12.84±0.05c	341.73±0.77a
	Microwave	41.53±0.82d	12.84±1.02d	-3.92±0.15c	13.43±0.94c	342.94±1.89a
	Freeze-drying	51.11±0.77c	13.36±1.50cd	-3.12±0.17c	13.73±1.40c	346.75±2.10a

Means with the different letter within a column are significantly different ($p < 0.05$).

Chemical and bioactive components

The chemical analysis results of orange and black carrot powders dried with three different methods are given in Table 2. Average ash, protein and fat amounts of carrot powders were determined as 5.77%, 8.232% and 2.34%, respectively. When the orange and black carrot powders were evaluated in terms of drying method, it was seen that the drying methods did not cause any change on the ash, protein and fat contents of the carrot powders. In general, black carrot powders had higher ash content than orange carrot powders.

Antioxidant activity and TPC of carrot powders obtained by different drying methods are presented in Table 2. Although the drying method is statistically insignificant on the amount of antioxidant activity of orange carrot powders, the lowest value was determined numerically in the samples prepared by the hot air drying method, and the highest value in the samples prepared by the freeze-drying method. When the black carrot powders were evaluated in terms of drying method, the carrot powders obtained by freeze-drying method had numerically higher antioxidant activity than the carrot powders dried in hot air and microwave. These changes may be resulted from the applied temperature during drying and long drying times. Similar results were found in other studies comparing the hot air method with other methods in drying carrots, and it was reported that the losses in microcomponents determined as a result of hot air drying were caused by the length of the process time, high temperature and oxidation (as a result of the presence of oxygen) (Chen et al., 2017; Cui et al., 2008; Lin et al., 1998).

The highest amount of TPC in carrot powders was determined in black carrot powder produced by freeze-drying method. Regardless of the drying method, black carrot powders yielded higher TPC than orange carrot powders. When the orange carrot powders were compared in terms of drying method, drying method did not change the TPC statistically, but the powders obtained by the hot air drying method had a lower TPC numerically. When the black carrot powders were evaluated among themselves in terms of drying method, it was determined that the TPC of black carrot powder obtained by freeze-drying method was significantly higher than the TPC of black carrot powder obtained by other methods. Presence of phenolic compounds in carrots contributes to sensory qualities such as color (Zhang et al., 2005), bitterness (Kreutzmann et al., 2008) and aroma (Naczk & Shahidi, 2003). Therefore, the response of phenolic compounds can be used as a good indicator to evaluate the quality of vegetables during processing and storage (Gonçalves et al., 2010). In general, it was determined that black carrot powders had higher antioxidant activity and TPC amount than orange carrot powders.

For the orange carrot powders, β-carotene content obtained by freeze-drying method (48.72 mg/100g) was found to be higher than that of powders dried in microwave and hot air (39.34 and 40.35 mg/100g) (Table 2). For black carrot powders, the highest amount of TA was determined in the freeze-drying powder (424.66 mgCGE/100g), followed by microwave (344.24 mgCGE/100g) and hot air (259.08 mgCGE/100g) dried samples. The amounts of β-carotene and TA of orange and black carrot powders obtained by freeze-drying method were found to be higher than powders obtained by other methods. By the fact that the application temperature of the freeze-drying process is low and the drying process takes place under a high vacuum, which reduces the oxidation and degradation of β-carotene and similar microcomponents (Cui et al., 2008; Lin et al., 1998).

Mineral contents

The mineral analysis results of orange and black carrot powders prepared with different drying methods are given in Table 3. Ca, Fe, K, Mg, P and Zn content of carrot powders ranged between 396.27-418.13 mg/100g, 1.29-1.92 mg/100g, 224.23-297.13 mg/100g, 288.98-368.74 mg/100g, and 1.42-2.92 mg/100g. The applied drying methods did not cause a statistical change on the mineral substance content of orange and black carrot powders. Fe, K, Mg and P content of black carrot powders had higher than that of orange one.

DISCUSSION

In this study, the color values, some chemical and bioactive components of orange and black carrot powders prepared using different drying methods were compared. When the results are evaluated in terms of carrot variety; black carrot powder had lower L*, a* and b* color values and generally higher ash, antioxidant activity, TPC and mineral substance amounts than orange carrot powder. In the comparison made according to the drying method; higher lightness, TPC, β-carotene and TA values were obtained in the carrot powders obtained by the freeze-drying method compared to other drying methods.

Table 2. Chemical and bioactive component results of carrot powders produced with different drying methods

Carrot varieties		Drying method	Ash (%)	Protein (%)	Fat (%)	Antioxidant activity (%)	TPC (mgGAE/100g)	β-carotene (mg/100g)	TA (mgCGE/100g)
Orange	Oven	Hot air	5.71±0.07b	7.93±1.95a	2.16±0.25a	75.16±5.40b	263.50±5.50d	40.35±1.12b	nd
		Microwave	5.69±0.07b	7.93±0.40a	2.08±0.14a	75.70±5.02b	430.00±17.80cd	39.34±0.85b	nd
		Freeze-drying	5.68±0.03b	7.97±0.21a	2.15±0.18a	76.60±4.74b	470.90±16.80cd	48.72±0.69a	nd
	Black	Hot air	5.88±0.92a	8.49±0.52a	2.55±0.76a	87.52±1.19ab	596.10±16.70bc	nd	259.08±9.76c
		Microwave	5.84±0.03a	8.54±0.28a	2.53±0.20a	87.69±0.89ab	742.80±21.70b	nd	344.24±6.67b
		Freeze-drying	5.83±0.03a	8.55±0.23a	2.59±0.20a	95.75±3.50a	1353.40±134.60a	nd	424.66±9.01a

Means with the different letter within a column are significantly different ($p < 0.05$). Results are dry matter basis. TPC: Total phenolic content. TA: Total anthocyanin.

Table 3. Mineral matters (mg/100g) of carrot powders produced with different drying methods

Carrot varieties	Drying method	Ca	Fe	K	Mg	P	Zn
Orange	Hot air	403.48±3.18a	1.30±0.06b	1630.90±58.8b	224.23±5.47b	293.19±4.16b	2.92±0.14a
	Microwave	396.27±8.40a	1.29±0.10b	1619.20±24.0b	234.23±7.10b	290.88±12.55b	2.84±0.17a
	Freeze-drying	396.27±4.65a	1.31±0.04b	1623.70±30.4b	235.44±8.75b	288.98±12.40b	2.83±0.23a
Black	Hot air	418.13±3.20a	1.89±0.03a	2296.90±101.0a	296.00±14.90a	361.48±8.79a	1.42±0.23b
	Microwave	414.94±7.13a	1.92±0.04a	2295.50±1.60a	287.07±4.31a	368.74±3.52a	1.50±0.03b
	Freeze-drying	417.69±7.25a	1.90±0.06a	2299.70±4.80a	297.13±7.28a	355.64±10.51a	1.47±0.04b

Means with the different letter within a column are significantly different ($p < 0.05$). Results are dry matter basis.

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NUTRITIONAL VALUE AND UTILIZATION POSSIBILITIES OF BULGUR INDUSTRY BY-PRODUCTS IN CEREAL PRODUCTS

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ABSTRACT

Bulgur is a semi-ready-to-eat cereal product with high nutritional and functional properties. Bulgur is commonly produced from *Tr. durum*. The bulgur production process basically consists of cooking, drying, grinding and classification stages. By-products rich in functional and nutritional components emerge in the intermediate stages of the bulgur production process. Some of these products are used as animal feed. However, bulgur by-products such as bulgur bran, bulgur flour and düğürcük are rich in protein, dietary fiber, bioactive components and mineral contents. In addition, phytic acid in bulgur bran and bulgur flour is quite low compared to untreated ones due to the application of pressure cooking during bulgur production. Bulgur by-products can be used in different ratios in the production of various cereal products such as bread, pasta, noodles, biscuits, crackers and tarhana, and contribute to the nutritional and functional properties of these products. In this study, the use of bulgur process by-products in various cereal products and their effects on the nutritional, functional and technological properties of these products were compiled.

Keywords: Bulgur, bulgur bran, bulgur flour, düğürcük.

INTRODUCTION

Bulgur, which has been consumed in Anatolia and Mesopotamia for centuries, is a gelatinized whole wheat product produced from durum wheat. Bulgur is also one of the first foodstuffs processed in the world and the main stages in its production are cleaning, cooking, drying, peeling, crushing, sieving and sorting.

Bulgur is an important wheat product with high dietary fiber content and low glycemic index, with 18.3 grams of dietary fiber per 100 grams. Its dietary fiber content is 3.5, 6.8, 1.8, 2.3, 2.3, 1.3 and 4.3 times higher than rice, wheat flour, oatmeal, wholemeal bread, soybeans and pasta, respectively (Bayram and Öner, 2007; Yıldırım et al., 2008a, 2008b).

Nutritionally, bulgur contains relatively high protein and fiber content, resistant starch, B vitamins, minerals and phytochemicals such as lutein and ferulic acid (Stone et al., 2020).

In the production process, especially in the cooking process; bulgur, in which nutrient loss is prevented by absorbing the vitamins, minerals and other nutrients that pass into the

cooking water into the wheat, is a food close to whole wheat in terms of essential nutrients and has a very high nutritional value (Öner, 2002).

As a result of the production technique, bulgur preserves the water-soluble nutrients carried to the inner parts of the grain more effectively, while the hard-glassy grain structure consisting of gelatinized starch and coagulated protein increases the resistance to long-term storage and pests by destroying biological and biochemical activity (Elgün et al., 1986).

During the processing of wheat into various products, high amounts of by-products are obtained. As a result of bulgur production, 20% of by-products such as bulgur bran, bulgur flour and bagels are produced. Bulgur bran is a by-product obtained as a result of stoning wheat in peeling machines after boiling and drying it in bulgur production. Bulgur flour is a by-product separated by passing through a 0.25 mm sieve in the classification process applied to wheat after the peeling and crushing processes. Simit (düğürcük) is a by-product that is separated by passing through a 0.75 mm sieve during the classification of crushed wheat (Hançer, 2010).

Cereal bran is a good source of dietary fiber. However, they contain high amounts of phytic acid, which can lead to some nutritional problems. In addition, cereal bran supplementation reduces the technological quality of food products. Reducing substances and proteolytic enzymes in the aleurone part of wheat bran weaken the gluten structure of dough and adversely affect the rheological properties of dough (Grosch and Wieser, 1999; Every et al. 2006). Bulgur bran does not contain an aleurone layer and its phytic acid content is low and its dietary fiber content is quite high due to the heat treatment applied during the production process (Balcı and Bayram, 2015). The total dietary fiber contents of bulgur flour, bulgur bran and düğürcük were found to be 56.2%, 69.0% and 21.1%, respectively (Hançer, 2010).

In this study, the use of bulgur bran, bulgur flour and düğürcük, which are produced as waste during bulgur production, in cereal products was reviewed.

USE IN CEREAL PRODUCTS

The by-products of bulgur, which is widely consumed in our country and in many countries around the world, have become more important with the determination of its nutritional value and have started to be used by researchers to increase the nutritional and functional value of the product to which it is added in many formulations, especially bread and pasta.

Baumgartner (2018) applied the microfludization process to bulgur and chickpea bran and used it in bread production. The addition of microfludized bran at an increasing rate negatively affected the rheological properties of the dough and the textural and sensory properties of the bread. However, the microfludization process slightly reduced the negative effects of the bran. As a result of this study, microfludized bulgur and chickpea bran were reported to be sources of dietary fiber with low phytic acid content. The addition of bran increased the dietary fiber, phenolic content and antioxidant activity of the bread depending on the amount added, and this increase was more pronounced in microfludized bran.

To develop short-cut pasta-type couscous, *Triticum durum* semolina and bulgur flour (sifted bulgur) were substituted at various concentrations. It was determined that weight gain, total flavonoid content, protein, ash and crude fiber content, hardness, stickiness, stickiness, gumminess, chewiness and elasticity values of the samples were affected by bulgur flour substitution, but elasticity, bulk density, total phenolic content and DPPH antioxidant activity values were not affected (Yüksel, Öner, & Bayram, 2017).

In a study conducted by adding bulgur bran of 3 different particle sizes (200μm, 400μm and 850μm) at different ratios (0, 5, 10, 15 and 20%) to two different biscuit flours (A and B),

bulgur bran significantly increased the total, soluble and insoluble dietary fiber amounts of biscuits depending on the ratio and particle size, and the highest values were observed in samples obtained by adding 20% of bulgur bran at 850μm size (Özkeser, 2015).

Wheat flour was used to make biscuits by replacing 10% of wheat flour with cereal by-products such as wheat, barley, oat and bulgur bran, poppy meal and germ. Some physical and sensory properties of the biscuits were measured and water absorption capacity and dough development time were slightly affected by the addition of these by-products. Apart from the addition of germ, the addition of cereal by-products mostly decreased the whiteness, spreading rate and general acceptability of the biscuit samples and increased the hardness (Yağcı, 2019).

In a study investigating the effects of bulgur by-products (bulgur flour: BU, bulgur bran: BK, düğürcük: D) on tarhana quality; bulgur by-products were used at the rates of 5%, 10%, 15%, 20%, 25% and 30%, the additions of BU, BK and D increased the protein and ash contents of tarhana, and the total dietary fiber contents of tarhana containing 20% BU and 15% BK, which were found to be sensory acceptable, were found to be 4 times higher than the total dietary fiber content of the control sample (Hançer, 2010).

In a study in which instant soup production was aimed by using düğürcük in tarhana, tarhana was produced by using two different proportions (50 and 100%) of düğürcük and the sensory properties and solubility values of the tarhana samples obtained were examined. It was concluded that tarhana containing 50% düğürcük could be used as instant soup and was liked in terms of appearance, structure and mouthfeel (Yurttaş et al., 2003).

RESULTS AND DISCUSSION

Wheat bran consists of the outer pericarp, inner pericarp, testa, hyaline layer, aleurone layer and some attached starchy endosperm residues (Hemery et al., 2011). However, bulgur bran is obtained from the outer layers of the bulgur grain, but unlike wheat bran, it does not contain the aleurone layer. While the phytic acid content of bulgur bran is very low due to the heat treatment applied during the production process (Balcı and Bayram, 2015), the dietary fiber content of bulgur bran is quite high compared to other cereal bran. Despite these advantages, studies on the inclusion of bulgur bran in foods are limited.

Bulgur flour, bulgur bran and düğürcük (simit) are by-products that occur during bulgur production. According to research (depending on the yield, process and technique used), the amount of by-products constitutes approximately 15% of the total amount of bulgur produced and the price is around 100-200 USD/ton. These products, which have high nutritional value, are currently used only as animal feed. It is important to increase the studies on their utilization in cereal products and to transform them into value-added products.

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UTILIZATION OF DILL, PARSLEY AND GREEN ONION POWDERS IN THE CRACKER FORMULATION

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ABSTRACT

In this study, dill, parsley and green onion powders were used in functional cracker production. After dill, parsley and green onions were dried and ground, they were sieved through a 500 μm sieve and used in the cracker formulation at four different ratios (0, 1, 3, 5%). The effect of dill, parsley and green onion powders on color, physical, textural and sensory properties of crackers was determined. Some quality characteristics of cracker samples containing dill, parsley and green onion powder were compared with control crackers prepared with refined wheat flour. L*, a*, b*, SI and Hue values of the cracker samples changed between 55.81-72.95, -6.37-2.22, 37.79-41.51, -87.47-86.63 and 37.86-41.58, respectively. All utilization levels of dill, parsley and green onion powder decreased the lightness of the cracker samples. As the proportion of dill, parsley and green onion powder increased in the cracker formulation, the thickness value of the cracker samples decreased, and the spread values increased. While the use of dill, parsley and green onion powder in cracker formulation did not have a significant effect on the hardness value of the samples, the fracturability values of the samples containing green onions were found to be higher.

Keywords: Cracker, ill powder, Parsley powder, green onion powder.

INTRODUCTION

Functional and nutritional products such as herbs, fruits and vegetables can be added to wheat flour to improve the nutritional value and health-promoting properties of food products (Dziki et al., 2014). Spices and herbs have been added to foods since ancient times, not only as flavoring agents but also as food preservatives. It has also been used in folk medicine (Kabić et al., 2008).

Anethum graveolens L. or European dill grows mostly in the European Mediterranean region, in the central and southern parts of Asia. India, Pakistan, USA, Mexico, Germany and the Netherlands are the top producers of dill (Kulkarni et al., 2012). Dill has long been used as a spice in different countries to season and flavor a variety of foods such as rice, sauces, salads, side dishes and soups (Jana and Shekhawat, 2010). A recent systematic review and meta-analysis of the effects of dill ingestion on glycemic index and lipid profiles and in adults shows that dill improves insulin resistance and serum low-density lipoprotein. A recent publication evaluating the effectiveness of dill supplementation on lipid profile in adults with

cardiovascular risk factors showed significant improvement in all lipid profile components: Triglycerides, low-density lipoprotein cholesterol, Total cholesterol, and high-density lipoprotein cholesterol (Mansoori et al., 2021).

Parsley (*Petroselinum Crispum Mill*.) is a popular vegetable of the Apiaceae family and is native to Southwestern Europe and Western Asia. Parsley is a medicinal herb and has been widely used in the Mediterranean for over 2000 years. It is currently grown as a spice for use in cuisines around the world (Punoševac et al., 2021). Parsley leaves are a rich source of essential oils, vitamins A and C, potassium, iron and ascorbic acid (Dobričević et al., 2019). Dirim and Koç (2019) investigated the properties of noodles enriched with parsley (2, 4, 6 and 8 weight percent); and reported that vitamin C, chlorophyll and carotenoid contents increased with the addition of parsley. They reported that noodles enriched with 2% parsley had the highest score in sensory evaluation. Sęczyk et al. (2015) reported that enrichment of pasta with dried and powdered PL increased the antioxidant capacity of pasta.

Onion has many medicinal properties such as antibiotic effect, lowering blood sugar and plasma cholesterol levels, antihyperlipidemia, thrombolysis, antiplatelet aggregation, prevention of rheumatoid arthritis and diuretic effects. Onions are also known to contain characteristic volatile substances that are produced enzymatically when tissue is injured. The substrates for the production of these volatiles are known to be alkyl cysteine sulfoxides and amino acid derivatives of cysteine. These derivatives cause various reactions to the sulfur-containing volatile substances of disulfides (Seguchi and Abe, 2003).

This study aimed to investigate the effects of dill, parsley and green onion powders on cracker's L*, a*, b*, Hue and SI color values, physical properties such as diameter, thickness and spread rate, as well as textural properties such as hardness and fracturability.

MATERIALS AND METHODS

Materials

Fresh dill, parsley and green onion, wheat flour (Hekimoğlu, Konya, Turkey), shortening, salt, powdered sugar, baking powder, and baker's yeast were obtained from the local bazaar in Konya (Turkey). Protease enzyme was procured from Vatan Enzyme (İstanbul, Turkey).

Preparation of the dill, parsley and green onion powders

The fresh dill, parsley and green onion were dried in a dry air dryer at 60 ± 2 °C for 12 hours. After drying, the dried dill, parsley and green onion samples were ground and sieved through a 212 μm sieve to obtain the dill powder, parsley powder and green onion powder.

Cracker production

Crackers were prepared according to a slight modification of the procedure reported by Davidson (2016). For control cracker preparation wheat flour (100 g), shortening (20 g), table salt (1.6 g), powdered sugar (1.5 g), baking powder (1.5 g), baker's yeast (0.2 g) and protease (0.01 g) were mixed in the mixer (Hobart N50, Canada Inc., North York, Ontario, Canada) until a homogeneous dough was obtained. The dough was fermented at room conditions for 20 minutes. Then, the fermented dough was formed into a 1 mm thick layer between two glass plates and shaped with a 50 mm diameter biscuit mold. It was baked in an oven (Vestel SF8401, Manisa, Turkey) for 11 minutes at 180°C. Other crackers were prepared by replacing wheat flour with 1%, 3%, and 3% levels of dill powder, parsley powder and green onion powder.

Color properties

Color measurement of cracker samples was performed using the Minolta CR 400 (Chroma Meter, Osaka, Japan). The measurement was made on the five different points on the surface of the crackers. L* (lightness, darkness), a* (red, green) and b* (yellow, blue) values were measured in cracker samples. Hue (color essence) value was calculated with arctan (b*/a*) formula and SI (saturation index) value was calculated with $(a^{*2}+b^{*2})^{1/2}$ formula.

Physical properties

The diameter, thickness, spread ratio and textural properties of the end products were determined. The diameter and thickness were measured using five sample pieces by a caliper (Mitutoyo, Tokyo, Japan) according to the AACC method 10-54 (AACC, 2010) and values were reported in millimeters. The cracker spread ratio was determined by dividing the diameter by thickness.

The hardness and fracturability value of the crackers were evaluated by three-point bending (HDP/3 PB) tests on a TA-XT plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with 5 kg loading cell. The measurement conditions of the texture analyzer were as follows: pre-test speed, 1.0 mm/s; test speed 1.0 mm/s; post-test speed, 10.0 mm/s. In the hardness value measurements, 5 measurements were made for each sample and it was studied in 2 replications.

Statistical analysis

All analyses were performed in duplicate. For statistical analysis, the JMP statistical program, version 10.0 (SAS Institute Inc., Cary, NC, USA) was used.

RESULTS AND DISCUSSION

Color properties

Color values of cracker samples prepared with dill, parsley and onion powder are given in Table 1. When the results were evaluated in terms of the type of powder used, the use of dill powder produced statistically darker crackers than parsley and onion powder ($p < 0.05$). However, the a* value of the cracker samples produced using onion powder was higher than the average a value of the samples with dill and parsley powder, and the b and SI values were numerically lower, but this difference was statistically insignificant. These observed effects might be related to the color properties of the raw materials (data not shown). Values of L*, a*, and b* attributes were found at 72.76, 2.22, and 37.57 for control crackers. With the increasing use of dill, parsley and green onion powder compared to the control, the L* and a* values decreased, while the b* value showed an increasing trend in the cracker samples ($p \leq 0.05$). Such color changes in crackers may be contributed by pigments such as chlorophyll a and b, which provide greenery in purslane, as well as β-carotene, which provides yellowness.

Table 1. Color values of dill, parsley and onion powder-enriched crackers

	n	*L**	*a**	*b**	Hue	SI
Powder type						
Dill	8	62.98±7.00b	-2.68±3.39a	39.33±1.60a	93.72±4.81a	39.54±1.78a
Parsley	8	67.32±4.96a	-1.99±2.82a	39.35±1.43a	92.79±4.07a	39.48±1.49a
Onion	8	65.71±6.25a	-1.99±3.14a	38.74±1.32a	91.73±3.30b	38.81±1.33a
Additive ratio (%)						

0	6	72.76±0.19a	2.22±0.34a	37.57±0.76c	86.60±0.43d	37.64±0.78c
1	6	68.27±2.87b	-2.21±0.22b	38.55±0.84b	93.10±0.49c	38.61±0.84c
3	6	62.02±2.67c	-3.47±0.76c	39.76±0.71a	94.80±1.05b	39.91±0.72b
5	6	58.30±2.89d	-5.40±1.73d	40.66±0.94a	96.50±2.23a	40.95±1.07a

[1]Means with the same letter within a column are not significantly different (p > 0.05). Hue: Hue angle. SI: Saturation index.

Physical properties of crackers

The effects of the dill, parsley and green onion powder on diameter, thickness and spread ratio and textural properties (hardness and fracturability) of the cracker samples are shown in Table 2. The diameter value of crackers containing parsley powder (48.12 mm)was higher than crackers prepared with dill and green onion powders (48.16 mm and 48.11 mm). Although there are numerical differences between the thickness and spread values of the crackers produced from different powders, they were found to be statistically similar. The increasing dill, parsley and green onion powder ratio from 0 to 5% increased the diameter and spread ratio value of crackers samples but decreased the thickness value. The diameter, thickness and spread ratio values of crackers ranged between 47.65 and 48.68 mm, between 3.85 and 4.63 mm and between 10.29 and 12.66, respectively. The spreading rate indicates the rising ability of the biscuits and is controlled by the viscosity of the dough as low viscosity allows the biscuits to spread more quickly (Ho et al., 2016; Zouri et al., 2016). Increasing the use of dill, parsley and green onion powder increased the spread rate, which may be associated with low gluten content. Ramashia, et al. (2021) reported that the incorporation of *P. curatellifolia* peel flour decreased the gluten protein of composite flours, and this caused a low viscosity of the dough. Similar results were observed by Nassar et al. (2008) where the inclusion of citrus peel flour improved the diameter of biscuits.

Table 2. Physical properties of dill, parsley and onion powder-enriched crackers

	n	**Diameter (mm)**	**Thickness (mm)**	**Spread ratio (W/T)**	**Hardness (g)**	**Fracturability (mm)**
Powder type						
Dill	8	48.16±0.39b	4.35±0.37a	11.15±1.05a	40104.82±84.21a	3.54±0.43b
Parsley	8	48.42±0.65a	4.22±0.35a	11.56±1.10a	40165.33±77.15a	3.76±0.40ab
Onion	8	48.11±0.34b	4.15±0.39a	11.68±0.93a	40109.04±108.58a	3.99±0.62a
Additive ratio (%)						
0	6	47.65±0.11c	4.63±0.17a	10.29±0.42d	40217.34±22.80a	3.39±0.38b
1	6	48.16±0.41b	4.36±0.38b	11.10±0.71c	40158.72±67.08ab	3.62±0.27b
3	6	48.46±0.23a	4.12±0.16bc	11.78±0.50b	40091.95±54.66bc	3.81±0.25ab
5	6	48.68±0.32a	3.85±0.09c	12.66±0.32a	40037.57±91.32c	4.23±0.67a

[1]Means with the same letter within a column are not significantly different (p > 0.05).

The textural properties of the samples are shown in Table 2. The hardness of crackers was not significantly affected by incorporating the dill, parsley and green onion powders The partial replacement of wheat flour with dill, parsley and green onion powders led to a decrease in crackers' hardness and a decrease in crackers fracturability. The hardness and fracturability values, which were determined as 40217.34 g and 3.39 mm in the control cracker samples, changed at 40037.57 g and 4.23 mm, respectively, with the use of 5% dill, parsley and green onion powders. Similarly, Sadeghzadeh Benam et al. (2021) reported that the hardness values (4.70-3.85 N) of bread enriched with 5% and 15% purslane leaf powder decreased depending on the increase in the enrichment level.

CONCLUSION

The findings of this study showed that dill, parsley and green onion powders can be used in cracker production. The powder type affected the L*, Hue, diameter and fracturability properties. The use of dill powder led to more dark crackers and green onion powder to lower the Hue value of crackers. When cracker samples were compared in terms of textural properties, no significant difference was observed between the hardness values of dill, parsley and green onion powder added samples. Usage of dill, parslane and onion powders, L* and a* values were decreased in the samples. While the cracker samples with 3-5% powder added showed higher diameter and spread rate and breakability values than the others, the addition of dill, increasing parsley and onion powder made the cracker samples thinner and softer.

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BIOACTIVE COMPAUNDS IN COMMON MEDLAR FRUITS (*Mespilus germanica* L.) IN DEPENDANCE OF RIPENESS STATE

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ABSTRACT

Medlar (*Mespilus germanica* L.) is a plant rich in phytonutrients and antioxidants as bioactive compaunds (BAC). It has numerous medical benefits on human health, especially in detoxification and purification from accumulated toxins. Its strong genetic potential and the ability to grow without using pesticides and similar chemical preparations gives it an advantage over almost all other fruits that can be consumed in the autumn and early winter days. In total 15 samples were purchased from the markets and the trade network, laboratory samples were prepared from them and the following parameters were analyzed in the laboratory, such as total solid soluble content (TSS, °Brix), total acidity (TA, %), ascorbic acid (AscA, mg 100 g^{-1}), total phenolics (TPh, mg GA/g FW), total flavonoids (TFl, mg CE/g FW) and anthocyanins (Ant, mg·g^{-1} FW). The laboratory analyzes were carried out in two phases of medlar ripening, in phase 1 when the stone cells were 35-40% softened and in a second phase with 75-80% softening. With the ripening/softening of the fruits, the content of the investigated parameters TSS (28.93 phase 1to 30.52 ph2), the content of TPh (6.72 phase 1 to 8.01 phase 2), the content of Tl (3.11 phase 1 to 3.72 phase 2) and the content of anthocyanins (2.224 phase 1 to 2.76 phase 2) increases. Only the total acidity and the content of ascorbic acid decrease as the ripening of the fruits progresses (0.91 phase 1 to 0.78 phase 2; 44.51 phase 1 to 36.25 phase 2), respectively. Fluctuations in phytonutrient and antioxidant content with fruit ripening are expected and research indicates that fruit maturity should be above 70-75% to utilize the maximum potential of medlar fruit.

Keywords: Medlar, BAC, phytonutrients, antioxidants, ripeness.

INTRODUCTION

The food production and processing industry continuously develops through modeling and optimization new, renewed and adapted technological processes to adapt to new trends. But the most important role consists in incorporating biologically active components either in the form of whole plants or their organs (root, stem, leaf, fruit, seed). For this purpose, the focus is on fruit and vegetable plants that are "forgotten", are not intensively cultivated, have no commercial value in terms of generating income, but are extremely adapted to local conditions and do not need additional agro-ecological measures, especially not from using chemical plant protection products (PPPs).

The medlar (*Mespilus germanica* L.), fam.*Rosaceae*, is consumed exclusively in the stage of technical maturity, because of the stone cells, which are similar to the quince (Hacıseferogŭlları et al., 2005). Medlar fruits have a rich and diverse vitamin composition, minerals, but are especially abundant in components that have antioxidant (Kamal et al., 2015), anti-inflammatory, antimicrobial effects according to scientific studies (Ayaz et al., 2019; Xianfei et al., 2007) and even in Alzheimer's disease (Baptista et al., 2014). Recognizable by the softened fruit with a wrinkled surface, they are ready to be consumed fresh, which is the most valuable feature to take advantage of active enzyme complexes (Gruz et al., 2011) and

biologically active components (BAC) such as phenols (Manach et al., 2004), flavonoids, tannins, anthocyanins, organic acids, and vitamin C (Glew et al., 2003). The medlar is one of the climacteric fruits in which, with the ripening that can take place by leaving the fruits on a branch, the content of total soluble solids content increases, and the titratable acidity decreases (Gómez-Caravaca et al., 2013). The health benefits of medlar are known, especially its antibacterial and antiviral effect (Safari and Ahmady-Asbchin, 2019), but also antidiabetic (Donnelly and Boland 1995; Bahadoran et al., 2013; Vinayagam and Xu, 2015), although the studies were conducted in vitro, which is why food technology has a particular interest in using their BACs, adding them to other food products to increase their nutritional and general value (Kris-Ethertonet al., 2002).

Precisely because of its rich nutritional and bioactive profile, medlar was chosen as the subject of research. The obtained results and information have potential health benefits (Żołnierczyk et al., 2021), and for the food industry they mean the isolation of certain antioxidants that keep the products fresh and unchanged for a longer time, and have a natural origin and an advantage over other chemically synthesized ones (Sadeghinejad et al., 2022). In addition, we believe that the changing consumer mentality will include in the fall procurement for fresh consumption.

MATERIAL AND METHOD

Genetic origin of the material. The medlar trees from which the fruits were taken are old and indigenous to the locality where they have been grown for more than 20 years in four regions of Macedonia, namely v.Volkovo-Skopje (v.Vol), v.Radozda-Struga (v.Rdz), v.Ribarci-Kavadarci (v.Rib) and v.Gorni Disan-Negotino (v.GD).They are mainly individual trees that are cultivated in the backyards of the owners. According to their statements, the trees are not treated with chemical means of protection and are mainly resistant to diseases, so they can be declared as organic fruits.

Plant sample. The fruits are collected in two phases, namely the first phase when they are 30-40% ripe and in certain parts along the peel they begin to soften, and the second phase when they are 70-80% ripe and softened and are considered technologically ripe. Until the moment of laboratory analysis, they were kept at +4°C in the refrigerator, and on the day of the analysis, they were adapted for 3 hours at room temperature +24°C. The fruits were mashed with a manual press and the peel and pulp were analyzed.

Qualitative Analysis. The qualitative analyzes refer to the determination of the taste, the surface of the exocarp, the shape of the fruit and the content of the obtained pulp (g/100g). The taste was determined by involving 15 local locals (testers) and a scale of 4 tastes was created, namely 1-sour, 2-sour-sweet, 3-sweet-sour, and 4-sweet. The surface of the fruits, i.e., their peel, was ascertained through touch by the same 15 testers and was declared as rough and mostly smooth, as well as the shape of the fruit oval-round, round, and properly round. The fruit diameter (FD) was determined with a Digital Caliper (6 Inch/ 150mm Vernier Caliper Measuring Tool) (mm), and the fruit pulp content weight (PCW), with manual maceration, removal of seeds and weight measurement on a digital scale (Globe Scientific GBP-602 Series GBP Toploading Portable Precision Balance, 600g x 0.01g).

Phytochemical Analysis. The biologically active components in the pulp are essential for the nutritional and antioxidant profile of the medlar fruit. The potential parameters are analyzed as follows: the content of total soluble solid compounds (TSSC) (Brix°±SD) by using refraktometer, the titrable acidity (TA) (%±SD) measured by titration with 0.1 N NaOH to pH 8.1 expressed as a percentage of citric acid (g/L), total phenols (TPh) by the Folin-Ciocalteu colorimetric method expressed as mg gallic acid equivalents GAE mg$\cdot 100^{-1}$ FW±SD, total flavonoids (TFlav) determined as milligram quercetin equivalent mg QE$\cdot 100^{-1}$), total anthocyanins (TAnt) (mg$\cdot 100^{-1}$ FW±SD) and ascorbic acid (AscA) (mg$\cdot 100^{-1}$ FW±SD).

Determination of correlation. The Röemer-Orphal table was used to determine the correlation between the content of total phenols and the content of total flavonoids, titratable acidity, ascorbic acid and the total soluble solid compounds (nc-no correlation, vwc-very weak, wc-weak, mc-moderate, sc-strong, vsc-very strong, cc-complete).

RESULTS AND DISCUSSION

According to the examined quality parameters of medlar fruit, interesting and very useful results were obtained, especially in terms of the utilization of nutritional properties depending on maturity. In the first phase (Table 1), when the fruit is 30-40% ripe, the amount of pulp obtained, total soluble solid content and titration acidity were examined. At the same time, the largest amount of pulp was obtained from medlars from v.Rdz (34.28 $g \cdot 100^{-1}$ FW), and the least from those from v.GD (27.92 $g \cdot 100^{-1}$ FW). TSSCs are the most represented in medlars from v.Vol. (10.05 Brix°), and the least in fruits from v.Rib (10.05 Brix°), where TA is the highest (1.15%). The fruits of v.GD have the lowest TA (0.79%). In the second phase, when the fruits were technologically ripe (70-80% ripened and softened), the taste, exocarp surface, shape and diameter were determined. The taste varies from sour-sweet (v.Vol) through sweet-sour (v. Rdz) to sweet (v. Rib and v.GD). The surface of the exocarp is rough in the fruits from v.Rib to mostly smooth in those from v.Rdz and v.GD, where they have the smallest diameter of 27.8mm (v.GD), and the largest in those from v.Rdz (32.7mm) (Hacıseferogulları et al., 2005). Regarding the first stage, it can be concluded that the amount of fruit pulp increases with ripening (29.62 $g \cdot 100^{-1}$ FW in c.GD to 38.49 $g \cdot 100^{-1}$ FW in v.Rdz. Although the amount of TSSC is slightly higher in the second stage, but still noteworthy and varies from 11.6 Brix° (v.Rib) to 15.3 Brix° (v.Vol).As ripening, the acidity decreases significantly, so the TA is quite low in fruits from the GD region (0.57%), and the highest in those from the Vol region (0.81%), which is quite expected for fruits that have a specific way of ripening (lodge, quince, pear) and are called climacteric fruits.

Table 1. Qualitative characteristics of medlar fruits in the two stages of examination

Characteristics	Region (village, nearest town)			
	Phase 1st			
	Volkovo, Skopje	Radozda, Struga	Ribarci, Kavadarci	G.Disan, Negotino
Exocarp surface	Rough	Almost smooth	Rough	Almost smooth
Fruit shape	Oval-round	Properly round	Round	Round
FD (∅)	29.5	32.7	28.3	27.8
PCW (g/100g)	31.57	34.28	28.15	27.92
TSSC (Brix°)	14.16	12.79	10.05	11.12
TA (%)	1.15	0.95	0.82	0.79
	Phase 2nd			
Taste	Sour-sweet	Sweet	Sweet-sour	Sweet
PCW (g/100g)	33.92	38.49	30.15	29.62
TSSC (Brix°)	15.3	14.9	11.6	12.9
TA (%)	0.81	0.63	0.66	0.57

The importance of fruits is due to the amount and representation of BAC (Table 2) which determine the antioxidant (İlhami et al., 2011; Nabavi et al., 2011) and nutritional profile (Slavin and Lloyd, 2012) and even antimicrobial profile (Zheng et al., 2018).). Total phenolic compounds in fruit peel range from 28.85 (v.Rib) to 34.12 GAE $mg \cdot 100^{-1}$ FW (v.Rdz), which is significantly more than in fruit pulp from 23.17 (v.Voll) up to 28.64 $mg \cdot 100^{-1}$ FW (v.GD).

The total representation of phenols in medlar fruit is the lowest in the region of Vol (53.45), and the highest in GD (62.11). Total flavonoids in fruit peel and fruit pulp vary in a relatively wide range, and in fruit peel from 2.12 (v.Rib) to 2.59 mg QE·100^{-1} FW (v.Rdz), and in fruit pulp from 1.98 (v.Rib) to 2.69 mg QE·100^{-1} (v.GD) which is similar to phenolic compounds. The total content of flavonoids in medlar fruit is 4.10 mg (v.Rib) to 5.22 mg QE·100^{-1} FW (v.GD). In the research study, the total anthocyanins were determined, which were found the least in c.Vol (0.152 mg·100^{-1} FW), and the most in c.Rdz (0.691 mg·100^{-1} FW). And while anthocyanins are the highest represented in v.Rdz, ascorbic acid is the lowest (0.152 mg·100^{-1} FW), and in v.GD the content is the highest (0.188 mg·100^{-1} FW).

Table 2. Biological active compounds of medlar fruits in the two stages of examination

BAC		Region (village, nearest town)			
		Phase 1			
		Volkovo, Skopje	Radozda, Struga	Ribarci, Kavadarci	G.Disan, Negotino
TPh in	Peel	28.42	32.76	29.38	30.64
	Pulp	22.81	26.43	23.58	27.89
TFlav in	Peel	2.12	2.35	2.07	2.48
	Pulp	1.97	2.01	1.83	2.04
TPh peel+pulp, GAE mg/100 g		53.45	61.06	55.18	62.11
TFlav		4.67	4.97	4.10	5.22
Anthocyanines CE, mg/100 g		0.528	0.691	0.663	0.684
Ascorbic acid, mg/100 mL		0.173	0.152	0.166	0.188
		Phase 2			
TPh in	Peel	30.28	34.12	29.85	33.47
	Pulp	23.17	26.94	25.33	28.64
TFlav in	Peel	2.46	2.59	2.12	2.53
	Pulp	2.21	2.38	1.98	2.69
TPh peel+pulp, GAE mg/100 g		51.23	59.19	52.96	58.53
TFlav		4.09	4.36	3.90	4.52
Anthocyanines CE, mg/100 g		0.629	0.935	0.716	0.792
Ascorbic acid, mg/100 mL		0.146	0.138	0.105	0.116

In the second stage (Table 2) when the fruit is technologically mature, the content of total phenols in both fruit peel and fruit pulp is somewhat lower than in the first stage. It is the lowest in the fruits from the Vol region (28.42 GAE mg·100^{-1} FW – peel; 22.81 GAE mg·100^{-1} FW – pulp), and the highest in those from the Rdz region (32.76 GAE mg·100^{-1} FW) in fruit peel, that is, in GD (27.89 GAE mg·100^{-1} FW) in fruit pulp. The average total representation of phenols ranges from 51.23 GAE mg·100^{-1} FW (v.Vol) to 59.19 GAE mg·100^{-1} FW (v.Rdz). Flavonoid compounds are less represented in fruit peel (2.07 mg QE·100^{-1} FW, v.Rib to 2.48 mg QE·100^{-1} FW, v.GD), while in fruit pulp the minimum content is higher in the second phase 2.04 mg QE·100^{-1} FW (c.GD) although insignificant, and lower the maximum 1.83 mg QE·100^{-1} FW (v.Rib). The total average representation of flavonoids varies from 3.90 mg QE·100^{-1} FW (v.Rib) to 4.52 mg QE·100^{-1} FW (v.GD) which means that it is lower in the stage of technological maturity. Anthocyanins are significantly more represented in the second phase and amount to 0.629 mg·100^{-1} FW (v.Vol) to 0.935 mg·100^{-1} FW (v.Rdz). The content of vitamin C is determined through the concentration of ascorbic acid, which is maximally

represented in 0.146 mg·100^{-1} FW in the fruits of v.Vol, and minimally (0.105 mg·100^{-1} FW) in those of v.Rib.

Greater variations (Figures 1 and 2) between total phenols and total flavonoids were found in relation to the representation in fruit pulp in the first phase, and almost insignificantly less in fruit peel in the second phase, where the variations in relation to the content of phenols and flavonoids. The variation in the average total content of these BACs is almost twice as small, unlike the others where the variations are much larger and more obvious on the graph for anthocyanins and less noticeable for ascorbic acid (Figure 1 and 2).

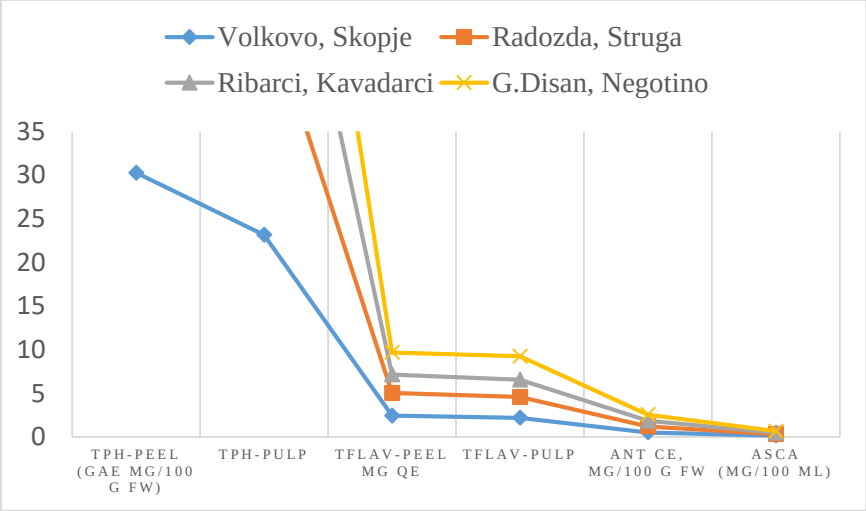

Figure 1. Variation in BAC level during investigation in 1st phase of maturity

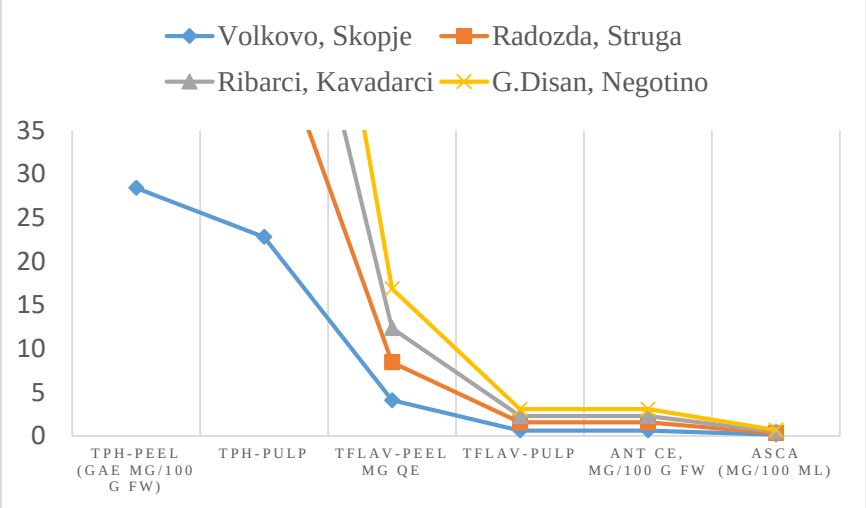

Figure 2. Variation in BAC level during investigation in 2nd phase of maturity

Such trends and the highly variable content of total phenols and flavonoids are also reflected in the correlative dependence between them and the rest of the examined BACs (Table 3). In the first phase of the research, a complete (absolute) correlation was found between total phenols and total flavonoids (0.902), a strong correlation between phenols and anthocyanins (0.763), a negative weak correlation with TSSC (-0.24) and no correlation with pso ascorbic

acid (-0.096). Total flavonoids were weakly correlated with anthocyanins (0.205), ascorbic acid (0.266) and TSSC (0.28). Anthocyanins have a strong negative correlation with TSSC (-0.73), and a very weak negative correlation with ascorbic acid (-0.117). A very weak negative correlation (-0.19) was found between TSSC and ascorbic acid. In the stage of technological maturity (second stage), total phenols are in very strong correlation with total flavonoids (0.802), strong correlation with TSSC (0.76), negative weak with ascorbic acid and independent of anthocyanin content. A weak correlation was found between total flavonoids and the content of anthocyanins, ascorbic acid and TSSC, respectively (0.208; 0.326; 0.41). The content of synthesized anthocyanins depends almost completely (0.93) on TSSC and not on ascorbic acid (0.020), while between TSSC and ascorbic acid there is a very weak negative correlation (-0.15).

As a characteristic fruit that shows climacteric ripening, the concentration of the quality properties and BAC content changes (Graph 1 and 2). As can be seen from Tables 1 and 2, with the ripening and softening of the fruits, the TSSC content increases (10.05-14.16 in the first stage; 11.6-15.3 in the second stage), and the titration acidity significantly decreases (0.79-1.15 in the first stage; 0.57-0.82 in the second stage) which coincides with the research of Selcuk and Erkan, 2015. Taking into account that medlar can suddenly soften and fail the fruit for consumption, it is recommended that at a stage like the second of this study when the fruits are 70-80% softened, to avoid a significant reduction in total phenolics and flavonoids. But the handling of these fruits should be careful to avoid mechanical injury (Zheng et al., 2018). With ripening and softening, the concentration of ascorbic acid also decreases significantly (0.152-0.188 in the first stage; 0.105-0.146 in the second stage). An increase in the concentration of anthocyanins is observed only with ripening (0.528-0.691 in the first phase; 0.629-0.935 in the second phase).

Table 3. Correlation coefficient between certain traits at pomegranate landraces

Traits	TFlav	AC	AscA	TSSC
	Phase 1st			
TPh	0.902cc	0.763sc	0.096nc	-0.24wc
TFlav	-	0.205wc	0.266wc	0.28wc
AC	-	-	-0.117vwc	-0.73sc
AscA	-	-	-	-0.19vwc
	Phase 2			
	TFlav	AC	AscA	TSSC
TPh	0.802vsc	0.045mc	-0.290wc	0.761sc
TFlav	-	0.208wc	0.326wc	0.413mc
AC	-	-	0.020nc	0.932cc
AscA	-	-	-	-0.154vwc

Conducted research indicates the fact that the polyphenolic and flavonoid profile of medlar fruits are higher in fruit peel compared to macerated fruit pulp, which coincides with previous research on medlar and quince (Żołnierczyk et al., 2023). The content of phenolic compounds is a genetic trait (Ayaz et al., 2008) and a stable trait that should be used in pre-selection and selection purposes and creation of new medlar genotypes (Ayaz et al., 2008). As the fruits ripen, the concentration of phenolic and flavonoid components decreases, which is why it is recommended to consume the fruits already at 70-80% ripeness, especially due to the fact that the reduced content is not drastic or much lower and the benefits of these compounds can be used (et al, 2002; Ayaz Dincer et al., 2002). Although ripening is recommended at

temperatures between 20-25°C, taking into account the genetic constitutional characteristic of the local populations, it can be concluded that the content of total phenols and flavonoids decreases minimally, hence it does not depend much on the temperature regime as in other fruits (Ayaz et al., 2008).

CONCLUSIONS

The examined genotypes differ among themselves in terms of qualitative properties and the content of biologically active components. With the ripening of medlar fruits, the content of total solid soluble content increases, and the acidity decreases. The medlar fruits originating from the region of Negotino, v.Gorni Disan village contain the most total phenols, total flavonoids and ascorbic acid, but also the fruits originating from the v.Radozda village have a high content of the examined parameters. As the medlar fruit ripens and softens, the concentration of total phenols, total flavonoids, and ascorbic acid decreases, so it is recommended to consume them at 70-80% ripeness. The indisputable quality and high content of biologically active components count medlar high on the list of fruits with health benefits.

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CHIA SEEDS (*Salvia hispanica* L.) IN DIETETIC REGIMES

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ABSTRACT

Chia (*Salvia hispanica* L.) is a very often used plant using seed material in the preparation of dietary meals. Apart from being rich in minerals potassium, calcium, magnesium, and iron, it has a favorable nutritional composition along with a wealth of dietary fibers, it is a prebiotic for GUT bacteria which increases the absorption of nutrients and maintains good health. Ten average seed samples of organically produced commercial cultivars (CV1-10) were used in the study obtained from local health food stores. Of the parameters determined by laboratory analyses, the focus is on the content of total carbohydrates, proteins, fats, and fibers. The average content of total carbohydrates is 40.58 g/100 g, and of total fat 32.89 g/100 g. The average protein content indicates the fact that chia meals are high in protein with an average protein content of 18.6 g/100 g. The fact that fibers are on average represented by 38.36 g/100 g is particularly important, which confirms the conclusion that the breakfast meal in the form of chia meals prepares the body for a good start of the day from an energetic, protein-structural, and prebiotic supported aspect.

Keywords: Chia seeds, Carbohydrates, Proteins, Fat, Fibers, Seeds, Dietetic.

INTRODUCTION

Today, chia, originating from southern Mexico and Guatemala, receives maximum attention from nutritionists, and it is increasingly common in daily meals in combination with other ingredients (milk, soy, nuts, fruit) (Ding et al., 2017; Breeson, 2009). Given its macro-thermal character and the difficulty of obtaining seeds, it complicates the production of local and indigenous genotypes, for which the commercial production of hybrid genotypes is part of selection programs that are very profitable especially due to the high demand in the market (Ayerza and Coates, 2009; Jambunsri et al., 2012). A major tool in the development of new genotypes is recombinant DNA technology focused on creating early-flowering genotypes (de Falco et al., 2017). Research studies point to the protective effect of chia seeds on the cardiovascular system, support in diabetes, reduction of metabolic syndrome and improvement of lifestyle (Peiretti and Gai, 2009; Bueno et al., 2010; Ayerza and Coates, 2011; Vedtofte et al., 2011; Martha et al., 2012). Chia seeds contain a very high content of saturated and unsaturated fatty acids, but also a representative amino acid composition. Black coloration of the seeds usually dominates, but white colored seeds are also found significantly (Ayerza 2013). In terms of biologically active components, chia shows similarities with sage seeds, primarily flavonoids, quercetin, genistein, caffeoyl derivatives and caffeic, chlorogenic and rosmarinic acids (Coelho et al., 2014; Mohd et al., 2012).

The rich fatty acid profile in chia seeds is increasingly being used in food technology, primarily as an additive and improver of the nutritional quality and composition of products and processing of animal origin (Porras-Loaiza et al., 2014; Scapin et al., 2015) with the so-called омега x-3 fatty acids, namely alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Of the oils of vegetable origin, the richest in ALA are those from flaxseed, canola, and soybean, while fish, fish products and seafood contain DHA and

EPA in significant quantities. The value of chia seeds is growing as a result of the exceptionally high content of vegetable fibers that means that they have a high water-holding capacity (Capitani et al., 2012). Chia mainly contains insoluble fibers, and very few soluble fibers (Ding et al., 2018), which is why they are widely used and added to the bakery and confectionery industry, as well as in meat processing (Sudha et al., 2007).

Considering that chia seeds contain bioactive components that directly affect health positively, they do not contain gluten and chia plants can be grown in arid areas (Bochicchio, 2015), the objective was challenging to investigate the quality of commercial seeds in the state. Hence, the recommendation from this research study is that chia seeds should be grown in the country, their health benefits should be used and it should be included in prepared meals daily.

MATERIAL AND METHOD

Origin of the material. The chia seed material was purchased from the commercial network, from 10 different organic food stores, spread over the territory of the city of Skopje. All ten commercial varieties (CV1-10) originate from imports and are available to citizens.

Plant sample. Purchased commercial varieties (CV1-10) chia seeds were dried at room temperature 20-22C for 48 hours. Then each CV is ground on a small laboratory mill, sifted through sieves ∅ 0.5mm, ∅ 0.2mm and ∅ 0.1mm and an average laboratory sample is prepared for analysis on an automatic laboratory separator. For the laboratory analyses, an extract was prepared in which the content of carbohydrates, crude proteins, crude fats and crude fibers was determined.

Qualitative Analysis. Total carbohydrates were determined with a refractometer and determining the refractive index (°Brix). The total protein content was determined by using the Kjeldahl method, and this method actually determines the total nitrogen content (N x 6.25 coeff.) and after is used to calculate the protein content as $g \cdot 100^{-1}$ DW±Sx (Varelis, 2016). The crude fat content was extracted by Soxhlet extraction ($g \cdot 100^{-1}$ DW±Sx. The determination of crude fibers was performed according to the Kürschner-Hanak method ($g \cdot 100^{-1}$ DW±Sx), therefore the moisiture content was measured by drying method for plants, and the plant material is dried 105 ± 2°C to constant weight (% DW±Sx).

Phytochemical Analysis. The quality of chia commercial varieties is complemented by the analysis of biologically active components (BAC), total phenols (TPh) and total flavonoids (TFlav), and the ratio between them TPh:TFlav is also determined. The total phenols (TPh) are quantified by the Folin-Ciocalteu colorimetric method expressed as mg gallic acid equivalents GAE $mg \cdot 100^{-1}$ DW±Sx, while total flavonoids (TFlav) arev determined as milligram quercetin equivalent mg $QE \cdot 100^{-1}$±Sx).

Determination of correlation. To determine the correlation dependence between certain parameters is used the Röemer-Orphal table. The correlation between the following parameters was examined: hygroscopic moisture (HM) and the content of carbohydrates (CH), crude proteins (CP), crude fat (CF), crude fibers (CFb); CH and CP, CH and CF, CH and CFb; CP and CF, CP and CFb. Correlative dependence is evaluated according to the above table as follows nc-no correlation, vwc-very weak, wc-weak, mc-moderate, sc-strong, vsc-very strong and cc-complete.

RESULTS AND DISCUSSION

The research study was conducted on commercially available varieties (CV1-10) of chia seeds that are available to consumers over the counter. The hygroscopic moisture, which is of particular importance for proper preservation and storage of the seed, is within the legally permissible limits, i.e. it does not exceed 16%, when the biochemical processes in the seed would be activated, reducing the amount of endosperm due to the respiration of the seed and its consumption. This would lead to declassified quality and reduced nutritional aspect. At the

same time, the average hygroscopic moisture found is 12.75%±0.06 (Table 1), and it varies from 11.6 - 13.67% (Figure 1) and a difference of 2.04% was found between CV1-10, which is also the smallest difference for the examined properties.

Chia meals are usually prepared at the beginning of the day or during a part of the day when there is an energy drop, because it contains polysaccharides whose degradation is gradual and provides a solid energy platform for the body's needs (Ixtaina, 2011). The average representation is high and amounts to 40.58 g·100^{-1} DW±0.05 (Table 1). The variation between the commercial varieties (CV1-10) ranges from 38.29 – 43.28 g·100^{-1} DW (Figure 2) with an evident highest difference between them of 4.99 which coincides with the results of Ayerza (2009).

Chia seeds have added value due to their high protein content, which averages 18.6 g·100^{-1} DW ±0.07 (Table 1), and the differences between genotypes is acceptable (3.56) given the different supply of traders and imports from abroad and varies within relatively narrow limits. 16.86 – 20.42 g·100^{-1} DW (Figure 3). The fatty acid profile in chia seeds is dominated by saturated fatty acids and the presence of omega x-3 fatty acids ALA, EPA and DHA, which belong to polyunsaturated fatty acids (PUFA) and are very important for lipid metabolism, the physiological functioning of the body and the dietary regime (Garg, 2006; Piretti and Gai, 2009). In CV1-10 fats are represented on average 32.89 g·100^{-1} DW ±0.04 (Table 1), with a rather high variation (4.56) from 30.69 to 36.25 g·100^{-1} DW (Figure 4).

Table 1. Qualitative characteristics of medlar fruits in the two stages of examination

Seed traits	**Moisture**	**Carbohydrates**	**Crude proteins**	**Crude fatts**	**Crude fiber**
CV1	12.57	41.37	20,42	33,18	37,09
CV2	13.25	38.75	18,91	31,47	39,24
CV3	13.67	42.28	19,32	30,69	40,14
CV4	12.79	42.42	16,86	32,51	38,07
CV5	11.63	39.57	17,34	31,77	37,16
CV6	12.09	38.29	20,03	34,18	38,21
CV7	13.42	42.16	18,41	32,29	39,24
CV8	12.89	43.28	17,17	33,37	39,17
CV9	13.53	41.09	17,62	33,79	37,79
CV10	11.68	38.46	18,22	35,25	37,22
x±Sx*	**12.75±0.75**	**40.58±12.95**	**18.6±5.96**	**32.89±10.49**	**38.36±12.18**

* Data are given as mean±standard error of the mean (n=3)

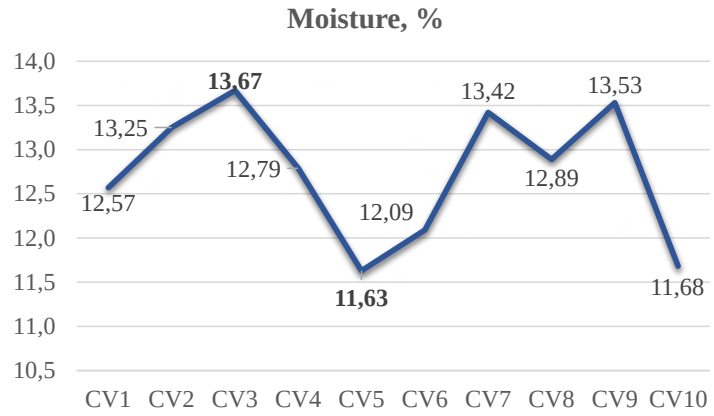

Figure 1. Variation in moisture content at investigated chia seed commercial varieties (CV1-10), %/DW

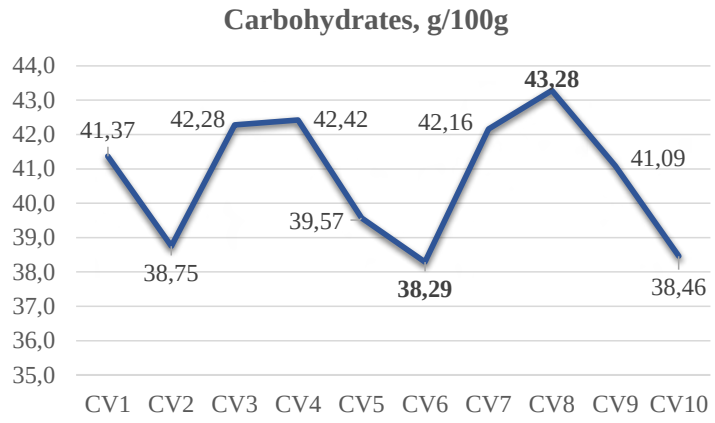

Figure 2. Variation in carbohydrate content at investigated chia seed commercial varieties (CV1-10), g·100^{-1} DW

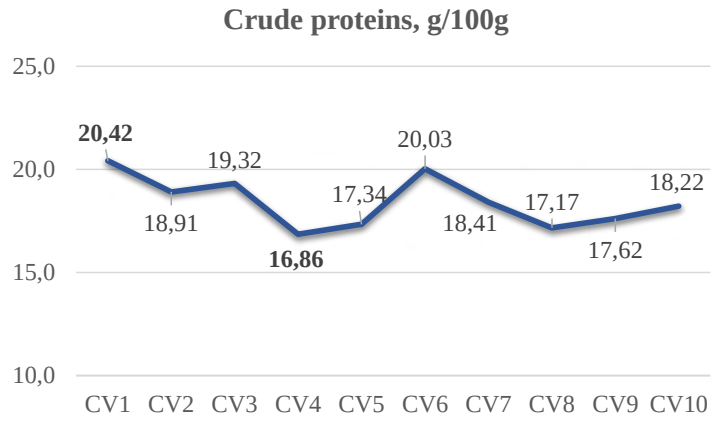

Figure 3. Variation in crude protein content at investigated chia seed commercial varieties (CV1-10), g·100^{-1} DW

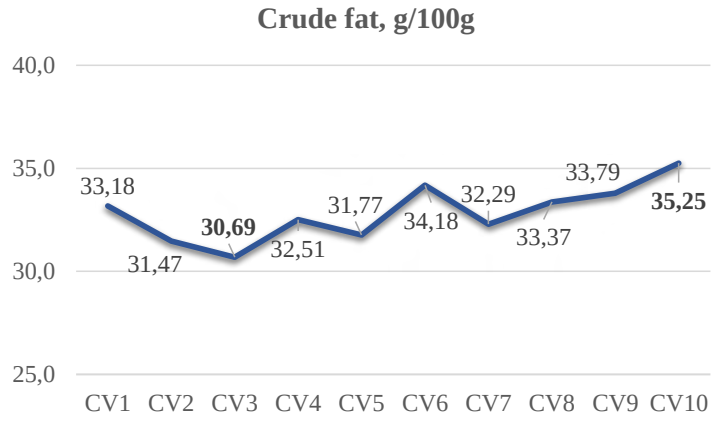

Figure 4. Variation in crude fat content at investigated chia seed commercial varieties (CV1-10), g·100^{-1} DW

Plant fibers present in chia seeds have a special significance for dietary regimes, whether it is for a healthy organism or with certain disorders/diseases of the cardiovascular system,

diabetes, metabolic syndrome or simply improving and managing one's own health by cultivating a correct lifestyle (Meineri and Peiretti, 2007). More than 80% are insoluble fibers, and according to some studies even more (Lairon et al., 2005), which serve as a prebiotic for probiotics in the GIT and maintain a rich intestinal microflora (Simopoulos, 2002). At the same time, it means immune support of the body, maintaining a stable level of glycemia and satiety for more than several hours (up to 5), which is why nutritionists must include chia in the daily menu (Marineli et al., 2015). The average content of crude fibers in CV1-10 is 38.36 g·100^{-1} DW ±0.03 (Table 1), and the variations between varieties range from 30.69 to 35.25 g·100^{-1} DW with a mutual difference of 3.05 (Figure 5).

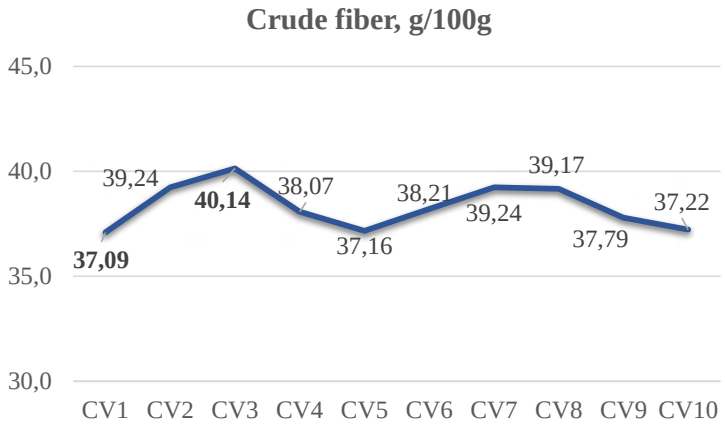

Figure 5. Variation in crude fiber content at investigated chia seed commercial varieties (CV1-10), g·100^{-1} DW

Between hygroscopic moisture (HM) and total carbohydrates (CH) as well as between HM and crude fibers (CFb) a strong correlation was found, respectively (0.61; 0.73), and between HM and CFb it was negative moderate (-0.49). Correlative dependence between CH and CFb is moderate (0.46). A weak negative dependence was found between CH and CP (-0.22), between CH and CF (-0.32), while between HM and CP (0.04), CP and CFb (0.02) and CP and CF (-0.04) have no correlative dependence, which indicates the fact that these properties (content of proteins, carbohydrates, fibers) are mainly genetically determined (Mohd et al., 2012) (Table 2).

Table 2. Correlation coefficient between certain traits at chia varieties

Traits	**CH**	**CP**	**CF**	**CFb**
M	0.61sc	-0.04nc	-0.49mc	0.73sc
CH	-	-0.22wc	-0.32wc	0.46mc
CP	-	-	-0.04nc	0.02nc

Biologically active components that show an antioxidant effect and affect the physiological state of cells by fighting free radicals such as total phenols (TPx) and total flavonoids (TFlav) are represented in high concentration in chia seeds and it is to them that the numerous health benefits are due (de Falco et al., 2017) in a continuous process of use and consumption. The average content of TPh is 228.17 GAE mg·100^{-1} DW±0.06, and it varies in wide ranges from 37.09 – 40.14 GAE mg·100^{-1} DW with a difference of 43.17. Total flavonoids (TFlav) varied within narrower limits of 158.29 – 188.59 QE mg·100^{-1} DW, a difference of 30.30 and an average concentration in CV1-10 of 179.38 QE mg·100^{-1} DW (Figure 6). By

determining the ratio between the content of total phenols and total flavonoids there is not always a linear relationship that indicates the antioxidant potential (Monroy-Torres et al., 2008). However, the ascertained high concentrations of TPH and TFlav serve to predict established synergistic relationships between these bioactive components that favor greater antioxidant activity. In the researched varieties, it is 1.28±0.08 with variations from 1.07 - 1.43 (Figure 7).

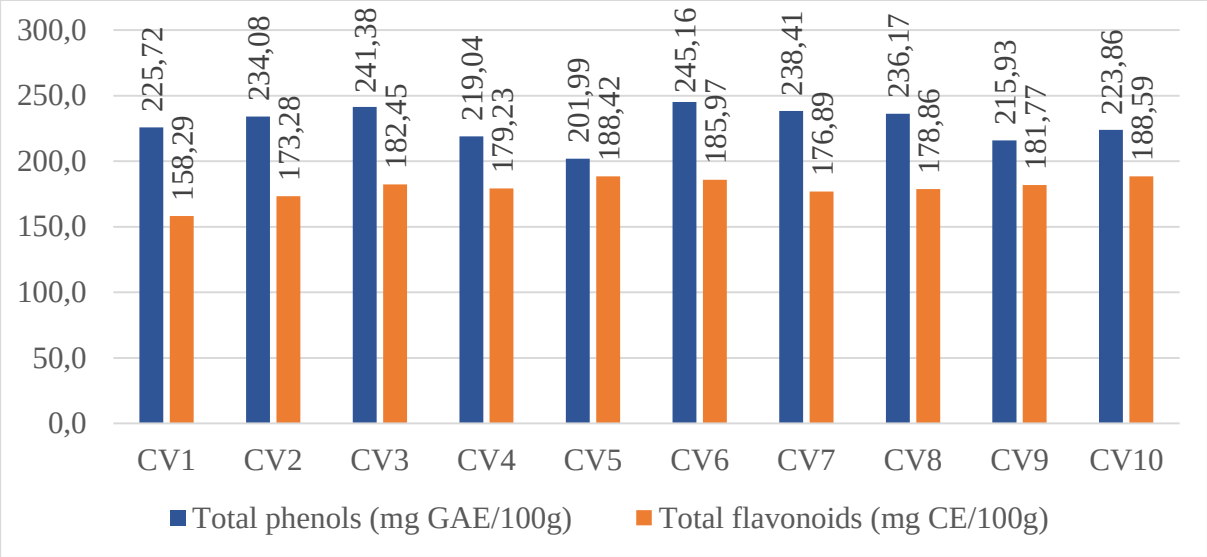

Figure 6. Content of total phenols and total flavonoids as biological active compounds in chia seeds (CV1-10)

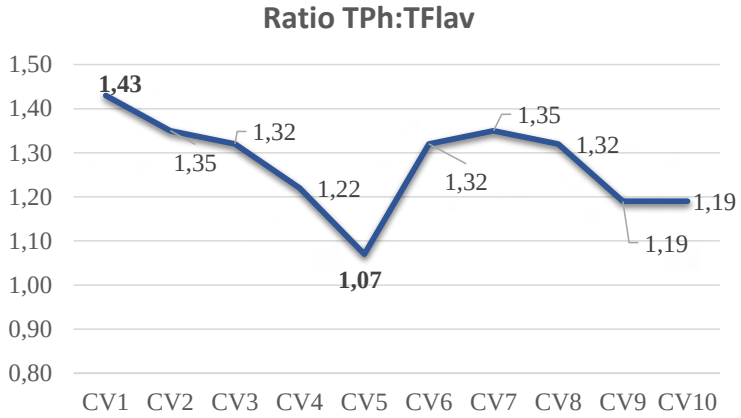

Figure 7. Ratio of total phenol (TPh) content and total flavonoids (TFlav), (CV1-10)

CONCLUSIONS

The research study was conducted in 10 commercial varieties (CV1-10) of chia seeds. The content of total carbohydrates (38.29 - 43.28 g·100^{-1} DW, average 40.58 g·100^{-1} DW, CV1-10), crude protein (16.86 - 20.42 g·100^{-1} DW, average 18.6 g·100^{-1} DW, CV1-10), crude fat (30.69 - 35.2 g·100^{-1} DW 5, average 32.89 g·100^{-1} DW, CV1-10) and crude fiber (37.09 - 40.14 g·100^{-1} DW, average 38.36 g·100^{-1} DW, CV1-10). The high content of total phenols (201.99 - 188.59 GAE mg·100^{-1} DW, average 228.17, CV1-10) and total flavonoids (158.29 - 188.59 QE mg·100^{-1} DW, average 179.38, CV1-10) as biologically active components make chia a superior food due to the fact that these components have an antioxidant effect and they improve and maintain the physiological state of the cells, and have numerous health benefits. For those

reasons, chia seeds are part of the daily menu prepared by nutritionists and accepted en masse by the healthy population that takes care of immune support for health and well-being, as well as by the population with certain diseases (cardiovascular, diabetes, anemia and metabolic syndrome.

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STUDY OF BIOFILM FORMATION IN *Pseudomonas Savastanoi* BY MICROTITER PLATE

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ABSTRACT

Olive tuberculosis is a disease caused by *Pseudomonas savastanoi*. This bacterium often coexists with non-pathogenic bacterial species, forming multispecific biofilms responsible for nodule formation. The development of this biofilm leads to the synthesis of higher levels of 3-indoleacetic acid (IAA), which can lead to a significant increase in nodule size and disease progression. The aim of this research was therefore to evaluate the formation of *P. savastanoi* B97 biofilm over a period of 6 days using the microtiter plate (MTP) method. The results obtained were classified by comparing the optical densities of the bacteria studied and those of the control. The optical density measured reflects the intensity of the biofilm attached to the walls of the microtiter plate. Our results showed that *P. savastanoi* B97 is capable to forming biofilms and that this ability increases with the incubation time ($0.086 < OD_{630nm} < 0.107$). Understanding the mechanisms of biofilm production by *P. savastanoi* B97, together with establishing the kinetics of biofilm formation on different parts of the olive tree, could help us to prevent and control the infection.

Keywords: Biofilm formation, *Pseudomonas savastanoi*, Olive tuberculosis, Microtiter Plate.

INTRODUCTION

Olive knot is a bacterial disease caused by *Pseudomonas savastanoi*. It is characterized by the appearance of nodules on the branches, leaves and fruits of the olive tree (Sisto et al., 2004). These nodules can vary in size from a few millimeters to several centimeters. Olive knot can cause significant damage to olive trees, up to and including the death of the tree (Godena et al., 2012). The nodules can block the flow of sap in the tree, leading to weakening of the tree and a decrease in its production (Lamichhane et al., 2014). The nodules can also facilitate the entry of other diseases and pests, which can aggravate the damage.

Olive scald is caused by a multi-species biofilm of *P. savastanoi* and other bacteria (Buonaurio et al., 2015). This biofilm, which is a community of bacteria living together, is responsible for the formation of nodules on the branches, twigs and trunk of the olive tree. These nodules are caused by indole-3-acetic acid (IAA), a substance produced by the bacteria in the biofilm (Smidt, M., and Kosuge, 1978; Surico et al., 1985). Indole-3-acetic acid stimulates plant cell growth and causes the formation of nodules. The progression of the disease is characterized by an increase in the number and size of the nodules, which can lead to a weakening of the olive tree and a decrease in its production.

The aim of this study was to quantify the biofilm formation of *Pseudomonas savastanoi* over a period of 6 days. We used the microtiter plate method, which allows us to measure the amount of biofilm formed on a solid surface.

MATERIAL AND METHOD

The bacterial strain used in this study was *P. savastanoi* B97, purchased from the Moroccan Coordinated Collections of Microorganisms (CCMM) of the National Centre for

Scientific and Technical Research (CNRST) in Morocco and isolated from the Beni mellal-Morocco region at 28 °C ± 2 for 24 h.

Stepanovic's method was used to grow biofilms in microtiter plates (Stepanović et al., 2007). The tests are performed in 96-well, flat-bottomed polystyrene microtiter plates. The concentration of the bacterial suspension is then measured at a wavelength of 630 nm and adjusted to 0.7-0.8. Under aseptic conditions, 200 µl of the adjusted bacterial suspension is added to each well. Plates are incubated at 28 °C. After 24 hours, the wells were rinsed three times with sterile water to remove non-adherent bacteria. The adherent biomass was exposed to 80 °C for 30 minutes to fix it. Biofilm formation was evaluated every 24 hours for 6 days. The negative control consists of wells filled only with liquid LB medium without bacterial cells.

The attached biomass was stained with 5% crystal violet for 5 minutes per well. Excess dye was removed by rinsing with tap water. The crystal violet bound to the biomass was then dissolved in an ethanol/acetone solution (80/20, v/v) at a rate of 200 µl per well. The concentration of bound biomass was determined by measuring the absorbance (OD) of the resulting solutions at 630 nm using a microplate reader. The amount of biomass bound to the microplate wells is proportional to the OD of the resulting solutions. The experiment was repeated three times to assess the reliability of the results.

Biofilm production was classified into four categories, based on the cutoff value, negative, weak, moderate, and strong, calculated according to the following formula (Folliero et al. 2021):

ODcutoff = Average OD of the negative control + (3 × standard deviation of the ODs of the three repetitions of the negative control).

The following criteria were used:

- $OD_{exp} \leq OD_{cut\ off}$ = Non biofilm former
- $OD_{cut\ off} < OD_{exp} \leq 2 \times$ ODcutoff = Weak biofilm former
- $2 \times OD_{cut\ off} < OD_{exp} \leq 4 \times$ ODcutoff = Moderate biofilm former
- $OD_{exp} > 4 \times OD_{cut\ off}$ = Strong biofilm former

RESULTS AND DISCUSSION

The figure shows the quantification of *P. savastanoi* B97 biofilm formation over 6 days. The absorbance of crystal violet is an indicator of biofilm strength. Biomass attached to the microtiter wells is stained with crystal violet as described in the Methods section. The crystal violet contained in the cells is extracted by dissolving in an ethanol/acetone mixture. The amount of biomass adhering to the well walls can be estimated from the absorbance of the mixture at 630 nm. Based on the Stepanovic classification, which refers to a comparison between Doc and DO_{test}, it is possible to describe biofilm formation as strong, moderate, weak or absent (Stepanović et al., 2007).

The *P. savastanoi* B97 strain is capable of forming a biofilm after 3 days of incubation. The intensity of the biofilm gradually increases with time and becomes strong after 6 days. The bacterium is able to strongly adhere to the wells, which facilitates biofilm formation. The colonization and kinetics of biofilm formation depend primarily on the surface properties of the bacterium and the walls of the microtiter plates.

The measured density of crystal violet is an indicator of the amount of biomass attached to the wall, which is composed of cells and extracellular matrix. Crystal violet is dissolved in ethanol/acetone and added to microtiter plate wells. A high optical density of the mixture indicates a large number of cells attached to the wells. The literature indicates that few studies have been conducted on *P. savastanoi* biofilms. In 2019, Moretti et al., characterized the formation of *P. savastanoi* biofilms using crystal violet staining. He observed that biofilm formation did not occur until the second day of incubation. These results contrast with our own observations, which show that biofilm formation begins on the first day of incubation. However,

P. savastanoi has been shown to form mutualistic biofilms when infected with the biofilm-forming bacteria *Pantoea agglomerans* and *Erwinia toletana*.

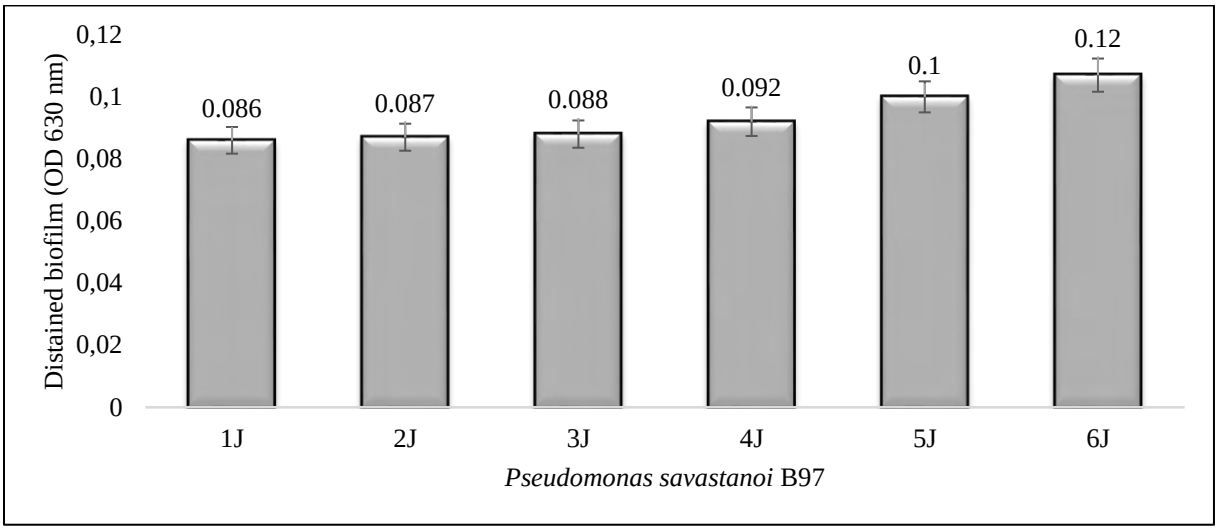

Figure 1. Quantification and following of biofilm formation *P. savastanoi* B97 strain using microtiter plate method

CONCLUSIONS

The microtiter plate assay showed that the strain *P. savastanoi* B97 is able to form strong biofilms from day one. The intensity of the biofilm increased with time. The optimized assay is an effective tool to evaluate the biofilm forming ability of *P. savastanoi* strains. This information is important for controlling the virulence of this bacterium.

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EVALUATION OF SACCHAROMYCES AND NON-SACCHAROMYCES YEASTS ISOLATED FROM ALBANIAN AUTOCHTHONOUS GRAPE VARIETIES FOR CRAFT BEER PRODUCTION

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ABSTRACT

In recent years, researchers have been working to create and expand the pool of yeasts for the brewery sector including the use of new strains isolated from non-brewing environments. In this context, the non-*Saccharomyces* and *Saccharomyces* yeasts use has attracted great interest from both researchers and commercial brewers for the production of novel beer styles. Recent research has shown that enzymatic activities of these non-conventional yeasts contribute during the fermentation process to the production of esters and higher alcohols that define the sensory characteristics of beer.

Therefore, the selection and use of new yeast strains with peculiar metabolic properties could represent the key point in differentiating products in the brewery sector, especially for local producers of craft beer.

Our study aims to evaluate some preliminaries fermentative characteristics of *Saccharomyces* and non-*Saccharomyces* strains isolated from the Albanian autochthonous grape varieties in the craft beer production. Specifically, the tested yeasts were isolated from the autochthonous grape varieties Kallmet and Shesh i Zi, collected from the Valias and Shkodra regions in Albania. Isolation was performed using WL as culture medium, followed by laboratory screening to assess the fermentation characteristics of the isolated yeasts. Our results showed that *Saccharomyces cerevisiae* SHZV3 strain has excellent aptitudes to be used as a new starter in craft beer production as it shows low levels of hydrogen sulphide (H_2S) production and also negative results in production of biogenic amines, conversely with other strains, which exhibited undesirable production of hydrogen sulphide (H_2S) and biogenic amines, rendering them unsuitable for beer production.

Keywords: *Saccharomyces cerevisiae, craft beer, fermentation characteristics, hydrogen sulphide, biogenic amines.*

INTRODUCION

The development of viticulture and oenology in Albania dates back to ancient times, due to the soil and favourable climatic conditions (Ruci et al ,2022). In the traditional context, grapes and wine have conventionally been employed for yeast isolation, primarily intended for application in the wine industry, with no consideration for their utilization in craft beer production. Many physiological parameters allow Saccharomyces to dominate grape juice fermentations, but its tolerance to high concentrations of ethanol is the principal feature of this yeast that allows its survival in this specific environment (Śtefânikovà et al, 2014). Non- Saccharomyces are the predominant yeasts species isolated at early stages of the spontaneous fermentation of Vitis vinifera L. Grape musts (Combina et al, 2005). As the fermentation progresses, the population of non-*Saccharomyces* species decreases and the wine yeasts *Saccharomyces cerevisiae* completes the fermentation process. The ability of *S. cerevisiae* to outcompete non-*Saccharomyces* species is associated with its higher fermentative power as well as its additional advantageous phenotypes that include alcohol tolerance and the secretion of killer-like compounds (Albergaria and Arneborg et al, 2016).

While many yeast strains are commercially available, the availability of new starter strains could be a useful differentiating factor among beers (Rossi et al, 2018). The aim of this study was to investigate yeast strains for their brewing ability and to test the promising strains in small brewing trials (Hutzler et al, 2019). For this reason, yeast isolated from grape must and finished fermented wine were tested in screening laboratory in order to discern new yeast strain possessing elevated abilities as a starter for beer production.

MATERIALS AND METHODS

Yeasts isolation

To carry out this study, two autochthonous varieties of black grapes (Vitis vinifera L.) were chosen such as: Shesh i Zi, from the area Valias in Tirana and Kallmet from the area of Kolplik in Shkodra. The samples, was harvest in optimal ripping and were transported to the Research Centre of the Faculty of Biotechnology and Food. Fermentation was carried out spontaneously in small vessels without adding Kalium Metabisulfite.

The progress of fermentation was checked daily (% sugar and temperature). Samples were taken every day during the fermentation. Yeast isolation was carried out in WL nutrient agar (Thermo Fisher Scientific, Waltham, MA, USA). Appropriate serial dilutions of each sample were made using a physiological saline solution (0.9% *w*/*v* NaCl). Subsequently, 0.1 mL of each dilution was distributed on Petri plates containing WL nutrient agar and incubated at 28º C for 1-3 days. After the incubation, 5 colonies from each sample were picked on the basis of typical *Saccharomyces* and non- *Saccharomyces* morphology. Purification of the strains was performed on the YEPD agar (Thermo Fisher Scientific, Waltham, MA, USA) by successive

subculturing. Stock cultures were maintained in YEPD broth (Thermo Fisher Scientific, Waltham, MA, USA) for daily use and were preserved in YEPD broth with 40% (v/v) sterile glycerol (Merck KGaA, Darmstadt, Germany) for long-term storage at −80 ◦C until further characterization.

Screening of Isolated Yeasts Strains

Morphological Yeast Features Evaluation

Evaluation of the cellular Morphological of the yeast was done by using optical Microscope (Zeiss Axiopoth, Carl Zeiss, Germany).

Pulcherrimin production.

Pulcherrimin production was evaluated according to method described by Pawlikowska et al 2020 using a specific culture media. Plates were incubated at 12°C and 28°C for 3-5 days. Positive result was given by the formation of a red pigment produced by the yeasts tested on the surface of the culture media.

Antimicrobial Activity.

Antimicrobial Activity was evaluated by Agar Well Diffusion method using BHI Agar medium (Brain Heart Infusion) as described by Iorizzo et al 2020 with some modification. The test was performed against beer spoilage bacteria, which: *Pediococcus damnosus* DSM 20289, *Levilactobacillus brevis* DSM 6235, *Lactiplantibacillus plantarum* DSM 20174 and *Fructilactobacillus lindneri* DSM 20690. Beer spoilage bacteria were provided by German Collection of Microorganism and Cell Cultures del Leibiniz-Institute DSMZ and were incubated in MRS broth at 28 °C 24h before the screening evaluation. Negative and positive control tests were performed in order to control the validity of the test. Plates were incubated at 28°C for 72h. After incubation, evaluation of the antimicrobial activity of each isolated yeast consisting in the measuring of the diameter (mm) of the clear zone of inhibition (ZOI) around the inoculated wells.

Hydrogen Sulphide (H2S) Production.

H_2S production of all the 8 yeast isolates was evaluated on BIGGY agar (Bismuth Sulphite Glucose Glycine Yeast; Thermos Fisher Scientific, Waltham, MA, USA). On this medium H_2S-negative strains showed white colonies, while H_2S-producing colonies were characterized by a brown or dark brown colour. Plates were incubated with 48-h yeast cultures at 12º C, 20º C and 28 °C for 3 days as described by Comitini et al 2011. For results, the following chromatic scale was considered: 0 (white colonies, no hydrogen sulphide production), 1 (cream colonies), 2 (light brown colonies), 3 (brown colonies), 4 (dark brown or black colonies, very intensive hydrogen sulphide production. The test was performed in triplicate.

Qualitative Screening of the β-Glucosidase Activity

β-Glucosidase activity was carried out into Esculin agar medium as described by Jose Juan Mateo et al 2023. The presence of the enzymatic activity was visualized as a dark halo around yeast growth.

Qualitative Screening of the β-lyase Activity

Qualitative screening of β-lyase activity was carried out on YCS with the addition of SMC (Yeast Carbone Base in addition S-methyl-l-cysteine) medium as described by Santos et al 2016.

. Plates were incubated for 48-72-h at 20 °C for 3 days. The presence of the enzymatic activity was visualized by yeast colony growth.

Biogenic Amines production

Biogenic Amines production was carried out in a modified YPD medium supplemented with 10 g/L of phenylalanine, tyrosine, lysine, ornithine and histidine (i.e., the decarboxylase medium) as described by Romano et al 2022 with some modification. The decarboxylation of the amino acid to the corresponding biogenic amine results in an increase in pH, detected by the change in color of the medium. While histamine, putrescine and cadaverine producing strains were identified by purple coloration, tyramine production was detected by the decolorization of the culture medium.

Cryotolerance.

The capacity to grow at 4 °C of all the 8 isolated yeast was evaluated by inoculating (10^6 cfu/mL) the yeast cultures into Erlenmeyer flasks (working volume of 100 mL) containing 80 mL of YEPD broth and maintained under stirring using a digital orbital shaker (Heathrow Scientific, IL, USA) set at 150 rpm as described by Iorizzo et al 2021.The growth was determined visually after 24 h of incubation.

Molecular identification

DNA extraction was carried out by using a DNA extraction kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's instructions as described in Iorizzo et al 2021.
Amplification of 28S rDNA region was carried out using U1 and U2 primers (Hertel et al 2003 and OIV-OENO 408-2011). PCR was performed using a Master cycler nexus gradient (Eppendorf, Hamburg, Germany).

RESULTS AND DISCUSSIONS

Eight yeast strains were isolated from autochthonous grape varieties, specifically Shesh i Zi and Kallmet, originating from the Tirana and Shkodër regions. These yeast isolates underwent comprehensive laboratory screening to assess their potential viability as starters for craft beer production. According to the morphological characteristics, and biochemical reactions only SHZV3 strain exhibited significant attributes suitable for utilization as a starter in the brewing

industry. After DNA sequencing, this yeast was identified as *Saccharomyces cerevisiae* isolated from Shesh i Zi variety after 6 days of fermentation.

a. Pulcherrimin Production

Pulcherrimin is an insoluble iron chelate formed via a non-enzymic reaction between Fe^{3+} and the water-soluble and diffusible pulcherriminic acid secreted by the cells of the antagonistic microorganism (Spiczki, et al 2020). Pulcherrimin production resulted positive in all selected yeasts in the Kallmet variety in both incubation temperature 12°C and 28°C as shown in Fig.1. In the Shesh i Zi variety only SHZV3T6 tested negative in both incubation temperature 12°C and 28°C and all other strains from Shesh i Zi tested negative. There have been numerous studies on the biocontrol ability and action mechanisms of large numbers of strains belonging to the *M. pulcherrima* clade, isolated from various substrates which exhibit antagonist activity against spoilage bacteria. (Kriegel et al 2022).

Table 1 Pulcherrimin production from isolated yeasts in both Shesh and Kallamet varieties at 12°C and 28°C

Cultivars	Area	Yeast	Pulcherrimin Production in 12°C	Pulcherrimin Production in 28°C
Shesh i Zi	Valias Tiranë	SHZV4T3	+	+
		SHZV2T6	+	+
		SHZV6T6	+	+
		SHZV1T6	+	+
		SHZV3T6	-	-
Kallmet	Koplik , Shkodër	KG1T6	+	+
		KG2T6	+	+
		KG5T6	+	+

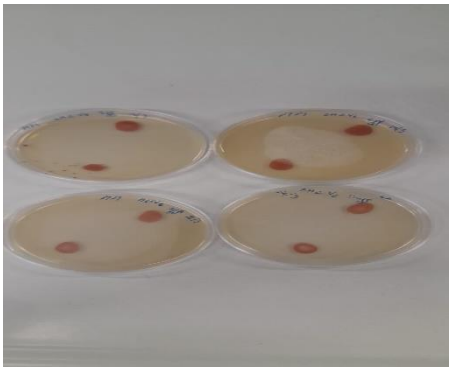

Figure 1. Pulcherrimin production by Yeasts strains from Shesh i Zi variety on Minimum Medium.

b. Antimicrobial Activity

In winemaking and brewing, an interesting application of biological activities is seen by the use of killer yeast to control the proliferation of spoilage microorganisms during the pre-fermentation phase (Comitini et al 2014). In past two decades, there are few research conducted to investigate the role of naturally occurring yeasts for inhibiting the growth of foodborne bacteria with various mechanisms (Younis et al 2017). As seen on Table 2 all conducted examinations yielded negative results, leading to the absence of any halo formation as shown in the Figure 2.

Table 2. Antimicrobial activity screening in BHI Agar medium

Cultivars	Area	Yeast	P. damnosus DSM 2030031	L. brevis DSM 6235	LB. plantarum DSM 2010074	FB. lindneri DSM 2060090
Shesh i Zi	Valias Tiranë	SHZV4T3	-	-	-	-
		SHZV2T6	-	-	-	-
		SHZV6T6	-	-	-	-
		SHZV1T6	-	-	-	-
		SHZV3T6	-	-	-	-
Kallmet	Koplik, Shkodër	KG1T6	-	-	-	-
		KG2T6	-	-	-	-
		KG5T6	-	-	-	-

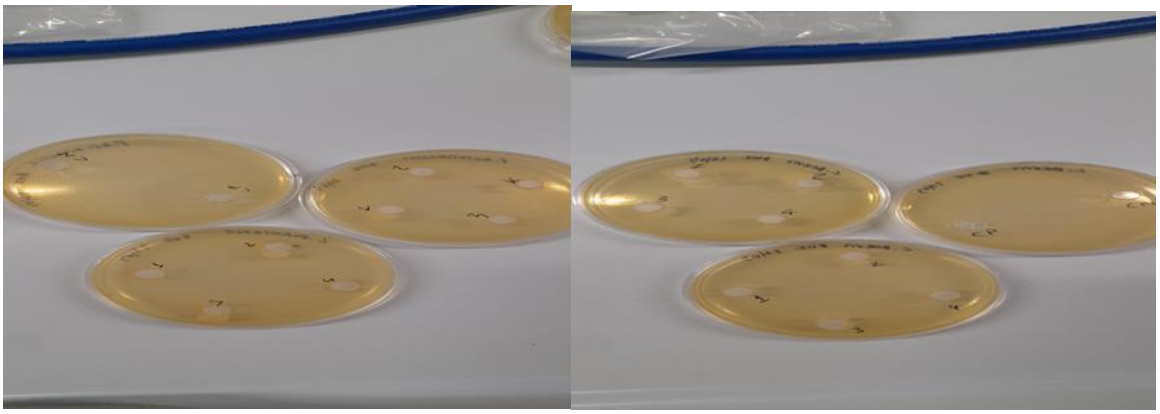

Figure 2. Antimicrobial activity in both Shesh i Zi and Kallmet varieties

c. Hydrogen Sulphide (H_2S) Production

Hydrogen sulphide (H2S) is produced by yeast during winemaking and brewing and possesses off-flavours reminiscent of rotten eggs (Kinzurik et al 2016). As H_2S is produced all along fermentation, it is necessary to trap this gas to assess the total production of one alcoholic fermentation (De Guidi et al 2021). Samples were put in thermostat respectively at 12°C, 20°C and 28°C to assess H_2S production as shown in table 3. SHZV3T6 did not produce detectable quantities of H_2S at 20°C and 28°C forming a light brown colony and white colonies at 12°C. All the other strains produced elevated quantitates of H_2S in 20°C and 28°C. Only at 12°C the yeasts should be considered as low producers of H_2S forming a light brown colony as shown in the Figure 3.

Table 3. Screening of H_2S production by isolated yeasts from Shesh i Zi and Kallmet varieties in Biggy Agar

Cultivars	Area	Yeast	Temp Incubation 12°C	Temp Incubation 20°C	Temp Incubation 28°C
Shesh i Zi	Valias Tiranë	SHZV4T3	2	3	4
		SHZV2T6	2	3	4
		SHZV6T6	2	3	4
		SHZV1T6	2	3	4
		SHZV3T6	0	2	2
Kallmet	Koplik, Shkodër	KG1T6	2	3	4
		KG2T6	2	3	4
		KG5T6	2	3	4

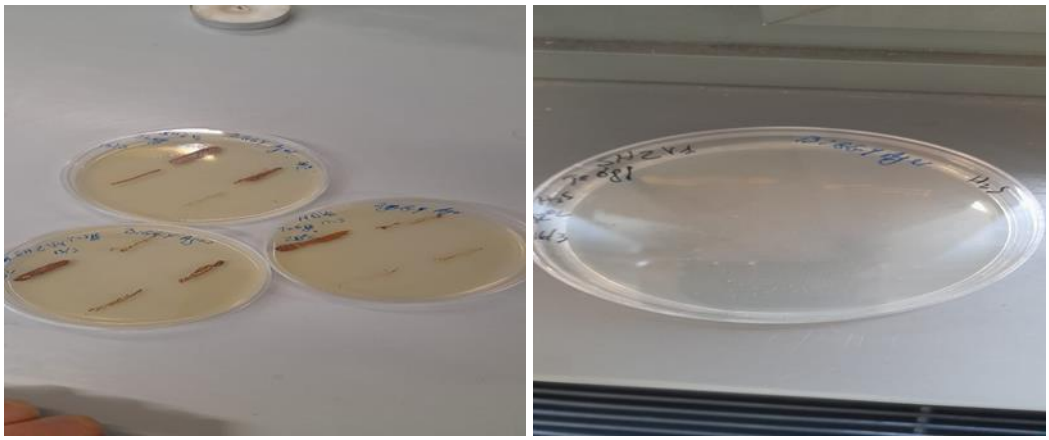

Fig.3 Dark brown colonies formed by some yeasts at 28°C and cream colonies by SHZV3T6 at 12°C

d. β-Glucosidase and β-Lyase assessment

The enzymatic ability of yeasts to hydrolyse glycosides due to β-Glucosidase activity (Han et al 2023) and to release thiols via the enzyme β-Lyase activity (Michell et al 2019) was performed in order to evaluate the enzymatic properties of isolated yeasts strains. β-Glucosidase activity gave positives results for 7 yeasts strains and negative results only for SHZV3T6. β-Lyase activity gave positive results only for SHZV3T6 as shown on Table 4.

Table 4 Screening of β-Glucosidase and β-Lyase activity on both Kallmet and Shesh i Zi isolated yeasts

Cultivars	Area	Yeast	β-Glucosidase activity	β-Lyase activity
Shesh i Zi	Valias Tiranë	SHZV4T3	+	-
		SHZV2T6	+	-
		SHZV6T6	+	-
		SHZV1T6	+	-
		SHZV3T6	-	+
Kallmet	Koplik, Shkodër	KG1T6	+	-
		KG2T6	+	-
		KG5T6	+	-

β-Glucosidase activity was visualized by yeast colony growth as shown in Figure 4.

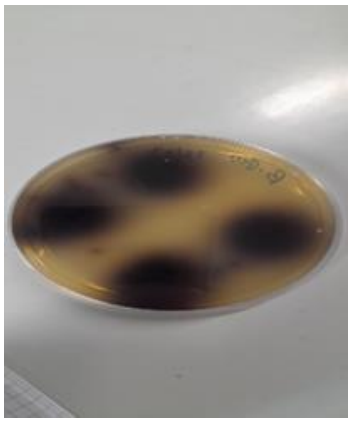

Fig. 4 β-Glucosidase activity of isolated yeast

e. Biogenic Amines Production

Yeast strain used for fermentation, can cause desirable as well as undesirable changes in beer as they could contain high concentrations of undesirable compounds, such biogenic amines (Bartkiene et al 2022). Laboratory screening was performed in order to evaluate Biogenic Amine production by isolated yeasts. Biogenic Amines screening gave positive results for most of the yeasts in YPD medium supplemented with 10 g/L of phenylalanine, tyrosine, lysine, ornithine and histidine producing biogenic amines respectively tyramine, cadaverine, putrescine and histamine. SHZV3T6 gave negative results in producing biogenic amines as shown on Table 5.

Table 5. Screening of Biogenic Amines production on both Kallmet and Shesh i Zi isolated yeasts

Cultivars	Area	Yeast	Phenylalanine	Tyrosine	Lysine	Ornithine	Histidine
Shesh i Zi	Valias Tiranë	SHZV4T3	+	+	+	+	+
		SHZV2T6	+	+	+	+	+
		SHZV6T6	+	+	+	+	+
		SHZV1T6	+	+	+	+	+
		SHZV3T6	-	-	-	-	-
Kallmet	Koplik, Shkodër	KG1T6	+	+	+	+	+
		KG2T6	+	+	+	+	+
		KG5T6	+	+	+	+	+

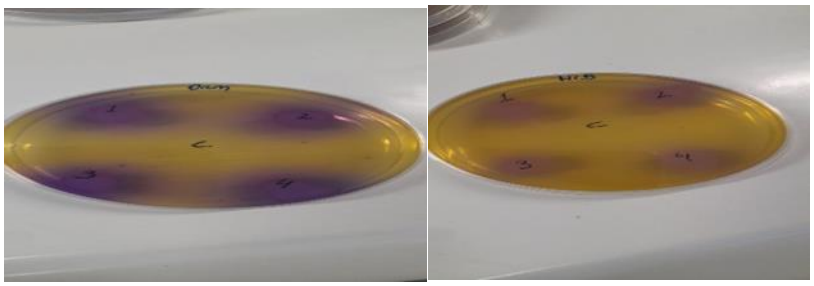

Fig. 5 Biogenic Amines screening test on both Shesh i Zi and Kallmet varieties.

f. Cryotolerance

Each strain of yeast exhibited the capacity for growth at a temperature of 4°C. This phenomenon was assessed through visual observation, wherein pellet formation at the base of the slant was observed. It is noteworthy, however, that the growth rate at 4°C was comparatively lower when contrasted with the growth rates observed at temperatures of 12°C, 20°C, and 28°C.

g. Molecular Identification

From all the strains isolated only SHZV3T6 was subject of molecular identification as the other were not suitable for beer industry. Based on the analysis of rDNA using the primers U1 and U2 performed in the laboratory of Technology and Microbiology in the Department of Agriculture, Environmental and Food Sciences at the University of Molise, Italy SHZV3T6 yeas isolate turned out to belong to the *S. cerevisiae* species.

CONCLUSIONS

The results shows that there are great possibilities isolating suitable yeasts from the Albanian autochthonous vineyard as potential starter in craft beer production even though most of the yeasts isolated during spontaneous fermentation were not suitable for beer production as they produce elevated quantities of H_2S and Biogenic Amines. This may be due to stress or H_2S may be generated through the degradation of sulfur-containing amino acids. These yeasts produce Pulcherrimin in Minimum Medium but didn't have any antibacterial activity against beer spoilage bacteria tested but may have for other bacteria. Although pulcherrimin itself does not exhibit antimicrobial activity, production of this metabolite leads to depletion of iron from the environment, which is the major mechanism of antimicrobial activity of the pulcherrimin producers. The ability to produce biogenic amines makes them not suitable for beer production. Only SHZV3T6 should be considered suitable for both Ale and Lager beer as didn't produce elevated quantities of H2S, especially in 12ºC for lager beer production and resulted negative in production in Biogenic Amines. This yeast has β-Lyase activity which enhanced ability to release hop-derived flavours through enzymatic activity. Future work will focus on the use of these yeasts in both lager and ale beer styles, particularly in the hoped beers such as IPAs to assess the fermentation characteristics and aroma profile of the finished beer.

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UV LIGHT-DRIVEN PHOTOCATALYTIC DEGRADATION OF METHYLENE BLUE USING TiO2, ZnO, and SnO2 as CATALYSTS: A COMPARATIVE STUDY

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ABSTRACT

The improper disposal of wastewater from the textile industry is a crucial global challenge that addresses various adverse effects on the aquatic environment. Methylene blue (MB) is a thiazine dye most widely used as a textile dye. Since MB is entirely stable, this dyestuff is very difficult to degrade in nature. Hence, different physical, chemical, and biological treatment technologies, including adsorption electrocoagulation, and electrochemical have been utilized to remove MB from contaminated water. Among them, heterogeneous photocatalysis is a cost-effective and sustainable method for the efficient breakdown of organic compounds into smaller and safer components without contributing to secondary contamination. For photocatalysis applications, TiO_2, ZnO, and SnO_2 are well-known semiconductors that play a vital role in mineralizing dyes into H_2O and CO_2 through the generation of reactive oxygen species.

In this study, the structural and morphological differences of TiO_2, ZnO, and SnO_2 nanoparticles were determined by FT-IR, XRD, SEM, and Raman spectroscopy. The photocatalysts were investigated comparatively for the degradation of MB dye under UV light irradiation. The percent degradation of MB in the presence of TiO_2 and ZnO was found to be 73% and 97 %, respectively, within 60 min irradiation. This value was lower for SnO_2 nanoparticles compared to TiO_2 and ZnO photocatalysts.

Keywords: Decolorization, heterogeneous photocatalysis, methylene blue, SnO_2, TiO_2, ZnO,

INTRODUCTION

The significant origin of wastewater management issues facing the world today is the heavy industries related to textiles (Islam et al., 2023). Textile wastewater contains various persistent pollutants such as non-biodegradable organics, dyes, heavy metals, surfactants, and phenols that detrimentally affect the environmental system (Kumar et al., 2023). Therefore, in order to comply with the national rules and international standards, dye wastewater must be treated before being discharged into any water bodies (Solayman et al., 2023). Although various efficient techniques have been reported to ensure the safe disposal of dye effluents, conventional wastewater treatment processes involving biological, physical, and chemical wastewater treatment processes have not been sufficient for the treatment of dye wastewater (Valli Nachiyar et al., 2023). However, advanced oxidation processes (AOPs) are cost-effective, environmentally friendly, and effective methods. These methods are extensively applied in dye wastewater treatment. Among AOPs, photocatalysis is the most popular and effective process using a semiconductor and UV light. A variety of photocatalysts have been used for the purification of wastewater in photocatalytic applications (Kurian, 2020; Solayman et al., 2023; Yadav et al., 2023). TiO_2 and ZnO have received much more attention compared to SnO_2 (Al-Hamdi et al., 2017).

In the present study, Fourier transform infrared spectrometer (FTIR) used with attenuated total reflection (ATR), Raman spectroscopy, X-ray diffraction (XRD), and Scanning electron microscopy (SEM) spectroscopic techniques were used to identify possible structural and morphological differences of TiO_2, ZnO, and SnO_2 nanoparticles. A comparative photocatalytic study was performed on the degradation of MB, a cationic dye, in the presence of TiO_2, ZnO, and SnO_2 photocatalysts under UV light irradiation.

MATERIAL AND METHOD

Tin(II) chloride dihydrate ($SnCl_2.2H_2O$), 25% ammonia solution (NH_3) and ZnO (Merck) powder were purchased from Merck. TiO_2 P-25 (Evonik) MB ($C_{16}H_{18}ClN_3S{\cdot}2H_2O$) was obtained from Merck. TiO_2 P-25 powder was a product of Evonik. All the other chemical reagents were analytical grade and used without further treatment. All aqueous solutions were prepared with distilled water (conductivity 2 μS/cm at 25 °C). The chemical structure of MB dye was given in Figure 1.

SnO_2 nanoparticles were prepared by precipitation method with reference to the methodology reported by Yousefi and colleagues with minor modifications (Yousefi et al., 2021). Briefly, NH_3 (8 mL) was added dropwise into a 0.1 M $SnCl_2.2H_2O$ (100 mL) solution in a flat-bottomed flask with vigorous stirring by a magnetic stirrer and stirring continued for 40 min. The white precipitate was obtained after the solution was kept at room temperature for 18 h. The precipitate was then filtered and washed thoroughly with distilled water and ethanol, respectively. Finally, the residue was dried in an air oven at 80 °C for 24 h, calcined in a muffle furnace at 500 °C for 3h. TiO_2 P-25 and ZnO powders were used as photocatalysts as provided by the supplier.

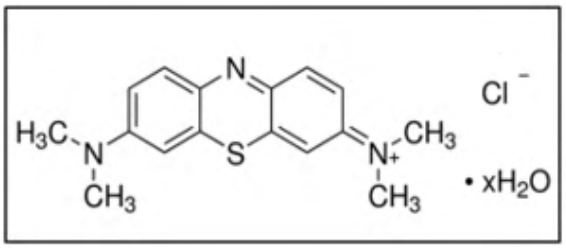

Figure 1. The chemical structure of MB (λ_{max}= 664 nm, MW= 319.85 g/mol).

FTIR spectroscopy was performed using Thermo Scientific Nicolet 6700 spectrometer equipped with an attenuated total reflection accessory. All spectra were obtained by 32 scans at a resolution of 4 cm^{-1} in the range of 4000–500 cm^{-1}. Dispersive Raman spectroscopic measurements were carried out by a Thermo Scientific DXR Raman Microscope with a spectral resolution of 4 cm^{-1}. The applied Ar^+ laser power was 10 mW at λ=532 nm. XRD diffractograms were acquired by a Rigaku-D/MAX-Ultima diffractometer with Cu Kα radiation (λ=1.54 Å) as X-ray source. The accelerating voltage and the applied current were 40 kV and 40 mA, respectively. The diffraction intensity was recorded in the range of 5-80° with a scan rate of 2° min^{-1}. SEM analysis was performed on FEI-Philips XL30 Scanning Electron Microscope with an accelerating voltage of 10 kV.

Testing of the photocatalytic activity was carried out in a cylindrical Pyrex reaction vessel. A 125W black light fluorescent lamp ($\lambda=_{max}$ 365 nm) was used as the light source and irradiated from top of the reactor. The light intensity reaching the reaction medium was I_o=1.65 × 10^{16} quanta/sec (Parker, 1997). The photocatalytic experiments were performed without pH adjustment. The catalyst dose amount used in experiments was 0.25 g/L and the initial MB concentration was 10 mg/L. The photocatalysts were dispersed in 50 mL of MB solution. The irradiated solution was immediately filtered through 0.22 μm cellulose acetate filters to remove

catalysts. The absorbance values of the samples were acquired by a Thermo Scientific Genesys 10S double beam spectrophotometer using 1 cm quartz cells.

RESULTS AND DISCUSSION

FTIR spectroscopy was used to identify the functional groups of the synthesized TiO_2, ZnO, and SnO_2 nanoparticles and the spectra was shown in Figure 2. A broad peak centered at around 3366 cm^{-1} and a medium peak at 1639 cm^{-1} located in the spectrum of TiO_2 corresponding to the stretching and bending vibration of OH groups on TiO_2 surface (Yalçın et al., 2010) (Figure 2 (a)). A wide peak centered at 3375 cm^{-1} attributed to the stretching of water molecules in ZnO spectrum (Moosavian and Moazezi, 2015) (Figure 2 (b)). Another peak observed at 1699 cm^{-1} related to H–O–H bending due to the presence of H_2O in the ZnO nanoparticles (Ashokkumar and Muthukumaran, 2014). The FTIR spectrum of SnO_2 was shown in Figure 2 (c). The weak bands at 3445 cm^{-1} and 1650 cm^{-1} were assigned to the OH stretching of adsorbed water molecules. The observed intense bands at 600 cm^{-1} and 516 cm^{-1} were related to the Sn-O and O-Sn-O stretching and bending modes of SnO_2 nanoparticles, respectively (Gnanamoorthy et al., 2021; Zhang et al., 2011).

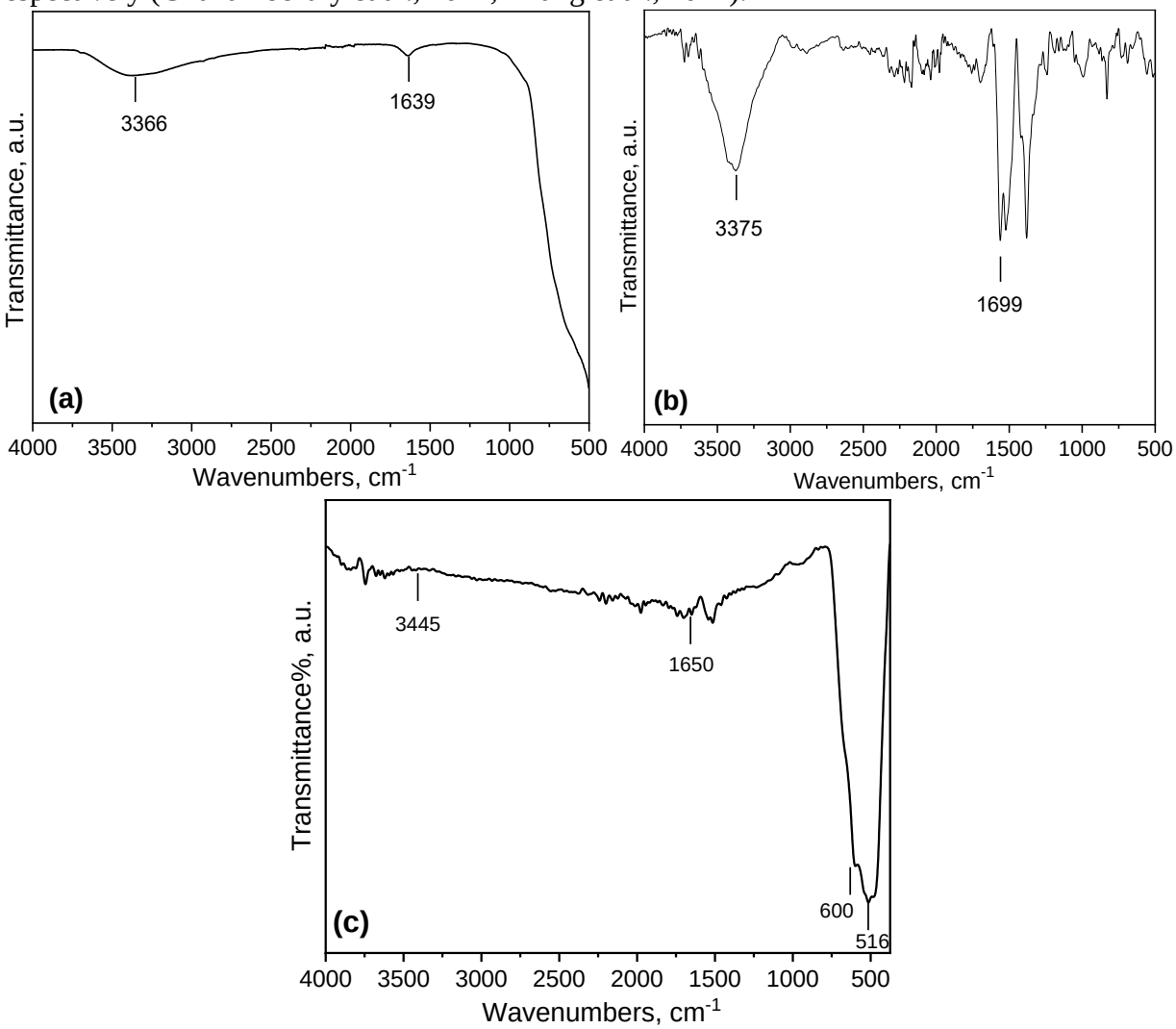

Figure 2. FTIR spectra of (a) TiO_2, (b) ZnO, and (c) SnO_2 nanoparticles.

Raman spectra of TiO_2, ZnO, and SnO_2 nanoparticles were presented in Figure 3. The Raman spectrum of TiO_2 (Figure 3 (a)) revealed five characteristic anatase bands at 630 cm^{-1} (E_g), 508 cm^{-1} (A_{1g}), 388 cm^{-1} (B_{1g}), 188 cm^{-1} (E_g), and 134 cm^{-1} (E_g) (Ohsaka et al., 1978).

The Raman spectrum of ZnO displayed a main and intense band located at 435 cm^{-1} corresponding to E_2 (high) mode (Figure 3 (b)). The other bands at 329 cm^{-1}, 384 cm^{-1}, 578 cm^{-1}, 663 cm^{-1} and 1151 cm^{-1} attributed to $2E_2$ mode, A_1 (TO) mode, A_1 (LO) modes, TA+LO, contributions of $2A_1$ (LO) and $2E_1$ (LO) modes, respectively (Dhingra et al., 2013; Pei et al., 2014; Šćepanović et al., 2010; Tao et al., 2007).

The Raman spectrum of SnO_2 nanoparticles was displayed in Figure 3 (c). The spectrum revealed three Raman bands at 766 cm^{-1}, 626 cm^{-1}, and 478 cm^{-1} corresponding to the tetragonal rutile structure SnO_2 active modes, B_{2g}, A_{1g}, and E_g, respectively (Sangeetha et al., 2011).

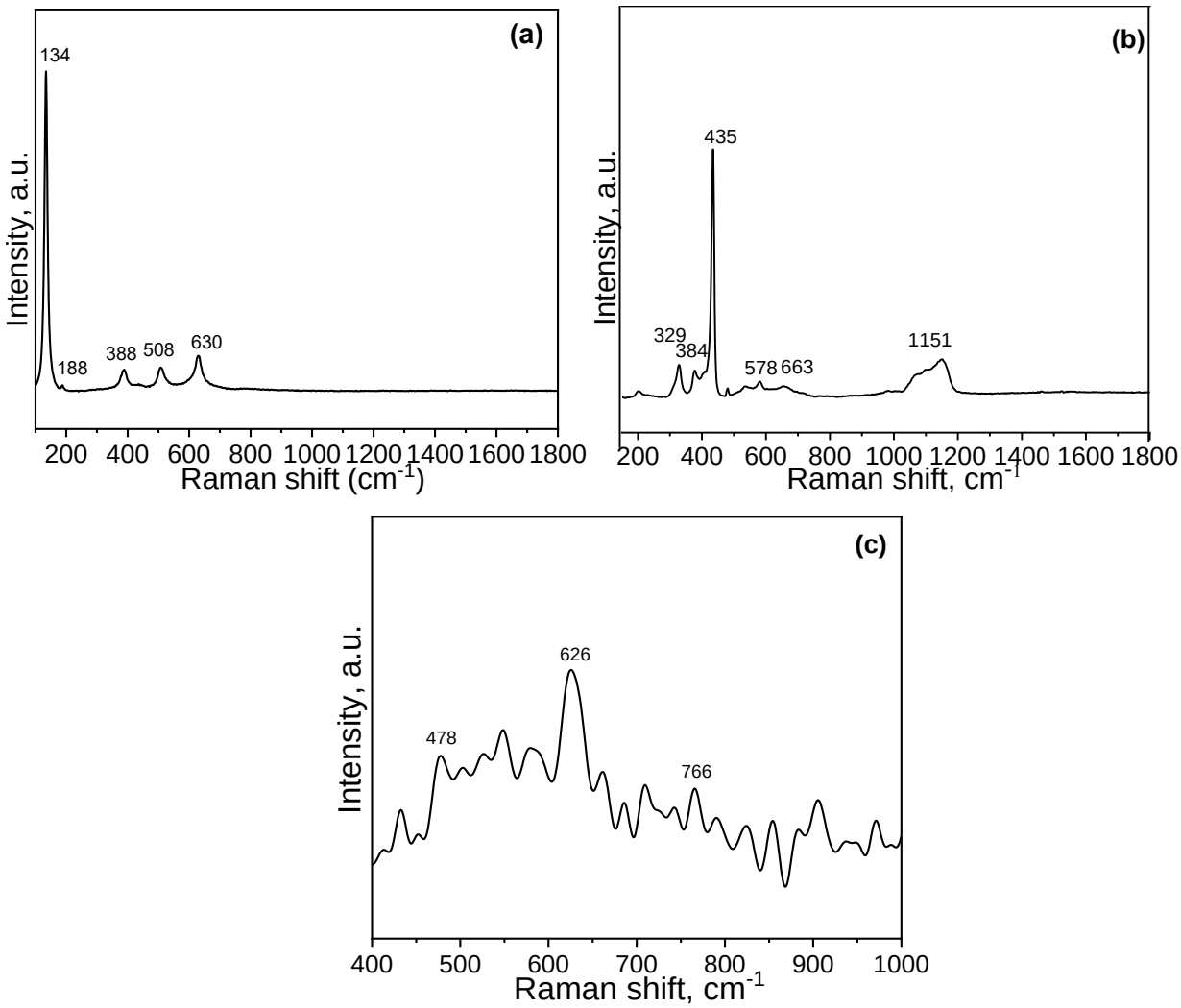

Figure 3. Raman spectra of (a) TiO_2, (b) ZnO, and (c) SnO_2 nanoparticles.

The XRD spectra of TiO_2, ZnO, and SnO_2 nanoparticles were presented in Figure 4. The crystallite phases of TiO_2 were presented by anatase (JCPDS No. 73-1764) and rutile (JCPDS No. 99-0090) crystal structures (Figure 4 (a)). A series of characteristic peaks at 2θ = 25.36º, 37.08º, 37.89º, 38.69º, 48.08º, 53.98º, 55.12º, 62.82º, 69.09º, 70.38º, 75.18º, 76.19º were attributed to (1 0 1), (1 0 3), (0 0 4), (1 1 2), (2 0 0), (1 0 5), (2 1 1), (0 0 2), (1 1 6), (2 2 0), (2 1 5), and (3 0 1) planes of anatase, while peaks at 2θ = 27.52º, 36.14º, 41.34º, 44.10º, 56.74º corresponded to (1 1 0), (1 0 1), (1 1 1), (2 1 0), and (2 0 0) planes of rutile, respectively. The diffractogram of ZnO (JCPDF 36-1451) exhibited the characteristic peaks at 2θ = 31.92°, 34.58°, 36.40°, 47.68°, 56.72°, 62.99°, 66.52°, 68.08° and 69.20° related to (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (2 0 0), (1 1 2) and (2 0 1) planes of zincite, respectively (Figure 4 (b)). The XRD diffractogram of SnO_2 nanoparticles was revealed in Figure 4 (c). The

observed diffraction peak positions were well matched with JCPDS No. 41-1445 and verified the tetragonal rutile structure of SnO_2. The diffraction peaks at 2θ = 26.58°, 33.88°, 37.90°, 51.80°, 54.80°, 57.98°, 62.00°, 66.08°, 71.40°, and 78.80 corresponded to the (110), (101), (200), (211), (220), (002), (310), (301), (202), and (321) planes (Tammina et al., 2018).

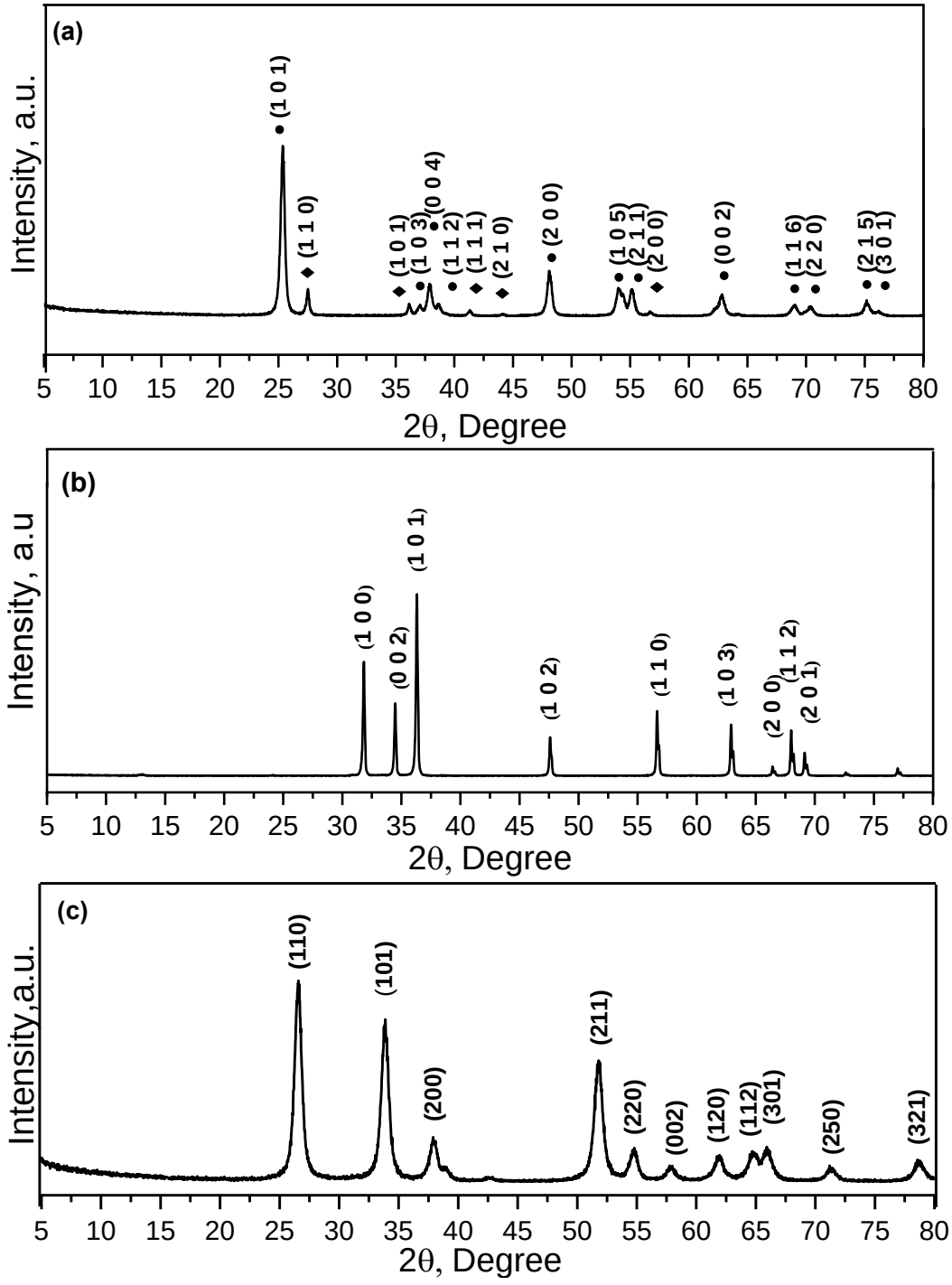

Figure 4. XRD spectra of (a) TiO_2, (b) ZnO, and (c) SnO_2 nanoparticles.

The morphologies of TiO_2, ZnO, and SnO_2 nanoparticles were examined by SEM analysis and SEM images were presented in Figure 5.

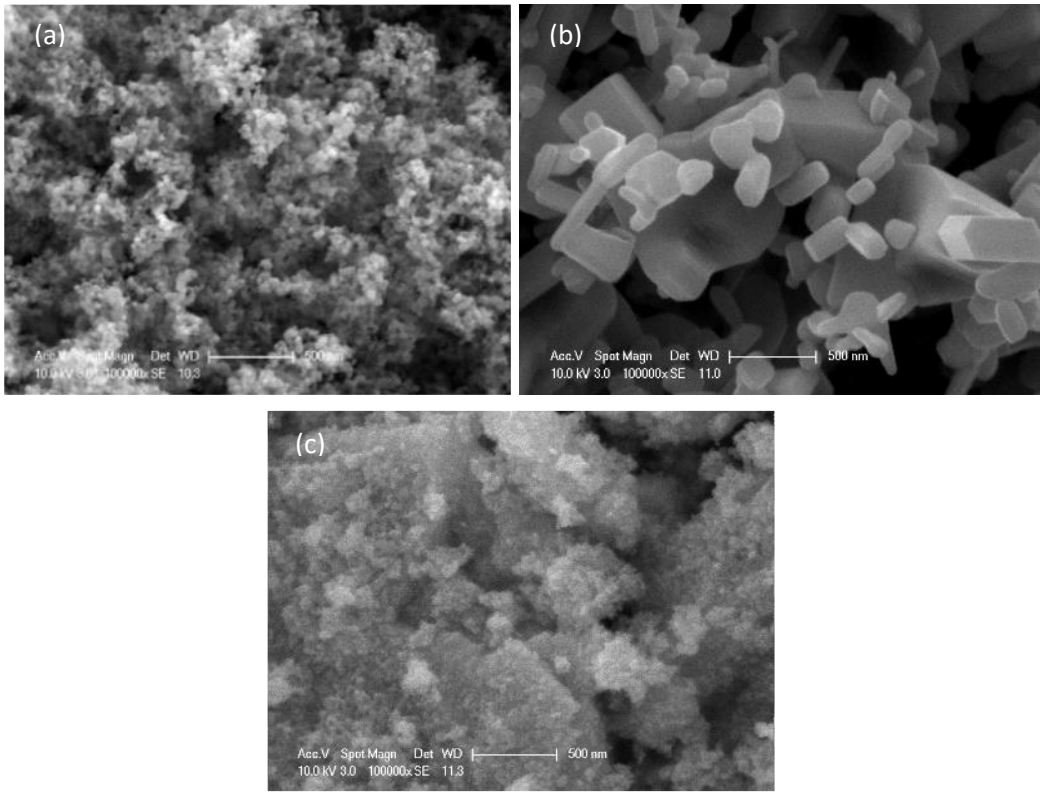

Figure 5. SEM images of (a) TiO_2, (b) ZnO, and (c) SnO_2 nanoparticles.

SEM image of TiO_2 nanoparticles (Figure 5 (a)) revealed almost spherical shapes with a slight agglomeration while ZnO consisted of a variety polyhedral shape (Figure 5 (b)). The SEM image of SnO_2 (Figure 5 (c)) showed that the nanoparticles were almost spherical, however the particle size and distribution were not homogeneous due to the presence of agglomeration (Habte et al., 2020).

The degree of MB decolorization by using TiO_2, ZnO, and SnO_2 nanoparticles (Figure 6) was calculated by the following equation (1).

$$\text{Decolorization, \%} = ((A_{,o} - A)/A_o)) \times 100 \quad (1)$$

where,

$A_{,o}$ = initial absorbance of MB and A = absorbance of MB at irradiation time t.

The percent degradation of MB in the presence of TiO_2 and ZnO was found to be 73% and 97 % respectively upon 60 min irradiation. TiO_2 and ZnO catalysts revealed a better photocatalytic efficiency than SnO_2 nanoparticles. An almost complete degradation of 93% and 99, respectively, was observed for TiO_2 and ZnO after 120 min under UV light. On the other hand, the removal efficiency of MB in the presence of SnO_2 was 22% even after 300 min irradiation.

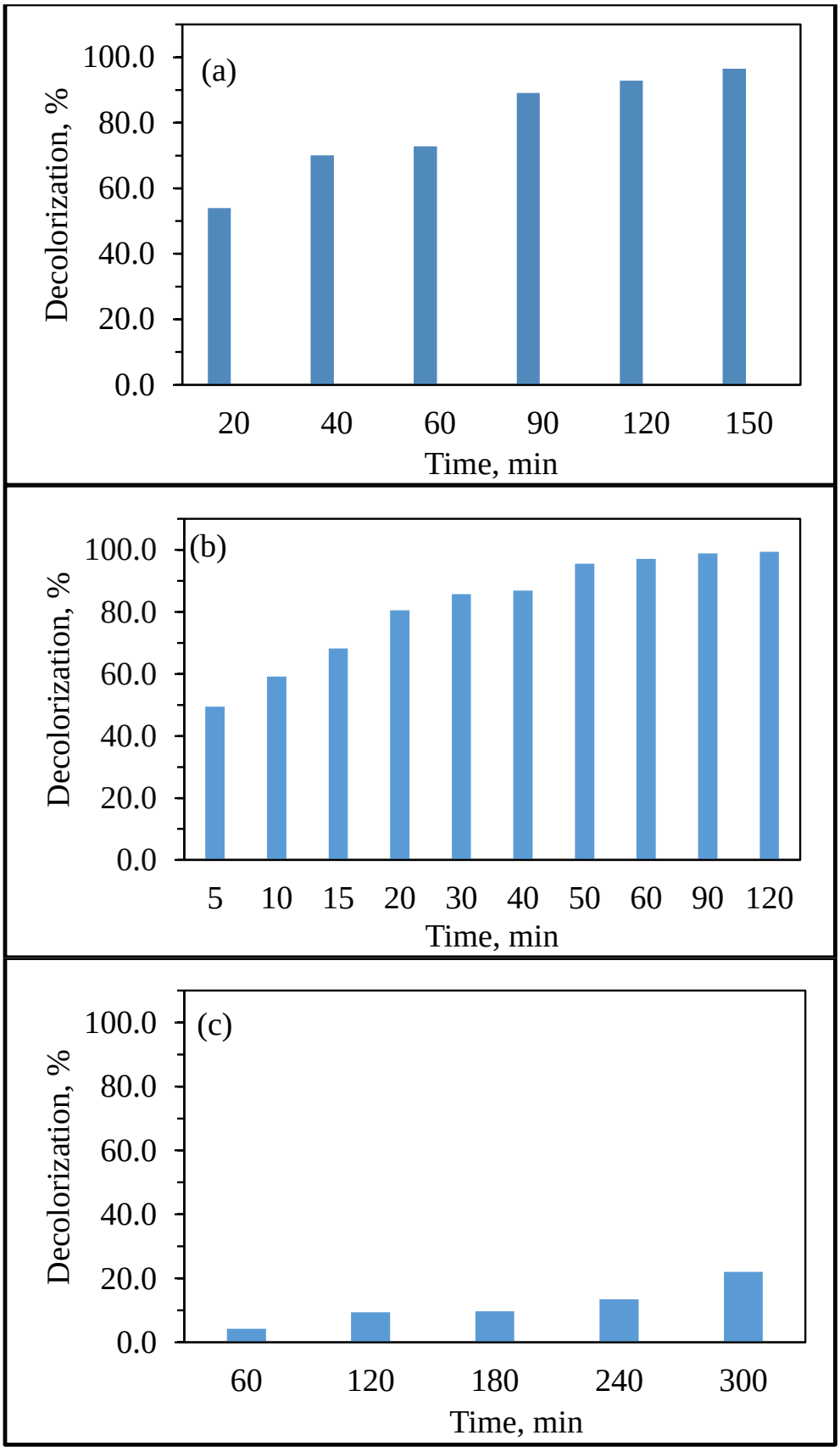

Figure 6. Removal efficiencies of MB upon using (a) TiO_2, (b) ZnO, and (c) SnO_2.

CONCLUSIONS

Based on the comparative study, SnO_2 nanoparticles were developed by using a facile precipitation method. FTIR spectra of catalysts confirmed the presence of functional groups of TiO_2, ZnO, and SnO_2 vibrational bands. XRD and Raman spectroscopy results indicated the evidence of tetragonal rutile structure of SnO_2. In addition, XRD and Raman confirmed the anatase and rutile phases of TiO_2 and the zincite phase for ZnO. The surface morphology of TiO_2, ZnO, and SnO_2 was identified by SEM analysis. TiO_2 and ZnO revealed an efficient MB removal compared to SnO_2.

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RECENT TRENDS AND FUTURE PROSPECTS ON THE POLYMERIC-BASED CATALYSTS FOR PHOTOCATALYTIC DEGRADATION

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ABSTRACT

Photocatalytic technology is viewed as an efficient wastewater treatment that is proficient in removing pollutants completely using UV/visible or solar energy. With this technology, there is an increasing interest in the development of polymer-based semiconductor materials, which leads to higher photocatalytic activity due to the modification of the structure. The main advantages of using polymers as catalysts in photocatalytic systems are their good photostability, moderating band gap, low-cost, and easy accessibility. Hence, the combination of various semiconductors with mostly preferred polyaniline, polythiophene, and polypyrrole conducting polymers appears to improve the photocatalytic performance. The recent synthesis of green polymeric catalysts composed of safe monomers makes them popular in photocatalysis. On the other hand, biopolymers also act as a support material for metallic oxides to avoid environmental toxicity from the use of chemical compounds. The current perspectives and prospects for advancement in green technology can be helpful in the potentiality of large-scale applications of polymeric-based catalysts that are utilized for photocatalysis.

Keywords: Biopolymers, catalyst, conducting polymers, green technology, photocatalysis,

INTRODUCTION

Water pollution is a global issue that can arise rapidly in developing as well as advanced countries due to the inappropriate disposal of chemicals, toxins, and microorganisms from industries. The contamination of carcinogenic and hardly biodegradable pollutants into water bodies can lead to either a short or long-term threat to the environment (Rafaie et al., 2023).

Conventional techniques are not sufficient for the water treatment of a variety of pollutants including dyes, pharmaceuticals, and various endocrine disrupting compounds (Srikanth et al., 2017). The most used physical methods are sedimentation, adsorption, activated carbon, ultra filtration, reverse osmosis, incineration, and membrane separation. In this inadequate water treatment method, typical physical processes are applied to remove organics that can cause a potentially harmful secondary effluent. Biological processes are applied in wastewater treatment by using activated sludge, microalgae, fungi, bacteria strains, and enzymes. The major drawbacks of biological processes are operating costs, the tendency of sludge swelling, and long pre-preparation cycles. Moreover, biodegradation is a slow and insufficient process for high chemical oxygen demand and total organic carbon removal of persistent pollutants. Chemical methods are expensive since they require a high dose of chemicals and the production of a large amount of sludge. Hence, it is necessary to improve effective processes that can destroy recalcitrant pollutants in wastewater as an alternative to conventional methods (Hansen et al., 2021; Ma et al., 2021; Zangeneh et al., 2015).

Advanced oxidation processes (AOPs) are promising novel, economically feasible, and widely applicable methods based on the formation of highly reactive and oxidizing free radicals. These reactive oxygen species such as hydroxyl radicals lead to change the chemical structure

of a wide diversity of contaminants (Zangeneh et al., 2015). AOPs can be classified into various types to generate hydroxyl radicals using chemical, photochemical, sonochemical, and electrochemical reactions (Oturan and Aaron, 2014). Photochemical AOPs are the most preferred, as the possible utilization of solar energy as a light source makes this process green and sustainable. Among the photochemical AOPs, photocatalysis and photo-Fenton processes are popular and modern technologies for the treatment of industrial effluents (Byrne et al., 2018).

PHOTOCATALYSIS

Photocatalysis is an effective AOPs and a photoinduced reaction occurs in the presence of a photocatalyst. In general, semiconductor-based oxides such as TiO_2, ZnO, CeO_2, ZrO_2, WO3, V_2O_5, Fe_2O_3, CdSe, ZnSe, and NiO are used. It has also been found that sulphides (MoS_2, ZnS, In_2S_3, CdS, etc.) and halides (AgCl, BiOI, etc.) are increasingly employed as photocatalysts. (Pastre et al., 2023; Srikanth et al., 2017; Zangeneh et al., 2015).

TiO_2 and ZnO are commonly studied photocatalysts due to their high stability and strength in photocatalysis. Besides, they exhibit a high photocatalytic activity without or with low toxicity and low-cost properties. However, a limitation is encountered in the utilization under the visible range of the solar spectrum irradiation, as it has a wide band. Consequently, the advance of new photocatalyst designs can overcome this limitation and improve their photocatalytic performance under solar light (Pastre et al., 2023; Puri and Gupta, 2023). In this respect, numerous studies have been performed to extend the optical absorption toward the visible region by using doping ions (cations or anions) or coupling semiconductors (Birben et al., 2017; Birben et al., 2016; Gurkan et al., 2017; Gurkan et al., 2012; Khaki et al., 2017; Turkten and Bekbolet, 2020; Turkten et al., 2019; Zhu and Zhou, 2019). Nowadays, polymers are used to modify and upgrade photocatalytic properties of catalysts (Ahuja et al., 2023; Mohammed et al., 2023).

CONDUCTING POLYMER BASED PHOTOCATALYSTS

Polyaniline (PANI), polypyrrole (PPy), and polythiophene (PTh) are prominent and well-studied conducting polymers (CPs) in photocatalysis. These members of the polymer class exhibit unique properties such as stability, ease of handling, charge carrier mobility, and compatibility that can qualify them to be employed as efficient photocatalysts. The coupling of CPs with a semiconductor could lead a synergistic effect to destroy aquatic pollutants (Mohammed et al., 2023; Taghizadeh et al., 2020; Turkten et al., 2021a).

Recently, the preparation of PANI based catalysts especially complexing with common semiconductors TiO_2 and ZnO have been employed to enhance the photocatalytic activity and reduce the rate of phogenerated electron-hole recombination. The photocatalytic properties of PANI-TiO_2 and PANI-ZnO photocatalysts are generally investigated by the degradation of dyes for example, methylene blue (Turkten et al., 2021a; Zia and Riaz, 2021). PANI-TiO_2 photocatalysts have been employed for photocatalytic degradation of bisphenol A (Sambaza et al., 2020), humic acid (Uyguner-Demirel et al., 2023), methylene blue (Lee et al., 2020; Rahman and Kar, 2020a; b; Wang et al., 2010; Yang et al., 2017), reactive black 5 (Jumat et al., 2017), rhodamine B and methylene blue (Radoičić et al., 2017; Radoičić et al., 2013), rhodamine B (RhB), methylene blue (MB) and phenol (Reddy et al., 2016), and sulfaquinoxaline (Sandikly et al., 2021). Photocatalytic performances of PANI-ZnO photocatalysts have been investigated by the degradation of Acid Blue 25 (Gilja et al., 2018; Gilja et al., 2020), (Nosrati et al., 2012), methylene blue (Turkten et al., 2021b), methylene blue and malachite green (Eskizeybek et al., 2012).

Several studies have been reported on the preparation and photocatalytic activity of PPy-TiO_2 (Baig et al., 2017; Gao et al., 2016; Kratofil Krehula et al., 2019; Luo et al., 2011; Silvestri

et al., 2020; Villabona-Leal et al., 2020; Yuan et al., 2020), PPy-ZnO (Balakumar and Baishnisha, 2021; Ceretta et al., 2020; González-Casamachin et al., 2019; Silvestri et al., 2019), PTh-TiO_2 (Ravi Chandra et al., 2015; Xu et al., 2011; Zhu et al., 2010), and PTh-ZnO (Khatamian et al., 2014) photocatalysts.

BIOPOLYMER BASED PHOTOCATALYSTS

In recent years, biopolymer based photocatalysts have gained increasing scientific interest due to their non-toxicity, compatibility, and flexibility, as well as their effects on pore size and surface morphology (Balakrishnan et al., 2022; Mohd Adnan et al., 2020).

Chitosan (CS) is one of the widely used natural biopolymers, which is the N-acetyl derivative of chitin found in the exoskeletons of crustaceans and obtained by deacetylation of chitin (Balakrishnan et al., 2022; Divya and Jisha, 2018). This biopolymer has been used with various semiconductors or sulphides to develop new photocatalysts, and their photocatalytic efficiencies have also been evaluated. There are many reports available on utilizing CS/nano-CdS composite (Zhu et al., 2009), CdS nanoparticles on CS microspheres (Zhang et al., 2014), CS/$CoFe_2O_4$ nanocomposite (Taleb, 2014), CS-ZS nanoparticles (Aziz et al., 2020), CS modified N, S doped TiO_2/ZnO (Farhadian et al., 2019), TiO_2/CS beads (Balakrishnan et al., 2020), CS/TiO_2 composite (Karthikeyan et al., 2017; Rejek et al., 2021), as catalysts for wastewater treatment. In general, CS-based photocatalysts could be promising candidates with superior photocatalytic functionalities in this field.

CONCLUSIONS

Based on photocatalysis studies, the development of polymeric-based catalysts results in various synergistic benefits on surface area, morphology, and photocatalytic properties. However, most of photocatalytic activity tests are performed in lab-scale applications. For future work, these photocatalysts are expected to be applied on an industrial scale for photocatalytic degradation of recalcitrant pollutants.

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POTENTIAL FOR RAINWATER HARVESTING IN LAYING HEN HOUSE: THE CASE OF BURSA

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ABSTRACT

Rapid industrial development, population growth, and climate change pressure on water resources. Existing water resources are depleted rapidly or become polluted and fall to a quality that cannot be used for consumption. Therefore, the creation and sustainability of alternative water resources have become essential for our future. Especially in the agricultural sector, the availability of water resources is vital for ensuring continuity in production and food supply. In addition, approximately 70% of clean water resources are used in agriculture. In addition to crop production, animal production is an important water user. Therefore, saving water in agricultural production is of great importance. The use of rainwater in both plant and animal production is one of the savings tools in recent years. Water consumption in livestock farms is realized as drinking water and water for daily use. This study determined the potential of rainwater harvesting from the roof of a layer hen house in the Bursa region, and the system design was made. As a result of the study, it was determined that rainwater harvesting from the poultry house roof was highest in December with 44,7 m^3 and lowest in August with 8,3 m^3. It has been determined that collected rainwater can supply an annual average of 35,5% of water consumption and usage in the poultry house. When a water tank with a capacity of 45 tonnes is designed to collect rainwater, the system will pay for itself in 24 years.

Keywords: Bursa, Drought, Water harvest, Water consumption, Laying hens

INTRODUCTION

Global climate change, which has impacted water resources significantly in recent years, poses severe threats for the future. In addition, the rapid development of industry, accelerated population growth, and increased consumption significantly impact water resources' pollution and rapid disappearance. For this reason, it is essential for our future to find alternative and sustainable water resources and to carry out studies and implement them.

Water resources' primary source is rainfall and related to how much rainwater can be captured and stored. Rainwater harvesting, which is an advantageous method for saving water and reducing surface runoff, especially for non-potable water uses, stands out as a good alternative that reduces the flow, peaks, and volumes of rainwater (Kilic and Yayli, 2019; Palermo et al., 2019). The utilization and supply of water resources are vital to ensure the continuity of production, especially in the agricultural sector. In farming, 70% of clean water resources are used in crop and animal production. Water consumption in livestock production is realized as drinking water and water for daily use. Therefore, studies on using rainwater harvesting in agricultural output are critical.

This study it is aimed to determine the potential of rainwater harvesting from the roof of a commercial laying hen house operating in the Bursa region and to design the system.

MATERIAL AND METHOD

This study investigated rainwater harvesting potential on the roof of a laying hen farm operating in Bursa Uludag University Faculty of Agriculture Agricultural Research and Application Center (Figure 1). There are 5500 laying hens in the henhouse. The roof area of the house was measured as 625 m^2 with a laser meter. The place where rainwater will be collected is directly proportional to the amount of rainwater harvested.

Figure 1. Satellite image of the laying hen house in this study

The rainwater harvesting potential of the poultry house considered in the study was calculated by Equation 1 (TEMA, 2023).

Rainwater capacity: Rain collection area * rainfall quantity * roof coefficient (1)

* filter efficiency coefficient

Rain collection area (m^2): It is the area where rainfall falls. In this study, the roof area of the poultry house was taken.

Rainfall quantity (mm): It is the amount of precipitation.

Roof coefficient: It expresses that not all the rain falling on the roof can be recycled. It is specified as 0,8 by DIN1989 German Standards (DIN, 1989).

Filter efficiency coefficient: The efficiency coefficient of the first filter passed for separation from solids indicates that some rain falling on the roof cannot pass through it. It is specified as 0,9 by DIN1989 German Standards (DIN, 1989).

The official website of the General Directorate of Meteorology was used for the average rainfall data of Bursa (Table 1) (MGM, 2023). The official website shows average values for the measurement period 1928-2022. The maximum precipitation fell in December, while the minimum precipitation was observed in August. The average annual rainfall is 707,4 mm.

Table 1. Bursa average annual precipitation (mm) (MGM, 2023)

Month	Average Monthly Total Rainfall (mm)
January	89,1
February	75,9
March	69,9
April	61,5
May	50,6
June	35,4

July	22
August	18,4
September	43,7
October	65,9
November	75,7
December	99,3
Total	707,4

The required storage volume was calculated by Equation 2 during the system design of the rainwater tank of the laying hen house considered in the study. The month with the highest rainfall is regarded as the rainfall parameter specified in Equation 2. For this study, December is taken into consideration.

Tank volume = rainfall volume * roof area * 0,8 * 0,9 (2)

RESULTS AND DISCUSSION

In the study, the rainwater harvesting potential is directly proportional to the rainfall. The most rainwater harvested was realized in December, while the least was in August (Table 2). The rainwater harvesting obtained from the roof of the investigated poultry house was approximately 320 m^3.

Table 2. Potential for roof rainwater harvesting (m^3)

Months	Rainwater capacity
January	40,1
February	34,2
March	31,5
April	27,7
May	22,8
June	15,9
July	9,9
August	8,3
September	19,7
October	29,7
November	34,1
December	44,7
Total	318,3

In calculating the daily water requirement of poultry, the amount of water required for each poultry was accepted as 0,25 L/day (Kilic and Abus, 2018). Drinking water consumption in the poultry house considered in the study is 1,4 m^3/day and 501,9 m^3/year. The domestic water consumption used for cleaning the poultry house is 396 m^3/year. The lowest rainwater harvesting potential is in summer, with 34 m^3. Coverage of water consumption with rainwater is directly proportional to the harvested rainwater harvest. Harvested rainwater meets 35,5% of the water consumption in the poultry house (Table 3).

Table 3. Rainwater harvesting and consumption coverage ratio by season

	Winter	Spring	Summer	Autumn	Total
Collected water (m^3)	118,9	81,9	34,1	83,4	318,33
Coverage ratio (%)	53,4	36,8	15,3	37,4	35,5

Rainwater Harvesting System Design

The system required to collect the calculated rainwater harvest has been designed, and its economic analysis has been made. The materials planned to be used in the system design and their numbers are given in Table 4. Underground and surface storage systems are used to collect rainwater. In this study, the storage design is planned to be located underground, and the galvanized steel water tank with a capacity of 45 tonnes can meet the need. The entire system design is calculated as 117,494 TL.

The annual water bill is approximately 8000 TL. The amount of savings obtained annually with the water obtained from rainwater harvesting is about 5000 TL. So, the new bill will be around 3000 TL. The rainwater harvesting design system will be able to amortize itself in 24 years.

Table 4. Economic analysis of rainwater harvesting system design

No	Material	Piece	Price
1	45 tonne galvanised steel water tank	1	35,249
2	Transport and installation costs	1	60,000
3	T pipe	1	100
4	11 Ø Pipe	50 meter	6495
5	Three-way valve	1	4000
6	Filter	1	11650

CONCLUSIONS

In the study, the potential of rainwater harvesting collected on the roof of a commercial hen house operating in the Bursa region has been theoretically demonstrated. To store the collected rainwater, a galvanized steel water tank with a capacity of 45 tons can supply the need. When the cost of the rainwater collection system and the annual cost of tap water are compared, the depreciation period of the system is found to be 24 years.

Rainwater harvesting is directly proportional to the collected roof area and the amount of rainfall. Therefore, the geographical region where rainwater is collected and the amount of rain received are also important parameters. Rainwater harvesting is an essential sustainable water resource alternative in the agricultural sector, constituting significant water use. More impressions should be placed on this alternative method by associating it with agriculture, and studies should be carried out for rainwater harvesting applications.

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RED DRAGON FRUIT: ALTERNATIVE USE OF FRUIT EXTRACTS IN VARIOUS INDUSTRIES

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Abstract

Plants known to have antimicrobial properties are very rich in secondary metabolites. Dragon fruit is one of the tropical fruits whose popularity has increased in recent years, especially in our country. Dragon fruit is known as a phytochemical store with bioactive components such as phenolic compounds, betacyanin, terpenoids and polysaccharides. In the present study, it was aimed to investigate the potential of using methanol extracts of red dragon fruit obtained from Turkey as a natural additive in various industries. The antimicrobial activity and photoprotective activity of the flesh and peel methanol extracts obtained by the hot water bath method were investigated in vitro. Firstly, the antimicrobial activity of the extracts was determined using disc diffusion and microdilution methods. Afterwards, the photoprotective activity was determined spectrophotometrically. The results determined that the highest inhibition zone diameter of the flesh extract was 11.84 mm against *P. aeruginosa* ATCC 27853 and *A. hydrophila* ATCC 19570. The highest inhibition zone diameter of the peel extract against the test microorganisms was determined as 11.38 mm in *C. glabrata* RSKK 04019. The minimum inhibition concentration (MIC) value was 10-40 µg/µl for flesh extract, it was determined in the range of 10-80 µg/µl for peel extract. The minimum bactericidal concentration (MBC) was determined in the range of 20->80 µg/µl for the flesh extract and in the range of 20-80 µg/µl for the peel extract. The sun protection factors of flesh and peel methanol extracts were determined as 10.64 and 14.34. These results revealed that methanol extracts obtained from dragon flesh have the potential to be used as natural additives in the pharmaceutical, food, feed, and cosmetic industries. At the same time, the data obtained from the study has the potential to lead to further studies for various industries.

Keywords: *Hylocereus polyrhizus*, Antimicrobial Activity, Sun Protection Factor, Natural Additive

INTRODUCTION

Plants are one of the alternative natural antimicrobial sources that can be used against various pathogens. Many plants with antimicrobial activity have been identified and more than 30000 natural antimicrobial compounds have been defined (Tajkarimi et al., 2010). The red dragon fruit (*Hylocereus polyrhizus*) is of great interest due to its remarkable red-purple color and biological activities (anti-obesity, anti-bacterial, antioxidant capacity and anti-inflammatory) (Celli and Brooks 2017; Liao et al. 2020). Dragon fruit is among the fruits rich in fiber and vitamins, which remove toxic substances such as heavy metals, control cholesterol and blood pressure, block diabetes, aid the digestive system (Gunasena et al., 2006).

Pathogens, which pose a great threat to human health, cause millions of infectious diseases in developed countries (Jin et al. 2009). The discovery of antibiotics, combined with significant advances in antimicrobial drug development, has become one of the most effective methods used in the treatment of microbial diseases. However, today, due to the misuse of antibiotic drugs, the resistance developed by microorganisms negatively affects the treatment of many diseases (Aminov, 2010; Tenover, 2006). Natural plant-derived substances can be a solution as an alternative to synthetic antimicrobials.

Solar UV rays are divided into three regions: UV-A (320–400 nm), UV-B (290– 320 nm), and UV-C (200–290 nm) (Mishra et al., 2011). Excessive exposure to sunlight can cause adverse diseases such as skin cancer (Kamell et al., 2011). Prolonged exposure to UV rays can cause sunburns, skin spots, skin aging and the onset of skin cancer (Ferrari et al. 2007). Chemical sunscreen creams cause various side effects such as allergic reactions in people with sensitive skin. In recent years, demands for using natural products to reduce or eliminate these side effects have increased (Mishra et al., 2011; Ahmady et al., 2020).

The purpose of our study is primarily the alternative use of red dragon fruit extracts as natural additives instead of synthetic additives in the pharmaceutical, food, feed, and cosmetics industries. Firstly, the antimicrobial activity of red dragon fruit extracts was investigated in vitro. Then, its alternative use as a natural preservative in sunscreens in the cosmetic industry was calculated spectrophotometrically.

MATERIAL and METHODS

Preparation of Red Dragon Fruit Methanol Extracts

The red dragon fruit was obtained from the red dragon fruit production greenhouse in Antalya (Kumluca). The flesh and peel parts of the red dragon fruit samples were separated from each other and thinly sliced. Afterwards, the sliced samples were airy dried separately in conditions. The samples were extracted every day (2 days) with a hot water bath for 9 hours. The red dragon fruit methanol extracts were dissolved in dimethyl-sulfoxide (DMSO) and then sterilized with a 0.22 μm syringe filter. The sterile extracts were stored under suitable conditions (at 4°C) until used.

Microbial Culture

Pseudomonas aeruginosa ATCC 27853, *Escherichia coli* O157:H7 and *Aeromonas hydrophila* ATCC 19570 were grown in Nutrient Broth (NB) for 24 hours at 37ºC. *Candida glabrata* RSKK 04019 was cultured at 30ºC for 24 hours in Yeast Peptone Dextrose (YPD).

Antimicrobial Assay of Red Dragon Fruit Extracts

Disc Diffusion Assay

The microorganism strains were washed twice in isotonic media and the concentration was adjusted to 0.5 McFarland. 100 µL of prepared microbial culture suspension (0.5 McFarland) was spread on specific agar medium. The sterile discs (6mm, Whatman no: 1) were placed on specific agar media. Afterwards, 20 µL (4 mg/disc) of red dragon fruit extract was dropped onto the discs. The prepared petri dishes were kept at +4 ºC for 2 hours and then incubated for 24 hours at appropriate temperatures. After incubation, the inhibition zone diameters formed around the discs were measured with a calliper and recorded.

Microdilution Assay

The minimum inhibitory concentration (MIC) and bactericidal or fungicidal concentrations (MBC or MFC) of red dragon fruit extracts were obtained by microdilution method. The red dragon fruit extracts were added to growth medium and diluted by a two-fold serial dilution method to obtain a final concentration of 80-5µg/µl. The microbial suspension was added to each tube and then incubated under the conditions required for each microorganism as mentioned above. After incubation, the extract concentration in the tube without microbial growth was determined according to turbidity and the lowest concentration was recorded as the MIC value. The MBC or MFC values were determined by inoculating samples from the mixture onto agar medium. The culture dishes were incubation period at the appropriate temperature for 24 hours. The lowest concentration without eventually growth of incubation was defined as MBC values.

Determination Sun Protection Factor of Dragon Fruit Extracts

The sun protection factor (SPF) of red dragon fruit extracts was determined by spectrophotometric method in vitro conditions. The extracts (0.002 g/ml) were mixed with 96% ethanol. The mixture was read in the UV-VIS spectrophotometer in the wavelength range of UV-B (290-320 nm). The Mansur equation was used to calculate the SPF value. Mansur's equation (Mansur et al., 1986):

Mansur's equation (Mansur et al., 1986)**:**

$$\mathbf{SPF} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times \mathrm{I}(\lambda) \times \mathrm{Abs}(\lambda)$$

CF is the correction factor (= 10);

EE(λ) is the erythemetogenic effect radiation wavelength (λ);

I(λ) is the intensity of sunlight at wavelength (λ);

Abs(λ) is the absorbance of extracts at wavelength (λ).

RESULTS and DISCUSSION

The biological activity of red dragon fruit methanol extracts was investigated on test microorganisms using disc diffusion and microdilution assays. The disc diffusion assay results of the extracts are presented in Table 1. The inhibition zone diameters of the flesh extract against the test microorganisms were determined in the range of 7.30-11.84 mm. The highest inhibition zone diameter of the flesh extract was determined against *P. aeruginosa* ATCC 27853 and *A. hydrophila* ATCC 1970. The inhibition zone diameters of the peel extract against the test microorganisms ranged from 7.52-11.38 mm. The highest inhibition zone diameter was determined against *C. glabrata* RSKK 04019.

Table 1. Disc diffusion assay results of red dragon fruit extracts.

Microorganism Strains	**Inhibition Zone Diameter (mm±sd)**		
	Extracts (2 mg/disc)		**Antibiotic**
	Flesh	**Peel**	**Kanamycin**
***P. aeruginosa* ATCC 27853**	11.84±0.00	10.71±0.02	20.37±0.20
***E. coli* ATCC O157:H7**	7.30±0.41	7.52±0.28	19.33±0.40
***A. hydrophila* ATCC 19570**	11.84±1.01	10.89±0.31	19.00±0.09
***C. glabrata* RSKK 04019**	11.35±0.30	11.38±0.02	**Fluconazole**
			20.35±0.10

In the study of Cheong et al. (2021), the inhibition zone diameter of the methanol extract obtained from *Hylocereus polyrhizus* fruit was determined as 14.10 mm against *E. coli* ATCC 25922. The zone of inhibition against *P. aeruginosa* ATCC 14149 could not be determined. In our previous study, the methanol extracts from *H. undatus* (white dragon) fruit and peel extracted with hot water bath were determined by disc diffusion method against test microorganisms (*E. coli* O157:H7, *P. aeruginosa* ATCC 27853, and *C. glabrata* RSKK 04019). As a result of the study, the inhibition zone diameters in the peel extract were determined as 7.81, 9.68 and 11.23 mm, respectively. It was determined as 7.86, 8.90 and 12.14 mm in fruit extract (Celik and Asan-Ozusaglam, 2023).

MIC is the lowest concentration that inhibits microorganism growth in vitro. The minimum bactericidal or fungicidal concentration is the lowest concentration that reduces the number of microorganisms in the medium containing the bacterial inoculum by 99.9 in vitro conditions (Kowalska and Dudek, 2021). In the current study, the MIC value of the flesh extract was determined in the range of 10-40 µg/µl and the MIC value of the peel extract was determined in the range of 10-80 µg/µl. The lowest MBC or MFC value (20 µg/µl) was determined for flesh extract on *P. aeruginosa* ATCC 27853 and for peel extract on *C. glabrata* RSKK 04019.

Table 2. Micro-dilution assay results of red dragon fruit extracts.

Microorganism Strains	Micro-dilution Assay			
	MIC (µg/µl)		MBC or MFC (µg/µl)	
	Flesh	Peel	Flesh	Peel
***P. aeruginosa* ATCC 27853**	10	40	20	80
***E. coli* ATCC O157:H7**	40	80	>80	80
***A. hydrophila* ATCC 19570**	40	40	40	40
***C. glabrata* RSKK 04019**	20	10	40	20

Yong et al., in their study (2012), determined the antimicrobial activity of red pitahaya methanol extract on *E. coli* ATCC 25922, *P. aeruginosa* ATCC 10145 and *P. aeruginosa* ATCC BAA-47 using the MIC assay. The results were shown as 12.5 mg/ml, 25 mg/ml, and 25 mg/ml, respectively.

The safety and toxic effects of chemicals in commercially produced sunscreen products are questioned by researchers (Yanishlieva vd., 2006). Investigations are carried out against the potential of using alternative natural preservatives against the dangers of the synthetic substances used (Korać ve Khambholja, 2011). In the present study, the sun protection potential of red dragon fruit extracts was determined as a result of spectrophotometrically measurement. The sun protection factor results of the flesh and peel methanol extracts are given in Table 3 and Table 4. SPF values of flesh and peel extracts were calculated as 10.64 and 14.34.

Table 3. SPF values of red dragon fruit flesh extract

Flesh			
Wavelength	$\mathbf{EE(\lambda) \times I(\lambda)}$	**Abs**	$\mathbf{CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)}$
290	0.0150	1.6783	0.2517
295	0.0817	1.3040	1.0653
300	0.2874	1.1500	3.3051
305	0.3278	1.0693	3.5052
310	0.1864	0.9743	1.8161
315	0.0839	0.9046	0.7572
320	0.0180	0.8256	0.1486
Sun protection factor (SPF)			**10.64**

Table 4. SPF values of red dragon fruit peel extract

Peel Extract			
Wavelength	$\mathbf{EE(\lambda) \times I(\lambda)}$	**Abs**	$\mathbf{CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)}$
290	0.0150	1.9633	0.2945
295	0.0817	1.7126	1.3992
300	0.2874	1.5233	4.3780
305	0.3278	1.4036	4.6012
310	0.1864	1.3136	2.4486
315	0.0839	1.2196	1.0208
320	0.0180	1.1390	0.2050
Sun protection factor (SPF)			**14.34**

In a study conducted by Celik and Asan-Ozusaglam (2023), SPF values of *H. undatus* (white dragon) peel and fruit extracts were determined as 25.92 and 24.84 for peel and fruit extracts, repectively. The literature studies on determination of SPF value of dragon fruit extracts are limited, therefore, more studies are needed.

CONCLUSION

In this study, antimicrobial activity and photoprotective activity of methanol extracts of red dragon fruit obtained from Turkey were investigated to determine their potential to be used as natural additives in various industries. The red dragon fruit extracts exhibited good activity against test microorganisms. In line with this conclusion, there is an alternative use of red dragon fruit extracts as natural additives in the pharmaceutical, food, and feed industries. In addition, the fact that the extracts have high SPF values is an indication that they have the potential to be used as a natural preservative in the cosmetic industry. As a result, it was determined that red dragon fruit extracts may have the potential to be used as natural additives instead of synthetic additives in various industries.

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RISKS RELATED TO PHYTOSANITARY PRACTICES OF APPLE GROWERS IN THE KHENCHELA REGION -ALGERIA

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Abstract

The main objective of the agricultural spraying process is to ensure optimum biological efficacy of the phytosanitary treatment under the constraints of technical aspects and economic considerations. In this context, mastering the techniques for applying plant protection products can help reduce their undesirable impact on the environment and on health. This may justify a search for the best technical conditions applicable to local conditions, with the aim of optimizing and rationalizing phytosanitary treatments.

Our survey-based study took place in 2019/2020 in the wilaya of Khenchela on 368 farmers spread over 08 communes belonging to 03 main daïra in agricultural activity. Our approach was to collect data on the phytosanitary practices of farmers in the region through a questionnaire used to estimate the risks associated with these practices.

The questions asked concerned the operator's ability to characterize the product packaging, protective measures (PPE), preparation of the spray mixture, product storage, climatic preference for treatment, information on the sprayer, type of product, formulation, dose and frequency.

The data collected was used to feed a mathematical model estimating farmers' exposure rates to plant protection products, and to assess the health risks associated with their practices. Most farmers neglect to wear PPE during spray preparation and crop treatment, which amplifies their exposure to the products and consequently increases the risk incurred. Indeed, the respondents declaring to have suffered at least one of the health problems are farmers who use their protective equipment little or poorly, or who do not respect product use instructions and directions for use, and mainly treatment doses.

Key words : Phytosanitary practices, farmers, exposure, risk, PPE.

INTRODUCTION

In industrial agriculture, an apple undergoes an average of 35 phytosanitary treatments. Herbicides, insecticides, fungicides... Apple growers can choose from over 2,500 toxic

products. Most pesticides are sprayed directly onto the fruit, leaving the skin saturated with chemicals **(Anonyme, 2016)**.

Phytosanitary applications refer to measures taken to prevent the spread of plant pests and diseases. Apple-growing is an important agricultural activity that requires phytosanitary measures to ensure the quality and safety of the produce **(Guy J. Hallman, 2011)**. Wireless monitoring systems can be used to reduce the level of pesticides while ensuring high-quality production **(Viani F. and *all,* 2016).** Fixed spraying systems have also been developed to improve working conditions, safety, timing, and performance of plan protection products' application in heroic viticulture areas **(Imperatore G. and *all,* 2021).** Additionally, beneficial fungi have been studied for their potential to manage phytosanitary problems in the thea agro-ecosystem **(Pandey A. K., 2021).** Overall, phytosanitary applications are crucial in apple-growing to ensure the safety and quality of the produce.

MATERIALS AND METHODS

our study was conducted from 2020 to 2021 (over a period of 14 months) during the main production and processing period for the cereal crop the data collection method is an individual farmer survey conducted at different sites in the study area, involving 368 producers, including 177 apple farmers in the Daïra Bouhmama and Daïra Kais represent. The questionnaire included questions on: Farm presentation, farmers' knowledge, use of phytosanitary products and methods of storage and personal protective equipment (PPE).

The data collected concerns the treatment methods used to control crop diseases and pests, the equipment used for spraying, as well as the various plant protection products used (formulation, dose, frequency, active ingredient, etc.) and protection measures (product-related risk to farmers). The list of products used was completed by examining empty packaging in the field, agricultural product vendors and packaging stored in the plant. Observations focused mainly on the preparation of the slurry, since this is a major risk phase for the operator (direct contact with the product).

RESULTS AND DISCUSSIONS

In order to obtain representative results for Khenchela, farms throughout the region were surveyed, including apple growing sectors in Daïra Bouhmama and Daïra Kais (48,10% apple producers).

The study shows that 92% of the 177 farms surveyed have a surface area of [0;10 ha], 3% have a surface area of [10;20 ha] and 1% have a surface area of [20;30 ha], while only 4% have a surface area over [+30 ha].

Yabous, Taouzient, Bouhmama and Chelia are the most productive apple-growing communes in the Khenchela region by successive order (24%, 21%, 18% and 16% growers).

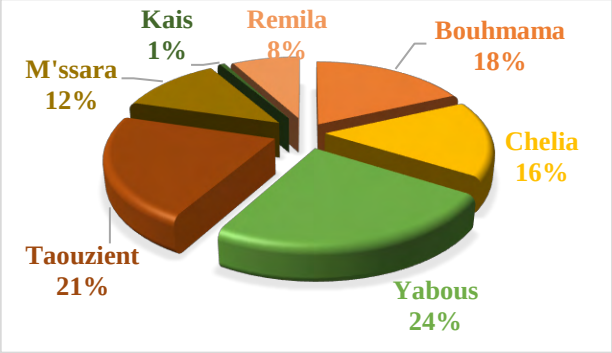

Figure 21 : Farms investigated of apple growing.

Application of phytosanitary products

As farm managers are responsible for spraying crop protection products, explaining treatment methods to applicators, they are the ones most exposed to potential health risks. In order to 51% of farmers wear personal protective equipment (fig. 02).

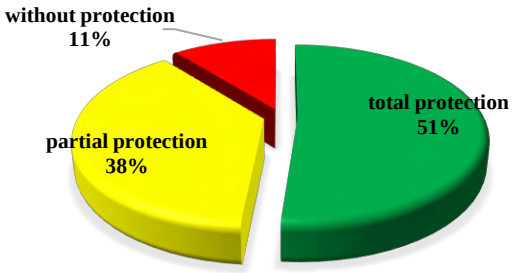

Figure 22 : Personal protection equipment.

Phytosanitary products for cereal treatments

A total of 177 farms surveyed in the region were found to use commercial specialties, all of them synthetic chemicals. Insecticides and fungicides were the most widely used, accounting for 66% and 33% respectively. Followed by herbicide-based products (1%).

The list of products was completed by examining stored packaging and the sellers of phytosanitary products.

The stady shows that the most useful products in the study area are: Voliam flexi 59 farmers (insecticide), Bayfidan by 34 farmers (fungicide), Score by 19 farmers (fungicide), Movento by 18 farmers (insecticide) and Voliam targo by 16 farmers (insecticide).

13 actives substance were inventoried, with Chlorantraniliprole et du Thiamethoxam (33,3%), Triadimenol with (19,2%), Difenoconazole with (10,7%), Spirotetramat 100 g/l with (10,2%) and 1,8% Abamectin et 4,5% Chlorantraniliprole with (9%).

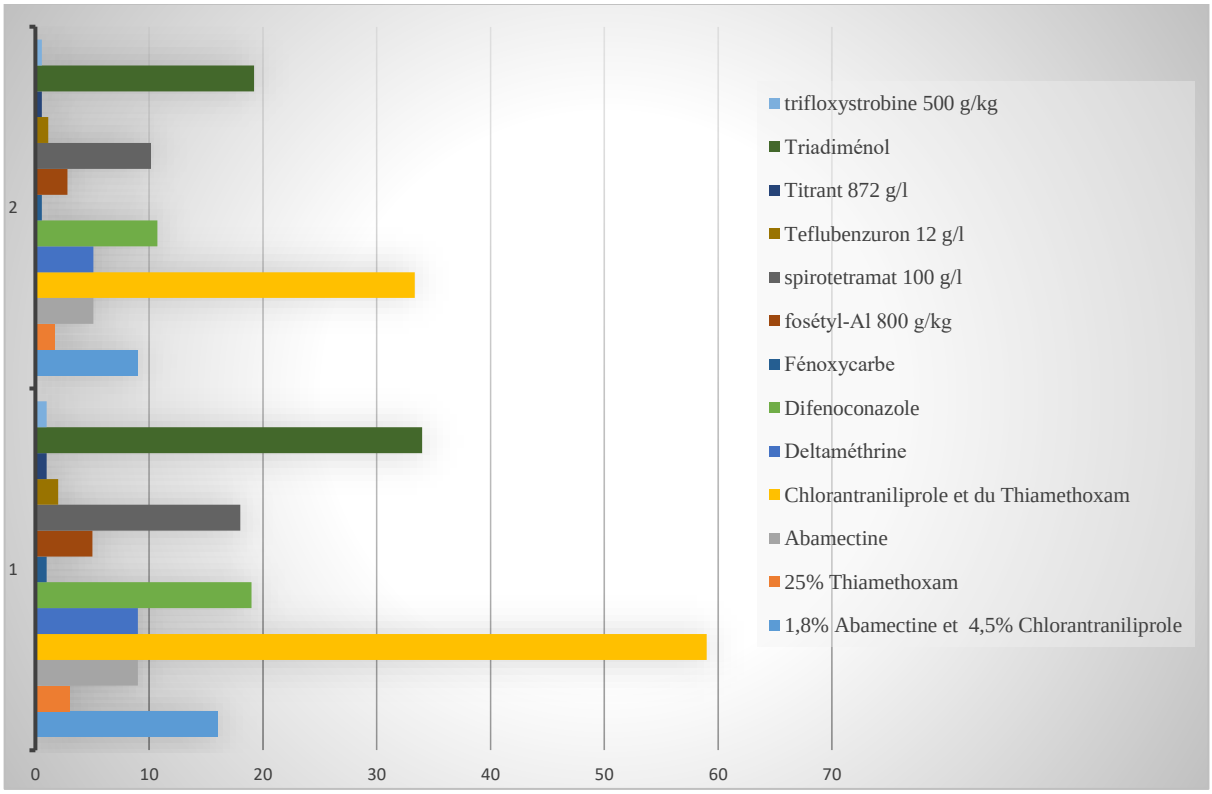

Figure 23 : Distribution of active material handled.

EXPOSURE RISK

In the Khenchela region, several noxious or toxic active ingredients have been identified on local markets, on packaging stored at growers' premises or on empty packaging burnt by local farmers after spraying. Estimation of potential exposure of growers over a working day (mg/kg body weight / day). The following data are integrated: application method, product name, active ingredient, concentration, formulation, PPE, dose and AOEL for each active ingredient. This model enables results to be compared with the AOEL (EU Pesticides database).

Estimated operator exposure without wearing protection, expressed as a percentage of the AOEL, represents 283% (without protection) of the AOEL for Spirotetramat, 179 % (without protection) of the AOEL for Abamectin, 107% (without protection) of the AOEL for Chlorantraniliprole (fig.04).

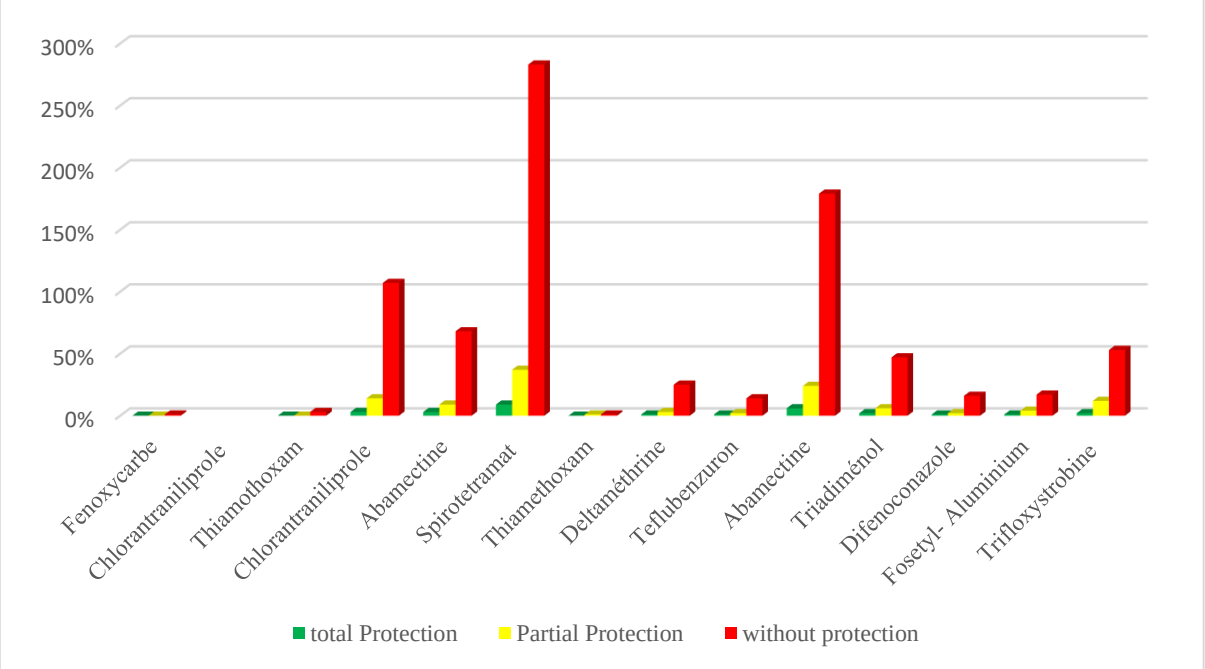

Figure 24 : Operator's exposure level.

The health risk for operators is considered unacceptable, without wearing protection during all phases of treatment. The risk diminutions when the operator dresses PPE.

CONCLUSION

Apple growers recognize the risks associated with poor phytosanitary practices: the supply of pesticides from informal channels, the use of toxic phytosanitary products, the total or partial absence of personal protective equipment, and the burning of empty packaging are all practices that expose these operators to danger.

The results tell us that some of the most frequently used active ingredients, mainly insecticides such as Spirotetramat (Movento) and Abamectin (Vertimec), exceed the tolerated limits. These active ingredients are known to be toxic and could have harmful effects after exposure, especially for farmers who fail to protect themselves when preparing bolls or applying plant protection products.

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SCREENING OF POLYPHENOL OXIDASE ENZYME IN LYCOPEN-RICH FRUITS

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ABSTRACT

Polyphenol oxidases (PPOs), which have been the subject of numerous studies in recent years and have attracted the attention of researchers, are enzymes that catalyze the oxidation of phenolic compounds. Although PPOs are generally found in plants, they are also found in many microorganisms and fungi. Therefore, PPOs have a wide distribution. PPOs are used in many fields, especially in medicine, cosmetics, and industry. The aim of this study was to investigate PPO by selecting fruits with high lycopene content. For this purpose, 9 different fruits (watermelon, bell pepper, fig, pomegranate, blood orange, grapefruit, cranberry, rosehip, potency pomegranate) were extracted in vitro using 13 different extraction methods (10 mM ascorbic acid in extraction buffer, 2 mM phenylmethylsulfonyl fluoride, 1% polyvinylpolypyrrolidone (w/v), 1% Triton X-100 (v/v), pH 7.0) and enzyme activity was measured spectrophotometrically.
Keywords: Polyphenol oxidase, Lycopene, Spectrophotometer, Enzyme purification, Enzyme isolation, Industry

INTRODUCTION

While a long shelf life is highly desirable for fruit and vegetable products, enzymatic browning is the main cause of quality loss in fruit and is generally considered nutritionally detrimental to food quality (Falguera et al., 2012). Therefore, enzymatic browning is a fundamental problem for the food industry. The degree of browning depends on the type and number of phenolic compounds, the presence of oxygen, reducing agents, metal ions, pH, temperature, and polyphenol oxidase (PPO) activity (Yoruk and Marshall, 2003).

The enzyme polyphenol oxidase (PPO) belongs to the oxidoreductase enzymes (EC 1) (Sekme, 2011). PPOs are divided into three main types according to their substrate specificity and mechanism of action. Laccases (EC 1.10.3.2, p-benzenediol: oxygen oxidoreductase), catechol oxidases (o-diphenol oxidoreductase; EC 1.10.3.1), and tyrosinases (EC 1.14.18.1, monophenol monooxygenase) (Griffith, 1994). These metalloenzymes belong to the oxidoreductase family (EC 1); they are found in the thylakoid membrane, cytoplasm, mitochondria, peroxisome, and chloroplast. Polyphenol oxidase is a copper ion containing enzyme. It can catalyze two different reactions using molecular oxygen. These reactions are hydroxylation of monophenols to o-diphenols (cresolase activity) and oxidation of o-diphenols to o-quinones (catecholase activity) (Spagna et al., 2005). cresolase and substances released because of catecholase activities cause the formation of brown, black and red pigments (Friedman, 1997; Sekme, 2011).

Polyphenol oxidases are used in many fields, such as the food industry, chemistry, pharmaceuticals, wine, beer, and fruit juice production (removal of phenolic substances), wastewater treatment, the plastics and paper industry, and melanin synthesis (Gasmalla et al., 2015; Maki et al., 2006).

Phenolic substances are natural antioxidants. Antioxidants act as radical scavengers and, due to these properties, prevent food components from reacting with oxygen. Thus, they delay the

spoilage of perishable products. To this end, natural antioxidants such as lycopene have gained importance in preventing food oxidation. Lycopene is a lipophilic carotenoid hydrocarbon pigment found in red, pink, and orange fruits and vegetables such as tomatoes, apricots, melons, and cranberries (Sevindik et al., 2021).

The purpose of this study was to investigate the presence of the enzyme polyphenol oxidase by selecting fruits with high lycopene content. For this purpose, 9 different fruits were extracted under in vitro conditions by 13 different methods and the enzyme activity was measured by the spectrophotometric method.

MATERIAL AND METHOD

Fruit samples were purchased at various public markets and markets in Kocaeli. The fruits were stored at -80°C.

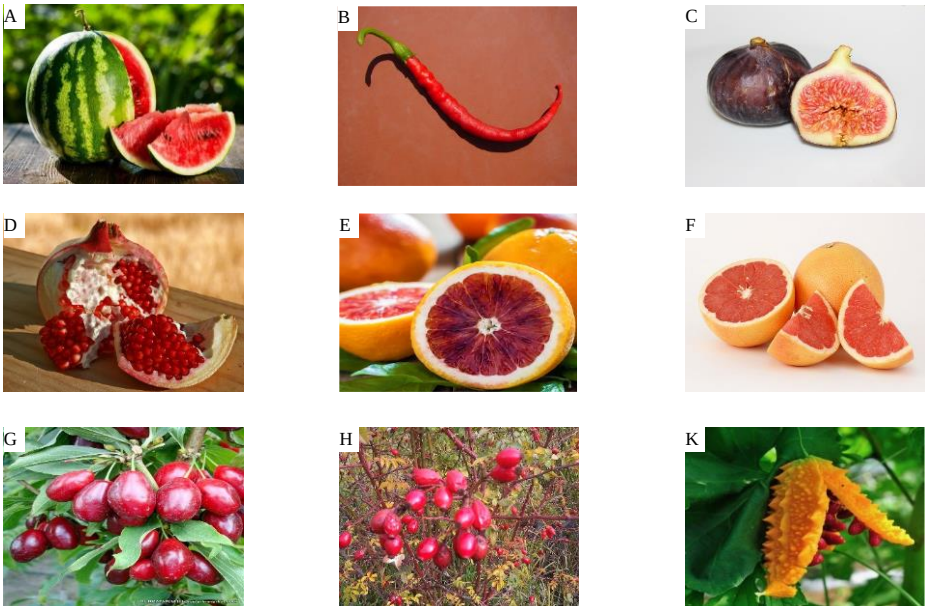

Figure 1. Citrullus lanatus (A), Capsicum annum (B), Ficus carica (C), Punica granatum (D), Citrus sinensis (Blood orange) (E), Citrus paradisi (F), Cornus mas (G), Rosa canina (H), Momordica charantia (K)

Extraction: the fruits were washed with distilled water (dH_2O). Then 200 g of the fruits were crushed with a blender and homogenized. 15 g of the pureed fruits were weighed and placed in 13 Falcon tubes. 15 ml of the prepared 0.1 M sodium phosphate buffer (pH 7.0) was added to them. Then, optimization was performed separately for each Falcon. 10 mM ascorbic acid, 2 mM for optimization phenylmethylsulfonyl fluoride, 1% polyvinylpolypyrrolidone (w/v) and 1% Triton X-100 (v/v) were used. The prepared mixture was shaken for 1 minute. The homogenate obtained was kept at +4 o C for 30 minutes and then at 10000xg for 30 minutes. After centrifugation, the mixture was filtered through a cheesecloth, the remaining pellet was removed, and crude enzyme extract was obtained.

Table 1. 13 methods used for extraction of PPO from various fruits.

		Ascorbic acid	**Polyvinylpolypyrrolidone (PVPP)**	**Phenylmethylsulfonyl fluoride (PMSF)**	**Triton X-100**
1	0.1 M Sodium phosphate buffer (pH 7.0)				
2			+		+
3		+	+	+	+
4			+		
5					+
6			+	+	+
7				+	+
8			+	+	
9				+	
10		+			
11		+			+
12		+		+	+
13		+		+	

It is important to minimize the formation of o-quinones during extraction. For this reason, PVPP and ascorbic acid were used to remove or reduce the phenolic compounds in the medium, Triton X-100 was used to dissolve the enzyme when bound to the cell organelle, and PMSF was used to increase PPO activity (Gauillard et al., 1997; Sojo et al., 1998).

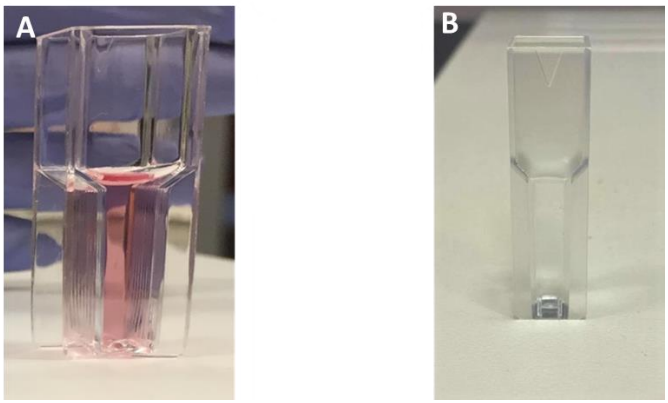

Figure 2. Enzyme activity assay. A – Sample cuvette, B –. Blank cuvette

To measure enzyme activity, the sample cuvette was prepared by adding 900 µl substrate (pyrocatechol) + sodium citrate buffer (0.1 M pH 5.4) and 100 µl enzyme solution with a final concentration of 100 mM. The empty control cuvette was prepared by adding 900 µl substrate (pyrocatechol) + sodium citrate buffer (0.1 M pH 5.4) and 100 µl sodium phosphate buffer (0.1 M pH 7.0). PPO activity was measured using a spectrophotometer at room temperature and 420 nm. The activity measurement was repeated twice in each experiment.

RESULTS AND DISCUSSION

PPO enzymes in, biosensor development, they are widely used in industry, especially in food, paper, textile, cosmetics, industrial wastewater treatment, and in the treatment of many diseases such as Parkinson's disease, cancer, and some infections. Due to their widespread use, their isolation and purification from various sources has gained great importance in recent years. Although PPOs are generally found in plants, they are also found in many microorganisms and fungi. PPO was isolated from these sources and purified using common purification methods (ammonium sulphate precipitation, temperature-induced phase separation (TIPS), three-phase

partitioning (TPP), aqueous two-phase extraction (ATPS), chromatographic purification). After the characterization studies of the enzyme, it was put into practice.

There are few studies in the literature investigating the relationship between lycopene, the antioxidant substance responsible for red color that occurs naturally in fruits, and PPO. Therefore, in this study, PPO activity was investigated using watermelon, red bell pepper, fig, pomegranate, blood orange, grapefruit, cranberry, rosehip, and bitter melon, which are rich in lycopene. The PPO activity of the fruits was compared with optimization performed by 13 differentmethods.

Table 2. PPO activity results

PPO Activity (U/mL)									
	Waterm elon	Red pepper	Fig	Pomegr anate	Blood orang e	Grapefruit	Cranberr y	Rosehi p	Potency pomegranat e
1	83	22	12 0	*	*	*	*	*	87
2	*	*	*	42	*	*	*	455	*
3	*	*	*	14	*	*	*	*	*
4	*	*	*	28	*	*	41	*	*
5	*	*	*	106	*	*	*	*	*
6	*	*	*	40	*	1135	66	*	95
7	*	*	*	29	*	*	*	*	*
8	*	*	*	23	*	*	123	*	*
9	85	26	91	8	*	*	*	*	*
10	60	20	*	*	*	1019	*	*	*
11	*	*	*	33	*	1774	*	*	115
12	*	*	*	12	99	*	*	*	*
13	85	13	60	*	*	*	*	*	*

(*= activity measurement could not be performed because a clear solution could not be obtained.)

CONCLUSIONS

Subsequently, crude enzyme extract samples obtained from fruits were prepared under optimal conditions and compared in terms of polyphenol oxidase activities. Accordingly, grapefruit fruit was found to have higher polyphenol oxidase activity towards catechol substrate at a concentration of 10 Mm ascorbic acid and 1% Triton X-100 compared to other fruits.

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LETHAL EFFECTS OF LAUREL, SENNA AND FENNEL PLANT EXTRACTS ON *TENEBRIO* MOLITOR

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ABSTRACT
This study was designed to determine the effects of laurel (*Laurus nobilis*), senna (*Cassia angustifolia*) and fennel (*Foeniculum vulgare*) plant extracts on lethality, larval, pupal and adult developmental period, adult longevity on *T. molitor.* Trials were carried out under laboratory conditions of 26±2 and 75±5% relative humidity. Five experimental groups were formed for each plant extract. 10 *T. molitor* larvae were placed in each experimental group (in petri dishes). Flour+wheat flour was added for nutritional needs and potatoes were added for water needs. On the first day, small cotton pieces impregnated with 10% plant extract were placed in the experimental groups and the petri dishes were wrapped with parafilm. After 24 hours, the numbers of surviving and dead larvae in the petri dishes were recorded. After pupae become adults, each one was taken into a separate petri dish to prevent intraspecific cannibalism. The longevity of the adult insects was calculated by recording the day they died. According to statistical analysis, the rate of dead larvae in the first 24 hours was 83.32±8.43% in laurel, 48.88±11.45% in cassia, and 62.00±18.54% in fennel. Pupal development time was 37.63±3.69 in laurel, 39.60±1.78 in senna, 34.05±1.05 in fennel, while the adult development time was 8.60±0.77 in laurel, 8.96±0.33 in cassia, 8.29±0.43 in fennel. The longevity was 37.87±2.52 in laurel, 28.28±2.71 in senna and 31.94±1.35 in fennel. No statistical difference was observed between other parameters except lethality percentages. This study reveals that plant extracts can be developed as new environmentally friendly control agents against stored product pests.
Keywords: *Laurus nobilis, Cassia angustifolia, Foeniculum vulgare,* Pests, Herbal Essential Oils, Ecological Insecticide

INTRODUCTION

Tenebrio molitor L. 1758 is a pest that causes very serious economic losses in stored products such as oats, barley, wheat, corn, bran and flour. *T. molitor* larvae feed on the product and cause deterioration of product quality with their feces. They usually hatch within 10-12 days and the larval development periods vary depending on temperature and humidity for 3-18 months. Pupal developmental periods can also take 5-10 days depending on temperature and humidity. After they become adults, they can live for 2-3 months and can reach a weight of 300 mg. Adult insects can lay eggs 4-17 days after mating (Baş and Ersoy, 2020). Adult females can lay up to 300-500 bean-shaped, white and sticky eggs (Rayees et al., 2021).

Producers try to fight against this damage caused by *T. molitor* to stored products with chemical methods. However, in recent years, with the understanding of the damage caused by chemical control to human and environmental health, producers are looking for new alternative control methods. Researchers are also working to produce biopesticides that are environmentally friendly and do not harm environmental health. Studies in this field with medicinal and aromatic plant essential oils and extracts have come to the fore in recent years (Rayees et al., 2021). As it is known, medicinal aromatic plants have been used in alternative medicine since ancient times, as well as insect repellent and insecticide. Plants produce some chemicals to adapt to

changing environmental conditions or to protect themselves from other harmful organisms (Aydın and Mammadov, 2017). Among these chemicals, which are called secondary metabolites, saponins, terpenoids, phenols, alkaloids, glycosides and tannins are very important and have been used for centuries against harmful insects. At the same time, they are used as raw materials in many sectors such as medicine, chemistry, food, textile, cosmetic industry and agriculture (Bourgaud et al., 2001). The effect of these metabolites is through contact, ingestion or inhalation. They provide protection to the plant by affecting the nervous system of insects. Or, they act as allelochemicals used to ensure interspecies interaction, causing the insect to move away from the plant, prevent its feeding or die (Ebadollahi et al., 2022). The laurel (*Laurus nobilis*) plant is an evergreen plant belonging to the Lauraceae family. The oil of the laurels, which are grown in abundance in the Sinop region and grown in the Sinop Province, is also extracted in the province of Samsun (Alacam district), which is close to the region. For this reason, it is economically very important in the region and is a serious source of employment. This is the main reason for choosing it in this study. In this study, it was aimed to contribute to the field of biological control by determining lethal, pupal, adult developmental periods and longevity on *T. molitor* of laurel (*Laurus nobilis*), senna (*Cassia angustifolia*) and fennel (*Foeniculum vulgare*).

MATERIAL AND METHOD

This study was designed to determine the effects of laurel (*Laurus nobilis*), senna (*Cassia angustifolia*) and fennel (*Foeniculum vulgare*) plant extracts on lethality, larval, pupal and adult developmental period, adult longevity on *T. molitor*. Trials were carried out under laboratory conditions of 26±2 ºC and 75±5% relative humidity.

Obtaining plant extracts: The laurel plant extract was taken from a factory that produces laurel oil in the Samsun-Alaçam region, while the others were obtained from herbalists. All plant extracts were dissolved in 95% (Merck) ethanol to create 10% (v/v) solutions. These 10% extract solutions of the plants were used in the experiments.

5 experimental groups were formed for each plant extract. 10 larvae (5x10=50) were placed in each experimental group. A total of 50 larvae were used for each plant extract. Flour+wheat flour was added for nutritional needs and potatoes were added for water needs. On the first day, small cotton pieces impregnated with 10% plant extract were placed in the experimental groups, and the petri dishes were wrapped with parafilm. After 24 hours, the numbers of surviving and dead larvae in the petri dishes were recorded. Lethality percentages were corrected and calculated according to the Abbott (1925) formula. Surviving larvae were left in the specified laboratory conditions and expected to pupae. Insects that died as pupae were also recorded on the day they died. The pupation day of the pupae was recorded. The surviving pupae were kept in the specified laboratory conditions to become adults. The day they became adult was also recorded. Each one was taken into separate petri dishes to prevent intraspecies cannibalism. The longevity of the adult insects taken into separate petri dishes were also recorded on the day they died, and their longevity were calculated. The same method was used for the control groups. Distilled water was used instead of plant extract in the control groups.

10 larvae (50 larvae) in 5 replicates for each plant, 10 larvae (50 larvae) in 5 replicates for the control groups, a total of 50x3= 150 for the experiments and 50 larvae for the control group were used.

Statistical analyzes: SPSS 22.0 program was used in data analysis. First of all, Abbott formula was used in determining lethality rates.

$[(A - B)\ A] \times 100$,

A, number of live insects in control group, %;

B, number of live insects in application group, %;

and corrected lethality rates (%) (Abbott, 1925).

Whether the data were normally distributed was evaluated according to Shapiro–Wilk test ($p > 0.05$). It was determined that all sample groups were normally distributed. One-Way Anova test was performed because the percentage of deaths while pupae, pupal developmental period, adult developmental period and the sample number of larvae and insects used in longevity experiments were not equal. Scheffe test was performed to determine between which groups there was a difference. Since the sample size of the larvae used for pupal development time was equal, the One-Way Anova test was applied to these groups, and the TUKEY-HSD test was performed to determine between which groups there was a difference. The significance level was taken as 0.05 for all statistical comparisons in the study.

RESULTS AND DISCUSSION

Table 1*. Lethality percentage of insects that die as larvae and pupa in the first 24 hours.

	Larvae	Pupae
Control	0.00±0.00 a	14.22±6.75 a
Laurel	83.32±8.43 b	70.77±7.70 b
Senna	48.88±11.45 c	43.73±9.86 ab
Fennel	62.00±18.54 b	30.47±6.66 a
	$F_{3,16}$= 9.142 p=0.001	$F_{3,12}$= 7.321, p=0.005

*There is a difference between values indicated by different letters in the same column $p<0.05$

Table 2*. Larval, pupal developmental periods and longevity of *Tenebrio molitor*.

	Larval Developmental Period	Adult Developmental Period	Longevity
Control	37.10±1.30 a n=50	9.32±0.35 a n=50	29.72±1.24 a n=50
Laurel	37.63±3.69 a n=8	8.60±0.77 a n=8	37.87±2.52 a n=8
Senna	39.60±1.78 a n=25	8.96±0.33 a n=25	28.28±2.71 a n=25
Fennel	34.05±1.05 a n=19	8.29±0.43 a n=17	31.94±1.35 a n=17
	$F_{3,97}$=1.523, p= 0.213	$F_{3,96}$ =1.024, p=0.386	$F_{3,96}$=2.192, p=0.94

*There is a difference between values indicated by different letters in the same column $p<0.05$

According to statistical analysis, the rate of dead larvae in the first 24 hours was 83.32±8.43% in laurel, 48.88±11.45% in cassia, and 62.00±18.54% in fennel. Lethality rates after pupae were 70.77±7.70 in laurel, 43.73±9.86 in cassia, and 30.47±6.66 in fennel (Table 1).

Pupal developmental period was 37.63±3.69 in laurel, 39.60±1.78 in senna, 34.05±1.05 in fennel, while the adult development period was 8.60±0.77 in laurel, 8.96±0.33 in senna, 8.29±0.43 in fennel. The longevity were 37.87±2.52 in laurel, 28.28±2.71 in senna and 31.94±1.35 in fennel. No statistical difference was observed between other parameters except lethality percentages (Table 2).

Crop and stored product protection mainly relies on the application of synthetic insecticides. However, in recent years, although pesticides have suppressed the population of pests, their harm to human and environmental health has been understood. For this reason, researchers have turned to alternative methods. One of these control methods is plant-derived extracts and essential oils obtained from environmentally friendly medicinal and aromatic plants. In fact, these essential oils are secondary metabolites produced in nature by many plant species to protect themselves against harmful plants and animals. It has also been known since ancient times that they exhibit toxic and/or repellent activity against various insects (Isman,

1995; Aydın and Mammadov, 2017). It is generally accepted that essential oils are promising active ingredients for biopesticides (Ebadollahi et al., 2022).

In the current study, the most sensitive life form to laurel from essential oils is the larva. In pupa form, the highest sensitivity was observed in senna plant extract. Plata-Rueda et al. (2017) in a study where they examined the lethal and repellent effects of 6 different concentrations of garlic oil on *T. molitor*, they found the highest lethal effect in the larval form. Adult and pupal forms followed this. They also determined that plant extracts had a repellent effect. Diallyl disulfide was found to have the most lethal effect among the components of garlic oil. Plata-Rueda et al. (2021) investigated the death, survival, respiratory and behavioral responses of thyme (Oregano) in a study with *T. molitor* larvae and pupae They determined that the main component in Oregano essential oil is carvacrol (25.6%). High mortality was observed in larvae (LD50 = 3.03 µg insect-1), pupae (LD50 = 5.01 µg insect-1) and adults (LD50 = 5.12 µg insect-1) treated with Oregano, with survival rates of 65-54%, respectively, 38-44%, 30-23% and 6-2%. Low respiratory rates were observed at different developmental stages of *T. molitor* after exposure to the oil. It also showed the behavioral avoidance response from the essential oil, causing a repellent repellent effect in larvae and adults.

Extracts and essential oils are obtained from many plant species belonging to Lamiaceae, Meliaceae, Asteraceae, Apiaceae, Labiateae, Piperaceae and Annonaceae plant families. These essential oils are used in many areas such as medicine, insect repellent, insecticide, cosmetics and raw materials in the pharmaceutical industry (Schoonhoven, 1982; Isman, 1995; Ramya et al., 2013). Plants of the Apiaceae family contain furanocoumarins, which have toxic effects against insects (Hekimoğlu and Altındeğer, 2006). Plant essential oils contain compounds such as terpenoids, alkaloids, and flavonoids.

Plant-based essential oils are not only toxic to harmful insects, but also have a feeding inhibitory and repellent effect (Adamski et al., 2016; Çetin and Elma, 2017). In the present study, there was no statistical difference between pupal and adult developmental periods of insects that did not die after being exposed to essential oils and became pupal stage. Pupal period was 37.63±3.69 in laurel, 39.60±1.78 in senna and 34.05±1.05 in fennel. Adult developmental periods were determined as 8.60±0.77 in laurel, 8.96±0.33 in senna and 8.29±0.43 in fennel. Longevity of insects was determined as 38.87±2.52 in laurel, 28.28±2.71 in senna and 31.94±1.35 in fennel (Table 2). There was no statistical difference between all these values and control groups. Therefore, we can say that these plants do not affect biological parameters such as longevity, pupal developmental period, adult developmental period, however, they definitely have a mortality effect in terms of lethality. The main components of laurel extract are eucalyptol and terpinyl acetate. The main components in the plant extract affect the tested parameters. Plant essential oils and extracts contain many compounds such as monoterpenoid, sesquiterpene, diterpenoid. While these compounds kill some insects directly, they may not kill others. However, the fact that these compounds do not kill insects does not mean that they are not effective. Essential oils can cause developmental disorders. It can also affect their activities such as laying eggs, courtship behavior or mating. The egg-killing effect of monoterpenoids occurs only when the nervous system begins to develop and is a neurotoxin (Wang and Wang, 2003; Campolo et al., 2018). Sönmez (2022) determined that eucalyptus (*Eucalyptus globulus*), thyme (*Thymus vulgaris*), laurel (*Laurus nobilis*) ve walnut (*Juglans regia*) extracts had an inhibitory effect on ovulation as well as lethality in a study on *Acanthoscelides obtectus* and *Callosobruchus maculatus* adults. Sönmez (2021) investigated the effects of fennel (*Foeniculum vulgare*), senna (*Cassia angustifolia*), St. John's Wort (*Hypericum perforatum*), ginger (*Zingiber officinale*) and zirnik (*Teucrium kotschyanum*) herb extract on the mortality rate and number of eggs laid by *C. maculatus*. As a result of the study, mortality rates were determined as 98.3 ± 1.6% in fennel and 5.00 ± 2.33% in senna. The number of eggs they laid was also found to be significantly lower than the control group.

Studies investigating the insecticidal, insect repellent, nutritional and growth inhibitory, and egg laying effects of plant-based essential oils against unwanted harmful insects are continuing rapidly (Ebadollahi et al., 2022). Buneri et al. (2019) investigated the lethal effect of Himalayan cedar (*Cedrus deodara*) on *T. molitor* larvae. They stated that the mortality effect was high, protein levels increased and feeding inhibitory activity was observed in insects treated with Himalayan cedar oil. Martinez et al., (2018) found that *T. molitor*, cinnamon and clove oil showed toxic activity in adult, larval and pupal stage insects in a study. They stated that the chemical composition of eugenol contained in the cinnamon plant showed the strongest toxic activity in all three life forms of the insect. Baş and Ersoy (2020) reported that essential oils obtained from *H. perforatum* plant increased the mortality rate of *T. molitor* in parallel with the increase in the concentration of essential oil exposed and has an important potential in the fight against this insect. Adamski et al., (2016) investigated the effects of *Solanum tuberosum* and *Lycopersicon esculentum* leaf extracts on *T. molitor* and *Harmonia axyridis*. It has been stated that different concentrations (1, 10, 100 and 1000 ppm) can be repellent or attractive, although they are not toxic to insects.

CONCLUSIONS

The insecticidal effect of the plant extracts used on insects has been proven by many studies. In addition, in recent years, studies on the effect of ovulation physiology and reduction of adult emergence are gaining momentum. In the present study, it was determined that laurel > fennel > senna plant extracts had a lethal effect on *T. molitor* larvae. Therefore, various plant extracts can be developed as new environmentally friendly control agents against harmful insects. In particular, long-release tablets can be developed to combat these insects both in the field and in warehouses.

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THE IMPORTANCE OF LAUREL PLANT EXTRACT IN FIGHTING HARMFUL INSECTS

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ABSTRACT
Stored product pests are responsible for the loss of significant amounts of product in silos. Pests cause a great deal of damage and economic loss to the harvested crop every year. Many control methods are used in the fight against these pests, both in the field and in the warehouses. Due to the easy and economically cheap application of pesticides, it forces the producers to figth with insecticides. However, in recent years, with the development of insect resistance to insecticides and the understanding of their damage to the environment, many producers and researchers are looking for alternative ways to figth these harmful insects. One of these alternative methods is the use of plant essential oils to figth harmful insect species. Aromatic plants have been used as insect repellent and killer since ancient times. Laurel (*Laurus nobilis* L.) belongs to the Lauraceae family and is an evergreen tree. Its leaves and fruits are used extensively in the pharmaceutical and perfumery industries. The most important product of laurel is its oil and essence. Its fruit contains 17-25% fixed oil. Fruits contain more oil than leaves. 41 different compounds were detected in essential oils obtained from laurel leaves. The main components of laurel extract are 1,8-cineole (52.88%), α-terpinyl acetate (11.77%) and sabinene (8.05%). Laurel essential oil has attracted a lot of attention in recent years due to the repellent and lethal effect of these compounds on harmful insects. In this study, the biological properties of the laurel plant and its extracts are emphasized, especially in the fight against harmful pests.

Keywords: Plant Essential Oils, Pests, Insecticide, Pesticide, Odor

INTRODUCTION

The laurel plant belongs to the subfamily Lauroideae of the Lauraceae family. There are two species: Mediterranean laurel (*Laurus nobilis* L.) and Azores laurel (*Laurus azorica* (Seub.) Franco). Laurel is a perennial, evergreen, about two meters tall and densely branched plant species (Sharma, 2017). The homeland of laurel is Anatolia and it is seen in many parts of Turkey's Mediterranean, Aegean, Marmara and Black Sea regions. The provinces where the plant is widely seen are Bursa, Balıkesir, Yalova, Istanbul, Kastamonu, Sinop, Zonguldak, Rize, Trabzon, Muğla, İzmir, Mersin, Antalya and Kahramanmaraş (Koçer and Ayanoğlu, 2021). Although it has been seen on the entire coastline of the Mediterranean since ancient times, today it spreads in the western Mediterranean Basin as well as in countries such as Turkiye, Greece, Romania, Algeria, Morocco, France, Libya, Belgium, Crimea, Mexico, Albania, Spain, Syria, Portugal, Canary Islands (Figure 1; Ayanoğlu et al., 2010). Its leaves are fragrant. Its fruit is similar to an olive. Apart from its black, chickpea-sized seeds, it has a very oily thin wall. The used part of the plant is the leaves and fruits. Laurel leaves and fruit are aromatic and stimulating. It has been determined that there is approximately 17-25% fixed oil in the fruit (Özer et al., 2019). 41 different compounds were detected in essential oils obtained from laurel leaves. These detected compounds constitute 90-94% of the herbal essential oil obtained from the laurel leaf. In essential oils obtained from laurel leaves, 1,8-cineol is the main compound, as well as pinene, sabinene, linalool, eugenol, eugenol acetate, methyleneeugenol, terpinol

acetate, phelandrene, other esters and terpenoids. Laurel essential oil is obtained from the leaves, bark or fruit of the plant by solvent extraction or steam distillation method. The chemical content of the obtained oil varies according to the harvest period, harvest time, geographical region and the organs from which the oil is extracted. In contrast to the sesquiterpenes in the wood part, monoterpenes such as α-pinene, β-pinene and 1,8-cineol were found to be the main components in the essential oil extracted from the bark part (Özer et al., 2019). It has been stated that the essential oil ratios of the laurel plant obtained from 100 locations in the 4 geographical regions (Black Sea, Marmara, Aegean and Mediterranean) where the laurel plant is most grown in Turkey vary between 0.4% and 4.5%, and the average essential oil ratio is 1.78%. The essential oil components are 1,8-cineole (31.87-67.56%), α-terpinyl acetate (4.09 - 22.22%), α-terpineol (0.94-16.08%), linalool (0.40 -13.04%), terpinene (2.31- 9.22%) and sabinene (0.56 -9.08%) (Karık et al., 2015). In another study comparing the chemical content of laurel essential oil from different locations in Hatay, 1,8-cineole (46.61-59.94%), α-terpinyl acetate (11.94-25.70%), α-pinene (3.66-2.61%) sabinene (14.05-7.83%), terpinene (1.82 - 2.20%) were determined (Sangun et al., 2007). As can be seen, many parameters such as the criteria during and after the harvest, the development period of the plant, the harvest time, the climatic conditions of the region where the plant is located, the direction of the plant cause the content of the oil obtained to change.

In addition to all these, the laurel plant has a special place in world cuisine and alternative medicine. The leaves, which are rich in essential oil, can be used as a spice to give a nice smell to the dishes and as a preservative in canned food. Laurel tea is also used extensively in alternative medicine. In addition to its appetizing, blood circulation and blood sugar regulator, carminative, digestive, diuretic, antipyretic, muscle relaxant properties, it is known to be good for tonsillitis and colds when taken by mouthwash. It is also known that laurel oil, which is used safely on dry, oily or allergic skin, nourishes the hair and gives softness and shine. Laurel oil is also used in the production of perfumed soap, in the food, beverage, pharmaceutical, chemical and cosmetic industries. It is the raw material of traditional laurel soap. 1 kg of laurel oil is obtained from approximately 10 kg of laurel seeds. The oil obtained from its leaves and fruits also finds use in the cosmetics, pharmacology and food industries (Sharma, 2017).

The leaves of the laurel plant are highly aromatic. Therefore, it is grown commercially. Among the countries that trade the laurel plant are Türkiye, Mexico, Portugal, Italy, Spain, France, Algeria and Morocco. In Turkey, the natural spread of the laurel plant is also quite common (Figure 2). It is the largest exporting country in the world (Yilmaz and Çiftçi, 2021). The Latin baccalaureus means laurel fruit. In ancient times, athletes who were successful in Olympic competitions were awarded with a crown made of laurel leaves on their foreheads. In the Roman period, in 342 BC, there was a wreath of laurel plants on the gold coins. It is known that the Greeks and Romans used wreaths made of the leaves of this plant as a crown in sports and war victories. It is also known that during the ancient Romans, laurel leaves were believed to have a protective effect against lightning strikes and that they had a laurel branch in stormy weather (Yilmaz and Çiftçi, 2021). The Romans believed that standing under a laurel tree would protect a person from plague infection. Laurel wreaths were worn by healers during healing ceremonies and when treating patients, to increase positive healing energy and to ward off negative energy that could circulate around the sick room. The laurel leaf was also burned in the sick room after the illness had passed to purify it and remove any remaining sickness vibrations. The Romans called it the plant of good angels. In the Middle Ages, laurel was believed to provide protection against both lightning and witches. In addition to these, the laurel plant has also been used in the treatment of many diseases. It has been used mainly as an aid to digestion and in the treatment of bronchitis and flu, to treat rheumatism, earaches, indigestion, sprains, and to promote sweating, as well as to treat various types of cancer.

In recent years, studies with alternative medicine and aromatic medicinal plants have shown that laurel can be used in many other areas in addition to its known benefits. Various pharmacological activities such as wound healing property, neuroprotective activity, antioxidant activity, antiulcerogenic activity, anticonvulsant activity, analgesic and anti-inflammatory, antimutagenic activity, immunostimulant activity, antiviral activity, anticholinergic activity, antibacterial activity, insecticidal and repellent, antifungal and acaricidal activity have been reported.

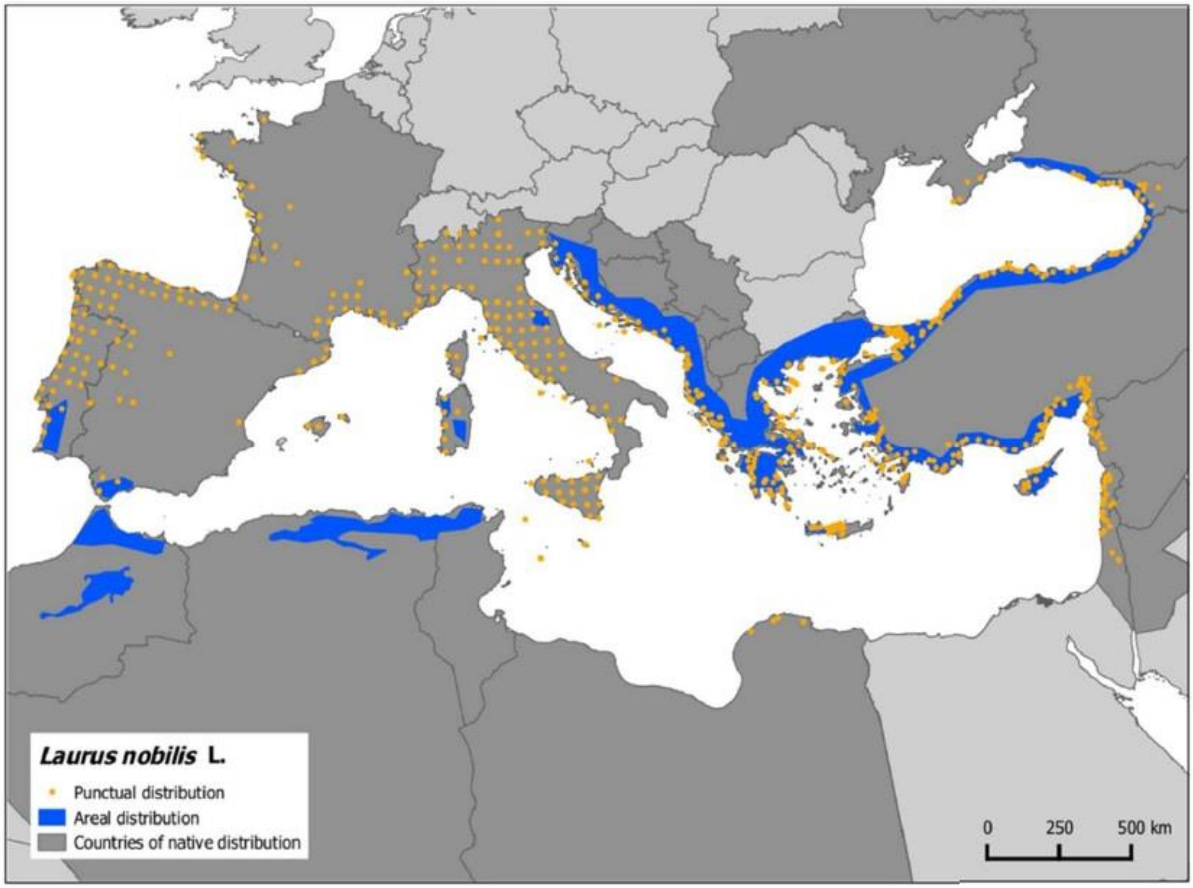

Figure 1: Distribution of *Laurus nobilis* L. (FAO)

Figure 2: Laurel (Provincial Basis) Distribution Map (OGM, 2022).

Major consumer countries in the world meet 95% of their laurel needs from Türkiye. Türkiye, dry laurel leaf in the world; It is the most important producer and seller. Laurel has a share of 10% in our country's natural plant exports.

Uses of laurel plant as insecticide and insect repellent

Approximately 7-50% of the crops harvested each year are destroyed by harmful insects. Tons of pesticides are used every year to prevent this yield loss. The use of pesticides is increasing every year in the world. 48.000 tons of pesticides are used in Germany, 24.000 tons in Poland, more than 18.000 tons in Great Britain, 62.000 tons in Italy and 1.7 million tons in China (FAOSTAT, 2017). Pests also harm the economy of many countries as they are responsible for the loss of harvested crops. Harmful insects can contaminate food products both in the field and in silos, pollute with their excrement, and cause weight loss as insect larvae feed on these products. Pesticides used can kill or suppress population size of these pest populations. But in addition to all these benefits, they cause serious damage to the ecosystem. Because insects develop resistance to these pesticides used every year, these pesticides leave residues on agricultural products, and pollute the soil and water, producers now prefer to use environmentally friendly methods. For example, methyl bromide and ethylene dibromide are banned in many countries due to their carsonogenic effects or their role in ozone depletion (Rajendran and Sriranjini, 2008). However, the ease of application of pesticides and the fact that they are quite cheap still cause them to be preferred. One of the methods that has attracted attention in this field in recent years is plant extracts and essential oils. In fact, since ancient times, aromatic plants have been used either by burning or their essential oils have been used as a killer or repellent against insects. With the increasing interest in organic agriculture, the use of these natural products is also increasing. Despite this, the percentage of use is still very low. Natural products make up a low percentage of available products. However, the use of plant-derived extracts in Integrated Pest Managements (IPM) has become one of the applied methods. Some researchers use the term biopesticide very cautiously. And excludes plant extracts from this term, while others include it. According to European Union regulations, plant extracts are evaluated in the group of bioinsecticides (Marchand, 2017). In addition, the United States Food and Drug Administration (FDA) has recognized plant essential oils (botanical pesticides) as safer than synthetic pesticides, which cause an increased risk of ozone depletion, neurotoxic, carcinogenic, teratogenic, and mutagenic effects in non-target organisms, and resistance in insects (Regnault-Roger et al., 2012).

It has been proven that plant essential oils stimulate the sense of smell and receptors of insects, keeping them away from agricultural products and giving positive results on the ecosystem (Hikal et al., 2017). At the same time, some essential oils prevent harmful insects from feeding, causing them to starve. Some have harmful effects on the growth and development of insects, reducing the weight of the larva, pupa and adult stages and prolonging the development stages (Spochacz et al., 2018). It reduces the survival rates of larvae and pupae as well as the adult emergence rates. Chemosterilants prevent insects from reaching sexual maturity by causing temporary or permanent sterility of one or both sexes and are chemicals used to control pest populations. Some plant essential oils are used as chemosterilants (Shaalan et al., 2005). For example, at the physiological level, azadirachtin blocks the synthesis and release of molting hormones from the prothoracic gland, causing inhibition of molting (ecdysis) in immature insects and leading to sterility in adult insects (Isman, 2006).

The aroma given to the laurel plant, especially by 1,8-cineol, which is the main compound of the laurel plant, causes insects to move away from this plant. The main components of laurel extract are 1,8-cineol (eucalyptol) and terpinyl acetate. The chemical compounds of laurel have been identified as 1,8-cineol, sabinene, α-terpinyl acetate, α-pinene and β-pinene. The chemical composition of laurel essential oils has been studied by many researchers. All researhers identified 1,8-cineol as the main component. The amount of 1,8-cineol varies between 31.4% and 56.0%, depending on the geographical location of the laurel plant and the country in which it is located (Karık et al., 2015; Alejo-Armijo et al., 2017; Anzano et al., 2022). The effects of plant essential oils on insects are manifested by contact, inhalation or ingestion. In particular, monoterpenoids paralyze the nervous system of insects and cause them to die. In addition, in

recent years, the effects of medicinal aromatic plants as well as laurel to prevent egg laying, prevent mating, and prevent courtship behavior have been determined, and they show promise in the fight against these harmful insects in the long term. Pinto et al. (2022), in a study they conducted against *Tuta absoluta* with essential oils including laurel plant, they found that laurel essential oil had an inhibitory effect on ovulation. Kanat et al. (2003), the essential oils of many plants against pine processionary beetle, *Thaumetopoea pityocampa* larvae, Jemaa et al. (2012) determined that *L. nobilis* essential oil has an insecticidal effect against *Rhyzopertha dominica* and *Tribolium castaneum*. Plant essential oils have different effects depending on the plant species or the physiological characteristics of insect species. Botanical insecticides can be classified into six groups; insect repellents, feed blockers, toxicants, growth retardants, chemosterilants and attractants (Rajashekar et al., 2012). Chahal et al. (2016) and Isikber et al. (2006) showed that essential oil obtained from the leaves of the laurel plant was associated with *T. castaneum*, Rozman et al. (2006) found that it had a toxic effect against *Sitophilus granarius*.

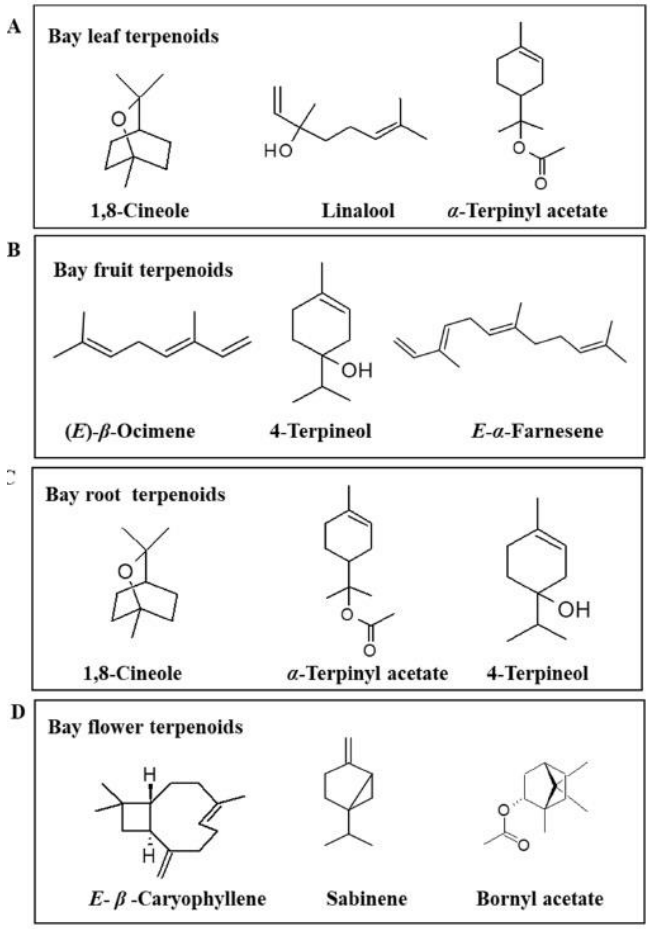

Figure 3. Representative terpenoids biosynthesized by Laurel (*Laurus nobilis* L.). (A) Laurel leaf terpenoids; (B) Laurel fruit erpenoids. (C) Laurel root erpenoid; (D) Laurel flower erpenoid (Paparella et al., 2022).

Kırpık et al. (2019) laurel and zahter (*Thymbra spicata* L.) essential oils on *R. dominica* and *Oryzaephilus surinamensis* in a study they found the fumigant toxicity rate of laurel to be 100% after the first 24 hours. Teke and Mutlu (2021) investigated 6 types of essential oils, including laurel, against *S. granarius* and *T. castaneum*. They found that all plant essential oils had fumigant, lethal and repellent effects, and there was a significant decrease in the F1 generation compared to the control groups. Papachristos and Stamopoulos (2002) conducted a repellent, toxic and reproductive inhibitory study of thirteen essential oils of plants, including *L. nobilis*, against *Acanthoscelides obtectus*. They found that laurel essential oil has a high

repellent and toxic effect, reducing fecundity and adult emergence. Regnault-Roger and Hamraoui (1994) in a study they conducted with the plant extracts of *L. nobilis* and *A. obtectus*, found that the life span of the adults, the number of eggs laid, the number of adults hatched and the adult hatching/egg-laying ratios differed according to the plant species, but all plant extracts provided a decrease in the specified parameters.

CONCLUSIONS

Essential essential oils obtained from medicinal aromatic plants have insecticidal properties. It is an excellent alternative to chemical pesticides to avoid the negative side effects of synthetic insecticides and protect crops from harmful insects. Laurel essential oil also has different forms of action on different types of insects. Therefore, it has the potential to be used safely to suppress or prevent harmful insect populations in products in forests, fields or warehouses.

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INFLUENCE OF POWDERS OBTAINED FROM DIFFERENT PARTS OF STINGING NETTLE (URTICA DIOICA L.) ON TECHNOLOGICAL PROPERTIES AND BIOACTIVE COMPONENTS OF NOODLES

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ABSTRACT
In this study, different parts of the stinging nettle (leaf and stem) were dried and ground to obtain stinging nettle leaf powder (SNLP) and stinging nettle steam powder (SNSP). Those powders were used in noodle production as replaced with wheat flour at 0, 2, 4, 6 and 8% ratios. Color values (raw and cooked), cooking properties (water uptake, volume increase and cooking loss), firmness, antioxidant activity (DPPH, FRAP and CUPRAC) and phenolic contents (free, bound and total) of noodles were determined. L*, a* and b* values of raw control noodle was found as 72.69, -0.52 and 31.72, respectively. Those color values were 35.63, -7.67 and 8.66 for raw noodles containing 8% SNLP and 52.75, -4.41 and 22.23 for raw noodles containing 8% SNSP, respectively. SNLP and SNSP addition significantly ($p<0.05$) affected all color parameters in raw and cooked noodle samples. Compared to the raw noodle samples, the yellowness value of SNLP and SNSP added noodles decreased with cooking. Water uptake and volume increase values of the noodles containing %4 and more SNLP and SNSP reduced compared to control. Even the lowest SNLP and SNSP addition ratio (2%) increased the free, bound and total phenolic content of the noodles, while antioxidant activity (measured by different methods) of noodles increased with 4% and more addition ratios.

Keywords: Stinging nettle, leaf powder, stem powder, noodle

INTRODUCTION
The stinging nettle (*Urtica dioica* L.) is a perennial herbaceous plant with spiny leaves, belonging to the nettle family (Urticaceae) (Bhusal et al., 2022). Stinging nettle leaves are good sources of protein, dietary fiber, minerals (calcium, iron, magnesium, manganese, zinc, phosphorus, potassium, copper and selenium), vitamins and bioactive compounds (Shonte et al., 2020). Stinging nettle leaves are generally used in dishes such as spinach. And also, it is added to soups, salads, herbal tea or decocted tea as well as in dried form for winter use (Guil-Guerrero et al., 2003). Stinging nettle has antiproliferative, anti-inflammatory, antioxidant, analgesic, anti-infectious, hypotensive, and antiulcer characteristics, as well as the ability to prevent cardiovascular disease, in all parts of the plant (leaves, stems, roots, and seeds) (Bhusal et al., 2022). In the literature, there are various studies in which nettle leaves and seeds are used as an ingredient in cereal products such as pasta, noodles, bread, biscuits, etc. (Adhikari et al., 2016; Alemayehu et al., 2016; Man et al., 2019; Đurović et al. 2020; Foret, 2021; Krawecka et al., 2021; Perez, 2022).

In this study, it was aimed to investigate the effects of powders obtained from the leaves and stems of stinging nettle on some technological and sensory properties noodle.

MATERIAL AND METHOD

Materials

Fresh stinging nettle (Urtica dioica L.) plant was hand-picked from Meram, Konya, Turkey. Raw materials (flour, egg and salt) used in noodle production were obtained from a local market in Konya.

Methods

Production of stinging nettle powders

The leaves and stems of fresh stinging nettle were separated and dried at 55 C until the moisture content reached below 10%. Dry leaves and steam ground a grinder (particle size below 500 μm) for obtaining stinging nettle leaf powder (SNLP) and stinging nettle steam powder (SNSP).

Noodle production

Control noodle was prepared from 100 g refined white flour, 40 g whole egg, 0.5 g salt and 30 ml distilled water. For other noodle formulations, refined wheat flour was replaced by SNLP and SNSP at 2, 4, 6 and 8 % levels. The noodles were made according to the procedure described by Özkaya et al. (2001). Briefly, noodle ingredients were mixed in the mixer (Hobart N50, Offenburg, Germany) at a low speed For 8 min and the dough obtained after mixing rested for 20 min. Noodle strips were obtained by passing the dough pieces through the noodle machine (Shule Pasta Machine, China), and the noodles were left to dry under room conditions for 3 days.

Color measurement

Color measurement was carried out by Minolta CR-400 (Minolta Camera, Co., Ltd., Osaka, Japan) in terms of L*, a* and b* values. Hue angle and saturation index (SI) values were calculated using a* and b* values. Hue angle; a*>0 and b*>0, if arctan [b*/a*]; a*<0 and b*>0, arctan [b*/a*] + 180°, SI; (a*2+b*2)1/2.

Cooking properties and firmness

Water uptake and volume increase values of gluten-free pasta samples were determined according to Oh et al. (1985) and Özkaya and Kahveci (1990). For determination of weight increase values, 20 g of noodle sample was cooked in 250 ml of boiling distilled water. The weight differences of raw and cooked noodle samples were determined as %. For the volume increase test, the cooked and filtered noodle samples were taken into measuring cylinders filled with pure water, the volume of the water they overflowed was determined, and the volume increase values were calculated from the values obtained. Cooking loss was determined after filtering 20 g of noodle sample cooked in 250 ml of boiling water. The filtrate water was dried in a drying cabinet at 135 ºC.

The firmness of the noodle samples was determined using a texture analyzer (Model TA-XT Plus, Stable Micro System Limited, Surrey, UK) based on the AACC Standard Method No: 66-50 (AACC, 2000).

Phenolic content and antioxidant activity

The free and bound phenolic content was determined based on Folin-Ciocalteu colorimetric method as described by Naczk and Shahidi (2004). Total phenolic content was calculated as the sum of free and bound phenolic content. Phenolic content was expressed as gallic acid equivalents (mg of GAE/100 kg). The antioxidant activity of samples was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Gyamfi et al., 1999; Beta et al., 2005), ferric reducing antioxidant power assay (FRAP) (Yilmaz, 2019) and cupric ion reducing antioxidant activity assay (CUPRAC) (Apak et al., 2008).

Statistical analysis

SPSS statistical program version 22.0 (SAS Institute Inc., Cary, NC, USA) was used for statistical data analysis. Mean values were compared with Duncan's multiple range test.

RESULTS AND DISCUSSION

Color values of noodle samples

Color values of raw and cooked noodles containing SNLP are given in Table 1. Increasing SNLP ratio in noodle formulation decreased L*, a* and b* color values of raw and cooked noodles compared to their control. The lowest L*, a* and b* values in raw noodle samples were obtained at the highest SNLP usage ratio. In the cooked samples, the lowest a* value was obtained at the ratio of 2-4% SNLP, and the lowest b* value was obtained at the ratio of 6-8% SNLP. When the raw and cooked noodle samples were compared among themselves, it was seen that the color values of L*, a* and b* decreased in general with the cooking process. This decrease may be related to the leaching of color pigments into the cooking water during cooking.

Table 1. Color values of raw and cooked noodle samples containing different ratios of SNLP

	Raw			Cooked		
	L*	***a****	***b****	***L****	***a****	***b****
Control	72.69±0.30a	-0.52±0.03a	31.72±0.63a	69.66±0.31a	-3.99±0.16a	23.35±0.32a
2% SNLP	56.55±0.40b	-3.56±0.07b	26.51±0.02b	47.66±0.13b	-8.18±0.28c	18.11±0.30b
4% SNLP	42.84±0.33c	-6.09±0.11c	16.33±0.13c	41.72±0.38c	-7.86±0.12c	14.22±0.15c
6% SNLP	37.08±0.17d	-6.60±0.09d	10.26±0.31d	32.39±0.88d	-6.95±0.19b	10.77±0.40d
8% SNLP	35.63±0.23e	-7.67±0.08e	8.66±0.12e	33.77±0.11e	-6.76±0.12b	10.37±0.37d

Means followed by the different letters within a column are significantly ($P < 0.05$) different.

Table 2. Color values of raw and cooked noodle samples containing different ratios of SNSP

	Raw			Cooked		
	L*	**a***	**b***	**L***	a*	b*
Control	72.69±0.30a	-0.52±0.03a	31.72±0.63a	69.66±0.31a	-3.99±0.16a	23.35±0.32b
2% SNSP	63.14±0.53b	-3.25±0.08b	27.73±0.36b	66.31±0.10b	-5.25±0.00b	25.72±0.14a
4% SNSP	61.78±0.16c	-4.59±0.06c	25.17±0.20c	61.56±0.33c	-5.15±0.51b	21.27±0.21c
6% SNSP	55.17±0.54d	-4.53±0.05cd	22.88±0.58d	55.93±0.63d	-5.08±0.10b	20.85±0.90c
8% SNSP	52.75±0.65e	-4.41±0.06d	22.23±0.75d	55.14±0.76d	-4.75±0.10b	20.67±0.85c

Means followed by the different letters within a column are significantly ($P < 0.05$) different.

The color values of the raw and cooked noodle samples prepared with SNSP addition are shown in Table 2. As in the samples containing SNLP, the addition of SNSP also reduced the all color values of the raw noodles. High SNSP usage ratios resulted in the lowest raw noodle color values. In the cooked noodle samples, the L* value decreased with increasing SNSP ratio. All SNSP utilization ratios gave a lower a* value in the cooked samples than the control sample. The use of 2% SNSP resulted in the highest b* value among cooked noodle samples. When the cooked and raw noodle samples were compared among themselves, cooked noodle samples had lower a* and b* values than raw ones.

L*, a* and b* values of wheat flour, SNLP and SNSP which are used as raw material in noodle formulation were 93.86, -5.03 and 13.99; 36.54, -7.67 and 11.66; 65.23, -7,50 and 25.72, respectively (data not shown). The lower L*, a* and b* color values of SNLP and SNSP compared to wheat flour were also reflected in the color values of the noodle samples. In studies where green leafy herbs (spinach, fresh mints) were included in the noodle or pasta formulation, it was reported that the color values of the product changed significantly (Dirim and Çalışkan, 2007; Shere et al., 2018). Sobota et al. (2020) used vegetable concentrates and powders in pasta production as natural coloring components and reported that the color of the products was unstable and less resistant to cooking.

Cooking properties and firmness of noodle samples

Cooking properties and firmness values of noodle samples containing SNSP are presented in Table 3. Water uptake and volume increase values of noodles prepared with %4 or more SNLP were found higher than control noodles. Cooking loss was not affected by the SNLP addition ratio. The use of 6-8% SNLP reduced the firmness values of the noodles.

All addition level of SNSP decreased the water uptake compared to the control (Table 4). Volume increase values ranged between 93.75 and 118.75%, and the SNSP usage above 4% decreased the volume increase. The highest SNLP ratio (8%) increased the cooking loss value significantly ($p<0.05$) compared to the control sample. The firmness value of the noodles using 2% SNSP was in the same group as the control sample, the use of 4% or more SNSP caused an increase in the firmness value of the noodles.

Table 3. Cooking properties and firmness values of noodle samples containing different ratios of SNLP

	Water uptake (%)	**Volume increase (%)**	**Cooking loss (%)**	**Firmness (g)**
Control	94.02±0.79a	118.75±1,74a	3.21±0.06a	1702±31.11a
2% SNLP	92.75±1.49ab	117.65±0.76a	3.23±0.16a	1709±29.70a
4% SNLP	90.91±0.65b	112.50±1.47b	3.22±0.14a	1649±45.25a
6% SNLP	83.90±0.41c	112.50±1.48b	3.36±0.11a	1411±15.56b
8% SNLP	82.44±1.02c	106.25±2.83c	3.48±0.24a	1132±18.38c

Means followed by the different letters within a column are significantly ($P < 0.05$) different.

Krawęcka et al. (2021) found cooking loss values and weight increase of pasta prepared 5% stinging nettle powder as 6.19% and 2.45, respectively. Those values were 3.74% and 2.21 in the control sample prepared with durum wheat semolina. In addition, the use of stinging nettle powder up to 5% ratio did not cause a statistical change in the firmness (determined with sensory analysis) values of the pasta samples. Teterycz et al. (2021) reported increasing dry matter losses by the utilization of hemp flour in pasta. Increasing cooking loss values may be due to the disintegration and weakening of the gluten network as a result of the incorporation of the high-fiber component (Krawęcka et al., 2021).

Table 4. Cooking properties and firmness values of noodle samples containing different ratios of SNSP

	Water uptake (%)	**Volume increase (%)**	**Cooking loss (%)**	**Firmness (g)**
Control	94.02±0.79a	118.75±1.74a	3.21±0.06b	1702±31.11d
2% SNSP	90.00±0.64b	118.75±2.49a	3.26±0.21b	1750±11.31d
4% SNSP	90.00±0.79b	112.50±1.30b	3.46±0.08ab	1842±18.38c
6% SNSP	87.05±0.75c	100.00±1.77c	3.49±0.11ab	2025±21.21b
8% SNSP	86.22±0.94c	93.75±1.15d	3.62±0.17a	2283±18.38a

Means followed by the different letters within a column are significantly ($P < 0.05$) different.

Antioxidant activities and phenolic contents of noodle samples

Antioxidant activities of noodle samples containing different ratios of SNLP are given in Table 5. DPPH antioxidant activity values increased with increasing use of SNLP in noodle samples. DPPH antioxidant activity value, which was 266.56 mg TE/kg in control noodles, increased up to 736.78 mg TE/kg in 8% SNLP added noodles. FRAP and CUPRAC antioxidant activity values of noodles increased with the use of 4% and more SNLP usage.

Table 5. Antioxidant activities of noodle samples containing different ratios of SNLP

	DPPH (mg TE/kg)	**FRAP (µmol TE/g)**	**CUPRAC (µmol TE/g)**
Control	266.56±5.41e	0.53±0.05d	92.89±3.44b
2% SNLP	318.78±4.71d	0.61±0.01cd	130.99±2.81b
4% SNLP	395.14±11.76c	0.67±0.03c	228.39±12.36a
6% SNLP	513.24±11.11b	1.11±0.11b	261.18±4.99a
8% SNLP	736.78±7.04a	1.74±0.09a	289.26±3.40a

Means followed by the different letters within a column are significantly ($P < 0.05$) different. DPPH: 2,2-diphenyl-1picrylhydrazyl radical scavenging (TE: Trolox equivalent). FRAP: Ferric reducing antioxidant power CUPRAC: Cupric ion reducing antioxidant capacity.

Table 6. Antioxidant activities of noodle samples containing different ratios of SNSP

	DPPH (mg TE/kg)	**FRAP (µmol TE/g)**	**CUPRAC (µmol TE/g)**
Control	266.56±5.41c	0.53±0.05c	92.89±3.44c
2% SNSP	268.32±4.39c	0.53±0.06c	112.78±8.26b
4% SNSP	311.79±9.29b	0.72±0.01b	132.78±8.24b
6% SNSP	327.56±4.68b	1.05±0.09a	157.83±1.10b
8% SNSP	370.36±15.77a	1.14±0.13a	276.97±9.12a

Means followed by the different letters within a column are significantly ($P < 0.05$) different. DPPH: 2,2-diphenyl-1picrylhydrazyl radical scavenging (TE: Trolox equivalent). FRAP: Ferric reducing antioxidant power CUPRAC: Cupric ion reducing antioxidant capacity.

Antioxidant activities of noodle samples containing different ratios of SNLP are presented in Table 6. DPPH and FRAP antioxidant activity values increased with the use of 4% or more SNSP in noodle samples while CUPRAC values increased at all SNSP usage ratios. When the noodle samples containing SNLP and SNSP were compared among themselves, noodles containing SNLP generally have higher antioxidant activity values than samples containing SNSP.

Phenolic contents of noodle samples containing different ratios of SNLP are shown in Table 7. Free, bound and total phenolic content of noodles ranged between 1945.58-2917.70 mg GAE/kg, 2918.38-4143.14 mg GAE/kg and 4863.96-7060.84 mg GAE/kg, respectively. Even the lowest SNLP usage ratio increased the free, bound and total phenolic content of the noodles. Noodles with 8% SNLP exhibited the highest free, bound and total phenolic content.

When the phenolic content of the noodles containing SNSP was evaluated, the amount of free, bound and total phenolic content increased with the increasing SNSP ratio (Table 8). Compared to the control noodle, with the addition of 8% SNSP, the amounts of free, bound and total phenolic content increased by 1.37, 1.30 and 1.33 times, respectively. When the noodle samples containing SNLP and SNSP were compared among themselves, noodles containing SNLP generally have higher free, bound and total phenolic content values than samples containing SNSP. Nettle leaves are rich in phyto-constituents, mainly polyphenols, flavonoids (kaempferol, isorhamnetin, quercetin, isoquercitrin and rutin) and phenolic acids (caffeic acid and chlorogenic acid), and carotenoids (β-carotene, hydroxyl-β-carotene, luteoxanthin, lutein epoxide, and violaxanthin) (Joshi et al., 2014). Maietti et al. (2021) reported that nettle leaf enrichment of bread provides an increase in total phenols and antioxidant activity. Đurović et al. (2020) determined that the total phenolic content and DPPH antioxidant activity increased significantly in the breads produced using stinging nettle leaf and stinging nettle extract at different ratios.

Table 7. Phenolic contents of noodle samples containing different ratios of SNLP

	FPC (mg GAE/kg)	**BPC (mg GAE/kg)**	**TPC (mg GAE/kg)**
Control	1945.58±11.29d	2918.38±5.22d	4863.96±13.00d
2% SNLP	2495.31±10.51c	3668.10±5.55c	6163.41±13.72c
4% SNLP	2634.87±3.22b	3557.07±4.33c	6191.94±10.42c
6% SNLP	2706.38±16.26b	3816.00±6.34b	6522.38±13.36b
8% SNLP	2917.70±22.21a	4143.14±6.72a	7060.84±16.05a

Means followed by the different letters within a column are significantly ($P < 0.05$) different. FPC: Free phenolic content, BFC: Bound phenolic content, TPC: Total phenolic content (GAE, gallic acid equivalent).

Table 8. Phenolic contents of noodle samples containing different ratios of SNSP

	FPC (mg GAE/kg)	**BPC (mg GAE/kg)**	**TPC (mg GAE/kg)**
Control	1945.58±11.29e	2918.38±5.22e	4863.18±13.00e
2% SNSP	2201.69±10.34d	3236.48±10.34d	5438.17±25.51d
4% SNSP	2456.61±10.95c	3316.42±10.95c	5773.02±25.72c
6% SNSP	2532.81±5.52b	3571.26±5.52b	6104.07±13.35b
8% SNSP	2671.27±5.31a	3793.20±5.31a	6464.47±12.95a

Means followed by the different letters within a column are significantly ($P < 0.05$) different. FPC: Free phenolic content, BFC: Bound phenolic content, TPC: Total phenolic content (GAE, gallic acid equivalent).

CONCLUSIONS

In this study, the usability of powders obtained from leaves and stems of stinging nettle in noodle production was investigated. The effects of SNLP and SNSP, which were used at different ratios in noodle production, on some technological properties and functional components of noodle were revealed. The use of SNLP and SNSP in noodle production was found significant ($p<0.05$) on all color values of raw and cooked noodles. The use of 4% and more powders obtained from different parts of stinging nettle decreased the water uptake and volume increase values of the noodles. High SNSP usage ratio (8%) resulted in a significant ($p<0.05$) increase in cooking loss. The use of 4% or more SNLP or SNSP increased the antioxidant activity values measured by all methods. On the other hand, even the lowest usage ratios of SNLP and SNSP improved the free, bound and total phenolic content.

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STUDY OF THE ANTIOXIDANT ACTIVITY OF EXTRACTS OF CONES OF CUPRESSUS SEMPERVIRENS

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Abstract

Substances such as antioxidants are found in abundance in plants. The objective of this study is to determine the content of phenolic compounds and the antioxidant potential of *Cupressus sempervirens* L. cones from the Terni forest (Tlemcen, Algeria). An assay of total polyphenol and flavonoid contents by spectrometry, estimation of antioxidant potential by the DPPH free radical trapping method are carried out on extracts of the cones. The yield was 98.12% and 81.22% for the two extracts obtained by ultrasound and maceration respectively; the average contents of total polyphenols and flavonoids were 534.65 ± 1.14 mg (EAG)/g (ES) and 335.75 ± 4.72 mg quercetin equivalents/g (ES) respectively for the ultrasonic extract. The mean EC50 values for the DPPH test were 0.109±0.030 mg/ml for the ultrasonic extract and 0.294±0.002mg/ml for the macerated extract. The quantitative estimation of flavonoids and total phenols by the colorimetric method showed that the extracts are rich in these compounds and have good antioxidant activity; it can be deduced that the cones of this species can be a source of antioxidant bioactive.

Keywords: *Cupressus sempervirens*, Antioxidants, total polyphenols, flavonoids.

INTRODUCTION

Polyphenols are natural compounds that are widely distributed in the plant kingdom and are of increasing importance, in particular thanks to their beneficial effects on health (Koechlin-Ramonatxo, 2006). They are also used as additives in the food, pharmaceutical and cosmetic industries (Bougandoura and Bendimerad, 2012). The main properties of phenolic compounds are mainly antiseptic (Epifano et al., 2007). Algeria, because of its varied climate and the nature of its soils, has an extremely rich flora in terms of medicinal and aromatic plants (Merdji and Guemache, 2023). To this end, and as part of the contribution to the enhancement of the Algerian flora, we were interested in the study of Cupressus sempervirens L. because this plant has a wide spectrum of therapeutic interests thanks to the polyphenols of its cones and needles. The green cypress is a conifer with fragrant evergreen foliage. The cypress family includes a large group of species, and it has many advantages. Research has confirmed that the fruits (cones) of green cypress are useful in many areas. Cypress nuts contain active ingredients with antiviral properties (Amouroux et al., 1998). These molecules have a direct action on the virus and thus suppress the infection (Amouroux et al., 1998; Bruneton, 1999). Cypress is also traditionally used to reduce the symptoms of 'venous insufficiency. In this context, our study focuses on the in vitro antioxidant activity and the determination of secondary compounds (total

polyphenols and flavonoids) of the extracts of *Cupressus sempervirens* L. cones from the Terni forest (Tlemcen, Algeria).

MATERIALS AND METHODS

Plant material

The species *C. sempervirens* was identified by Benabadji N. (2002), Professor at the Laboratory of Ecology and Management of Natural Ecosystems, University Abou Bakr Belkaid-Tlemcen (Algeria).

The cones of *C. sempervirens*, family Cupressaceae were harvested in the spring of 2022 according to a random sampling at the level of the forest of Terni, they were cleaned then dried in a dry, ventilated place sheltered from direct sunlight light (Cecchini, 2003). After drying, the samples were ground. Then passed through a sieve to obtain a homogeneous powder. The powder obtained was put in glass pillboxes and stored until extraction.

Preparation of extracts

There are several methods for extracting polyphenols. The most effective method that was chosen for this study is solid-liquid extraction using two techniques, ultrasonic extraction and cold extraction or maceration (Penchev, 2010).

A sample of 1g of the dry cones is subjected to maceration in 10 ml of Methanol/water (8:2 v/v) for 24 hours at room temperature and in the dark (Extract maceration) and a sample of 1g of the dry cones is added to 10 ml of a mixture of water/formic acid/nitrile acetate led to ultrasound (ultrasound Extract); the extracts obtained are filtered, then concentrated under vacuum using a rotary evaporator at 45°C.

Yield

Extraction yield is calculated by the following formula (Falleh et al., 2008):

$$R\ (\%) = 100*(Mext/Mech)$$

R: the yield in (%).
Mext: the mass of the extract after evaporation in mg.
Mech: the dry mass of the plant sample in mg.

Dosage of total phenols

The dosage of total phenols is determined by the Folin-Ciocalteu reagent according to the method of (Singleton et al., 1999). The reagent is reduced during the oxidation of phenol, in a mixture of blue oxide of tungsten and molybdenum, the absorption is measured using a spectrophotometer at 765nm. A calibration curve is produced using gallic acid as a positive control in order to express the contents in milligram (mg) equivalents of gallic acid per gram of dry matter (mg EAG / g DM).

Dosage of flavonoids

The flavonoid dosage is determined using the technique of (Zhishen et al., 1999). A calibration curve is produced in parallel under the same operating conditions using catechin as a positive control. The flavonoid content is expressed in milligram (mg) catechin equivalents per gram of dry matter (mg EC/g DM).

Evaluation of antioxidant activity

The evaluation of the antioxidant activity was carried out according to the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. In this test, antioxidants reduce the purple-colored 2,2-diphenyl-1-picrylhydrazyl to a yellow compound, diphenylpicrylhydrazine. Free radical scavenging activity was measured as shown (Lee et al., 2003). The percentage decolorization of the DPPH radical was calculated from the following formula:

Anti-radical activity = 100 (1- sample absorbance / control absorbance).

Statistical analysis

All experiments were performed in triplicate. Results expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The phenolic compound extraction yield results of the two extracts obtained from the cypress cones are shown in Figure 1. They are expressed as a percentage. The extraction yield is the ratio of the quantity of natural substances extracted by the extractive action of a solvent to the quantity of these substances contained in the plant material (Gélébart, 2016). It depends on several parameters such as: the solvent, the pH, the temperature, the extraction time and the composition of the sample (Nait Sidi Ahmed, 2012).

The results showed that the best yield is recorded for the extract obtained by the Ultrasonic extraction technique; it is 98.12% and for the extract obtained by maceration is 81.22%, these yields are very close to the mass of dry plant matter used.

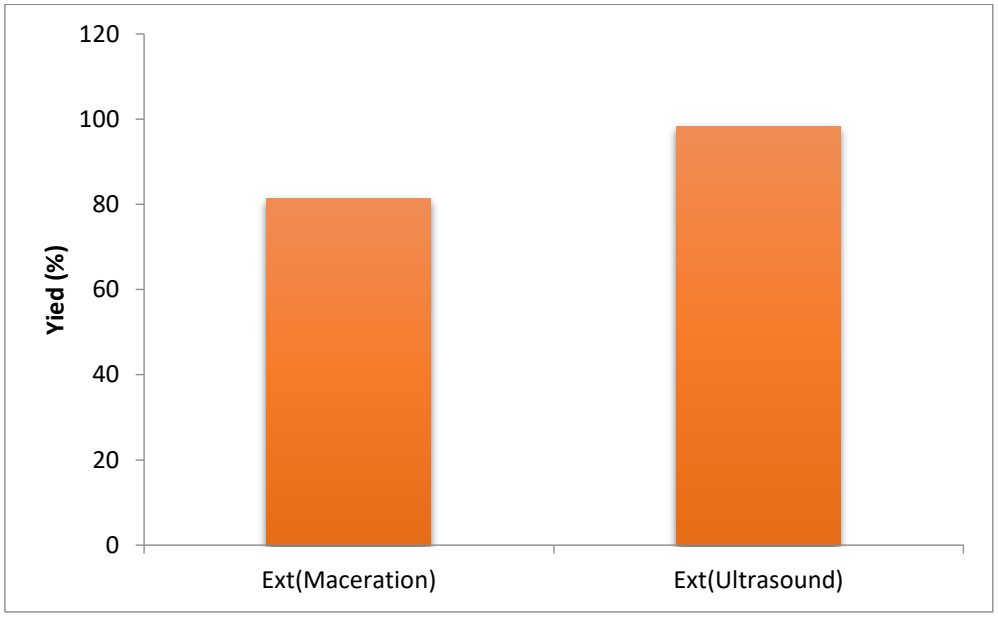

Figure 1. Extraction yield of phenolic compounds from two extracts of green cypress cones.

The results of the content of total polyphenols, flavonoids of the two extracts obtained are illustrated in Figure 2.

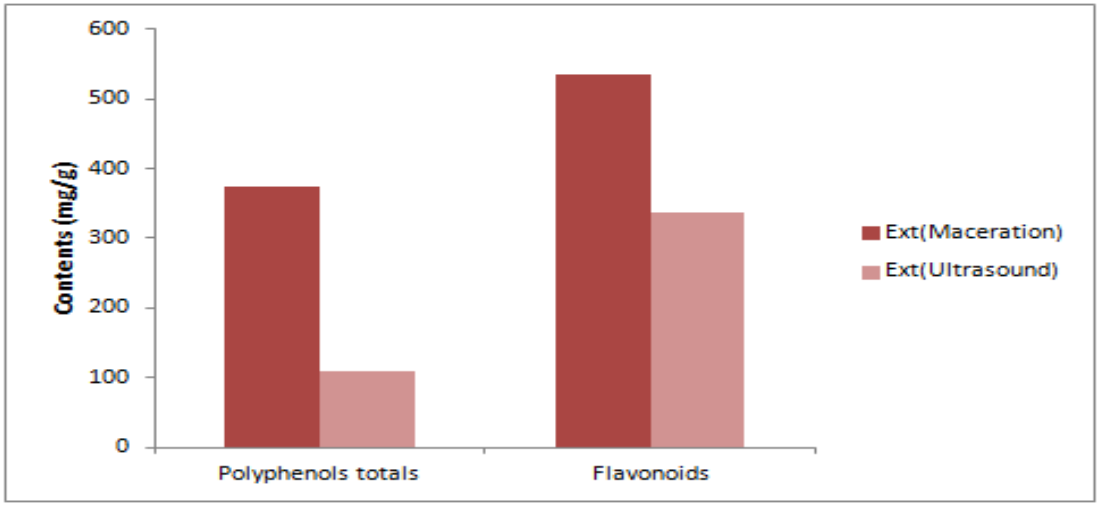

Figure 2. The content of total polyphenols and flavonoids of the two extracts (Ext) obtained from cypress cones.

The quantification of phenolic compounds in the two extracts obtained from the evergreen cupressus cones showed that the best average contents of total polyphenols and flavonoids were 534.65 ± 1.14 mg (EAG) / g (ES) and 335.75 ± 4.72 mg equivalents of quercetin/g (ES) respectively are obtained by ultrasound and 374.5±1.37 mg (EAG)/g (ES) and 108.88±4.33 mg equivalents of quercetin/g (ES) are also obtained by maceration. Polyphenols are commonly found in plants (Kim et al., 2003). Flavonoids and phenolic compounds constitute a beneficial effect on human health (Basalan et al., 2011). The comparison of our results with those of (Aloui et al., 2020). which aims to study the phytochemical composition of the methanolic and aqueous extracts and to evaluate their antioxidant potential in the needles and cones of *C. senpervirens*, shows that the contents of total polyphenols and flavonoids are lower than our results in both case.

The antioxidant activity of the extracts of *C. sempervirens* cones was evaluated in vitro by the DPPH test and the result was expressed in terms of percentage reduction of DPPH/concentration of the extracts. The values obtained are shown in figure 3, the efficiency of the two cone extracts in trapping the DPPH radical, expressed by the inhibition rate (I %) as a function of the different concentrations.

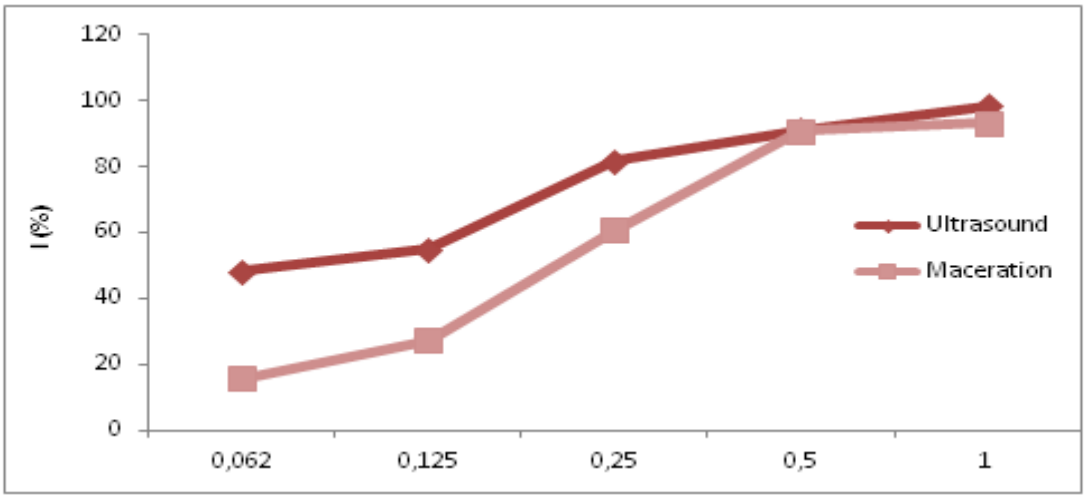

Figure 3. Inhibition (I %) as a function of different concentrations of two extracts.

These results showed that the two extracts of cypress cones obtained by the two extraction methods have antioxidant activity. The anti-radical activity revealed an EC50 of 0.109 ± 0.030 mg / ml for the extract obtained by ultrasound, the latter has a more intense free radical scavenging activity than the EC50 0.294 ± 0.002 mg / ml of the extract obtained by maceration. Polyphenols and flavonoids play an important role in the defense against free radicals (Govindarajan et al., 2006) and they are considered among the most powerful antioxidants. According to these results, cypress cones are a natural source of antioxidants.

CONCLUSION

The present study proposed to carry out the quantification of polyphenolic compounds (total phenols, flavonoids) by spectrophotometry of the extracts of the cones of *Cupressus sempervirens* L. of the Cupressaceae family from the region of Terni Mont of Tlemcen (Algeria) obtained by two methods of extraction (maceration and ultrasound) whose study results have shown that the best method of extraction of phenolic compounds is ultrasound. Several studies have been carried out on the quantification of secondary metabolites and especially polyphenols including flavonoids. Given the importance of these metabolites in biomedical, biochemical and other research. The antioxidant activity of these extracts evaluated in vitro by the DPPH test showed that the cypress cones studied are characterized by a strong antioxidant power. The application of natural antioxidants is a very promising field in full development. This leads to more and more research, aimed at diversifying the resources of its natural substances. The results obtained by our study allow us to deduce that cypress cones constitute a natural source of antioxidants which remain to be exploited for future use in the fields of food, cosmetics and health. It is necessary to complete this research with other methods to identify, isolate and purify the constituents of this organ (cone).

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CYTOTOXICITY OF ORANGE PEEL (*Citrus sinensis*) ESSENTIAL OIL NANOEMULSIONS ON THE RAINBOW TROUT GONADAL CELLS

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ABSTRACT

The citrus industry holds a significant position in the agricultural industry. However, it also generates substantial amounts of orange peel (*Citrus sinensis*) wastes. Essential oil production is one of the widely used bio-economical methods for the evaluation of these wastes. Essential oils are evaluated below their potential for use in different sectors due to their volatile nature and low stability against environmental stress conditions, which the nanoemulsion can overcome. Therefore, this study aimed to form and characterize of the nanoemulsion of orange peel essential oil (OPEON) and investigate its cytotoxicity on the rainbow trout gonadal (RTG-2) cells. The OPEON (0.1:0.3:0.6:99 w/w, EO: Tween 80: Ethanol: water) was successfully created using an ultrasonic homogenizer. The OPEON was characterized using TEM (~100 nm), zeta sizer (the ζ-potential value of -12.6 mV, and the polydispersity index of 0.657, conductivity of 0.00547 mS/cm), and FT-IR analysis. Treatments of 125, 250, 500, 1000 ppm of the OPEON have statistically significant toxic effects on the RTG-2 cells after 24 hours of exposure. Based on the study results and considering the toxic effect on cells, there is a potential for effective use of nanoemulsion forms of essential oils, especially in the pesticide industry.

Keywords: Orange peel essential oil, Nanoemulsion, Zeta sizer, Cytotoxicity, Rainbow trout gonadal cells

INTRODUCTION

Citrus fruit, a valuable plant grown worldwide, has a crucial position in fruit consumption, fruit juice, marmalade, jelly, canned food, and essential oil production. In terms of usage area, orange (*Citrus sinensis*) is the most used citrus species (Sandhu et al., 2021). However, up to 60% of the processed fruit weight emerges as solid waste containing highly valuable bioactive compounds (essential oils, flavones, polyphenols, etc.), which creates an economic burden (Omran et al., 2018; Victor et al., 2021). Considering the rich functional components of orange peel, essential oil extraction is one of the widely used bioeconomic methods in the evaluation of these wastes (Gavahian et al., 2019; Siddiqui et al., 2022).

Essential oils are concentrated hydrophobic liquids and complex compounds characterized by a strong odor and composed of various plant metabolites. Essential oils are clear and soluble in lipid/organic (ether, alcohol, fixed oils) solvents and have less density than water (Kar et al., 2018). The biological activities of essential oils vary depending on the chemical composition, which varies according to the plant parts used for extraction, the extraction method, the phenolic stage of the plant, the harvest season, the age of the plant, the nature of the soil and environmental conditions (Said-Al Ahl et al., 2017). Citrus essential oils are widely used in sectors such as beverages, ice cream, cookies, biscuits, cakes, room fresheners, household products, perfumes, pharmaceuticals, aromatherapy, and detergents (Geraci et al., 2017; Hanif et al., 2019). However, there are some drawbacks that limit the high usage potential of essential oils. The high volatility and sensitivity of essential oils to chemical conversion or degradation reactions such as oxidation, isomerization, polymerization and

rearrangement depending on environmental parameters such as temperature, light and atmospheric oxygen limit their potential for use in the field (Pavoni et al., 2020; Oladipupo et al. al., 2022). In addition, essential oils have poor physico-chemical properties, such as fast half-life and low solubility in water. As a way to deal with this, nano formulations are being developed that can retain essential oils without interfering with their bioactivity, provide deeper tissue penetration, increase bioactivity as they allow easier cellular uptake, and achieve the desired slow release (Pavoni et al., 2020; Mustafa and Hussein, 2020).

Nanoemulsions are two-phase dispersion of two immiscible liquids in nanosizes, which are water-in-oil (W/O) or oil-in-water (O/W) formulations and droplets stabilized by amphiphilic surfactants. Nanoemulsions have droplet sizes of 20–200 nm in diameter. The large surface area provided by their nanometric size provides higher loading capacity and improved solubility, resulting in increased bioavailability of poorly soluble compounds. Nanoemulsions are kinetically stable (Feng et al., 2018; Barradas and de Holanda e Silva, 2021; Sharma et al., 2022). Although there are a few studies in which nanoemulsion forms of orange peel essential oil (OPEON) are obtained by different methods and its antifungal, antibacterial and larvicidal activities are shown, there is no study showing toxicity *in vitro* according to our best knowledge (Azmy et al., 2019; Das et al., 2020; Farouk et al., 2022). Based on these observations, this study was modeled to generate data to create and characterize of the OPEON and its cytotoxicity on rainbow trout gonadal (RTG-2) cells.

MATERIAL AND METHOD

The orange peel essential oil was purchased from a local company, BIOMESI Bioagrotechnology R&D, located in Adana, Turkey (Durmuş et al., 2023). The RTG-2 cell line (Registration Number: 95121808) was purchased from Türkiye ŞAP Enstitüsü (Ankara, Turkey) (Çiçek, 2023).

The OPEON was formed using an ultrasonic homogenizer (BANDELIN electronic GmbH & Co. KG, Berlin, Germany) following the method described by Durmuş (2020) with minor changes. 30 µL of essential oil, 90 µL of ethanol, 180 µL of Tween 80 and 29.7 µL of distilled water (0.1:0.3:0.6:99 w/w) were placed in a glass beaker and exposed to an ultrasonic homogenizer. Ultrasonic homogenizer operating conditions were set as 15 min, 70 amplitude, 20 kHz and 500 W, and a titanium probe (2 mm diameter and 1950 mm height (MS72)) was used. In addition, ice was used around the beaker to avoid thermal effects during the process (Durmuş, 2020).

Transmission electron microscope (TEM) (Hitachi High Tech HT7700, Japan), zeta sizer (Malvern Zeta sizer Nano ZSP, Malvern Instruments Pvt Ltd, UK) and Fourier transform infrared spectroscopy (FTIR) (Bruker VERTEX 70v brand, Germany) was used to determinate of surface morphology, zeta potential and molecular structure of the OPEON, respectively (Sogan et al., 2023). These analyzes were carried out with service procurement at the Eastern Anatolia High Technology Application and Research Center (DAYTAM, Erzurum, Turkey).

The RTG-2 cells were cultured 89.5% Eagle's minimal essential medium (EMEM: with L-glutamin medium, ATCC 30-2003) supplemented with 10% fetal bovine serum (Biowest S1810-500) and 0.5% penicillin-streptomysin (Sigma P4333) in 25 cm^2 culture flasks (Isolab 120.11.025) at 23.7 ˚C without CO_2 respiration (Çiçek, 2023).

The OPEON were prepared at different concentrations (125, 250, 500, and 1000 ppm) by dissolving in ethanol:distilled water solution prepared in a 1:1 ratio. Experimental groups were applied on the RTG-2 cells seeded 24 hours ago at a density of $3x10^4$ cells/well. For the control groups, ethanol: distilled water and Tween 80: ethanol were used. Then, a cell viability test was carried out after 24 hours of incubation.

Sulforhodamine B test was performed for cell viability testing. Briefly, after 24 hours of incubation with the experimental groups, 100 µL of cold 10% trichloroacetic acid solution (CAS No: 76–03–9, Sigma Aldrich, USA) was applied to the RTG-2 cells (4 °C, 1.5 hours).

Following washing 5 times with distilled water and air drying, the cells were fixed with 50 µL of 0.4% SRB dye (CAS No: 3520–42-1, Sigma Aldrich, USA) was prepared in 1% acetic acid (CAS No: 64–19–7, Sigma Aldrich, USA) (30 min in the dark). Washing was done 5 times with 5% acetic acid solution and air drying. Then, 150 µL of 10 mM Tris base (CAS No: 77–86–1, Sigma Aldrich, USA, pH 10.5) was added to each well and kept in an orbital shaker (15-20 min, 150 rpm). The absorbance values were read in a micro-plate reader (EpochTM, BioTek, USA) at 564 nm (Vichai and Kirtikara, 2006).

The study data (n= 6 independent experiments) were evaluated using the GraphPad Prism 9.00 Statistical Software (GraphPad Software, Inc., California, USA). Experimental groups were analyzed using One-way analysis of variance (ANOVA) and the statistical significance was accepted at $p \leq 0.05$ level (Çiçek, 2023).

RESULTS AND DISCUSSION

Detailed characterization is necessary to confirm the presence of nanostructures in the production of nanomaterials. Therefore, in this study, TEM, Zetasizer, and FTIR were utilized for the characterization of the OPEON. During the nanoemulsion process, the reaction mixture turned milky white (as observed macroscopically) after ultrasonication process. Eventually, this mixture became translucent and dispersed. TEM images of the OPEON are shown in Figure 1 at different scales. According to TEM images, the OPEON consisted of spherical droplets and was obtained in sizes of 100 nm and above. The Ostwald maturation phenomenon, in which a possible solubility in the aqueous phase of the emulsion system transferred from small droplets to large droplets, may have led to the obtaining of nanoemulsions of different sizes (Farouk et al., 2022). In this study, negative staining (uranyl acetate) was used to remove water from the outer layer of nanoemulsion droplets and visualize it (Klang et al., 2012; Somala et al., 2022). TEM images show that the OPEON has no physical deformation, no agglomeration and a smooth structure.

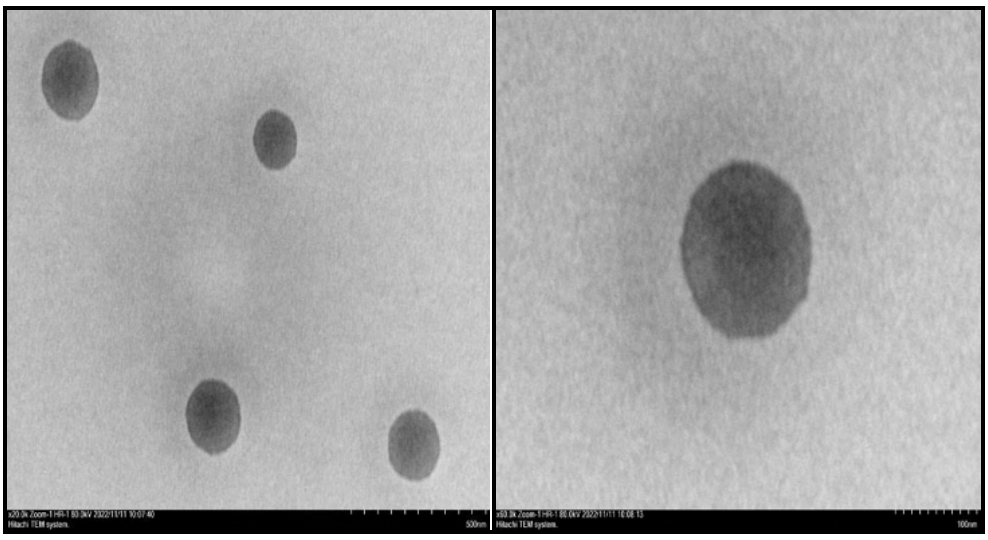


Figure 1. TEM images of the OPEON at 500 nm and 100 nm scales

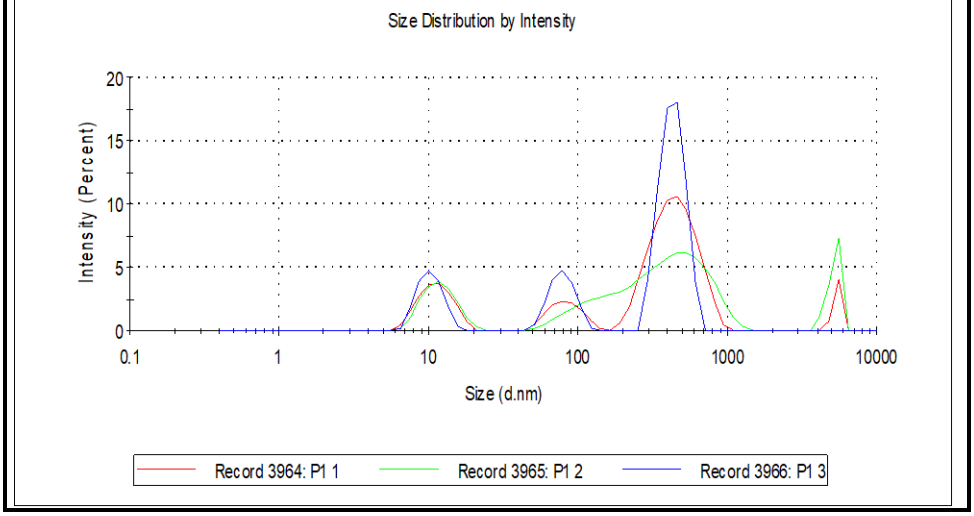
Zetasizer analysis was performed to measure the particle size, zeta potential values, and polydispersity indexes of the OPEON droplets at a constant temperature of 25°C (as shown in Figure 2). The average droplet particle sizes of the OPEON ranged from 405 to 1291 nm, with a 701.2 nm of mean droplet particle. Considering the size distribution graph, it is understood that nanoemulsions with sizes around 10 nm and 100 nm are obtained at the same density percentage, and there are also emulsion forms, with sizes around 1000 nm and above. This explains the visualization of nanoemulsions with sizes around 100 nm in TEM analysis.

Increases in the size of the emulsion structure may depend on the type of surfactant and its chemical composition in the essential oil (Mohammad et al., 2019).

The mean zeta potential (ζ-potential) value of the OPEON was determined as -12.6 mV. High negative and positive ζ-potential values may indicate that repulsive forces are more dominant than attractive forces. In this study, the OPEON was obtained using an ultrasonicator. Mechanical stress occurring during the ultrasonication process can cause the release of free -OH and -COOH groups from the essential oil, leading to an increase in the negative charge on the surface of the nanoemulsion. The ζ-potential value of nanoemulsion, which is considered electrostatically stabilized, is expected to be in the range of ±30 mV (Gurpreet and Singh, 2018). Therefore, based on the mean ζ-potential value, it can be assumed that the OPEON has a suitable shelf life and can be used effectively and with long-term effect in various areas for different purposes (Farouk et al., 2022).

The mean polydispersity index (PDI) of the OPEON was determined as among 0.509-0.943. The PDI shows a narrow distribution of nanoemulsions size if it is below 0.2 or 0.25 (Kaci et al., 2018). In this study, the OPEON was obtained in different sizes, and the dispersions of nanoemulsions between 10 nm and 100 nm are less than the dispersions of nanoemulsions with sizes close to 1000 nm. This explains the high PDI values. If the PDI value is greater than 0.5, the system is called broad size distribution (Golfomitsou et al., 2018). Tween 80 concentration is the most crucial factor affecting the PDI value of nanoemulsions (Pongsumpun et al., 2020). This suggests that the concentration of Tween 80 used in this study should be kept higher in other studies.

Sample	Z-avarage (d.nm)	ZP (mV)	PDI
P11	405	-14.8	0.509
P12	407.5	-13.1	0.519
P13	1291	-10	0.943
Mean	701.2	-12.6	0.657
Std. Dev.	510.8	2.43	0.248



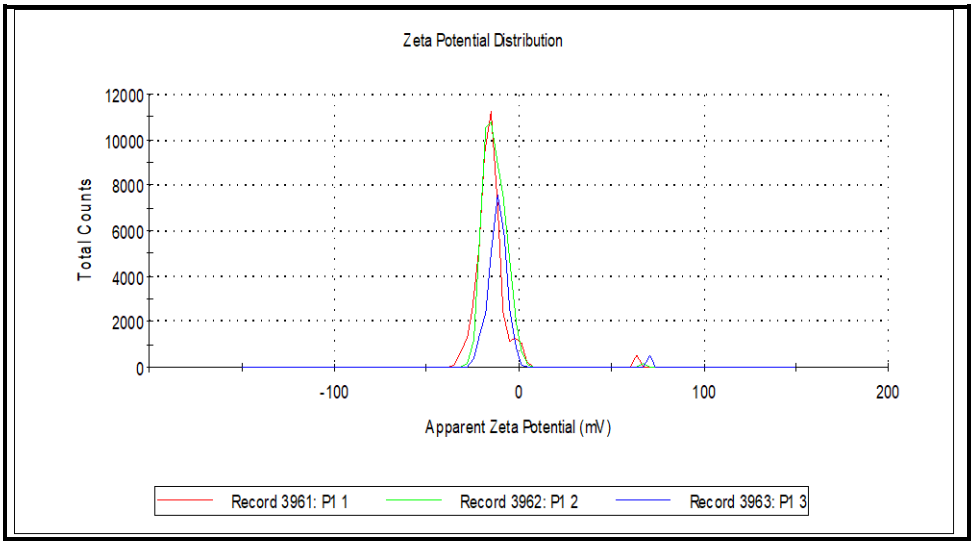


Figure 2. The droplet particle size distribution, zeta potential distribution, polydispersity indexes of the OPEON (P11, P12, P13: three repetitions; ZP: Zeta potential, PDI: Polydispersity index)

FTIR analysis was performed to characterize the molecular structure and functional groups of the OPEON, as shown in Figure 3. The peaks observed at 3753 cm^{-1}, 3496 cm^{-1} and 3481 cm^{-1} corresponds to O-H stretching of alcohol, phenol, and hydroxyl groups, the peak at 2921 cm^{-1} indicates to C-H and O-H stretches of the alkanes, and the peak at 2856 cm^{-1} is -C-H aldehydic stretching and -C-H stretch, as well as carboxylic acid O-H stretch (Opoku et al., 2021; Soni et al., 2022). An absorption band at 2368 cm^{-1} indicates the O=C=O stretching of carbon dioxide. In this study, the C=O ester groups at 1733 cm^{-1} may be associated with the ester groups found in Tween 80 (Osanloo et al., 2022). The peak was observed at 1652 cm^{-1} corresponds to vibratory stretching bonds of groups C=O (Amide type I), the band at 1558 cm^{-1} indicates amide II and N-H bending (Hosseinnia et al., 2017; Al-Hilifi et al., 2022). The peaks observed at 1458 cm^{-1}, 1350 cm^{-1}, and 1297 cm^{-1} are related to CH_2 bending vibration, NO_2 stretch and CH_3 bend, respectively (Zhang et al., 2017; Michelina et al., 2019). The peak at 1247 cm^{-1} represents the C-O-C stretch, while the peak at 1099 cm^{-1} indicates the C–O stretching. The peaks among at 500-950 cm^{-1} refer to C-H and C=C bends (Min et al., 2021).

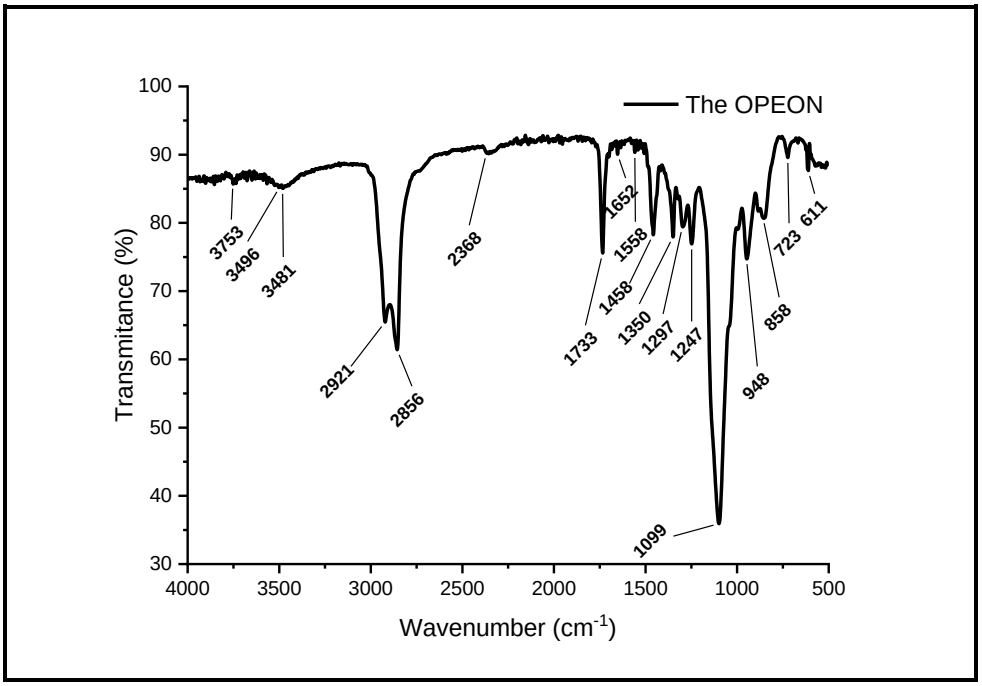


Figure 3. The FTIR spectra of the OPEON

The cytotoxic effect of the OPEON prepared at different concentrations on the RTG-2 cells after 24 hours of exposure is shown in Figure 4. Treatments of the OPEON (125, 250, 500, and 1000 ppm) showed significantly higher cytotoxic effects compared to the control group. Although 1:1 ethanol and Tween 80 treatments showed toxic effects compared to the control group, they did not reduce the RTG-2 cell viability as much as the OPEON treatments. The cytotoxic effects of nanoemulsion forms of essential oils are generally higher than that of free essential oils, depending on surfactant, nano size, electrical properties, chemical composition of the essential oil, dose, and time. In addition, the interaction between the surface charges of nanoemulsions and the charges of cell membranes can increase the cytotoxic effect (Yoon et al., 2018; Marchese et al., 2020).

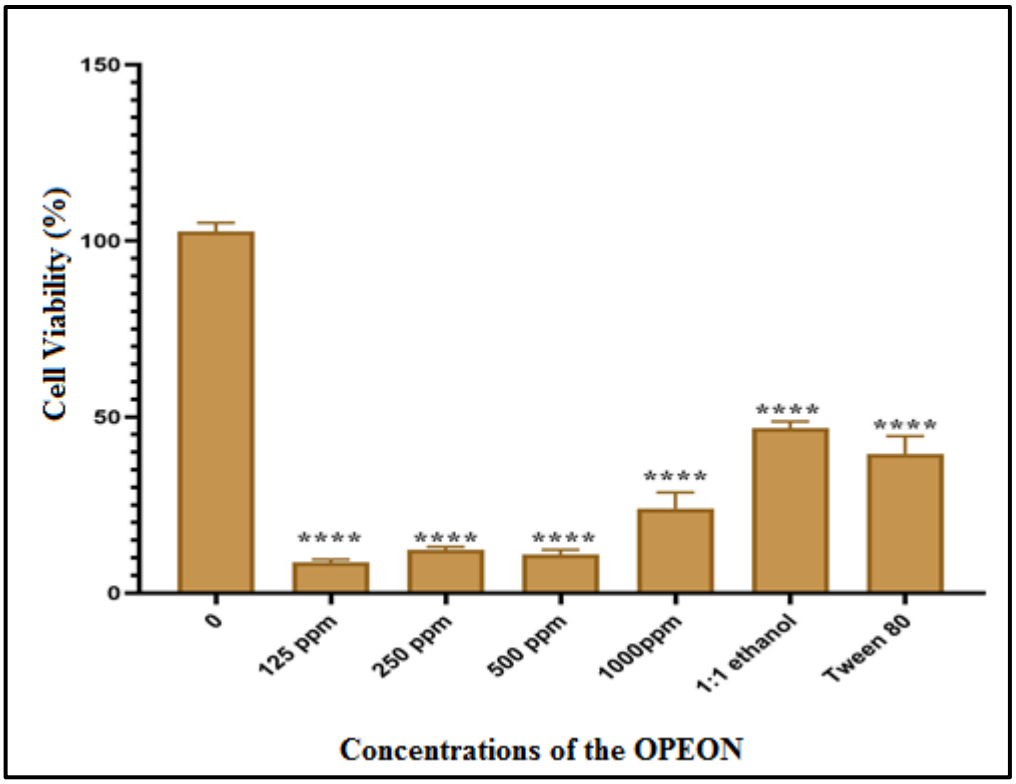


Figure 4. The cytotoxic effects of the OPEON on the RTG-2 cells for 24 hours

CONCLUSIONS

This study demonstrated that the OPEON was successfully obtained at different sizes. However, for long-term stability, it is recommended to change the formulation ratios for subsequent processes. Considering the toxic effect of the OPEON on the RTG-2 cells, future studies may focus on its potential for use in fields such as pesticide production, antibiotic agent.

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THE EFFECT OF DIFFERENT PROBIOTIC MIXTURES ADDED TO THE LAYING HENS DIET ON PERFORMANCE AND EGG QUALITY PROPERTIES

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ABSTRACT

This study was conducted to determine the effect of adding two different types of probiotics and their mixtures to the diets of laying hens on performance and egg quality. In the study, a total of 105 laying hens at 59 weeks of age were fed with five different diets created by adding *Bacillus megaterium* ($1x10^{10}$ g/cfu) and *Bacillus amyloliquefaciens* ($1x10^{10}$ g/cfu) to the corn-soybean meal-based diet (positive control) and the diet containing 35% barley (negative control) at a level of 0.5 g/kg and equal amounts (0.25+0.25 g/kg) of these probiotics. The study was conducted for 42 days with 7 replicates in 5 treatment groups. As a result of the study, the initial body weight, final body weight, body weight change, feed intake, egg production, egg mass and feed conversion ratio of the treatment groups were statistically insignificant ($P>0.05$). Egg weight was significantly higher in groups containing *Bacillus megaterium* and *Bacillus amyloliquefaciens* compared to the positive control group ($P<0.01$). The rate of damaged eggs was higher in the group containing *Bacillus megaterium* than in both control groups ($P<0.05$). The addition of *Bacillus megaterium* and *Bacillus amyloliquefaciens* to laying hen diets did not statistically affect the lightness index (L*) and b* value from egg yolk color criteria, Haugh unit, shell breaking strength, egg shell weight, egg shell thickness, and egg shell ratio examined while a* value was lower in all barley-containing groups ($P<0.01$). According to the results of the study, it can be said that adding both probiotics alone to the diet may be beneficial in terms of increasing egg weight in laying hens.

Keywords: *Bacillus amyloliquefaciens, Bacillus megaterium*, Performance, Laying hen, Egg quality

INTRODUCTION

Large-scale poultry farming facilities expose poultry to stressful conditions such as ration problems, diseases, and inadequate environmental conditions. Since these difficulties lead to significant economic losses, such problems should either be prevented beforehand or effectively controlled. Various feed additives are used in poultry nutrition to prevent such problems, including probiotics.

Probiotics are live microorganisms that are known to provide benefits to the host when given in appropriate amounts. These benefits include improving the intestinal structure, strengthening immunity against pathogens, increasing the intestinal microflora, suppressing pathogen colonization, and/or regulating intestinal colonization by symbiotic bacteria, thereby affecting the health, physiology, or production performance of poultry (Callaway et al., 2008; Gaggia et al., 2010; Xiang et al., 2019). *Lactobacillus, Streptococcus, Saccharomyces, Aspergillus,* and *Bacillus* are some of the probiotic species that have been used in poultry nutrition in the past (Tannock, 2001). Previous findings have shown that adding probiotics to the diet of laying hens not only increases egg production but also improves feed utilization, performance, and eggshell quality (Mikulski et al., 2012; 2020).

Grains are the most important and commonly used feed components in poultry nutrition. Barley is commonly used in poultry feed as an energy source. However, the carbohydrates in barley, especially non-starch polysaccharides such as beta-glucans, are not as easily digestible

as those in corn. The β-glucans contained in barley are released from cell walls and bind with water in the intestine to form a gel, which increases the viscosity of the intestinal contents. This increase in intestinal viscosity adversely affects the digestion and absorption of nutrients (Zielke et al., 2017). Hooda et al. (2011) found that high viscosity prevented mixing of the intestinal contents and altered the transport properties of nutrients on the mucosal surface. Therefore, barley β-glucans are considered anti-nutritional factors that limit the nutritional quality of barley (Stein et al., 2016). Enzyme addition to barley-based diets for poultry reduces intestinal viscosity and increases the utilization of barley. Enzymes also improve litter quality in poultry fed barley-based diets (Smits and Anisson, 1996). Probiotic addition to barley-based diets can also increase the digestibility of nutrients as an alternative to enzymes. The addition of oligosaccharides and *Lactobacillus* to broiler diets has been reported to increase the activity of β-glucosidase, alpha-galactosidase, aminopeptidase, maltase, and alkaline phosphatase enzymes (Mehrabadi and Jamshidi, 2019).

Bacillus species of probiotics are suitable feed additives for poultry health due to their ability to produce various enzymes such as protease, amylase, and lipase and their stability in the presence of stress.

Bacillus amyloliquefaciens is an effective among *Bacillus* species and is a probiotic isolated from soil. It has been reported in various studies that it can be used as an alternative to antibiotics to modulate the intestinal flora of broiler chickens, improve the intestinal epithelial barrier, and enhance immune function (Du et al., 2018; Wang et al., 2021). In another study, the addition of *Bacillus amyloliquefaciens* to layer diets reduced stress and improved their immune systems, thereby increasing their performance and egg quality (Zhou et al., 2020). *Bacillus amyloliquefaciens* can improve egg production, sperm production quality, egg quality/hatchability, and slow down the reproductive aging of chickens (Prazdnova et al., 2019). However, the effects of *Bacillus amyloliquefaciens* on the performance and healthy status of laying hens remain uncertain.

Among *Bacillus* species, *Bacillus megaterium* has become a research material as a probiotic due to its unique properties such as resistance to stress conditions and high temperature and easy storage (Vary et al., 2007; De Vos, 2009). *Bacillus megaterium* is a gram-positive bacterium that can produce spores as a new type of microecological additive isolated from chicken manure. Ding and Wang (2015) found that adding 100 mg/kg *Bacillus megaterium* to the diets of laying hens significantly increased egg weight and egg production compared to the control group, while significantly reducing fecal ammonia nitrogen and uric acid contents. Some researchers have suggested that the addition of Bacillus megaterium to the diets of broiler chickens can improve feed consumption, live weight, feed conversation ratio, and growth performance (Ding et al., 2016; Chen et al., 2016).

The aim of this study is to evaluate the effects of *Bacillus amyloliquefaciens* and *Bacillus megaterium* separately and mixture as feed additives on the performance and egg quality characteristics of laying hens fed barley-based diets. We are aim to inform the researchers in this direction according to the comparison of data between these two probiotics.

MATERIAL AND METHOD

In the study, 105 Tinted laying hens aged 59 weeks were used as animal material. The experiment was carried out in a total of 35 subgroups, consisting of 5 different treatments and 7 replications for each treatment, according to the randomized design. Laying hens were randomly distributed as 3 in each sub-group. Feed and water were supplied to hens as ad libitum, and the lighting program is 16 hours of daylight was applied during the experiment. Hens were fed with five different diets created by adding *Bacillus megaterium* ($1x10^{10}$ g/cfu) and *Bacillus amyloliquefaciens* ($1x10^{10}$ g/cfu) to the corn-soybean meal-based diet (positive control) and the diet containing 35% barley (negative control) at a level of 0.5 g/kg and equal

amounts (0.25+0.25 g/kg) of these probiotics. The study was carried out for 42 days (two periods of 21 days). The trial diets were prepared according to the requirements reported by NRC (1994) for laying hens (Table 1).

Table 1. Ingredients and calculated nutrient composition of diets with and without barley used in the study

Ingredients	**Control (+)**	**Control (-)**
	%	**%**
Maize	56.00	23.20
Barley	-	35.00
Soybean meal (% 47 CP)	18.75	14.00
Sunflower meal (% 28 CP)	10.00	10.00
Vegetable oil (8800 ME/kg)	3.90	6.45
Limestone	9.15	9.20
Dicalcium phosphate	1.60	1.50
Salt	0.25	0.25
Premix[1]	0.10	0.10
L-Lysine	0.10	0.15
DL-Methionine	0.15	0.15
Total	**100**	**100**
Calculated Chemical Composition		
Metabolizable energy (kcal/kg)	2748.70	2748.70
Crude Protein (%)	15,81	15.79
Crude Fiber (%)	4,49	5.05
Calcium (%)	3,93	3.92
Available phosphorus (%)	0,41	0.39
Lysine (%)	0,78	0.76
Methionine (%)	0.38	0.37
Methionine + Cystine (%)	0,61	0.43

[1]Premix is supplied per kg of diet; vitamin A, 4 mg; vitamin D_3, 0,055 mg; vitamin E, 11 mg; vitamin B_{12}, 0,66 mg; nicotine acid, 44 mg; calcium-D-pantothenate, 8,8 mg; riboflavine, 5,8 mg; thiamine, 2,8 mg; folic acid, 1 mg; biotine, 0,11 mg; coline, 220 mg; manganese: 60 mg; iron: 30 mg; zinc: 60 mg; copper: 5 mg; iodine: 1 mg; selenium: 0,1 mg.

The body weights of hens were determined by weighing them on a scale with a sensitivity of 1 g at the beginning and end of the experiment, and the body weight gain were calculated from these data. Egg production (%) was calculated using the formula (Total number of eggs in the period (pieces) / Number of animals in the group (pieces)) × 100. Egg weights were determined by weighing all eggs collected from each subgroup during the last two days of each 18-day period on a digital scale with a sensitivity of 0.01 g. Egg mass was calculated by multiplying the percentage egg yields for each period by the average egg weights and dividing by 100. Hens were weighed and fed in groups, and daily feed intake was calculated. The feed conversion ratio was calculated by dividing the average daily feed intake per chicken for each period by the egg mass for that period (g feed/g egg mass). Broken, cracked, and damaged eggs were recorded daily and calculated as a percentage of the total number of eggs.

The eggshell breaking strength, eggshell membrane weight and thickness, albumen index, yolk index, Haugh unit, and yolk color were determined in a total of 6 eggs collected from each subgroup during the last two days of each 21-day period. The eggshell breaking strength was measured using an Egg Force Reader (Orka Food Technology, Israel). The eggshell weight (without contents) was determined by washing the eggs thoroughly after they were broken and separated, then drying them for 3 days at room temperature and weighing them on a precise digital scale. The eggshell ratio was calculated by dividing the eggshell weight by the egg weight. Eggshell thickness was determined by taking the average of measurements made with a digital micrometer on the mass, pointed, and equatorial regions of broken eggshells. The egg quality parameters were also determined in the eggs used to determine the eggshell quality parameters. For this purpose, the eggs were broken on a glass table and the egg

internal quality parameters were determined. The height of the yolk and albumen was measured with a digital height gauge, while the yolk diameter and egg albumen length and width were measured with a digital caliper (Mutitoyo, Japan). The yolk index = (yolk height/yolk diameter) × 100; Albumen index = (albumen height/(albumen length + albumen diameter)) × 100; Haugh unit = 100 × log (albumen height + 7.57-1.7 × egg weight$^{0.37}$) was calculated using a formula. Yolk color was measured as CIELab (L*, a*, b*) values using a Konica Minolta CR-200 colorimeter. Egg internal quality analyses were completed within 12 hours after the eggs were collected.

To determine whether the treatments had an effect on the parameters examined, one-way analysis of variance (ANOVA) was applied to the data obtained using Minitab 17 statistical package program, and Duncan Multiple Comparison Test was applied to determine differences between treatment groups (Düzgüneş et al., 1987).

RESULTS AND DISCUSSION

According to Table 2, the effects of adding 0.5 g/kg *Bacillus amyloliquefaciens* (1x10^{10} cfu/g), 0.5 g/kg *Bacillus megaterium* (1x10^{10} cfu/g), and these probiotic mixtures to the diets of laying hens on initial body weight, final body weight, body weight gain, egg production, feed intake, and feed conversion ratio were not statistically significantly different from those of the control group (P>0.05).

According to Table 2, the addition of probiotics only affected egg weight and damaged eggs among the performance parameters (P<0.01; P<0.05). The addition of *Bacillus megaterium* and *Bacillus amyloliquefaciens* to the diets of laying hens significantly increased egg weight compared to both the control group (+) and the probiotic mixture (BM+BA) (P<0.01). The enhancing effect between these two probiotic species on egg weight was the same (P>0.05). According to Table 2, the addition of *Bacillus megaterium* to the diets of laying hens has significantly increased damaged eggs compared to both of the control groups (P<0.05).

Previous studies have shown that probiotics affect numerous performance parameters of laying hens. These parameters include the dynamics of body weight (body weight gain), feed conversion ratio, egg production, and egg quality (improved shell thickness, egg weight) (Lei et al., 2013; Chaucheyras-Durand and Durand, 2010; Smith, 2014; Bai et al., 2016). *Bacillus* type probiotics are commonly used in poultry feeding and have emerged as a promising approach to improving poultry health (Jia et al., 2016). These probiotics exhibit resistance to different climatic conditions and have a long shelf life. *Bacillus* species, including B. amyloliquefaciens, are found in normal intestinal microbiota and have the ability to grow and produce spores in the gastrointestinal system (Cartman et al., 2008; Cutting, 2011; Barbosa et al., 2005).

Furthermore, their ability to form biofilms is medically important (Ushakova et al., 2009). Our study's findings have shown that adding different types of probiotic mixtures to the diets of laying hens has a significant effect on egg weight and the rate of damaged eggs. According to previous studies, Tsai et al. (2023) compared the addition of 0.3% *Bacillus subtilis* with 0.1% *Bacillus amyloliquefaciens* to the diets of laying hens and reported that *Bacillus subtilis* increased final body weight and body weight gain more than Bacillus amyloliquefaciens and the control group (P<0.05). They attributed this to *Bacillus subtilis'* better promotion of nutrient absorption. Same researchers found that *Bacillus amyloliquefaciens* did not affect egg yield, feed intake, egg mass, and the rate of damaged eggs. In contrast, Mazanko et al. (2018) reported that adding various *Bacillus* species probiotics to the diets of laying hens; among species *Bacillus amyloliquefaciens* increased egg production of hens. However, Weili et al. (2014) claimed that *Bacillus megaterium* improved the performance of laying hens and reduced ammonia emission in feces.

The results of the studies reported are partially consistent with the results of our study. However, the reason why the performance parameters reported in our study were not affected by treatments may be due to the difference in the levels of probiotics used, the absence of any stress factors in the animals, and the provision of optimal conditions (temperature, humidity, ventilation, etc.) within the coop.

Table 2. The effect of different probiotic mixtures in the diets of laying hens on performance

Performance parameters	CON (+)	CON (-)	BM	BA	BM+BA	P-Value
IBW, g	1784,14±48,52	1867,29±40,71	1838,29±54,71	1820,00±52,80	1818,86±44,00	0,811
FBW, g	1805,86±49,55	1875,14±32,34	1821,00±36,13	1793,43±44,97	1836,57±38,14	0,661
BWG, g	21,57±13,55	7,57±18,34	-17,14±23,32	-26,86±15,22	17,86±17,54	0,243
FI, g/hen/day	107,14±1,36	106,27±1,83	107,55±1,99	108,00±1,11	110,96±0,43	0,228
Egg production, %	91,20±1,30	85,93±3,24	85,69±2,58	84,25±1,40	91,05±2,03	0,101
Egg weight, g	64,13±1,11^{B}	66,36±1,05^{AB}	69,15±1,57^{A}	69,64±0,73^{A}	65,36±0,85^{B}	0,004
Egg mass, g/day	58,43±0,98	57,19±2,71	59,10±1,26	58,63±1,04	59,46±1,12	0,871
FCR, FI/EM	1,84±0,03	1,90±0,08	1,82±0,01	1,85±0,04	1,85±0,02	0,789
Damaged eggs, %	6,45±0,85^{b}	4,45±0,48^{b}	14,77±3,32^{a}	8,47±2,52^{ab}	10,41±1,10^{ab}	0,024

$^{a, b}$: Means with different superscripts in the same row were significantly different (P<0,05),
^{A,B}: Means with different superscripts in the same row were significantly different (P<0,01),
CON (+): Barley-free control; CON (-): Barley based control; BM: *Bacilllus megaterium* (0,5 g/kg); BA: *Bacillus amyloliquefaciens* (0,5 g/kg); BM+BA: *Bacillus megaterium* + *Bacillus amyloliquefaciens* (0,25 g/kg + 0,25 g/kg); IBW: Initial body weight; FBW: Final body weight; BWG: Body weight gain; FI: Feed intake; EM: Egg mass; FCR: Feed conversion ratio

Table 3 shows the effect of adding 0.5 g/kg *Bacillus amyloliquefaciens* ($1x10^{10}$ cfu/g) and *Bacillus megaterium* ($1x10^{10}$ cfu/g) and their probiotic mixtures to the diets of laying hens on egg quality characteristics.

According to the table, the values of albumen index, yolk index, Haugh unit, eggshell breaking resistance, eggshell weight, eggshell thickness, eggshell ratio, L*, and b* values were not significantly different from those of the control group for the mixtures of *Bacillus megaterium* and *Bacillus amyloliquefaciens* (P>0.05).

Adding probiotic mixtures to the diets of laying hens of different types only statistically significantly affected the a* values of yolk color measurements (P<0.01) compared to the control group (+) and significantly reduced them. The addition of *Bacillus megaterium* and *Bacillus amyloliquefaciens* to the diets of laying hens significantly reduced a* values compared to the control group (+), and the most reducing effect between these two probiotics was detected in the group with *Bacillus amyloliquefaciens* addition. Additionally, the effect of the probiotic mixture on a* value was the same as that of the control group (-) (P>0.05).

Egg quality generally encompasses various parameters such as eggshell weight, albumen and yolk quality. Egg quality has a genetic basis and varies among breeds of laying hens. However, egg quality is also affected by the housing conditions, age, and diets used for the hens (Jha et al., 2020). In their studies examining egg quality characteristics of laying hens, Abd El-Hack et al. (2017) reported that *Bacillus subtilis* increased yolk index, yolk color, and eggshell thickness compared to the control group. However, it did not affect Haugh unit values. However, Tsai et al. (2023) reported that *Bacillus subtilis* and *Bacillus amyloliquefaciens* did not affect eggshell thickness, eggshell weight, and yolk color. In another study, *Bacillus amyloliquefaciens* significantly improved eggshell quality, and researchers attributed this to the increase in calcium absorption due to the increase in nutrient utilization. This is possible because probiotics create an acidic environment suitable for mineral ionization and lower intestinal pH, which is necessary for the dissolution and optimal absorption of both calcium and phosphorus (Resta-Lenert and Barrett, 2003).

Haugh unit is an important measure of egg protein quality. A higher value indicates increased egg albumen viscosity and, therefore, better quality. Lei et al. (2013) reported that

the addition of *Bacillus amyloliquefaciens* at different doses resulted in different increases in Haugh unit, which could improve egg quality by increasing egg protein metabolism. Although our findings do not match those of various researchers reported so far, it is thought that this may be due to the absence of any stress factors in the animals. Jia et al. (2016) supported this idea by stating that probiotic addition could be more effective than normal conditions when animals are stressed, thus reducing the negative effects of mycotoxins on laying performance and effectively improving egg quality while reducing the accumulation of aflatoxin residues in eggs.

Table 3. The effect of different probiotic mixtures in the diets of laying hens on egg quality characteristics

Egg quality parameters	**CON (+)**	**CON (-)**	**BM**	**BA**	**BM+BA**	**P-Value**
Albumen index, %	9,81±0,42	8,58±0,37	8,90±0,41	8,79±0,25	9,09±0,36	0,185
Yolk index, %	42,25±0,37	41,61±0,47	42,74±0,31	42,83±0,65	42,96±0,48	0,265
Haugh unit	86,21±1,55	80,46±1,75	81,46±1,62	80,95±1,07	82,50±1,27	0,068
Eggshell breaking strength, kg	4,04±0,14	4,09±0,15	3,77±0,18	3,84±0,13	3,92±0,11	0,498
Eggshell weight, g	6,01±0,18	6,42±0,09	6,31±0,14	6,64±0,13	6,26±0,16	0,061
Eggshell thickness, mm	0,351±0,008	0,370±0,005	0,356±0,007	0,379±0,008	0,386±0,014	0,498
Eggshell ratio, %	9,37±0,20	9,68±0,16	9,15±0,25	9,54±0,20	9,58±0,19	0,382
Yolk color traits						
L*	46,95±0,34	47,72±0,46	47,39±0,32	47,59±0,48	47,92±0,41	0,515
a*	8,33±0,48^{A}	3,78±0,39^{BC}	4,31±0,35^{B}	3,15±0,32^{C}	3,26±0,28^{BC}	0,000
b*	31,91±0,55	30,70±0,48	30,94±0,42	30,25±0,71	30,49±0,43	0,240

^{A,B}: Means with different superscripts in the same row were significantly different (P<0,01),
CON (+): Barley-free control; CON (-): Barley based control; BM: *Bacilllus megaterium* (0,5 g/kg); BA: *Bacillus amyloliquefaciens* (0,5 g/kg); BM+BA: *Bacillus megaterium* + *Bacillus amyloliquefaciens* (0,25 g/kg + 0,25 g/kg)

CONCLUSIONS

In conclusion, it has been determined that the addition of 0.5 g/kg *Bacillus megaterium* and 0.5 g/kg *Bacillus amyloliquefaciens* and their mixtures to the diets of laying hens has a statistically significant effect on egg weight, the rate of damaged eggs, and the a* value of yolk color measurements. While the individual addition of these two probiotics significantly increased egg weight and it reduced the a* value. *Bacillus amyloliquefaciens* increased egg weight the most at a level of $1x10^{10}$ cfu/g compared to the control group. According to the results, *Bacillus amyloliquefaciens* can be added to laying hen diets due to its ability to increase egg weight in 59-week-old hens.

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THE EFFECTS OF DIFFERENT ROW SPACING ON AGRICULTURAL CHARACTERISTICS OF SAFFLOWER (*Carthamus tinctorius* L.) GROWN IN DRY CONDITIONS

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ABSTRACT

This research was carried out to determine the effects of different row spacing on agricultural characteristics of some safflower varieties under dry conditions in the Ölmez District of Konya Province during the 2022 safflower growing season (April-August).Three safflower varieties, namely Göktürk, Olein, and Servetağa, were used as experimental materials, with different row spacings of 20 cm, 30 cm, and 40 cm. The intra-row spacing was maintained at a constant 10 cm in all parcels. The experimental design was "Randomized Complete Block Design in Split Plots" with three replications. In the study, row spacings were placed in main plots and varieties were placed in sub-plots for ease of application. The seed yield, plant height, first branch height, number of branch per plant, number of head per plant were examined, and significant interactions were noted among number of head per plant and seed yield for row spacings × varieties. According to variety averages in the study, the highest yield of 125.9 kg da^{-1} was obtained from the Servetağa variety, while the lowest yield of 103.6 kg da^{-1} was obtained from the Olein variety. When the average values were taken into consideration, the highest seed yield was found in 30 cmxGöktürk as 138.1 kg da^{-1}. When looking at the average of interactions of row spacings × varieties; there were determined that the plant height was between 65.7-87.7 cm, the first branch height was 37.5-52.9 cm, the number of branch per plant was 5.3-6.9 pieces, the number of head per plant was 8.1-15.0 pieces, seed yield 63.3-138.1 kg da^{-1}. In addition, the research revealed the relationships between agricultural characteristics by both correlation and principal component analysis. A significant and positive relationships were found between same characteristics according to both analyzes. Consequently, the Göktürk variety demonstrated superior performance compared to other varieties under the rain-deficient conditions of the Ölmez District in Konya Province, and a row spacing of 30 cm was found to be the most suitable for this region.

Keywords: Safflower, *Carthamus tinctorius* L., row space, yield, agricultural characteristics.

INTRODUCTION

The safflower plant is one of the important oil plants that can be grown in nearly 60 countries worldwide, including marginal cultivation areas. Among the advantages of safflower cultivation are its deep-rooted structure, which helps improve soil quality, its ability to grow in arid conditions, its high-water retention capacity in heavy soils, and its lower demand for irrigation and fertilization compared to other oil crops such as sunflower and soybeans (Gilbert et al., 2008). The safflower plant is used in the vegetable oil industry; its flowers are used in dyeing food, fabrics, rugs, etc., for medicinal purposes and as animal feed. Safflower is an annual, multi-branched cultivated plant that can grow between 30-150 cm. Its flower colors can vary from yellow, orange, and red, and its oil content can vary from 20% to 45% (Dajue & Mündel, 1996). Safflower oil, which is also used as biodiesel, contains 90% unsaturated fatty

acids, including oleic and linoleic, and 10% saturated fatty acids (Velasco & Fernandez-Martinez, 2001; Emongor & Emongor, 2023).

Vegetable oil consumption per capita in our country increases with the increasing population, which causes the import figures of vegetable oil to increase yearly. Sunflower is one of our country's most cultivated oil plants, followed by cotton, soybean, peanut, rapeseed, and safflower plants. Safflower is among the priority oil crops in order to close the vegetable oil deficit of our country, as it enables the cultivation of safflower in arid areas reserved for fallow. In addition, the importance of safflower agriculture has increased daily with government subsidies (Yilmaz et al., 2021).

Yield and yield factors in safflower cultivation vary according to genotype, climatic conditions, soil structure, and agronomic practices (Eryiğit et al., 2015). It has been recorded in many studies that plant density within agronomic practices has a significant effect on the development and yield elements of a safflower plant (Ivanova et al., 2017; Gürsoy et al., 2018; Arslan & Güler, 2022; Sefaoğlu & Özer, 2022).

In this study, it has aimed to determine the effects of different row spacing on agricultural characteristics in some safflower varieties under dry conditions.

MATERIAL AND METHOD

The experiment was conducted in the 2022 growing season (April-August) in the farmer's field in Ölmez District of Altınekin District of Konya Province. Soil samples taken from different points of the test field at a depth of 0-30 cm before sowing are given in Table 1. As can be understood from the examination of Table 1, the soil texture of the experimental area is loamy, with a slightly alkaline soil pH, very low salt content, moderate organic matter content, and high lime content. The phosphorus content is sufficient, and the potassium level is high. In addition, trace elements such as Mg, Cu, and Mn are adequate, while Ca and Zn are abundant, and the iron (Fe) content is at a sufficient level.

Table 1. Soil analysis of the trial area

Texture (%)	48.28	**Mg (me/100 g)**	3.52
pH	7.46	**Ca (me/100 g)**	18.91
EC (mhos cm^{-1})	0.15	**Cu (ppm)**	0.63
$CaCo_3$ (%)	72.66	**Fe (ppm)**	3.48
Organic Matter (%)	2.32	**Mn (ppm)**	21.02
P_2O_5 (kg P_2O_5 da^{-1})	11.34	**Zn (ppm)**	4.96
K_2O (kg K_2O da^{-1})	79.69		

**Soil analysis was performed in BSK analysis laboratories.*

The average temperature (°C), total precipitation (mm), and relative humidity (%) values for the Altınekin District of Konya Province, covering the growing season of the research (April-August), are provided in Table 2. When we look at the average temperature values during the research period, it can be observed that the data for the year 2022 are close to the long-term averages. In terms of total precipitation, the values for April, May, and June in 2022 (5.4 mm, 32.9 mm, and 30.5 mm, respectively) were recorded much higher compared to the long-term averages (24.0 mm, 44.3 mm, 57.5 mm, respectively) (Table 2). The relative humidity values for the long-term average were found to be lower than the values for the year 2022, except for April (Table 2).

Table 2. Climatic data of the growing seasons (April-August) in which the experiment was conducted

	Average Temperature (°C)		Total Rainfall (mm)		Relative Humidity (%)	
Months	Long Years**	2022	Long Years	2022	Long Years	2022
April	11.6	13.6	24.0	5.4	55.5	43.9
May	16.4	14.9	44.3	32.9	50.8	59.0
June	19.6	19.6	57.5	30.5	57.4	59.5
July	23.5	21.4	9.2	7.9	42.6	47.5
August	23.4	20.0	15.7	9.8	44.1	45.4
Total/Average	18.9	17.9	150.7	86.5	50.1	51.1

**The data was obtained from the records of the Konya Regional Directorate of Meteorology, and the data for Altıekin District in Konya Province is provided. **Long-Term Average: It covers the years 2014-2021.*

The study was established with three replications according to the "Randomized Complete Block Design in Split Plots" experimental design, with different row spacings (20 cm, 30 cm, 40 cm) in the main plots and varieties (Göktürk, Olein, Servetağa) in the sub-plots. The Göktürk variety used in the study is thorny in plant type, with flowers initially appearing in yellow and gradually turning orange. Additionally, it is registered by Konya Bahri Dağdaş International Agricultural Research Institute. The Olein variety is of thorny type, with orange-colored flowers, and it was developed by the Department of Field Crops at Isparta Applied Sciences University Faculty of Agriculture. The Servetağa variety is also thorny in type, with yellow flowers, and it was developed by the Department of Field Crops at Selçuk University Faculty of Agriculture.

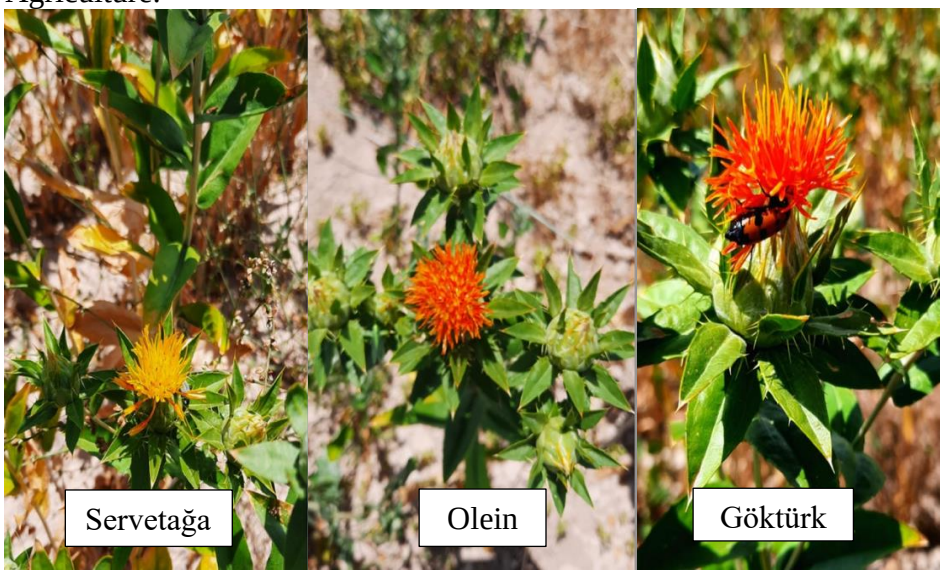

Figure 1. Images of the varieties used in the experiment during the flowering period.

In the study, each plot was 5 meters long and arranged in 5 rows with a spacing of 70 cm between plots and 1 meter between blocks. Planting was performed manually on April 1, 2022, with row spacing determined by markers at 3-5 cm depth. When the plants reached a 15-20 cm height, they were thinned to achieve a row spacing of 10 cm along with the first hoeing. During this period, the seedhead weevil (*Bangasternus planifrons*) pest was detected in the experiment field, and two insecticide applications were made using an insecticide containing 25% Malathion to control this pest. Harvesting was done by hand on August 25, 2022, when the crown leaves of the plants had dried, the seeds had whitened, the leaves had turned brown, and the head had completely dried. Before planting, 8 kg da^{-1} of phosphorus and 6 kg da^{-1} of nitrogen

were applied to the subplots as DAP (Diammonium Phosphate) and Ammonium Sulfate (%21 N) along with the planting. Prior to harvest, parameters of plant height (cm), first branch height (cm), number of head per plant, number of branch per plant were measured and calculated from 10 randomly selected plants from each plot, and values of seed yield (kg da^{-1}) from each parcels were detected and recorded.

The analyses of variance were done by JMP (8.1) statistical software package (Anonymous, 2009), and mean values were compared using multiple ranges of the Least Significant Difference (LSD) test at a 5% and 1% significance level using the MSTAT-C package program (Anonymous, 1993). In addition, Pearson's correlation and the principal component analysis was performed by JMP (8.1) software's.

RESULTS AND DISCUSSION

The variance analysis regarding the agricultural characteristics of different row spacings in some safflower varieties has been provided in Table 3.

Table 3. The variance analysis for agricultural characteristics of different row spacings in some safflower varieties

Source of variations	df	F Values				
		Plant height	First branch height	The number of brach per plant	The number of head per plant	Seed yield
Replication	2	1.77	0.17	3.41	1.72	0.29
Row spacing (R.S.)	2	0.49	12.71*	0.11	20.24**	7.84*
$Error_1$	4	-	0.95	-	-	-
Variety (V)	2	8.26**	4.03*	15.26**	7.89**	4.64*
R.S. x V	4	0.89	1.10	0.41	3.86*	3.95*
$Error_2$	12	-	-	-	-	-
Total	26	-	-	-	-	-

**p<0.01, *p<0.05

Plant Height (cm)

According to Table 3, concerning plant height, variety differences were statistically significant at the 1% level, while row spacing and row spacing x variety interaction were found to be insignificant. In this study, where variety differences in plant height were significant, the Servetağa variety led the first group (a) with 81.3 cm, followed by the Göktürk variety in group (ab) with 71.2 cm, and the Olein variety in group (b) with 68.2 cm. Although statistically insignificant, with respect to row spacing, the narrowest row spacing, 20 cm, resulted in the lowest plant height (70.4 cm), while the highest plant height values (75.5 cm) were obtained at 30 cm row spacing, and they were in the same group. When numerically evaluated in the row spacing x variety interaction, the longest plant height was determined at 40 cm x Servetağa interaction (87.7 cm), while the shortest plant height was recorded at 20 cm x Olein interaction (65.7 cm) (Table 4).

Table 4. Mean values (cm) of plant height determined in different row spacings in safflower varieties and groupings

Plant height				
	Row spacing x Variety			
Variety	20 cm	30 cm	40 cm	Means of variety**
Göktürk	70.3	74.1	69.2	71.2 ab
Olein	65.7	71.3	67.6	**68.2 b**
Servetağa	75.1	81.2	87.7	**81.3 a**
Means of row spacing	70.4	75.5	74.8	73.6
CV	11.88%			
LSD	Row spacing: n.s.; Variety: 10.34; Row spacing x Variety: n.s.			

As seen in Table 4, plant height values increased from 20 cm to 30 cm then decreased again at 40 cm, except for the Servetağa variety. In a study conducted by Köse and Bilir (2017), various row spacings (15 cm, 30 cm, 45 cm) and sowing rates (1.5 kg ha^{-1}, 3.0 kg ha^{-1}, 4.5 kg ha^{-1}, 6.0 kg ha^{-1}, 7.5 kg ha^{-1}) were applied. The researchers reported that plant height values also increased as row spacing and sowing rates increased. They suggested that this may be due to the ability of plants to utilize temperature and sunlight better with decreasing row spacing and increasing sowing rates, leading to increased competition. On the other hand, in another study conducted by Sefaoğlu and Özer (2022), row spacings were noted at 20 cm, 40 cm, and 60 cm, and sowing rates at 20 kg ha^{-1}, 40 kg ha^{-1}, 60 kg ha^{-1}. When examining plant height values, it was determined that as row spacing increased, plant height values also increased. The lowest value was obtained at the narrowest row spacing (20 cm) with 64.9 cm, followed by 66.3 cm at 40 cm and 69.6 cm at 60 cm. Factors such as the genetic structures of varieties, differences in cultural practices, and the climate and soil conditions in which they are grown can explain the variability in plant height values obtained from safflower in many studies (Öztürk et al., 2009; Gürsoy et al., 2018). However, the plant height values recorded in this study were close to the upper limit of the values recorded in studies related to different row spacings, such as Atakan (1992) (55.80-69.07 cm), Sefaoğlu (2017) (64.87-69.63 cm), and Erpay (2022) (60.9-67.0 cm); they were considerably lower than the findings of Gürsoy et al. (2018) (101.8-126.1 cm) and Arslan and Güler (2022) (173.7-170.5 cm).

First Branch Height (cm)

According to Table 3, variety differences were statistically significant at the 1% level, while row spacing and row spacing x variety interaction were found to be insignificant. Among row spacing averages, the tallest first branch height was recorded at 30 cm with 49.9 cm, followed by 46.6 cm at 40 cm row spacing within the same group. The shortest first branch height was observed at 20 cm row spacing, measuring 39.4 cm in length. In the study, concerning first branch height, the highest value according to variety averages was recorded in the Servetağa variety (48.3 cm) and classified in group (a). The Olein variety was categorized as group (ab) with 45.3 cm, while the lowest first branch height was determined at 42.2 cm in the Göktürk variety, representing group (b). Although statistically insignificant, when examining the row spacing x variety interaction for first branch height, the longest first branch height was recorded at 52.9 cm in the 40 cm x Servetağa interaction, while the shortest first branch height was observed at 37.5 cm in the 20 cm x Göktürk interaction (Table 5).

Table 5. Mean values (cm) of first branch height determined in different row spacings in safflower varieties and groupings

First branch height				
	Row spacing x Variety			
Variety	20 cm	30 cm	40 cm	Means of variety*
Göktürk	37.5	48.3	40.7	**42.2 b**
Olein	39.0	50.8	46.1	**45.3 ab**
Servetağa	41.7	50.4	52.9	**48.3 a**
Means of row spacing*	**39.4 b**	**49.9 a**	**46.6 a**	**45.3**
CV	14.5%			
LSD	Row spacing: 5.89; Variety:4.73; Row spacing x Variety: n.s.			

In a study conducted by Erpay (2022) on safflower varieties with different row spacings (15, 30, 45 cm), it was stated that row spacing and first branch height values increased proportionally. The lowest row spacing (15 cm) was recorded as 28.3 cm, while 30 cm had 30.2 cm, and 45 cm had 34.3 cm. In this study, as row spacing increased in the Servetağa variety, the first branch height increased, while in other varieties, there was an increase in values from 20 cm to 30 cm, followed by a slight decrease after 30 cm. The first branch height is crucial for mechanical harvesting (Atan et al., 2019). The values obtained for the first branch height in our study are in line with the findings of Aydın (2012) (33.85-40.95 cm) and Erpay (2022) (28.3-34.3 cm).

Number of Branch per Plant (pieces)

Variety differences in terms of the number of branch per plant were found to be statistically significant at the 1% level, while row spacing and row spacing x variety interaction were detected insignificant (Table 3). When examining the numerical values of row spacings in the study, the lowest value of 6.3 pieces was obtained from 20 cm and 40 cm, while the highest value of 6.4 pieces was recorded at 30 cm. Regarding varieties, according to the LSD test results, the first group (a) was determined to have 6.7 pieces in the Göktürk and Servetağa varieties, with the lowest number of branch per plant, 5.5 pieces, counted in the Olein variety. Although statistically insignificant, when the row spacing x variety interaction for the number of branch per plant was examined, it was observed that varieties responded differently to row spacings, with only the Göktürk variety showing an increase in the number of branch per plant as row spacing increased (Table 6).

A study by Kaya et al. (2015) conducted with safflower lines and varieties under Eskisehir conditions reported the highest number of branch per plant as 4.6. In a study by Koç and Güneş (2021) aimed at determining the relationships between flower yield and some morphological characteristics in different safflower genotypes, the values for the number of branch per plant were found to range from 6.0 to 13.2. In a study by Sefaoğlu and Özer (2022), the number of branch per plant was recorded as follows: 3.5 at 20 cm, 4.0 at 40 cm, and 5.0 at 60 cm row spacing. Generally, an increase in row spacing is expected to lead to an increase in the number of branch per plant, and there is also a close relationship between the number of branch and the number of head (Weiss, 2000). The variation in the number of branch observed in this study across different row spacings is thought to be due to variety characteristics, the location of the research, and ecological factors. The values for the number of branch per plant

determined in this study are in line with the findings of Erpay (2012) (5.8-7.8) and Sefaoğlu and Özer (2022) (3.54-5.02).

Table 6. Mean values number of branch per plant determined in different row spacings in safflower plant varieties and groupings

Number of branch per plant				
	Row spacing x Variety			
Variety	20 cm	30 cm	40 cm	Means of variety**
Göktürk	6.6	6.8	6.9	**6.7 a**
Olein	5.4	5.8	5.3	**5.5 b**
Servetağa	6.9	6.7	6.7	**6.7 a**
Means of row spacing	6.3	6.4	6.3	6.3
CV	13.17%			
LSD	Row spacing: n.s ; Variety: 0.79; Row spacing x Variety: n.s			

Number of Head Per Plant (pieces)

As can be observed from Table 3, significant differences were statistically determined at the 1% level for the number of head per plant concerning row spacing and varieties, while the row spacing x variety interaction was found to be significant at the 5% level. According to row distance, the highest number of head per plant, 14.2, was recorded in the 40 cm and 30 cm row spacings, representing group (a). The lowest number of head per plant, 11.7, was observed in the 20 cm row spacing and included in group (b). Significant differences among varieties were also noted in the study, with the highest number of head per plant being 14.5 head from the Göktürk variety (group a), followed by 13.8 head from the Servetağa variety (group ab), and 11.9 head from the Olein variety (group b). Regarding the row spacing x variety interaction, the lowest value was 8.1 in the 20 cm x Olein interaction, while all other interactions represented group (a).

In their study examining the effects of different row spacings and planting densities on safflower plants, Köse and Bilir (2017) reported an increase in the number of head with increasing row spacing and decreasing planting density. They found that the number of head varied between 10.6 and 20.9 depending on row spacing and planting density, with the highest value obtained from applications with a 45 cm row spacing (16.8) and a 1.5 kg planting density (17.2). In a study conducted by Arslan and Güler (2022), as row spacing increased, the number of head also increased and was found to range between 12.29 and 16.14 head. The close relationship between the number of branch and the number of head in the plant was reported in many studies, suggesting that as row spacing increases and the number of branch increases, the number of head also increases, indirectly positively affecting seed yield (Weiss, 2000; Moghaddasi & Omidi, 2015). The variation in the number of head among the varieties used in this study can be attributed to not only variety differences but also ecological factors. Furthermore, the number of head is considered one of the most important selection criteria for determining seed yield in safflower plants, with an expected increase in seed yield as the number of head increases (Uysal et al., 2006).

Table 7. Mean values number of head per plant determined in different row spacings in safflower varieties and groupings

Number of head per plant				
	Row spacing x Variety*			
Variety	20 cm	30 cm	40 cm	Means of variety**
Göktürk	**13.8 a**	**15.0 a**	**14.7 a**	**14.5 a**
Olein	**8.1 b**	**13.9 a**	**13.7 a**	**11.9 b**
Servetağa	**13.3 a**	**13.7 a**	**14.3 a**	**13.8 ab**
Means of row spacing **	**11.7 b**	**14.2 a**	**14.2 a**	13.4
CV	16.77%			
LSD	Row spacing: 2.06; Variety: 2.05; Row spacing x Variety: 2.54			

Seed Yield (kg da^{-1})

In the study investigating the agricultural characteristics of different row spacings in safflower varieties, the variance analysis results for seed yield are presented in Table 3, while the mean values and groupings are provided in Table 8.

Table 8. Mean values (kg da^{-1}) of seed yield determined in different row spacings in safflower varieties and groupings

Seed yield				
	Row spacing x Variety*			
Variety	20 cm	30 cm	40 cm	Means of variety*
Göktürk	104.6 b	**138.1 a**	116.7 ab	119.8 ab
Olein	**63.3 c**	125.6 ab	121.8 ab	**103.6 b**
Servetağa	126.5 ab	135.1 ab	116.2 ab	**125.9 a**
Means of row spacing*	**98.1 b**	**132.9 a**	118.2 ab	116.4
CV	21.76%			
LSD	Row spacing: 24.49; Variety: 16.53; Row spacing x Variety: 28.63			

In the study, row spacing, variety, and row spacing x variety interaction were found to be significant at the 5% level for seed yield (Table 3). Concerning row spacing, the highest seed yield was observed at 30 cm with 132.9 kg da^{-1}, while the lowest seed yield was obtained at 20 cm with 98.1 kg da^{-1}. According to variety averages, the highest seed yield of 125.9 kg da^{-1} was determined for the Servetağa variety, and the lowest seed yield was obtained from the Olein variety (103.6 kg da^{-1}). Regarding row spacing x variety interactions, the highest seed yield was calculated for the 30 cm x Göktürk interaction (138.1 kg da^{-1}), while the lowest seed yield was recorded for the 20 cm x Olein interaction (63.3 kg da^{-1}).

Seed yield is a parameter that can be affected by cultural practices and environmental conditions, as well as being a cultivar characteristic of safflower (Polat, 2007). Ozlem and Bilir (2017) reported values obtained from different row spacings in their studies as 1451.9 kg ha^{-1} at 15 cm, 1997.1 kg ha^{-1} at 30 cm, and 1875.9 kg ha^{-1} at 45 cm, respectively. In research conducted by Gürsoy et al. (2018), considering values obtained from different row spacings, the lowest seed yield was recorded as 121.4 kg da^{-1} for the 20 cm x Ayaz interaction, while the

highest value was determined as 157.7 kg da^{-1} for the 20 cm x Ayaz interaction. In another study focused on different row spacings as the sole variable, seed yield was found to be 146.2 kg da^{-1} at 15 cm, 144.3 kg da^{-1} at 30 cm, and 105.4 kg da^{-1} at 45 cm (Erpay, 2022). In a study conducted by Sefaoglu (2017), it was reported that as planting distances increased, the decrease in seed yield due to increased soil evaporation was more pronounced compared to denser planting. Therefore, practices such as the appropriate row spacing and plant density per unit area are of utmost importance for seed yield.

On the other hand, one of the main reasons for the low yield of safflower, which has difficulty competing with other oilseed crops such as sunflower, canola, and soybean, is that the average safflower yield in our country is around 90 kg da^{-1}. With breeding efforts and cultural practices such as appropriate planting frequency, it is expected that safflower yield will increase day by day (Bayramin, 2006). The seed yield values in this study partially align with the findings of the researchers mentioned above, but the responses to different row spacings have shown variation. Row spacings increased from 20 cm to 30 cm, and decreases in yield were observed at 40 cm.

Pearson's Correlation Estimation

Table 9. Pearson's correlation estimation between agricultural traits in some safflower varieties cultivated under dry conditions and different row spacing.

	PH	**FBH**	**NB**	**NH**	**SY**
PH	1				
FBH	0.706**	1			
NB	0.487**	0.082	1		
NH	0.380	0.392*	0.530**	1	
SY	0.477*	0.564**	0.378	0.689**	1

* $p < 0.05$, ** $p < 0.01$ (PH: Plant height, FPH: First plant height, NB: Number of branch, NH: Number of head, SY: Seed Yield)

Pearson's correlation estimation (Table 9) showed that plant height had a significant positive correlation with the first plant height (r= 0.706**), the number of branch per plant (r= 0.487**) and seed yield (r= 0.477*). The first plant height showed a significant positive correlation with the number of head per plant (r= 0.392*), seed yield (r= 0.564**). The number of branch per plant showed a highly significant positive correlation with the number head per plant (r= 0.530**). The number of head per plant had a highly significant positive correlation with seed yield (r= 0.689**).

Principal Components Analysis

The principal component (PC) axes, eigenvalues, variation, and cumulative variation ratios were obtained as a result of Principal Component Analysis (PCA) and factor coefficients indicating the weight values of principal components based on traits are presented in detail in Table 10. As a result of the analysis, 3 independent principal component axes were obtained concerning the 5 traits examined. The eigenvalues of the first 3 basic components were found from 0.74 to 3.06. The first principal component axis accounts for 61.23% of the total variation. The second and third principal components cover 21.07% and 14.86% of the total variation, respectively (Table 10).

Table 10. Eigenvalue, variation and principal component axes of the traits examined as a result of principal component analysis

Eigenvalue	3.06	1.05	0.74
Variation (%)	61.23	21.07	14.86
Cumulative Variance (%)	61.23	82.30	97.16
Traits	**PC1**	**PC2**	**PC3**
Plant height	0.44	0.17	**0.71**
First plant height	0.39	**0.71**	0.02
Number of branch	0.38	**-0.66**	0.34
Number of head	0.50	-0.19	-0.44
Seed Yield	0.51	-0.00	-0.44

It is known that the features examined in principal component analysis have significant weight when the weight values in the components are 0.6 and above (Jeffers, 1967). As a result of the analysis, the first plant height and the number of head per plant were determined as features with high factor coefficients on the second PC axis, which covers 21.07% of the total variation. The third PC axis represents only the plant height feature. In order for the biplot plot to adequately explain the total variation, the total principal component ratio must be greater than 50% (Kroonenberg, 2016). In this study, this value was 61.23%. A loading plot graph was created for the evaluation of 3 safflower varieties with 3 different row spacing using PC1 and PC2 components (Figure 2). A score plot was created to evaluate 3 safflower varieties with 3 different row spacing using PC1 and PC2 components (Figure 3). It was reported that if the angle between the vectors in the figure is $<90^o$, there is a positive relationship, if it is $>90^o$, there is a negative relationship, and if the angle between the vectors is 90^o, there is no significant relationship (Seymen et al., 2019). Plant height, first branch height, number of head and number of branch when the angle is less than 90° (Figure 2). Accordingly, there is a positive relationship between plant height and first branch height, and between the number of branch and the number of head. The results observed in the loading plot graph (Figure 2) and the results in the Pearson's correlation estimation (Table 9) largely support each other. The stability of the safflower varieties with different row spacings in terms of agricultural traits are given in Figure 3. While the varieties to the right of the line that represents the average agricultural traits according to the average center of the coordinates and cuts the axis in the middle gave higher data than the average, the varieties to the left gave lower data than the average. Genotypes close to the line that crosses the origin horizontally are also considered the most stable genotypes (Karaman, 2019). Accordingly, the 30*Göktürk variety is the interaction closest to the stability line, the lowest results were obtained from the 40*Olein and 20*Göktürk interactions, and good results were obtained from the other interactions.

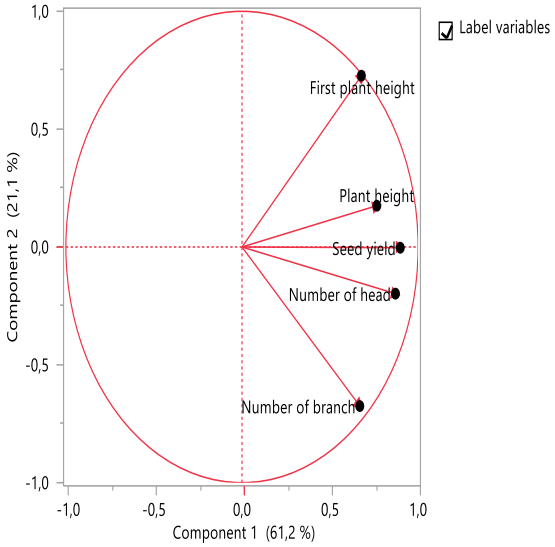

Figure 2: Loading plot graph obtained from PC1 and PC2 as a result of PCA

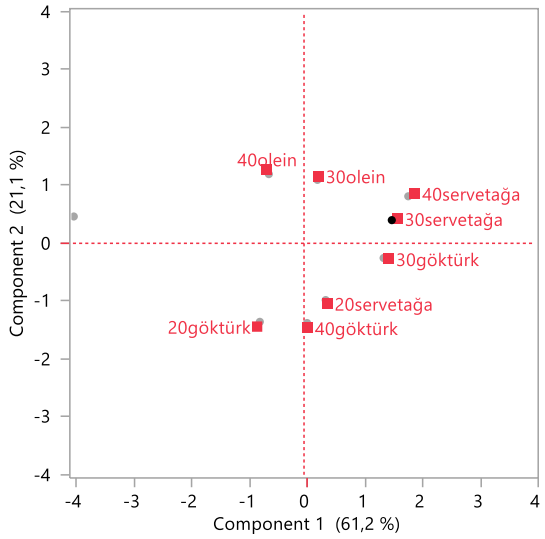

Figure 3: Score plot graph obtained from PCA result PC1 and PC2

CONCLUSIONS

In this study conducted under dry conditions in Ölmez District, which is located in Altınekin District of Konya Province, with the aim of determining the agricultural characteristics of different row spacings in some safflower varieties, the best overall results in terms of the examined characteristics were obtained from the Göktürk variety at a spacing of 30 cm. Particularly, research on the appropriate row spacing for the safflower plant, which can be cultivated in dry areas, is of great importance since it directly affects seed yield. Conducting experiments in different climates and soil conditions, especially in locations with higher rainfall and for a minimum duration of two years, is essential to improve seed yield and other agricultural parameters and to facilitate better interpretation.

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COMPARISON OF TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENTS OF BOTH AQUEOUS AND METHANOLIC EXTRACTS OF *MARRUBIUM VULGARE* L.

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ABSTRACT

Marrubium vulgare L. *is* a member of the Lamiaceae family, which is significant in medicine. It was defined by Paracelcus as the "doctor of the lung". Traditionally; it was applied externally to treat skin conditions as well as ingested for disorders of the mouth, respiratory system, and digestive system, jaundice, menstrual pain and as a stimulant. In the past, In Tunisia, this plant is used to treat hypertension, diabetes, and heart disease; in Mexico, its decoction is known for its antidiabetic properties. İn our country, it is traditionally used as a diuretic, carminative, pain killer, antipyretic, and appetite stimulant. The major compounds of the plant are known to be the phenolic compounds and flavonoids, which are categorized as natural sources of antioxidants and the goal of this study is to determine the total phenolic and total flavonoid contents of both aqueous and methanol extracts of aerial parts of *M. vulgare* L.

Total phenolic content was calculated by using the Folin Ciocalteu method. The absorbance of the samples was measured at 750 nm using a spectrophotometer. The results were given as gallic acid equivalents. The total flavonoid content was measured using the aluminum chloride colorimetric method and was calculated as quercetin equivalent. The absorbance of the mixtures was measured at 415 nm in a spectrophotometer.

In conclusion; it was determined that the methanolic extract of the aerial part of the plant had a higher phenolic content (38.60 mg ± 4.8 mg GAE/g) than the aqueous extract (27.83 mg± 4.61mg GAE/g). On the other hand, it was determined that the aqueous extract of the plant (4.02 mg± 0.1mg QE/g) had a higher total flavonoid content than the methanol extract (3.25 mg ± 0.19mg QE/g).

Keywords: *Marrubium vulgare* L., total flavonoid, total phenol content

INTRODUCTION

M.vulgare L belongs to the medicinally important family Lamiaceae. *Marrubium* L genus has total of 23 taxons in our country. Since 14 of them are endemic and their endemic rate is high, so they are valuable in terms of genetic diversity (Bakış, Babac, & Uslu, 2011). Originating Central Asia and the Mediterranean, this plant is common throughout the Americas, Central Europe, Australia and South Africa (Fleming, 2000). İt spread along the Black Sea, Marmara, Aegean, Mediterranean, Southeast, and Central Anatolia coasts in our country and commonly found in the provinces of Amasya, Antalya, Balıkesir, Bursa, Çanakkale, Denizli, Eskişehir, Hatay, İçel, İzmir, Karabük, Konya, Manisa ve Şanlıurfa (Bakış, Babac, & Uslu, 2011). Among the people, names such as ''gray grass, Karaderme, false nettle, dog grass, dog fly, kukas grass and mayasıl grass,, are given (Baytop, 1994). Scientific studies have also established the effects of the plant, which have been widely used historically and culturally. These effects include anti-inflammatory, analgesic, antinociceptive, antiedematous, antispasmodic, gastroprotective, antihypertensive, hepatoprotective, antioxidant, antihyperlipidemic, antimicrobial, anticancer, immunomodulation, parasitic, antiprotozoal, and

neuroprotective. (Bühring, 2005; Fleming, 2000; transl. Beck, 2005; Lodhi, Vadenere, & Sharma, 2017).

Phytochemicals are bioactive substances produced by the secondary metabolism of plants that are not edible but have health benefits (Demir & Akpınar, 2020). The major compounds of this plant are known to be the phenolic compounds and flavonoids, which are recognized secondary compounds, have a significant impact on the antioxidative pathway. It shows this effect by neutralizing free radicals and as a positive potentiator (Demir & Akpınar, 2020; Pukalskas, Venskutonis, Salido, de Waard, & van Beek, 2012; Vanderjagt, Ghattas, Vanderjagt, Crossey, & Glew, 2002; Feyerer, 2021). This study was conducted to measure the *M. vulgare* L. aerial parts' total phenolic and total flavonoid contents of the methanol and aqueous extracts.

MATERIAL AND METHOD

Marrubium vulgare L. plant was collected during the vegetation period, the aerial parts were separated and dried under suitable conditions, and it was taken to Ankara Yıldırım Beyazıt University Herbarium with the barcode number Koç 3634. The dried samples were pulverized in the mill for processing.

Methanol and water extracts were prepared for each of the plants. To prepare the methanol extract, 10 g of a ground plant sample was added to 150 ml of MeOH and the extraction was carried out in an ultrasonic bath for 1 hour. After extraction, the methanol extract was filtered through filter paper and then the solvent was removed using a rotary evaporator. To prepare the water extract, 10 g of the ground plant and 150 ml of water were added and the extraction was carried out in an ultrasonic bath for 1 hour. Then the water extract was filtered and frozen and the water removed by lyophilization. All extracts were stored at +4 °C until analysis.

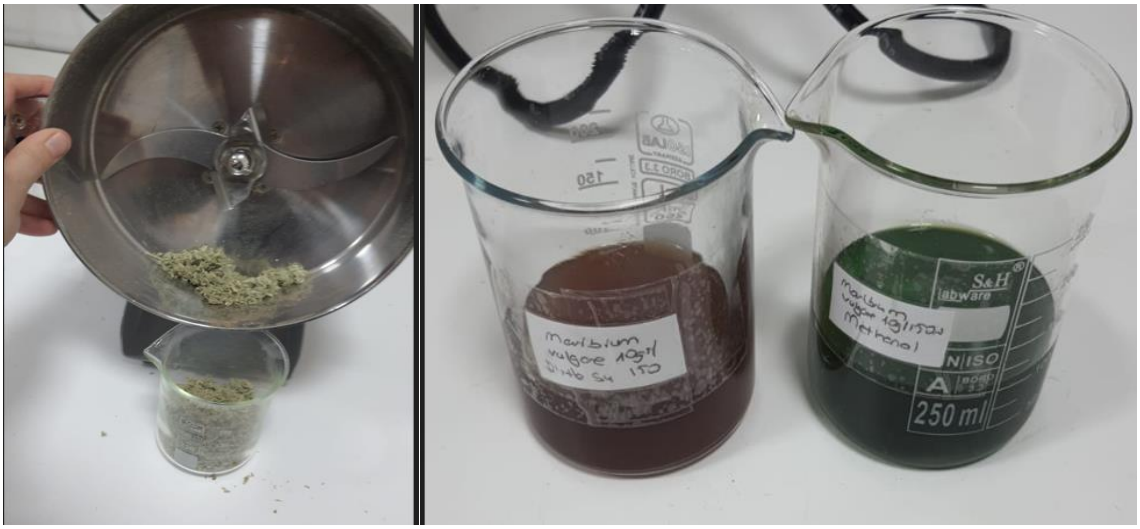
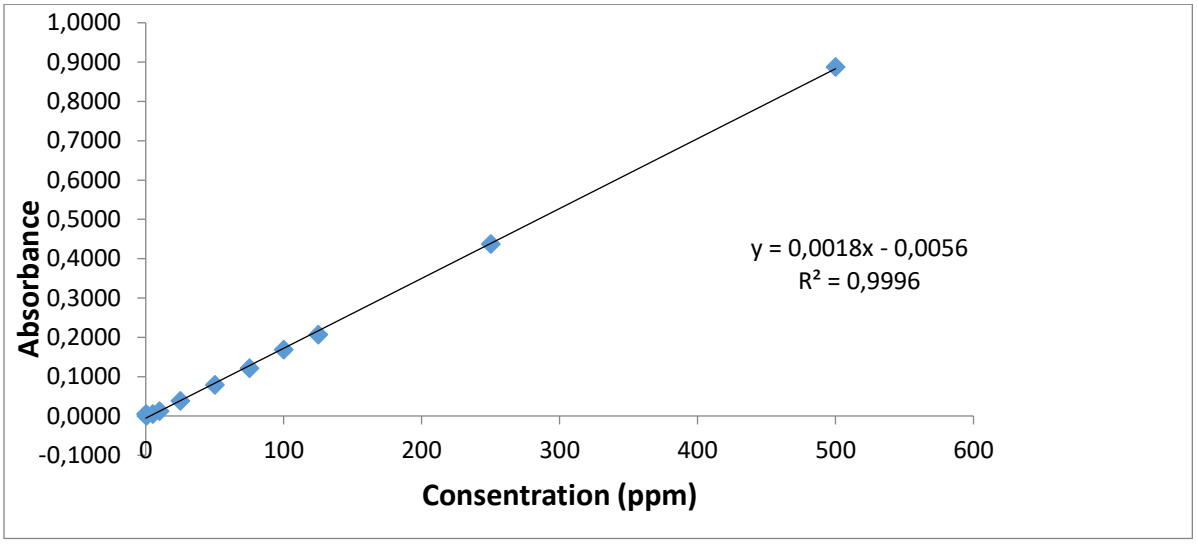


Figure 1. Methanol and water extracts of *Marrubium vulgare* L.

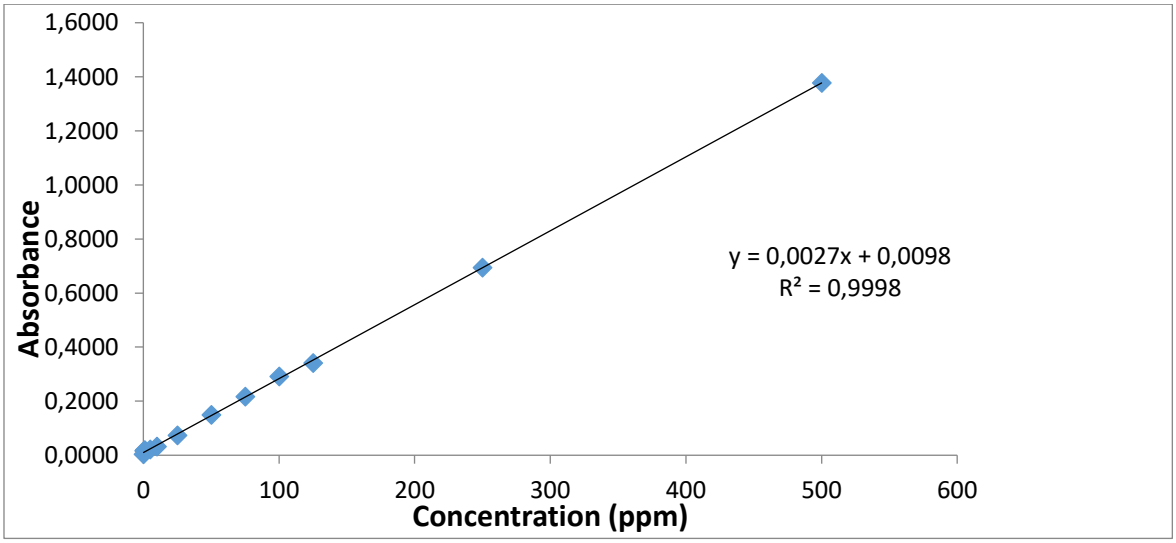
Total phenolic content was calculated by using the Folin Ciocalteu method(modified) (Kabach, Mrid, Bouchmaa, Bouargaine, & Nhiri, 2010). 150 ml of (2% Na2CO3) Sodium carbonate and 100 ml of extract were combined with 250 ml of the Folin-Ciocalteu reagent. After being vortexed, the mixture was left at room temperature for 30 minute. The absorbance of the samples was measured at 750 nm using a spectrophotometer. A calibration curve for

gallic acid was created using various gallic acid concentrations (0.1, 0.5, 5, 10, 25, 50, 75, 100, 125, 250, 500), and the equation Y = 0.0018X - 0.0056 (R^2 determined = 0.9996) was used to calculate the amount of phenolic compounds in the extracts as acid equivalents (mg GAE/g).



Graphic 1. Gallic acid calibration curve

The total flavonoid content was measured using the aluminum chloride colorimetric method (modified) and was calculated as quercetin equivalent (Khaled-Khodha, Boulekbache-Makhlouf, & Madani, 2014). The following ingredients were combined with 200 µl of extract; 600 µl ethyl alcohol, 200 µl aluminum chloride, 200µ l sodium acetate, and 2 ml distilled water. After being vortexed, the mixture was left to cool for 30 minutes. Different quercetin concentrations (0.1, 0.5, 5, 10, 25, 50, 75, 100, 125, 250, 500) were used to generate the quercetin calibration curve, and the flavonoid content of the extracts was converted to quercetin equivalent using the equation Y = 0.0027X - 0.0098 (R2 =0.9998) (mg of QE/g). The absorbance of the mixtures was measured at 415 nm.



Graphic 2. Quercetin calibration curve

RESULTS AND DISCUSSION

The results of the study with repeated measurements are as shown in the tables;

Total Phenol Experiment									
Control	**A. Measure**	**A. Blank**	**A. Sample**	**x (µg/ml)**	**X(mg/ml)**	**Equivalent of the Gallic acid (mg GAE/g)**	**Average (mg GAE/g)**	**STD EV**	**KTFe±SD**
***Marrubium vulgare*-MeOH**	0,122	0,067	0,055	33,440	0,033	33,44	38,60	4,80	38,60±4,80
***Marrubium vulgare*-MeOH**	0,121	0,055	0,065	39,450	0,039	39,45			
***Marrubium vulgare*-MeOH**	0,122	0,051	0,072	42,918	0,043	42,92			
***Marrubium vulgare*-Aqueous**	0,103	0,067	0,035	22,718	0,023	22,718	27,83	4,61	27,83±4,61
***Marrubium vulgare*-Aqueous**	0,102	0,055	0,047	29,112	0,029	29,112			
***Marrubium vulgare*-Aqueous**	0,102	0,051	0,051	31,665	0,032	31,665			

Table 1. Results of total amount of phenolic content

Total Flavonoid Experiment									
Controll	**A.Measure**	**A. Blank**	**A. Sample**	**x (µg/ml)**	**X(mg/ml)**	**Equivalent of the Quercetin (mgQE/g)**	**Average (mgQE/g)**	**STD EV**	**KTF±SD**
***Marrubium vulgare*-MeOH**	0,083	0,065	0,018	3,035	0,003	3,04	3,25	0,19	3,25±0,19
***Marrubium vulgare*-MeOH**	0,081	0,062	0,019	3,403	0,003	3,40			
***Marrubium vulgare*-MeOH**	0,082	0,063	0,019	3,309	0,003	3,31			
***Marrubium vulgare*-Aqueous**	0,086	0,065	0,021	4,004	0,004	4,00	4,02	0,10	4,02±0,10
***Marrubium vulgare*-Aqueous**	0,083	0,062	0,020	3,937	0,004	3,94			
***Marrubium vulgare*-Aqueous**	0,084	0,063	0,021	4,126	0,004	4,13			

Table 2. Result of Total amount of flavonoid content

As a result, the methanolic extract obtained from the aerial part of *Marrubium vulgare* L. had a richer phenolic content (38.60 mg ± 4.8 mg GAE/g) than the aqueous extract. The total phenolic content of the aqueous extract (27.83 mg± 4.61mg GAE/g) was measured. However, it was obtained that the aqueous extract (4.02 mg± 0.1mg QE/g) had a higher total flavonoid content than the methanol extract (3.25 mg ± 0.19mg QE/g).

Marrubium vulgare L.	Total amount of phenolic content	Total amount of flavonoid content
Aqueous extract	27.83 mg± 4.61mg GAE/g	4.02 mg± 0.1mg QE/g
Methanol extract	38.60 mg ± 4.8 mg GAE/g	3.25 mg ± 0.19mg QE/g

Table 1. Comparison of total phenolic and total flavonoid contents of both aqueous and methanolic extracts of *Marrubium vulgare* L.

CONCLUSIONS

With sales of more than 146 million USD in the USA in 2018, *Marrubium vulgare* L, a plant with a great potential and widespread use, is also the one of the most popular herbal dietary supplement (Sağlam, 2019). It is important to evaluate this plant, which has a wide distribution in our country and has antioxidative activity.

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EXPERIMENTS ON REVEALING EFFECTS OF DIFFERENT MEDIA STRENGTH, AND SUCROSE-DEPENDENT IN ADVENTITIOUS ROOT CULTURES OF RADISH *(Raphanus Sativus* L.)

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Radish (*Raphanus sativus* L.), one of the important vegetables of the *Cruciferae* family, has attracted attention due to its nutritional content and health-improving properties. The adventitious root culture technique not only supports the propagation of medicinally valuable plants but also offers an alternative method to harvest valuable bioactive components from plants. In the current study, hypocotyls obtained after germination of red and black radish seeds were used as explant material, the effect of MS basic medium at different strengths (¼, ½, ¾, and 1) and the effect of different amounts of sucrose (20, 25, 40 and 50 mg L^{-1}) on adventitious root formation were evaluated in terms of biomass formation. Within the framework of the results obtained, it is thought that adventitious root cultures of radish can be used as a complementary method in the large-scale production of valuable bio-compounds to be used in the pharmaceutical industry.
Keywords: *in vitro,* root cultures, medium strength, biomass

INTRODUCTION

Plants are used as a model system and a taxonomic marker for the discovery of new compounds. Increasing awareness of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs has increased interest in the use of plants and plant-based medicines. The complex structure of the compounds and the high cost of chemicals make the synthesis of bioactive compounds a difficult process. However, the production of these compounds is also affected by some factors, such as the growing period of the plant and the season in which it is collected, species and variety differences, the plant being in a certain growth and development period, and the lack of adequate methods for the production and standardization of the plant (Ahmad et al., 2015).

As an alternative solution to such problems faced by the phytopharmaceutical industry, biotechnological approaches, especially plant tissue culture methods, attract a lot of attention. Since plant tissue culture methods offer a controlled supply of biochemicals independent of plant availability with consistent product quality, it is thought that they could provide a great potential to boost the conventional agriculture for the industrial-scale production of bioactive plant metabolites (Ramachandra Rao and Ravishankar, 2002; Ahmad et al., 2015). Plant cell, tissue, and organ culture techniques are promising techniques that have the potential to be used in obtaining valuable plant metabolites, including pharmaceuticals (Murthy et al., 2008; Ahmad et al., 2015). These techniques both provide a reliable source for plant-based pharmaceutical products and can be used for large-scale cultures of plant metabolites. The increasing commercial importance of secondary metabolites in recent years has led to great interest in the diversification of the production of bioactive plant metabolites through tissue culture technology. At this point, the adventitious root culture system provides an alternative approach for the improvement and development of plant-based pharmaceutical compounds (Ahmad et al., 2015).

Plant roots serve as a source of bioactive molecules, including a surprising variety of metabolites, agrochemicals, flavors, dyes, and fragrances (Fulzele et al., 2002). Recently, organ culture, especially adventitious root culture, has been applied to many medicinal plants due to their rapid growth as well as stable production of secondary metabolites of pharmaceutical and

nutraceutical interest (Murthy et al., 2008). Adventitious roots induced under aseptic conditions in a suitable nutrient medium supplemented with plant growth regulators have a good growth rate and have a potential for accumulation and sustained production of secondary metabolites (Hahn et al., 2003).

Adventitious root formation is a complex process involving various endogenous and exogenous factors (Sorin et al., 2005). The adventitious root formation process is divided into four stages: (a) the pre-emergence stage of the root, which includes the molecular and biochemical process changes that occur prior to any cytological formation up to the emergence of primordial roots, (b) the early stage of root development, (c) the root growth stage (weight and volume increase) and (d) the final stage of root configuration (emergence of the first root) (Zhang et al., 2017). The initiation and differentiation process during the physiological stages of rooting can be triggered by changes in endogenous auxin concentrations and external addition of specific auxins (Praveen et al., 2009).

Adventitious roots show high stability, high growth rate, and continuous secondary metabolite production when stimulated in an aseptic artificial nutrient medium supplemented with optimal phytohormone (Hahn et al., 2003). These roots produce high amounts of alkaloids, terpenoids, and phenols in cell and tissue spaces and show high stability and growth rates; they can be easily produced in a suitable hormone-supplemented environment with a low amount of inoculum (Sivakumar et al., 2006). Adventitious root cultures also provide an experimental system to study the link between primary and secondary metabolism (Ahmad et al., 2015).

Radish (*Raphanus sativus* L.), one of the important vegetables of the *Cruciferae* family, is one of the functional foods that have an important place in meeting people's fresh vegetable needs and attracts attention with its nutritional content and health-improving properties (Akan et al., 2013) such as antibacterial, antioxidant, antimutagenic, hepatoprotective, nephroprotective, antidiabetic, anti-inflammatory activities (Rosés et al., 2023).

In the current study, hypocotyls obtained from red and black radish seeds germinated *in vitro* were used as starting material, and Murashige and Skoog (MS) basic medium at different strengths (¼, ½, ¾, and 1) and various amounts of sucrose (20, 25, 40 and 50 mg L^{-1}) were used to reveal their effect on adventitious root formation regarding biomass accumulation.

MATERIALS AND METHODS

The study was carried out in the Tissue Culture Laboratory of Akdeniz University, Faculty of Agriculture, and Department of Horticulture.

Explant and Media Preparation, Explant Culturing

Seeds of red and black radish cultivars were used in the study. Seeds of the cultivars were subjected to surface sterilization before being cultured. The seeds were first kept in 70% ethanol solution for 1 minute and then sterilized in 50% sodium hypochlorite solution for 12 minutes, and then, it was rinsed 3 times with sterile distilled water.

Sterilized seeds were cultured in MS (Murashige and Skoog, 1962) medium (MS0) containing 30 g L^{-1} sucrose and 6 g L^{-1} agar, without the addition of plant growth regulators, to germinate under *in vitro* conditions. Cultured seeds were incubated under 24±2 °C temperature and 16/8 hour light/dark photoperiod conditions. For seed germination under *in vitro* conditions, 15 glass jars with a volume of 660 mL were used for both cultivars, and 15 seeds were planted in each jar.

The 15-day-old hypocotyls from the germinated plantlets were used as starting material for the adventitious root culture study. Hypocotyls were cut into 0.5 - 1 cm pieces in a laminar flow and cultured in the combinations of media presented in Table 1 for the initiation of adventitious root cultures. For each cultivar, 90 mm 5 petri dishes/medium were used while 15 hypocotyl explants were cultured in each petri dish and the study was carried out in 3 replications.

Table 1. Media combinations used in the study

Media Codes	Media Combinations			
	MS	**IBA (mg L^{-1})**	**Sucrose (g L^{-1})**	**Agar (g L^{-1})**
1	¼	2	30	6
2	½	2	30	6
3	¾	2	30	6
4	1	2	30	6
5	¾	2	20	6
6	¾	2	25	6
7	¾	2	40	6
8	¾	2	50	6
Control	1	-	30	6

Establishing and Propagating of Adventitious Root Cultures

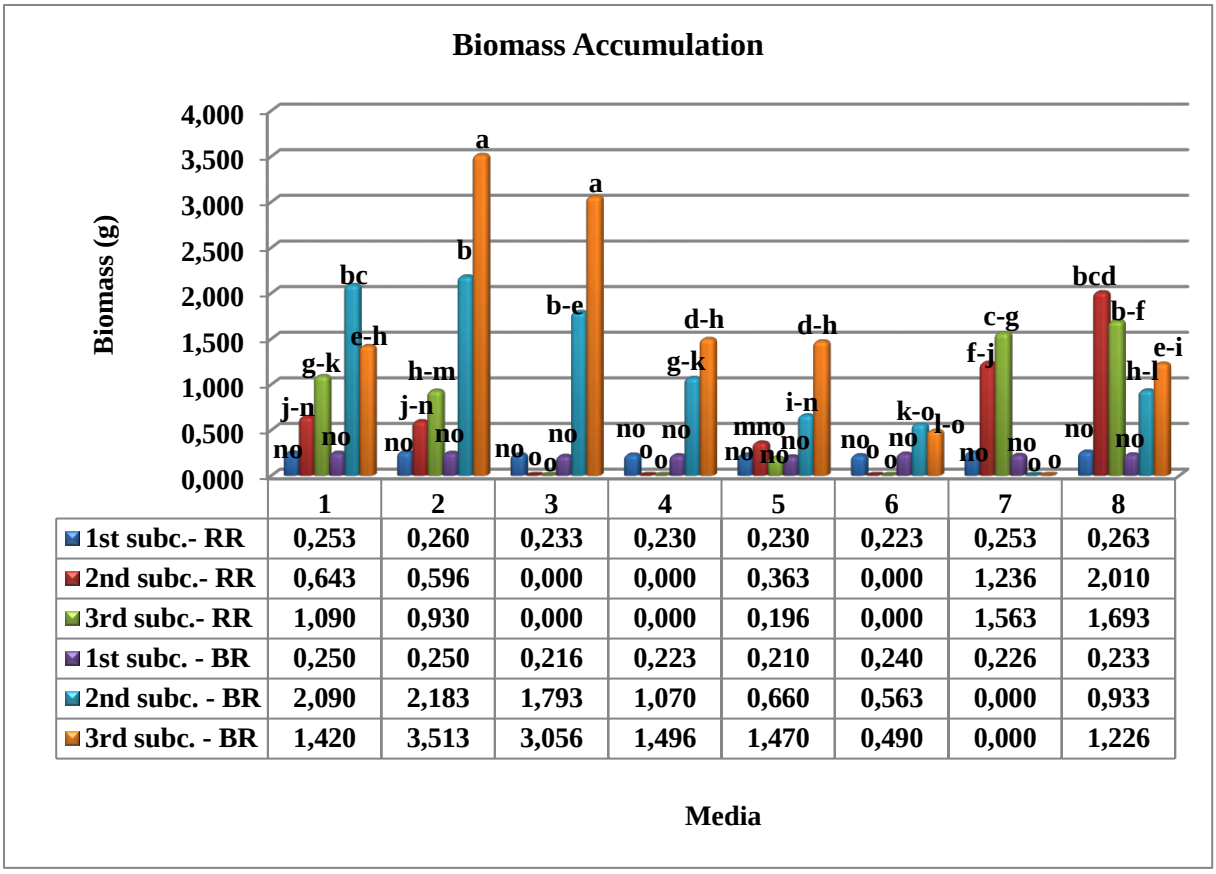
Adventitious root cultures were initiated with 2-month-old roots consisting of hypocotyl explants cultured in different combinations of media. Adventitious roots (0.15 - 0.20 g/petri) obtained from hypocotyls of black and red radish cultivars were inoculated into 100 mL conical flasks containing 30 mL of nutrient medium. The medium combinations used in the initiation of adventitious root cultures were used as liquid medium without adding agar for adventitious root propagation. Cultures were shaken at 130 rpm on an orbital shaker under dark conditions at 24±2°C for 4 weeks. Three subcultures with 4-week intervals were conducted in the study, and the weights of the proliferating adventitious roots at the end of each subculture were measured and recorded.

Statistical analysis

The experiments of the current study were conducted in three replicates with a completely random factorial design. The data obtained were made in the JMP package program and the differences between the averages were determined by the 'least significant difference' (LSD) test, and the differences were found to be statistically significant at the $p < 0.05$ level.

RESULT AND DISCUSSION

In this study, the effects of MS media of various strengths and sucrose at different concentrations on the initiation of adventitious root cultures and biomass accumulation in 2 different radish cultivars were evaluated (Figure 1 - 3).



	1	2	3	4	5	6	7	8
1st subc.- RR	0,253	0,260	0,233	0,230	0,230	0,223	0,253	0,263
2nd subc.- RR	0,643	0,596	0,000	0,000	0,363	0,000	1,236	2,010
3rd subc.- RR	1,090	0,930	0,000	0,000	0,196	0,000	1,563	1,693
1st subc. - BR	0,250	0,250	0,216	0,223	0,210	0,240	0,226	0,233
2nd subc. - BR	2,090	2,183	1,793	1,070	0,660	0,563	0,000	0,933
3rd subc. - BR	1,420	3,513	3,056	1,496	1,470	0,490	0,000	1,226

Figure 1. Biomass accumulation in radish cultivars regarding different media and subcultures
(1): Different letters among cultivars, media and subcultures denote significant differences (LSD test, p<0.05).
(2): LSD cultivars* = 0.115; LSD media* = 0.231; LSD subculture* = 0.142*; **(b)** LSD cult. x media x subc.* = 0.568
(3): Abbreviations; subc.= subculture; RR = Red Radish; BR = Black Radish

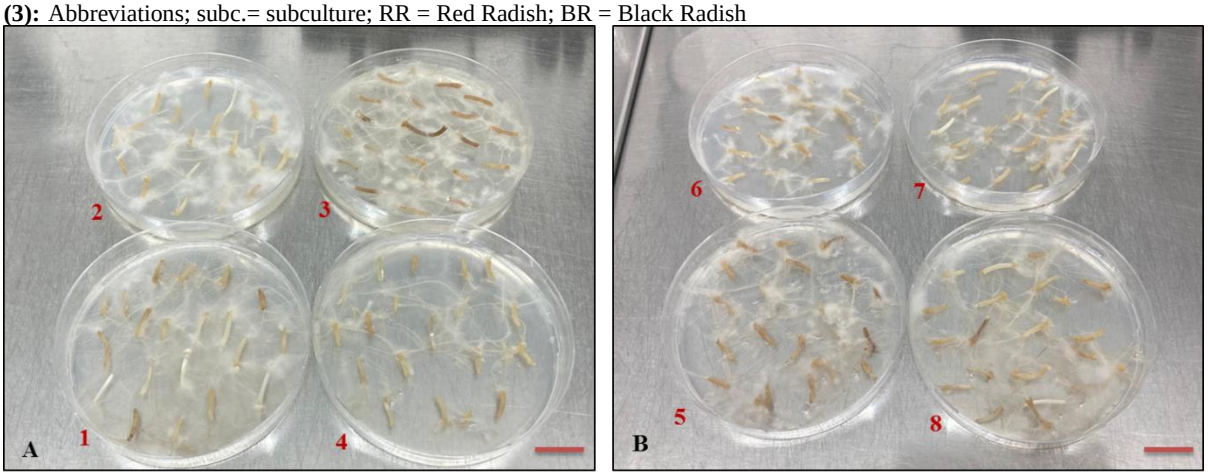


Figure 2. Adventitious root formation from hypocotyls of black (A) and red radish (B) cultivars in different media

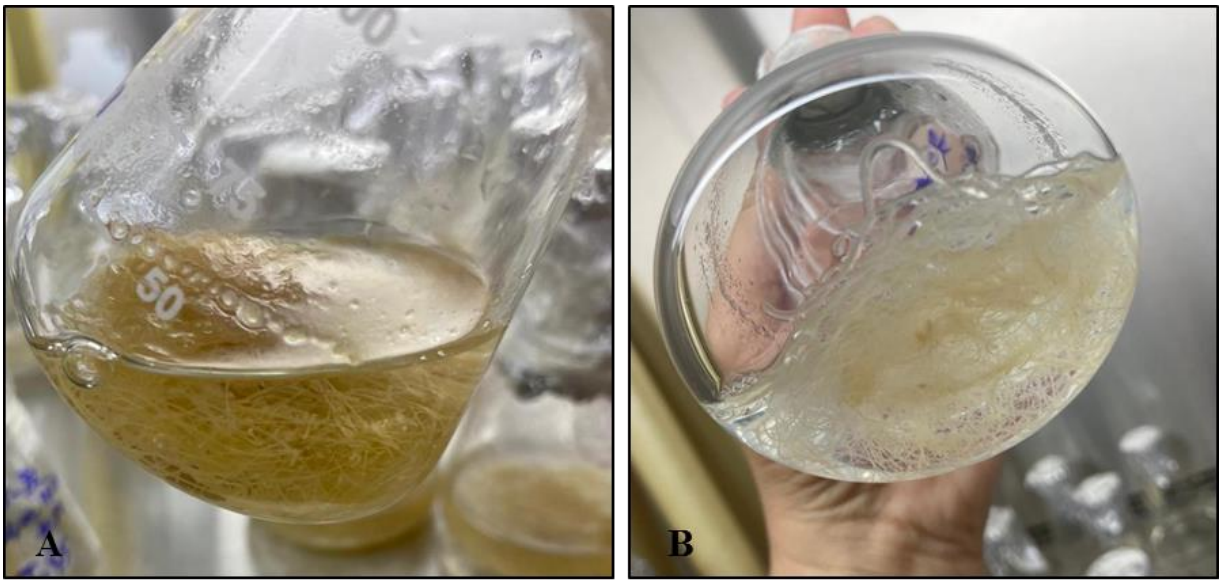


Figure 3. Adventitious root cultures of black (A) and red radish (B) cultivars in different media

According to the findings, statistical differences were determined regarding biomass accumulation between radish cultivars. The responses of the cultivars to adventitious root cultures were found to be different in terms of biomass accumulation, and it was observed that the black radish cultivar provided higher biomass accumulation than the red radish cultivar.

Differences were detected in terms of the nutrient media combinations evaluated. The combination of media in which the ½ MS strength supplemented with 30 g L^{-1} sucrose (medium combination No. 2) gave the most positive value in terms of biomass accumulation, which was followed by the medium No. 8, contained ¾ MS strength and 50 g L^{-1} sucrose.

Among various plant cell and organ cultures, adventitious root culture is an alternative and complementary tool to whole plant cultivation to produce high-value phytochemicals (Hao and Guan, 2011; Baque et al., 2012b; Sivanandhan et al., 2013), a wide range of physiological It has a complex molecular process involving several factors (Sorin et al., 2005).

Previous studies have reported that auxins play a very important role in the adventitious root formation process, and that exogenous auxin applications as well as endogenous auxins are important in promoting the initiation and division of adventitious roots (Baque et al., 2009; Hussein et al., 2012). Indole butyric acid, IBA, is known as synthetic auxin and is among the first plant hormones used to increase root formation in plant stems (Deloso et al., 2020). For example, in a study, it was reported that IBA was more effective than IAA and NAA in stimulating adventitious roots from hypocotyl explants of *Psoralea coryfolia* (Baskaran and Jayabalan, 2009). In current study, IBA (2 mg L^{-1}) was used and had a positive effect on adventitious root development.

Success in adventitious root cultures is affected by various factors. As stated before, in addition to the effect of auxins, the salt strength of the medium, sucrose concentration, or explant type are among the determining factors for success in adventitious root cultures.

There are many studies showing that low salt strength may boost root induction and growth. Wu et al. (2006) found that *Echinacea angustifolia* adventitious roots required low levels of ambient salt strength for the production of root biomass. Baque et al. (2010) reported that a gradual decrease in fresh and dry weight was observed as the salt strength of the nutrient medium increased in the adventitious root culture study of *Morinda citrifolia*. Zhang et al. (2011) reported that a medium containing low salt strength provided a good performance in terms of induction and growth of adventitious root cultures of *Periploca sepium*. It is thought that one reason why low salt strength produces positive results on root biomass in different plant species may be that low salt strength leads to a decrease in osmotic pressure in cultures,

which may be suitable for root primordium induction and growth (Zhang et al., 2011). Another possible reason is that favorable interactions between nutrients in low salt strength cultures make it easy ions to reach to the roots (Wu et al., 2006).

In vitro plantlets require carbon sources in their artificial media for biological processes such as survival, growth, development and accumulation of bioactive compounds under aseptic and controlled conditions. Carbohydrates are an important source of carbon and energy in plant cell and organ cultures. Sugar added to the culture medium not only acts as a carbon source but also plays a role in the osmotic regulation of water stress (Hilae and Te-chato, 2005). The growth rate of biomass is directly associated with sugar consumption. It is thought that the increase in growth in adventitious roots may be due to the adventitious roots needing high sucrose for growth at the differentiation stage and structural integrity (Tremblay and Tremblay, 1995). Muthoharoh et al. (2019) investigated the effects of different sugar types and concentrations on root biomass in *in vitro* adventitious root culture of *Gynura procumbens* (Lour) Merr. Researchers obtained the highest fresh root biomass when 5% and 3% sucrose were used as carbon sources, respectively. Similarly, Manuhara et al. (2017) reported that an increase in fresh root weight was observed by increasing sucrose concentration, and the highest root weight value was obtained with the use of 5% sucrose. In another study, Ahmad et al., (2021) reported that there was an increase in biomass accumulation in stevia (*Stevia rebaudiana*) by adding a sucrose concentration as high as 50 g L^{-1} to the growing medium.

In an adventitious root culture study conducted by Adil and Abbasi (2019) in leaf cabbage (*Brassica oleracea* var. *acephala*) they found that the sucrose concentration significantly affected the adventitious root formation. Researchers, who revealed maximum adventitious root formation in MS medium supplemented with 40 g L^{-1} concentration of sucrose, reported that increasing the sucrose concentration to a level higher than 40 g L^{-1} adversely affected adventitious root formation in cotyledon explants. Similar results were reported by Wang and Weathers (2007) on *Artemisia annua* L. and by Baque et al. (2012a) on the plant *Morinda citrifolia* (L.).

Different explants, including leaves, stems, petioles, roots, and hypocotyl parts, can be used for the induction of adventitious roots *in vitro* in many industrially important plant species (Paek et al., 2009; Sharma et al., 2013; Kawakami et al., 2015; Khan et al., 2015; Khan et al., 2017; Saeed et al., 2017). For this reason, it is thought that the different results obtained in terms of biomass in adventitious root cultures of various plant species may be also due to differences in species, varieties, and explants.

CONCLUSION

In light of the results obtained from the study, differences were determined among the cultivars. Decreasing the MS strength to a certain extent or increasing the sucrose concentration added to the medium with decreasing MS strength led to an increase in the biomass obtained from adventitious root cultures in radish. Considering that adventitious root cultures are an important alternative method for producing high-value phytochemicals, it is thought that within the framework of the results obtained, adventitious root cultures of radish can be used as a complementary method in the large-scale production of valuable phytochemicals to be used in different industries such as pharmaceutical industry.

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RECENT OBSERVATIONS ON *TURSIOPS TRUNCATUS* (DELPHINIDAE) AT THE SEA-CAGE FISH FARMS IN THE TURKISH AEGEAN SEA

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ABSTRACT

This paper reports on the recent observations on *Tursiops truncatus* displaying the opportunistic feeding behaviour at a sea-cage fish farm in the Aegean Sea. On 27 March 2022, at least ten specimens of *Tursiops truncatus* were observed during the SCUBA diving beneath a sea-cage of a fish farm in Gerence Bay, İzmir at a depth of about 60 m. The dolphins were taken by an underwater camera video. For details, we interviewed with diver as a staff of the fish farm. Occasionally, during the cleaning of the dead reared fish bag at the bottom of the sea-cage, bottlenose dolphins and bluefin tunas (*Thunnus thynnus*) are retrieved together around the diver. A part of dolphins and bluefin tunas seem to be cooperatively fishing. Then, bluefin tunas swim in the lower layer, while dolphins swim in the epi-layer. Namely, dolphins outperform in the race for grabbing bait versus tunas. Additionally, dolphins push wild fish into the cage net in a coordinated manner, and then, rip them out of the net and eat them one by one.

Keywords: Bottlenose dolphin, bluefin tuna, feeding behaviour, SCUBA, İzmir

INTRODUCTION

Thirteen cetacean species are known to exist along the Turkish waters, of which 8 species among them are considered regular: *Delphinus delphis, Tursiops truncatus, Phocoena phocoena, Stenella coeruleoalba, Grampus griseus, Physeter macrocephalus, Ziphius cavirostris* and *Balaenoptera physalus* (Öztürk and Tonay, 2019). Among them, only *D. delphis* and *T. truncatus* are existed in all Turkish seas, while only three odontocete species (*D. delphis, T. truncatus,* and *P. phocoena*) occur in the Black Sea and the Sea of Marmara (Öztürk and Öztürk, 2002).

The bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821) is pelagic, oceanodromous at a depth range of 0 - 1316 m (Palomares and Pauly, 2022). They are distributed in tropical and temperate waters of all oceans, as well as in semi-enclosed seas such as the Gulf of Mexico, the Gulf of California, and the Mediterranean, Black and Red Seas (Notarbartalo di Sciara, 2002). Specifically, it is the highest common cetacean in the Mediterranean Sea. Key areas of distribution include the Alboran, Balearic, Adriatic Seas, the Algerian coastal waters, Tunisia and Malta, the Aegean Sea, the Turkish straits system, and it is widely distributed along the Black Sea continental shelf as well, where, according to some authors it is represented by the sub-species *T. truncatus ponticus* (Notarbartalo di Sciara, 2002).

Today, the interaction between coastal marine mammals and anthropogenic-related activities along the coastline is increasing due to intensive settlements (i.e. urbanization) and some marine jobs (marine traffic, fisheries, mariculture, etc.). The sea-cage fish farms as an element of mariculture have been well-developed in the Mediterranean since the 1980s. Sea-cages as floating structures can be a type of FADs (fish aggregation devices) and can ever serve

as mega FADs (Dempster and Taquet, 2004; Sanchez-Jerez et al., 2007). Therefore, wild fish species (mostly pelagics) are attracted to fish farms. Probably, the primary attractive mechanism is food availability, either in the form of direct predation on stock, or an indirect trophic level in the form of farm waste (Fernandez-Jover et al., 2008; Barrett et al., 2018). As a secondary attraction effect (i.e. predation), wild fish populations around the sea-cage fish farms attract some sea mammals (Ceyhan et al., 2020). Beveridge (2001) stated that there was an enormous range of predatory species such as squid, fish, sea turtles, birds, and marine mammals at Mediterranean fish farms. These predators can kill or wound fish, damage equipment, resulting in losses through escapes (Beveridge, 2001).

Nowadays, some studies on predators (e.g. seabirds, seals and dolphins) near sea-cage fish farms have been documented (Güçlüsoy and Savaş, 2003; Nelson et al., 2006; Díaz López, 2006, 2017; Díaz López and Shirai, 2007; Gerovasileiou et al., 2017; Aguado-Giménez et al., 2018; Ceyhan et al. 2020). However, there are many unknown behaviours of marine mammals regarding anthropogenic-related activities that need explanation. The paper aims to contribute toward a more detailed understanding of the relationships between bottlenose dolphins and sea-cage fish farms in the Aegean Sea.

MATERIAL AND METHOD

On 27 March 2022, at least ten specimens of *Tursiops truncatus* (in Turkish, '*afalina*' or '*şişe burunlu yunus*' Figure 1) were observed during the SCUBA diving beneath a sea-cage of a fish farm in Gerence Bay, İzmir (Coordinates: 38°26' N - 26°27' E) at a depth of about 60 m. Opportunistic video recordings were made to document and verify group size, presence of immatures and behavioural interaction. Videos were taken by GoPro Hero 7 underwater camera. For details, we interviewed with diver, who is a staff of fish farm.

Figure 1. *Tursiops truncatus* and *Thunnus thynnus* (BFT) specimens beneath a sea-cage in Gerence Bay, İzmir (photo: G. Subakan)

RESULTS AND DISCUSSION

At least ten specimens of *Tursiops truncatus* were observed beneath a sea-cage fish farm in İzmir, northern Aegean Sea. According to G. Subakan (pers. comm.), *T. truncatus* were usually existing with 10-12 individuals grouping around the fish farm, all year round. It is obviously that *T. truncatus* swimming together with crowd groups in the Aegean Sea. Dolphins

(here *T. truncatus*) that come to fish farms to feed have gotten used to divers, swim with them and never harm the divers (G. Subakan, pers. comm.).

Occasionally, during the cleaning of the dead reared fish bag at the bottom of the sea-cage, bottlenose dolphins and bluefin tunas (*Thunnus thynnus*, BFT) are retrieved together around the diver. However, a part of dolphins chases BFT in coordination. Then, BFTs swim in the lower layer, while dolphins swim in the epi-layer. Namely, dolphins outperform in the race for grabbing bait versus BFTs. Additionally, dolphins push wild fish into the cage net in a coordinated manner, and then, rip them out of the net and eat them one by one. Rarely, dolphins can damage the net of a cage. Regarding to Subakan (pers. comm.), a few years ago, a dolphin entered in fish farm cage in Muğla region, southeastern Aegean Sea, which allowed to fish escape. Diaz-López (2006) mentioned that bottlenose dolphins appear capable of modifying their hunting tactics according to the abundance of prey. On the other hand, Ceyhan et al. (2020) reported that dolphins had the highest interaction rate (68.1%) with sea-cage fish farms as predators (following 47.8% for BFT), no attack into the sea-cages has been observed or reported by fish farmers during the study. Moreover, Ceyhan et al. (2020) emphasized that the dolphins showed cunning behaviour, and wild fishes such as *Boops boops*, *Scomber colias* and *Sardinella aurita* swimming around sea-cages were chased by dolphins towards the protection nets in order to entanglement their operculum, then they ate prey easily.

CONCLUSIONS

This paper presents the first photographic record of *T. truncatus* that swims with BFT and diver at a sea-cage fish farm in the southeastern Aegean Sea. Additionally, as opportunistic feeding behaviour, dolphins are chasing the wild fish towards the protection nets in order to entanglement their operculum. This feeding behaviour was confirmed the second time with this study.

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DYNAMICS OF THE APHID POPULATION ON TOBACCO IN PRILEP REGION

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ABSTRACT

Leaf aphids are among the most important pests on tobacco. They directly affect tobacco yield and quality. Investigation was carried out in 2017-2021 on tobacco plants in Prilep, by application of Method of survey of 20 randomly selected tobacco stalks infested with aphids. During the summer 2017-2021, aphids developed many parthenogenic generations of apterous aphids on tobacco, which depends primarily on temperature fluctuations and precipitation, as well as on the physiological state of the plant and soil nitrogen fertilization. Aphid infestations were often found first along the field margins nearest the direction of prevailing winds. Aphid colonization begins with the movement of few winged females into tobacco fields that give birth to live nymphs. These offspring will become mature, wingless aphids that in turn will deposit more live nymphs and make collonies on top tobacco leaves and flowers. *M. persicae* was present on tobacco plants from the beginning of July until the end of October. Following the dynamics of the aphid population in Prilep region in 2017-2021, the most intensive attack of aphids on tobacco occurs in August. The maximum incidence of aphids was on the 10th of August 2017, on the 20th of August 2018-2020 and on the 1st of August 2021, when aphids form large, dense colonies at the growing points. On the examined stalks, in 2017 were observed 70.707 aphids, 48.527 in 2018, 54.036 in 2019, 59369 in 2020 and 20738 aphids in 2021.

Keywords: Sunflower, Sustainable production, Drought tolerance, Hybrid, Yield traits, Yield performance,

INTRODUCTION

The green peach aphid, *M. persicae*, is a highly polyphagous species, colonizing over 500 species of host plants from at least 40 different families (Blackman and Eastop, 2000, cit. Srigiriraju, 2008; Grigorov, 1979).

The holocycle of *M. persicae*, with sexual reproduction and overwintering of eggs on Prunus, occurs in the temperate regions of every continent, and although anholocycly is widespread in warm climates there are indications that the potential for sexual reproduction may be retained throughout the whole range of the species (Blackman, 2009).

During each annual cycle, cyclically parthenogenetic *M. persicae* aphids reproduce asexually several times on herbaceous plants (secondary hosts) and once sexually on peach trees -*Prunus persica* L. (Guillemaud et al. 2003).

In field conditions of Macedonia, it has a holocyclic life cycle where the sexual phase is completed on a peach and asexual phase occurs on tobacco and other secondary host species (Janusevska, 2001; Krsteska, 2007).

In addition to damaging the field, it easily attacks vegetables and ornamental plants grown in greenhouses (Krsteska, 2016).

Aphid diet causes damages on tobacco leaves and reduction of carbohydrates, soluble sugars and glucoses. They may also cause water stress and reduced growth rate of tobacco plant (Todoroski, 1965; Todoroski and Maceljski, 1983; Srigiriraju et al., 2010; Maric and Camprag, 1982).

The main goal of the investigations was to perform analysis of population dynamics of aphids in tobacco fields.

MATERIAL AND METHOD

Investigations were carried out during 2017-2021, on tobacco plants in Prilep. The observations were made with application of Method of survey of 20 randomly selected tobacco stalks infested with aphids.

Tobacco stalks were sampled from the whole area of the trial at 10-days interval, starting from June 1, up to the beginning of October. The investigations were performed on parts of tobacco (leaves, tobacco flowers, seed capsules).

Table 1. Observation of tobacco leaves 2017-2021
Method of survey of 20 tobacco stalks

Date of control	N^0 of tobacco leaves/year				
	2017	**2018**	**2019**	**2020**	**2021**
01.07.	326	287	257	221	247
10.07.	362	351	362	376	356
20.07.	517	501	505	498	508
01.08.	598	536	566	562	561
10.08.	642	611	602	623	622
20.08.	699	678	692	687	658
01.09.	724	713	717	705	679
10.09.	655	619	609	613	601
20.09.	617	596	587	520	515
01.10.	587	567	569	548	534
Total	**5727**	**5459**	**5466**	**5353**	**5281**

10 checks were made by this method in each of the years of investigations, i.e. 200 stalks per year, or 1000 stalks in total.

The investigation included a total of tobacco leaves (5727 in 2017, 5459 in 2018, 5466 in 2019, 5353 in 2020, 5218 in 2021).

RESULTS AND DISCUSSION

The aphid attack commercial varieties of *Nicotiana tabacum* L. and they appear in all tobacco producing regions in Macedonia.

During investigation of the species of Aphididae family, tobacco was attacked only by *Myzus persicae* Sulzer (Figure 1).

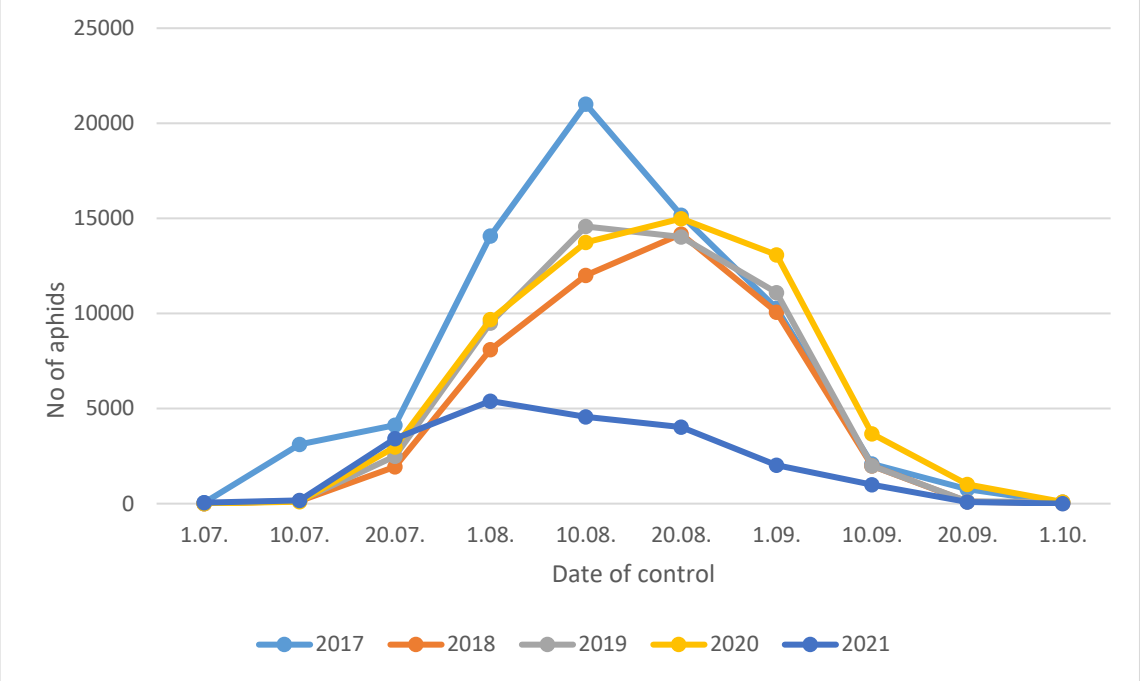
Figure 1. Aphid collonies on top tobacco leaves and flowers

M. persicae developed many parthenogenic generations of apterous aphids on tobacco, which depends primarily on temperature fluctuations and precipitation, as well as the physiological state of the plant and soil nitrogen fertilization.

Aphid infestations were often found first along the field margins nearest the direction of prevailing winds. Aphid colonization begins with the movement of few winged females into tobacco fields that give birth to live nymphs. These offspring will become mature, wingless aphids that in turn will deposit more live nymphs and make collonies on top tobacco leaves (Figure 2) and flowers.

Figure 2. Aphid collonies on tobacco leaves

Following the dynamics of the aphid population in Prilep tobacco producing region during 2017-2021, the most intensive attack of aphids on tobacco occurs in August (Graph. 1). *M. persicae* was present on tobacco plants from the beginning of July to the beginning of October.



Graph. 1 Dynamics od aphid population on tobacco

Climate conditions lower the plant defense system against insect pests, and cause tobacco plant to be more vulnerable to pests attack.

Aphids were identified in large quantitative representation in 2017, 70.707 aphids were found on the examined stalks (Table 1). In 2018 aphid population decreased and 48.527 aphids were observed. In 2019 a slightly higher population of aphids was determined compared to the previous year i.e. 54.036 aphids. This increasing trend continued in the following year and 59369 aphids were found in 2020. Unfavorable climatic conditions caused the development of a smaller population of aphids in 2021 when a total of 20738 aphids were determined.

The maximum incidence of aphids was on the 10th of August 2017, on the 20th of August 2018-2020 and on the 1st of August 2021, when aphids form large, dense colonies at the growing points on tobacco plants.

M. persicae make colonies on young tobacco leaves, buds and flowers and in strong attack, they are dried and covered with an abundance of honeydew, which is populated with black sooty mold. Infected tobacco plants lag behind in growth and are susceptible to attack by other plant pests and pathogens.

On tobacco leaves 2017-20121 the apterous (wingless) aphids have various shades of green, orange, red or yellow color with oval body, approximately 2.15 mm long (Figure 3).

Figure 3. Wingless aphids on tobacco

This color morphism in *M. persicae* results from the presence of a series of glycosides in the aphid hemolymph (Blackman, 1974).

According to Capinera (2020) the wingless (apterous) aphids are yellowish or greenish in color. They measure about 1.7 to 2.0 mm in length. A medial and lateral green stripes may be present. The cornicles are moderately long, unevenly swollen along their length, and match the body in color. The appendages are pale.

According to Blackman, Eastop (2017) adult apterous parthenogenetic female are small to medium sized, pale greenish-yellow or various shades of green, pink red or almost black.

On tobacco leaves nymphs resemble parthenogenetic, apterous aphids and their color is green, yellow or red (Fugure 4).

Figure 4. Nymphs on tobacco leaves

According to Capinera (2020) nymphs initially are greenish, but soon turn yellowish, greatly resembling viviparous (parthenogenetic, nymph-producing) adults. The nymphs that give rise to winged females (alatae) may be pinkish.

According to Blackman, Eastop (2017) immature female alatae are often red or pink, and immature males are always some shade of yellow or yellow-green.

As aphid densities on tobacco increase 2017-2021, winged forms are produced to aid dispersal. Alate aphids have a black head and black-redish thorax, and a yellowish green abdomen with a large dark patch dorsally. Their body is oval and although they looked bigger than apterous aphids (because of the wings) they measure approximately 2.05 mm in length.

Capinera (2020) winged (alate) aphids have a black head and thorax, and a yellowish green abdomen with a large dark patch dorsally. They measure 1.8 to 2.1 mm in length.

Table 2. Quantitative representation of aphid population on tobacco

Date of control	N⁰ of aphids/year				
	2017	**2018**	**2019**	**2020**	**2021**
01.07.	54	-	-	7	56
10.07.	3111	132	154	98	167
20.07.	4120	1938	2488	2987	3421
01.08.	14072	8098	9492	9678	5397
10.08.	21005	12004	14574	13745	4567
20.08.	15176	14176	14023	14990	4021
01.09.	10272	10073	11098	13082	2021
10.09.	2090	1982	1995	3678	1001
20.09.	766	107	127	1021	87
01.10.	41	17	85	83	-
Total	**70707**	**48527**	**54036**	**59369**	**20738**

CONCLUSIONS

During investigation of the species of Aphididae family, tobacco was attacked only by *M. persicae*. It has high potential for reproduction and development.

M. persicae was present on tobacco plants from the beginning of July until the beginning of October. The most intensive attack of aphids on tobacco occurs in August when aphids form large, dense colonies at the growing points of tobacco plants.

In tobacco biocenosis in the region of Prilep 2017-2019, *M. persicae* developed many generations with high quantitive representations of aphids. Due to the unsuitable climate conditions in 2021, the number of its generations on tobacco was reduced.

On the examined stalks, in 2017 were observed 70.707 aphids, 48.527 in 2018, 54.036 in 2019, 59369 in 2020 and 20738 aphids in 2021.

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STUDY OF THE ADHESIVE BEHAVIOR OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* ON GLASS

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ABSTRACT
Bacterial adherence and growth on biotic and non-biotic surfaces, are considered to be the primary steps leading to pathogenic biofilms formation responsible of bacterial infections and contaminations in many fields. Therefore, the prevention of bacterial attachment remains the best strategy to face these problems. In this context, this in vitro investigation aimed to study predictive and experimental adhesion of two of the most common strains; *Escherichia coli* CIP54127 (Gram-negative) and *Staphylococcus aureus* CIP5315 (Gram-positive) on microscope slides, using the contact angle method. The physicochemical properties (hydrophobicity, electron donor/acceptor properties and free energy) were calculated by the XDLVO theory. From the results, both strains were classified as qualitatively hydrophilic with relatively low contact angles with water ($33.4° < \theta w < 48.3°$). In addition, *E. coli* CIP54127 showed a positive free energy value of the interaction between the bacterium and water ($\Delta Giwi = 56.6$ mJ.m-^{2}), while a negative free energy value was observed for *S. aureus* CIP5315 ($\Delta Giwi = (-4.5)$ mJ.m-^{2}). As a result, *E. coli* CIP54127 and *S. aureus* CIP5315 were characterized quantitatively hydrophilic and hydrophobic respectively. The electron donor (base) character of the bacterial surface of *E. coli* CIP54127 ($\gamma- = 63.7$ mJ.m-^{2}) was more prominent than the *S. aureus* CIP5315 character ($\gamma- = 25.5$ mJ.m-^{2}), yet the electron acceptor (acid) characters of both strains were weak (0 mJ.m-$^2 < \gamma+ < 1.6$ mJ.m-^{2}). Moreover, theoretical adhesion suggested that glass colonization by the tested bacteria was thermo-dynamically favorable in the case of *E. coli* CIP54127 ($\Delta GTot = (-49.20)$ mJ.m-$^2 < 0$) and relatively unfavorable for *S. aureus* CIP5315 ($\Delta GTot = 5.79$ mJ.m-^{2}). Subsequently, experimental adhesion on microscope slides revealed that the percentage of surface occupied by *E. coli* CIP54127 (51%) was higher than that occupied by *S. aureus* CIP5315 (26%). Thus, predictive and experimental adhesions were in the same direction. These results could later be applied in research aiming to understand and control interfacial phenomena in order to prevent biocontamination of various surfaces.
Keywords: Adhesion, Physicochemical properties, *Escherichia coli*, *Staphylococcus aureus*, Contact angle.

INTRODUCTION
Biocontamination of biotic and abiotic surfaces by microbial biofilms is a natural phenomenon. In particular, negative biofilms cause significant damage in many vital fields such as medicine (Monteiro et al., 2009; Odo et al., 2021) biotechnology (Jullien et al., 2003; Arreguin-Campos., 2023) and the environment (de Kerchove and Elimelech, 2007; Elgoulli et al., 2021).
Biofilm formation always starts with microbial adhesion resulting from the combination of physicochemical and energetic interactions between the surface of the microorganism and the support (Hamadi et al., 2005, 2009; Soumya et al., 2011; Hamadi et al., 2012; Cheng et al., 2019). The XDLVO approach considers Van der Waals (dispersive) interactions electrostatic interactions and acid-base (non-dispersive) interactions (Roosjen et al., 2006; Boks et al., 2008;

Gardner et al., 2008) to play the most important role in estimating of the total energy of interaction between two entities. These interactions are crucial in the adhesion process and therefore in the formation of biofilms. Thus, it seems more efficient to understand and control the physicochemical parameters involved in the initial steps preceding the attachment of bacteria to the surface in order to prevent bio-adhesion and biofilm formation.
The primary objective of this work was to predict the adhesion of two model bacteria *Escherichia coli* CIP54127 (Gram-negative) and *Staphylococcus aureus* CIP5315 (Gram-positive), known for their pathogenic potential (Valaperta et al., 2010; Fetsch et al., 2014; Tanih et al., 2015; Elmonir et al., 2018; Silva, 2023), on one of the most countlessly used materials: glass. According to the XDLVO theory, the surface physicochemical properties, including hydrophobicity, donor-acceptor components and the total interaction energy of the surfaces were characterized using contact angle measurements (sessile drop method). Secondly, to evaluate the adhesive behavior of the studied strains studied by quantifying the adherent cells on the glass supports.

MATERIAL AND METHOD

1. Preparation of bacterial suspensions

In this study, *Escherichia coli* CIP54127 and *Staphylococcus aureus* CIP5315 were cultivated in a solid Luria Bertani (LB) medium at 37°C. After 24 hours of incubation, precultures were suspended in a KNO_3 (0.1 M) solution and washed twice by centrifugation at 5 000 *g* for 15 min (Hamadi et al., 2014). The optical densities of each bacterial suspension were then adjusted between 0.7 and 0.8 at 600 nm (approximately 10^8 FCU/ml) ((Busscher et al., 1984). Afterwards, the adjusted suspensions were filtered under negative pressure using a 0.45 µm cellulose acetate membrane filter, in order to obtain a uniform layer of bacterial cells. Contact angle measurements repeated separately in triplicate (Hamadi and Latrache, 2008).

2. Theoretical adhesion

2.1.Contact angle measurement

The physicochemical characterization of the surface was carried out by the contact angle method using a goniometer (GBX instruments, France). Contact angles were standardized using three liquids as reference (Table.1): diiodomethane (non-polar), formamide (polar) and distilled water (polar) (Van Oss., 1993; Van Oss., 1997). A drop of each solvent was deposited on solid substrate or bacterial layer, and three to six contact angle measurements were taken.
The approach of Van Oss et al (1988) enabled us to determine: Lifshitz-Van der Waals (γLW), electron donor (Lewis base (γ−)) and electron acceptor (Lewis acid (γ+)) components of the support (glass) and the bacterial surface. In this approach, Young's formula (A) was used to express the contact angle (Θ) where (L) and (S) denote the liquid phase and the solid surface respectively:

(A) $$cos\,\theta = -1 + 2\frac{\sqrt{\gamma_S^{LW}\gamma_L^{LW}}+\sqrt{\gamma_S^{+}\gamma_L^{-}}+\sqrt{\gamma_S^{-}\gamma_L^{+}}}{\gamma_L}$$

The surface free energy is defined as: $\gamma_S = \gamma_S^{LW} + \gamma_S^{AB}$; where the Lewis acid-base component of the surface tension is expressed by (B):

(B) $$\gamma_S^{AB} = 2\sqrt{\gamma_S^{-} \times \gamma_S^{+}}$$

Surface hydrophobicity is generally represented by the free energy of interaction ΔGiwi between two entities of a given material (i) immersed in water (w) (Van Oss et al., 1988). The surface is considered hydrophobic if the surface free energy was negative (ΔGiwi<0). In contrast, the surface is considered hydrophilic, if the ΔGiwi was positive. This free energy ΔGiwi can be estimated through the following equation (C):

$$\textbf{(C)} \qquad \Delta G_{iwi} = -2\gamma_{iw} = -2\left[\left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 + 2\left(\sqrt{\gamma_i^+\gamma_i^-} + \sqrt{\gamma_w^+\gamma_w^-} - \sqrt{\gamma_i^+\gamma_w^-} - \sqrt{\gamma_w^+\gamma_i^-}\right)\right]$$

Table 1. Contact angle solvents energetic properties (Van Oss., 1997).

Solvents	γ^{LW} (mJ.m^{-2})	γ+(mJ.m^{-2})	γ-(mJ.m^{-2})
Diiodomethane	50.5	0.7	0.0
Formamide	38.7	2.3	39.4
Water	21.6	25.4	25.4

γ^{LW}: Lifshitz-van der Waals components of the surface tension; γ−: electron donor surface tension component; γ+: electron acceptor surface tension component

2.2.Adhesion prediction

According to the XDLVO theory (Van Oss., 1997), the measurement of the total free energy of interaction (ΔGTot) predicts the adhesion between different surfaces and colloids by calculating the sum (D) of the Liftshiz Van-der- Waals (LW), acid-base (AB) and electrostatic double layer (EL) interactions (Missirlis and Katsikogianni, 2007):

$$\textbf{(D)} \qquad \Delta G^{Tot} = \Delta G^{Lw} + \Delta G^{AB} + \Delta G^{EL}$$

The electrostatic double layer interactions were neglected, since our studied bacterial mass was previously treated with high ionic strength KNO_3 solution (0.1 M). Adhesion could be favorable or unfavorable if this energy is negative (ΔGTot <0) or positive (ΔGTot >0), respectively.

3. Experimental adhesion

3.1.Glass coupons preparation

In this study, glass was chosen as the substrate because of its hydrophilic nature and simple molecular structure. Glass coupons were obtained by cutting microscope slides into small squares (1 cm x 1 cm). The substrates were disinfected by soaking in ethanol 70% (vol/vol) for 15 min, and washed six times with sterile distilled water and then autoclaved at 120°C for 15 min (Hamadi et al., 2014).

3.2.Adhesion assay

The already adjusted bacterial suspensions (10^8 FCU/ml) of *E. coli* CIP54127 and *S. aureus* CIP5315 were incubated for 3 h at 25 ◦C, in Petri dishes containing the sterilized glass coupons. After incubation, the glass surfaces were carefully rinsed with sterilized distilled water to remove non-adherent cells (Hamadi et al., 2014). The adherent bacteria were stained by Gram coloration and each surface was observed by optical microscopy (×100). Each experiment was performed in triplicate.

The percentage of the surface occupied by the adherent cells, was obtained by treating microscope images using an open software for processing and analyzing scientific images. This Java-based program was developed at the National Institute of Health and the Laboratory for Optical and Computational Instrumentation (LOCI, University of Wisconsin).

RESULTS AND DISCUSSION

Generally, microbial adhesion is governed by surface physicochemical properties of both the cells and the substrates, these properties are commonly measured by contact angle method (Bellon-Fontaine., 1996; Li and Logan, 2004). Tables 2 and 3 presented the surface physicochemical characteristics of the glass and the bacteria studied respectively, where total surface free energy, acid-base, non-polar and polar contribution to surface free energy, were calculated. Based on the study results, glass is a hydrophilic (ΔGiwi=16.2 mJ.m-^{2}), strong electron donor (γ- = 40.36 mJ.m-^{2}), and weak electron acceptor (γ+ = 1.54 mJ.m-^{2}) materiel (table.2). As shown in Table 3, *E. coli* CIP54127 had a hydrophilic character (θw = 33.4°; ΔGiwi = 56.6 mJ.m-^{2}), while *S. aureus* CIP5315 surface was relatively more hydrophobic with a higher value of θw = 48.3° and a negative surface free energy (ΔGiwi = (-4.5) mJ.m-^{2}). According to the literature, it is highly acceptable to correlate the physicochemical properties of a bacterial surface with its chemical composition (Mozes et al., 1988; Van der Mei et al., 1989; Mozes et al., 1989; Latrache et al., 1994; Van der Mei and Busscher, 1997; Boonaert and Rouxhet, 2000; El Ghmari et al., 2002; Hamadi et al., 2005, 2008, 2012). Indeed, the decrease in the water contact angle (θw) could be due to the interaction between oxygen from surface functional groups (OH, CO-C) and water molecules through hydrogen bonding, which subsequently reduces the hydrophobicity of the cell surface. (Latrache et al., 1994 ; Van der Mei and Busscher, 1997 ; Latrache et al., 2002). This is the case for E. coli, whose hydrophilicity has been explained in particular by the concentration of polysaccharides on its surface (Latrache et al., 2002). Moreover, both strains surfaces showed an electron donor property between γ- = 63.7 mJ.m-^{2} and γ- = 25.5 mJ.m-^{2} and a limited electron acceptor parameter (0 mJ.m-^{2} < γ+ < 1.6 mJ.m-^{2}) (table.3). This result could be explained by the fact that most bacteria are likely to have negatively charged surfaces with a dominant donor character (Van Der Mei et al., 1998).

According to thermodynamic model of Van Oss (1997), the adhesion total free energy of the investigated bacteria had been calculated (Table.4). Therefore, predictive adhesion of *E. coli* CIP54127 to glass was favorable (ΔGTot = (-49.20) mJ.m-^{2}< 0), and relatively unfavorable for *S. aureus* CIP5315 (ΔGTot = 5.79 mJ.m-^{2}). In addition, during the experimental adhesion examination on glass (Figure.1), *E. coli* CIP54127 also presented the most important number of adherent cells (51%) verses a lower percentage in the case of *S. aureus* CIP5315(26%). There is no doubt in the literature that the adhesion of bacteria depends on the nature of the surface. In general, hydrophobic bacteria tend to adhere to hydrophobic surfaces and hydrophilic cells tend to adhere to hydrophilic substrates (Boulang´e-Petermann et al., 1997; Kerr et al., 1999; Cerca et al., 2005; Krasowska and Sigler, 2014). This may explain the ability of *E. coli* CIP54127 (hydrophilic) to adhere better to glass (hydrophilic) than *S. aureus* CIP5315 (hydrophobic). However, it is highly probable that in addition to the combination of surface hydrophobicity and acid-base interactions which play an important role in microbial adhesion

process (Otto et al., 1999; Garrett et al., 2008), ionic strengths could also directly influence this phenomenon by altering the surface properties (Hamadi et al., 2005).

Table 2. Contact angles, tensions and free energy of microscope slide surface

Substrate	Contact angles (°)			Surface tension (mJ.m-^{2}): components and parameters			ΔGiwi (mJ.m-^{2})
	Θ Diiodomethane	Θ Formamide	Θ Water	γ^{LW}	γ+	γ-	
Glass	**46.6 (1.4)**	**45.8 (3.3)**	**36.5 (3.1)**	**36.3**	**1.54**	**40.36**	**16.2**

Standard deviations were given in parentheses. Contact angle (Θ) of the glass surface with tree solvents: diiodomethane, formamide and water; γ^{LW}: Lifshitz-van der Waals components of the surface tension; γ-: electron donor surface tension component; γ+: electron acceptor surface tension component; ΔGiwi: The free energy of interaction between glass and water

Table 3. Contact angles, tensions and free energies of *E. coli* CIP54127 and *S. aureus* CIP5315 surfaces

Strains	Contact angles (°)			Surface tension (mJ.m-^{2}): components and parameters			ΔGiwi (mJ.m-^{2})
	Θ Diiodomethane	Θ Formamide	Θ Water	γ^{LW}	γ+	γ-	
***E. coli* CIP54127**	**51.1 (0.27)**	**5.03 (0.27)**	**33.4 (0.19)**	**33.7**	**0**	**63.7**	**56.6**
***S. aureus* CIP5315**	**43 (0.04)**	**30.4 (0.27)**	**48.3 (0.31)**	**38.1**	**1.6**	**25.5**	**-4.5**

Standard deviations were given in parentheses. Contact angle (Θ) of the glass surface with tree solvents: diiodomethane, formamide and water; γ^{LW}: Lifshitz-van der Waals components of the surface tension; γ-: electron donor surface tension component; γ+: electron acceptor surface tension component; ΔGiwi: The free energy of interaction between the surface of the bacteria and the water

Table 4. Total free energy of adhesion between strain surfaces and glass

Strains	ΔG^{LW} (mJ.m-^{2})	ΔG^{AB} (mJ.m-^{2})	ΔG^{Tot} (mJ.m-^{2})
***E. coli* CIP54127**	-3.19	-46.02	-49.2
***S. aureus* CIP5315**	-4.2	9.99	5.79

ΔG^{LW}: Lifshitz-Van der Waals interactions; ΔG^{AB}: Lewis acid–base interactions; $\Delta G^{Tot:}$ total free energy of interaction

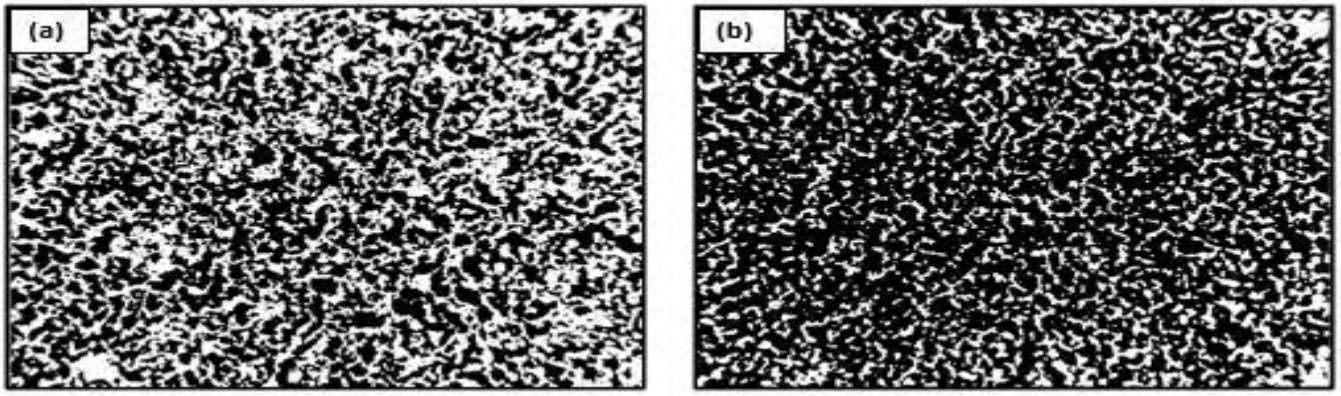

Figure 1. Imagej treated images (×100) of *E. coli* CIP54127 and *S. aureus* CIP5315 after three hours of adhesion to glass. Adhered bacterial cells are in white. (a): the area occupied by E. coli (51%); (b): the area occupied by S. aureus (26%)

CONCLUSIONS

This study investigated, the predictive and the experimental adhesion of two common and widely distributed bacteria to glass. Experimentally, *E. coli* CIP54127 showed a greater ability to adhere to glass surfaces than *S. aureus* CIP5315. Based on our results, the XDLVO thermodynamic model associated this affinity with the hydrophilic nature of the *E. coli* CIP54127 surface compared to a relatively hydrophobic character for *S. aureus* CIP5315. However, both strains were electron donors. Thus, *E. coli* CIP54127 has a higher affinity for hydrophobic surfaces than *S. aureus* CIP5315. The process of microbial adhesion to a surface involves interactions between the bacteria, the substrate and the surrounding environment. Further research is needed to understand the interfacial parameters that control this phenomenon so that contamination can be reduced or even prevented in many applications.

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ASHWAGANDHA(*Withania somnifera*) AS A MEDICINAL PLANT

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ABSTRACT

Ashwagandha (*Withania somnifera*) is a medicinal plant species in the Solanaceae family. *Withania somnifera* contains therapeutically important secondary metabolites, alkaloids, and withanolides Among the secondary metabolites found in the plant, phenolic compounds, sterols, glycovitanolides, and flavonol glycosides can be counted. The most potent part of *Withania somnifera* (L.) is its roots, which are rich in alkaloids, steroidal lactones, and saponins. The leaves of the plant are bitter and have some medicinal uses for fever and painful swelling. The flowers are astringent, depurative, diuretic and aphrodisiac. The seeds are anti-helminthic and remove white spots on the cornea. The fruits have traditionally been used as a topical treatment for tumors, tuberculous glands, carbuncles, and skin ulcers. This review study searched and presented literature on ashwagandha morphological features, secondary metabolites, and usage areas.

Keywords: Ashwagandha, secondary metabolites, root, medicinal plant

Introduction

Ashwagandha (*Withania somnifera*) is a small shrub belonging to the *Solanaceae* family, which also includes tomatoes, potatoes, and peppers. The history of Ashwagandha dates back thousands of years and has played a significant role in India's medical and cultural traditions (Singh et al., 2011). Traditionally, the plant has been believed to help reduce stress, increase energy, support the immune system, and improve overall health (Raut et al., 2012). The plant has small greenish-yellow flowers and small orange-red fruits. It is native to India, the Middle East, and some regions of Africa, with its roots being the most commonly used part for medicinal purposes. *Withania somnifera* is a short, perennial shrub that can grow to a height of approximately 30 to 150 cm. Its leaves are simple and arranged oppositely along the stem. The stem is woody, cylindrical, and grayish-brown in color. Ashwagandha's flowers are small and bell-shaped, typically ranging from greenish-yellow to pale green. The fruit turns from orange to red when ripe and contains numerous seeds. The roots of Ashwagandha vary in color from pale tan to brown.



Figure 1. The general appearance of the Ashwagandha plant.

Ashwagandha is commonly used in alternative medicine. Some common uses of Ashwagandha include reducing stress and anxiety, increasing energy levels, supporting neuroprotective properties, balancing the immune system, supporting adrenal glands, enhancing muscle strength, and regulating blood sugar (Chandrasekhar et al., 2012; Sharma & Arora, 2006).

Botanical Features

The plant *Withania somnifera* is a short, perennial shrub that can grow to a height of approximately 30 to 150 cm. Its leaves are simple and arranged oppositely along the stem. They are oval to lance-shaped, with lengths ranging from approximately 5 to 10 cm. The leaves are green and possess a slightly rough texture. The stem is woody and cylindrical, covered with a grayish-brown bark. It branches out and can grow both upright and in a spreading manner. Ashwagandha's flowers are small and bell-shaped, usually ranging from greenish-yellow to pale green. They are arranged in clusters called cymes and are found in the leaf axils.

Figure 2. The overall appearance of *Withania somnifera* L. plant with flowers, fruit, and seeds.

The flowers have a unique, somewhat tubular appearance. The plant's fruits produce small, round fruits that start green and turn orange-red when ripe. Each fruit contains numerous small seeds. The seeds are small, flat, yellow, kidney-shaped, approximately 2 mm long, 1.5-2 mm wide, and 0.5 mm thick (Rajeswara Rao et al., 2012). The roots of *Withania somnifer*a are the plant's most well-known and commonly used part. They are fleshy, long, and conical, resembling carrots, and their color ranges from light tan to brown.

Chemical Characteristics

The Ashwagandha (*Withania somnifera*) is a rich source of various bioactive compounds. The medicinal content of this plant includes various alkaloids, steroidal lactones (withanolides), flavonoids, and other bioactive compounds. Here are some key compounds found in Ashwagandha and their roles:

Withanolides: The most significant compounds found in Ashwagandha are withanolides. These compounds are associated with various pharmacological effects of the plant, such as helping the body cope with stress, supporting the immune system, exhibiting anti-inflammatory effects, and having antioxidant activity (Ames, 1993).

Alkaloids: Ashwagandha contains alkaloids, including somnin, somniferin, anaferin, and tropin. These alkaloids may contribute to the plant's pharmacological effects (Atasü & Petters, 1981).

Flavonoids: The flavonoids present in Ashwagandha contribute to its antioxidant properties. Antioxidants neutralize harmful free radicals, protecting the body against oxidative stress (Gupta & Rana, 2007).

Phytosterols: Phytosterols found in Ashwagandha may contribute to its anti-inflammatory and immune-supporting effects (Singh et al., 2011).

Areas of Usage

Ashwagandha, thanks to its adaptogenic properties, may help manage stress. Research suggests that it can help balance the levels of the stress hormone cortisol and reduce anxiety (Chandrasekhar et al., 2012).

Ashwagandha can also assist in increasing energy levels and promoting overall well-being. Its use in enhancing both physical and mental performance is widespread (Raut et al., 2012).

The plant possesses immune-boosting properties. It can be used to strengthen the immune response and protect the body against infections (Mishra et al., 2000).

There is evidence of Ashwagandha's neuroprotective effects. It is used to protect nerve cells and support brain health. Due to its adaptogenic properties, Ashwagandha is also studied for its potential to increase muscle strength (Wankhede et al., 2015).

Ashwagandha is one of the herbal remedies used for thyroid disorders. In India, different parts of the plant such as leaves, roots, flowers, seeds, and bark are traditionally used in folk medicine for liver tonic, anti-inflammatory agents, bronchitis, asthma, ulcers, emaciation, insomnia, and dementia. The use of the plant in anxiety, cognitive and neurological disorders, inflammation, and Parkinson's disease is supported by clinical research. Steroidal lactones isolated from the leaves of the plant (Withaferin-A, Withanolide D, Vitanolide G) are antitumoral and are based on the biological effects of the plant (Verma & Kumar, 2011).

Research and studies on the inhibition and reduction of tumor growth by *Withania somnifera* provide promising evidence that this impressive plant may be highly effective in the treatment of tumor-related diseases, including cancer (Singh et al., 2011).

There are dozens of studies demonstrating that *Withania somnifera* slows down, halts, reverses, or eliminates neurotic activity. Therefore, *Withania somnifera* can be used for the treatment of Alzheimer's, Parkinson's, and other neurodegenerative diseases at any stage, even before a diagnosis is made, such as in cases of mild forgetfulness (Verma & Kumar, 2011).

Withania somnifera's anti-inflammatory effect has been observed in studies conducted on mice. *Withania somnifera* has suppressed inflammation by affecting the levels of inflammatory markers (Gupta & Rana, 2007).

Conclusion

Ashwagandha (Withania somnifera), a plant belonging to the Solanaceae family, holds significant importance in India's medical and cultural traditions. With a history spanning thousands of years, it has been believed to offer a range of benefits, including stress reduction, energy enhancement, immune system support, and overall health improvement. Ashwagandha is widely utilized in alternative medicine. It is used for reducing stress and anxiety, increasing energy levels, supporting neuroprotective properties, balancing the immune system, supporting adrenal glands, enhancing muscle strength, and regulating blood sugar. Key compounds found in the plant include withanolides, alkaloids, steroidal lactones, flavonoids, and tannins. Withanolides are associated with Ashwagandha's adaptogenic properties, while alkaloids may have effects on the nervous system. Steroidal lactones, on the other hand, possess adaptogenic and anti-stress qualities.

In conclusion, Ashwagandha is a medicinal plant of significant importance in both traditional and modern alternative medicine. Its adaptogenic properties, stress-coping abilities, energy-boosting effects, and immune system support have made it a popular herbal supplement. To further its integration into agricultural practices and pharmacological studies, research on Ashwagandha's adaptation, cultivation techniques, and pharmacological activities should be conducted, leading to its inclusion in the list of Medicinal Plants by the Ministry of Agriculture in our country.

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WHITE OLEANDER: INVESTIGATION OF POTENTIAL USAGE IN AQUACULTURE AND COSMETICS INDUSTRIES

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ABSTRACT

Plants have been used since ancient times to treat many diseases and are the source of therapeutic agents. In this study, biological activity of acetone and ethanol extracts from white oleander flowers and leaves was determined against fish pathogens. The disc diffusion and microdilution assays were used to determine the biological activity of the extracts on fish pathogens. The highest zone diameter of inhibition was determined as 12.67 mm in flower acetone extract against *Vibrio anguillarum* A4. The lowest inhibition zone against *Lactococcus garvieae* for leaf ethanol extract was determined as 7.09 mm. The MIC value was between 5-40 mg/mL for white oleander extracts against the fish pathogens. The lowest value of MBC was determined as 5 mg/mL in leaf ethanol extract against *Aeromonas hydrophila* ATCC 19570. In addition, sun protection factor (SPF) values of white oleander extracts were evaluated. The SPF values of the extracts were obtained as 25.72-26.52. The extracts from white oleander which is used as an ornamental plant can be used as natural additives in the aquaculture and cosmetics industries.

Keywords: Antimicrobial activity, Cosmetic, Fish pathogens, *Nerium oleander,* SPF

INTRODUCTION

Nerium oleander belong to the Apocynaceae family. It spreads in the warm and subtropical regions of the Mediterranean. It is an evergreen plant that grows spontaneously in dry stream beds and can reach up to five meters in height. Oleander, a plant species with pink or white flowers that is usually grown as a garden ornamental plant, is a perennial plant that can be grown all over the world (Abdou et al., 2019). Although oleander is known as a poisonous plant species, it has been reported to have important bioactive compounds according to toxicological, pharmacological, biochemical, and ethnobotanical studies in various dose studies (Bavunoğlu et al., 2016; Farkhondeh et al., 2020). In recent years, it has been determined that the *Nerium oleander* has many effects, for example, antioxidant, antileukemia, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, diuretic, antimicrobial, immunomodulatory effects (Hase et al., 2016).

The decrase of fish from natural water sources and the increasing demand for aquaculture by consumers are the two main factors for the expansion of aquaculture today (Zeybek et al., 2008). Various infections causing epidemics in fish are a major concern in the aquaculture industry, possibly causing significant economic loss due to disease and death (Irshath et al., 2023). Antibiotics have been used for many years in the treatment of bacterial infections in fish (Haniffa and Kavitha., 2012). One of the main problems in the use of antibiotics is that fish pathogens are resistant to antibiotics and cause water pollution (Rahman et al., 2017). Plants can produce active molecules with many different biological activities and act against pathogens. Thus, it is expected that plant extracts will influence drug-resistant pathogenic bacteria (Amenu, 2014). There are many plants used for therapeutic purposes in society (El Sawi et al., 2010).

It has been reported that plant phenolics have a photoprotective role against UV light (Del Valle et al., 2019). Overexposure to UV radiation causes health problems such as eye diseases,

immunosuppression, and skin cancer (Norval., 2006; Hussein., 2005). UV-B rays from the UV class can cause adverse effects such as sunburn on the skin. In addition, UV-B can cause disturbances of the immune system and problems such as photo carcinogenesis (DeBuys et al., 2000). UV-A and UV-B rays cause skin aging and release of toxic radicals (Farrukh et al., 2014). UV-C has a high energy level. Its damage is quite high, but it cannot reach the earth by being swallowed by ozone and oxygen in the stratosphere (Allen and Bain., 1994).

In the current study, it was aimed to determine the biological activity of acetone and ethanol extracts obtained from the leaves and flowers of *Nerium oleander* on fish pathogens to obtain usage potential as natural antimicrobials for the aquaculture industry. Then, the sun protection factor (SPF) of the extracts was determined for cosmetic industry.

MATERIAL-METHOD

Plant Material

White oleander flower and leaf samples were obtained from Alata Horticultural Research Institute (Mersin/Turkey) in June 2021.

Preparation of White Oleander Flower and Leaf Extracts

The white oleander flower and leaf samples were washed with distilled water and dried at room temperature, and the dried samples were ground. The extraction, 50% acetone and 50% ethanol were added separately to the plant material. The extracts were obtained in 3 repetitions for 30 minutes with a sonication device (Hielscher). Then, the crude extract was obtained by evaporating of solvents. The obtained extracts were stored at 4°C during use.

Fish Bacterial Pathogens

The fish bacterial pathogens as test microorganisms (*Vibrio anguillarum* A4, *Aeromonas hydrophila* ATCC 19570, *Vibrio alginolyticus, Yersinia ruckeri* and *Lactococcus garvieae*) were used. *A. hydrophila* ATCC 19570 in Nutrient-Broth (NB) at 37°C, *L. garvieae* and *Y. ruckeri* in Tryptic-Soy-Broth (TSB) at 25°C, *V. anguillarum* A4 and *V. alginolyticus* in TSB with 2% NaCl at 25°C were cultured.

Determination of Antibacterial Activity

Disc Diffusion Assay

The disc diffusion assay was applied to determine the biological activity of the acetone and ethanol extracts from the white oleander flower and leaf. 100 µl of suspensions of fish pathogenic test microorganisms (0.5 McFarland) were spread on agar medium. 20 µl (2 mg/disc) extract-impregnated discs (No: 6 mm) were placed on solid agar in triplicate. After incubation for 24 hours, the inhibition zones were measured using a caliper.

Micro-dilution Method

The minimum inhibition (MIC) and bactericidal concentration (MBC) values of white oleander leaf and flower extracts were determined using a micro-dilution method. Bacterial suspension (0.5 McFarland) was added to each tube containing the extract and nutrient medium. Tubes with different extract concentrations were incubated. At the end of the incubation, the concentration of the extract in the tube without bacterial growth was recorded as MIC values. Samples taken from the tubes were inoculated into solid medium using the spot cultivation method. After incubation, the extract concentrations without bacterial growth on solid media were determined as the MBC values of the extract.

Determination Sun Protection Factor (SPF) of White Oleander Extract

The sun protection factor (SPF) of white oleander extracts was determined using the in-vitro method (spectrophotometrically). The extract was stirred in 96% ethanol. The absorbance

values of the homogeneous mixture were measured using a spectrophotometer (Beckman-Coulter) (wavelength: 290nm and 320 nm). Absorbance values were calculated using Mansur's equation (1) (Mansur et al., 1986).

Mansur's equation:

$$\textbf{SPF: CF} \times \sum_{290}^{320} \boldsymbol{EE}(\boldsymbol{\lambda}) \times \boldsymbol{I}(\boldsymbol{\lambda}) \times \boldsymbol{Abs}\ (\boldsymbol{\lambda}) \qquad \textbf{(1)}$$

Correction Factor (10) **(CF),** Erythemetogenic Effect (λ) **(EE)**, Intensity of Sunlight at Wavelength (λ) **(I),** Absorbance of Extracts at Wavelength (λ) (**Abs).**

RESULTS AND DISCUSSION

Antibacterial activities of white oleander flower and leaf extracts were investigated on fish pathogens by disc diffusion and microdilution experiments. Inhibition zone diameters formed by the extracts on the test bacteria are given in Table 1. The inhibition zone diameters were determined in the range of 7.09 mm and 12.67 mm. The flower acetone extract (WOFA) has the highest zone diameter of inhibition against *V. anguillarum* A4.

Table 1. Inhibition Zone Diameters of White Oleander Extracts

Microorganisms	**Inhibition zone diameter (mm)**			
	WOFA	**WOFE**	**WOLA**	**WOLE**
V. anguillarum A4	12.67±0.66	12.06±0.77	11.98±0.83	11.18±0.41
A. hydrophila ATCC 19570	11.40±0.50	10.57±0.91	11.11 ±0.66	10.36±0.35
V. alginolyticus	12.01±0.98	11.80±0.01	12.53±0.52	9.56±0.53
Y. ruckeri	11.17±1.01	10.01±0.13	10.68±0.40	9.14±0.23
L. garvieae	7.96±0.65	8.35±0.99	7.68±0.43	7.09±0.16

*WOFA: White Oleander Flower Acetone WOFE: White Oleander Flower Ethanol WOLA: White Oleander Leaf Acetone WOLE: White Oleander Leaf Ethanol

In a study conducted with oleander acetone extract, the highest disc diffusion diameters were determined against *Pseudomonas* spp. and *Candida albicans* as 17.23 mm and 15.75 mm (ThamaraiSelvi and Deepthi, 2018). In a study by Boyraz and Koçak (2006), the oleander methanol extract prepared by the soxhlet method determined its antimicrobial activity on fungal microorganisms (*Alternaria mali, Sclerotinia sclerotiorum, Colletotrichum circinans, Fusarium oxysporum* and *Botrytis cinerea*). It has been indicated that oleander extract has an antifungal effect on all phytopathogenic fungi at doses of 1% and 2%.

The MIC values of white oleander extracts are given in Table 2. According to the MIC value results of white oleander flower and leaf extracts, among the test microorganisms, the highest effect was detected against *A. hydrophila* ATCC 19570 and *V. anguillarum* A4 at a concentration of 5 mg/mL. The MIC values of the extracts varied from 5 mg/mL to 40 mg/mL.

Table 2. MIC Values of White Oleander Flower and Leaf Extracts.

Microorganisms	**MIC (mg/mL)**			
	WOFA	**WOFE**	**WOLA**	**WOLE**
V. anguillarum A4	10	5	10	20
A. hydrophila ATCC 19570	20	10	5	5
V. alginolyticus	20	40	40	40
Y. ruckeri	40	40	40	20
L. garvieae	40	40	40	20

*WOFA: White Oleander Flower Acetone WOFE: White Oleander Flower Ethanol WOLA: White Oleander Leaf Acetone WOLE: White Oleander Leaf Ethanol

MBC values in white oleander extracts are given in Table 3. The lowest concentration of MBC on *A. hydrophila* ATCC 19570 was determined in leaf ethanol extract (WOLE) with 5 mg/mL. In general, the lowest MBC value against fish pathogens was determined in leaf ethanol extract.

Table 3. MBC Values of White Oleander Flower and Leaf Extracts

Microorganisms	**MBC (mg/mL)**			
	WOFA	**WOFE**	**WOLA**	**WOLE**
V. anguillarum A4	20	10	10	20
A. hydrophila ATCC 19570	20	10	10	5
V. alginolyticus	40	40	40	40
Y. ruckeri	40	40	40	20
L. garvieae	40	40	40	20

*WOFA: White Oleander Flower Acetone WOFE: White Oleander Flower Ethanol WOLA: White Oleander Leaf Acetone WOLE: White Oleander Leaf Ethanol

If the MBC/MIC ratio of the determined extract is ≤4, it indicates bactericidal activity, and if the MBC/MIC ratio is >4, it has bacteriostatic activity (Krishnan et al., 2010). The MBC ratio was found below 4. White oleander extracts have a bactericidal effect against all tested pathogens (Table 4).

Table 4. MBC/MIC Results of White Oleander Flower and Leaf Extracts

Test Microorganisms	**MBC/MIC**			
	WOFA	**WOFE**	**WOLA**	**WOLE**
V. anguillarum A4	2	2	1	1
A. hydrophila ATCC 19570	1	1	2	1
V. alginolyticus	2	1	1	1
Y. ruckeri	1	1	1	1
L. garvieae	1	1	1	1

*WOFA: White Oleander Flower Acetone WOFE: White Oleander Flower Ethanol WOLA: White Oleander Leaf Acetone WOLE: White Oleander Leaf Ethanol

Sun Protection Factor (SPF)

SPF values of the oleander extracts determined by spectrophotometric method are given in Table 5. The SPF values of the white oleander leaf extracts were found to be close to each other, between 25.72 and 26.52. The highest SPF value belongs to WOFE extract.

Table 5. SPF Values of White Oleander Flower and Leaf Extract.

Extracts	**SPF Value**
WOFA	26.49±0.04
WOFE	26.52±0.17
WOLA	25.72±0.02
WOLE	26.23±0.01

*WOFA: White Oleander Flower Acetone WOFE: White Oleander Flower Ethanol WOLA: White Oleander Leaf Acetone WOLE: White Oleander Leaf Ethanol

In the literature, *Nerium oleander* leaf extract showed significant cytotoxic activity against tumor cells and a potent antioxidant effect against non-cancer cells (Mouhcine et al.,

2019). Therefore, white Oleander extracts can be used as safe photoprotective agents in the cosmetic industry.

CONCLUSION

Antibacterial activities of flower and leaf extracts from white oleander prepared with two different solvents (ethanol and acetone) against fish pathogens and also SPF were investigated. It has been determined that the flower and leaf extracts of white oleander have antibacterial activity against all the tested fish pathogens. The SPF values of the white oleander flower and leaf extracts were high. Suitable concentrations of flower and leaf extracts of white oleander can be used as natural ingredients in cosmetic and feed industries.

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PREPARATION AND COMPARISON OF WATER-SOLUBLE MAGNETIC NANOPARTICLES MODIFIED WITH DIFFERENT FLUORESCENT DYES

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ABSTRACT

Nanoparticles have a very important role in the field of science and technology today. It is defined in the literature as a particle with a size in the range of 0-100 nm. In today's conditions, the main goal is to achieve high efficiency and low toxicity with less material and energy. In this direction, it is adopted to prepare particles with a sustainability approach and use them in applications. Particles in metallic, polymeric, or hybrid structures can be prepared by chemical and physical methods. They have different properties according to both the structure of the material they are prepared and their size and shape. It is used and will be used in many application areas such as biomedicine, electronics, environment, agriculture, and energy. In R&D studies, existing nanostructures are functionalized and made more useful. The weak optical properties of magnetic nanoparticles, which are very attractive thanks to their magnetic properties, cause weakness in many application areas. To eliminate this deficiency, magnetic particles can be modified with molecules or particles with optical properties such as dyes, molecules, or quantum dots. In this study, cobalt ferrite magnetic nanoparticles were modified with chitosan to make their surfaces suitable for functionalization, and then the attachment of different dye molecules (eosin y, fluorescein, and rhodamine B) to the particle surface by bonding reactions were examined with spectrophotometric methods.

Keywords: Magnetic Nanoparticles, Biomedical Applications, Fluorescent Dyes

INTRODUCTION

Nanotechnology is a trendy issue in the field of science right now. The products and techniques that have arisen because of these studies make daily living more efficient. The need for sustainable energy, the detection and treatment of new diseases, the rising demand for food because of population growth, and other factors are driving the advancement of nanoscience. Nanostructures are the fundamental building blocks for the scientific approach to product development. Because of their unique features, nano-sized materials are very useful in biomedical research(Khan et al., 2019).

Magnetic nanoparticles have properties especially suitable for use in both imaging and treatment methods. The fact that they have high contrast in the MR imaging system and are suitable for magnetic targeting processes gives these particles an important role in the diagnosis of cancer disease(Cabuil, 2015; Frey et al., 2009a; Laurent et al., 2010). In addition, the heat release of magnetic particles under an alternating magnetic field makes them a very effective tool for the application of hyperthermia therapy in cancer treatment. So much so that while the currently used hyperthermia treatments are only effective in cancer cells near the surface, in the magnetic hyperthermia approach, cancer cells in deep regions also undergo apoptosis thanks to magnetic nanoparticles agglomerated by targeting within the tumor(Hoopes, 2013; Salunkhe et al., 2014).

They can be prepared with a functional surface of different shapes and sizes, so this feature makes them preferred in biomedical applications. However, the absence of optical properties of magnetic nanoparticles is an important feature. This makes it difficult to monitor

the instantaneous state of magnetic particles in both experimental and applications(Cabuil, 2015; Frey et al., 2009b). This problem can be handled by modifying magnetic particles with optical properties of dyes (fluorescent or chromophore dye etc.) and nanostructures (quantum dots or plasmonic nanoparticles etc.). The resulting nanostructures have both magnetic and optical properties. Especially in intracellular studies, magnetic particles with optical properties are becoming a unique tool for determining particle or drug concentration(Sun et al., 2016; Wei et al., 2018).

Rhodamine b is a water-soluble molecule with fluorescence used for staining. It is used for labeling muscle and other tissue to image sub-cellular structures and for imaging in liquid with a fluorescence microscope. While its excitation value is 545 nm, it emits strongly at 575 nm(Chen & Wood, 2009). The Eosin yellow dye is frequently employed in the identification of bacterial species as a gram staining type due to the red hue and significant absorption by red blood cells. Its excitation and emission wavelengths are 488 nm and 537 nm, respectively(Thabet & Ismaiel, 2016).

MATERIAL AND METHOD

1.) Co-precipitation was used to produce $CoFe_2O_4$ nanoparticles (MNPs). In a flask, a 1:2 mole solution of $CoCl_2$ and $FeCl_3$ salts was made, and it was combined with a NaOH solution at 80°C for one hour. With the use of a magnet, the resultant black particles were collected, and three magnetic washing procedures were carried out using a solution of ethanol, 2-propanol, methanol, and water. It was then dissolved in ionized water(Aşik et al., 2016).

2.) MNPs and chitosan solutions were combined in order to cover the surface of MNPs with chitosan. 1.0 mL of MNPs solution was diluted to 50.0 ml with DI water and its Ph was adjusted to about 10 by using sodium hydroxide solution. Then this solution was added to 3mg, 5.0 mL of chitosan(in 0.5 M acetic acid) solution, drop by drop. The mixtures were stirred for 12 hours, chitosan-MNPS was collected by using a magnet, and the supernatants having excess chitosan were discarded. Final particles were suspended in di water.

3.) Aqueous solutions of $1x\ 10^{-5}$ M, 10.0 ml of Rhodamine b, Fluorescein and Eosin Y were prepared separately. EDC/NHS reactant solutions of solutions were added and mixed for 30 minutes. Then the MNPs solution (10.0 ml chitosan-MNPs + 5.0 ml DI water) was added dropwise to the previous solutions. and stirred for 2 hours. The mixtures were washed 5 times using the magnetic washing technique. In the last step, chitosan -MNPs particles with dye molecules attached to the magnet were dispersed in 10.0 ml of DI water. Procedure photography is given in Figure 1 and Figure 2(Sahoo et al., 2017).

Figure 1. a) Dye solutions, b) Dye solutions after chitosan-MNPs addition

Figure 2. Magnetic washing procedure of Dye-MNPs a) 1st washing, b) 2nd washing.

4.) After stability observations the optimization study was performed by using Rhodamine B dye at different concentrations. The procedure used in the previous step was followed by using $2x10^{-6}$ M, $6x10^{-6}$ M, $1x10^{-5}$ M, $1.4x10^{-5}$M, and $2x10^{-5}$ M rhodamine B solutions.

5.) After the optimum value was determined, the concentration of the dye molecule attached to the surface was determined as an approximate value by drawing a 5-point calibration curve.

RESULTS AND DISCUSSION

FTIR Spectroakopiai and Zeta Potential measurement methods were used to characterize the prepared Chitosan-MNPs particles.

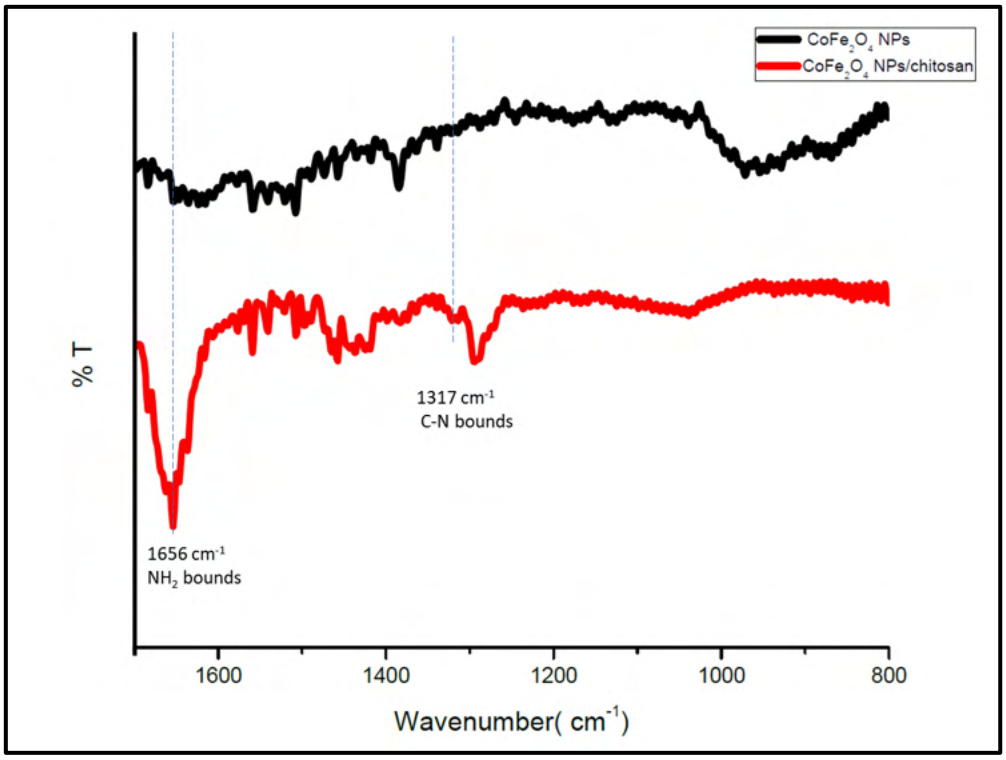

Figure 3. FTIR spectra of MNPs($CoFe_2O_4$ NPs), and chitosan-Chiotosan-MNPs

In Figure 3, the FTIR spectra of bare MNP and Chitosan-coated MNP particles are given. The spectrum in black represents MNPs, while the red one represents chitosan-coated MNPs. In the red spectrum, the NH_2 group bend scissoring peak at 1656 cm^{-1} and C-N stretching peak at 1317 cm^{-1} are seen(Pineda et al., 2014). These peaks are not seen in the spectrum of bare MNPs, proving the presence of the amine group in chitosan. According to these FTIR spectra, the surface was coated with chitosan.

According to zeta potential measurements, the values changed from -0.0775± 0.0561 mV in bare-MNPs solution to 37.8468± 2.3337 mV after coating with chitosan. This change in surface charge indicates that the surface of MNPs particles is coated with chitosan and that the chitosan-MNP particles are homogeneously dispersed in the solution obtained after coating.

The solutions obtained as a result of the experimental 3rd part studies showed similar emission colors with the dye solutions when viewed under a UV lamp at 365 nm. Figure 4

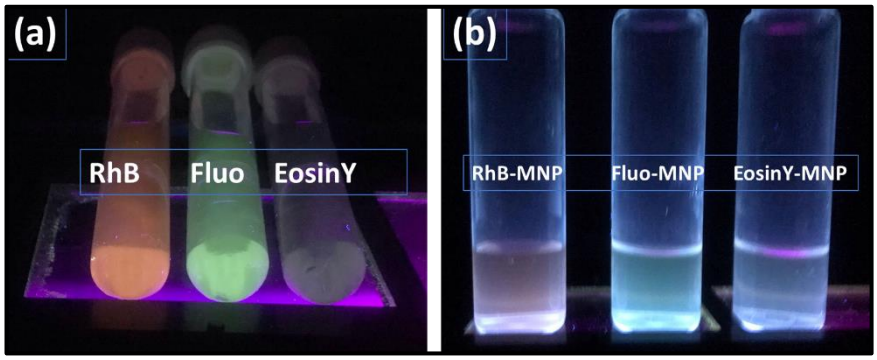

Figure 4. Image of a.) dye solutions, b.) dye-MNPs solutions under UV light.

In Figure 4 a) the image of the dyes under UV light(365 nm) is given, while in Figure 4b) the image of the dye-attached MNPs particle solutions is given. Emissions belong to dye-attached particles, as the dye-attached particles were magnetically washed five times and the supernatants were discarded.

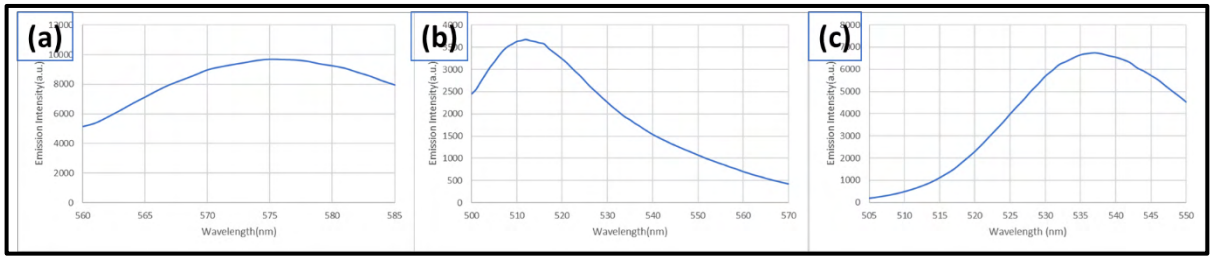

Figure 5. Spectrum of $2x10^{-3}$M a.) Rhodamine B solutions, b.) Fluorescein solutions, and c.) Eosin Y solutions.

In Figure 5, the fluorometric measurements of the dye solutions were taken and it was seen that they were compatible with the literature(El Kurdi & Patra, 2018; Herbrik et al., 2020; Slyusareva et al., 2011). Figure 5. a) Rhodamine B, Figure 5. b) fluorescein and Figure 5. c) eosin y dye emission spectra are given.

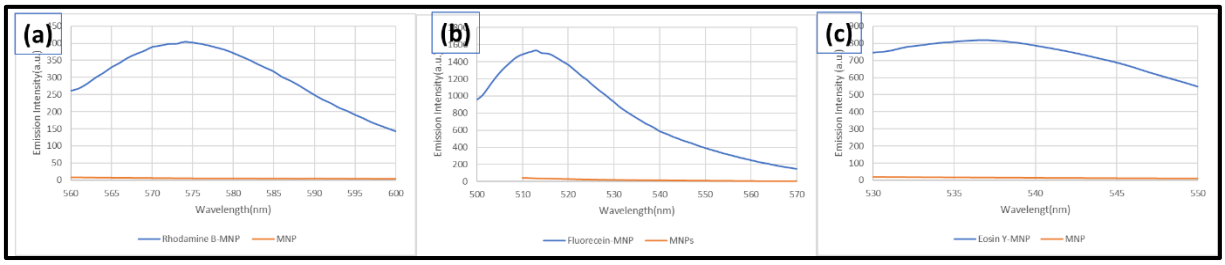

Figure 6. Spectrum of a.) Rhodamine B-MNPs solutions, b.) Fluorescein- MNPs solutions, and c.) Eosin Y- MNPs solutions, which are prepared by adding $1x10^{-5}$M dye solutions

Spectrums of spectrofluorometric measurements of the solutions obtained in the experimental part 3 were made and are given in Figure 6. Orange lines in the spectra belong to MNPs (given as background.) Blue spectra belong to Figure 6. a) rhodamine B-MNPs, b) fluorescein-MNPs, and c) eosin y -MNPs, respectively. When the individual spectra are compared, it is seen that the dyes are attached to the surface of the MNPs. The emission values obtained coincide with the wavelength values obtained in the dyes. The highest intensity value was obtained from the particles using fluorescein dye (b spectrum).

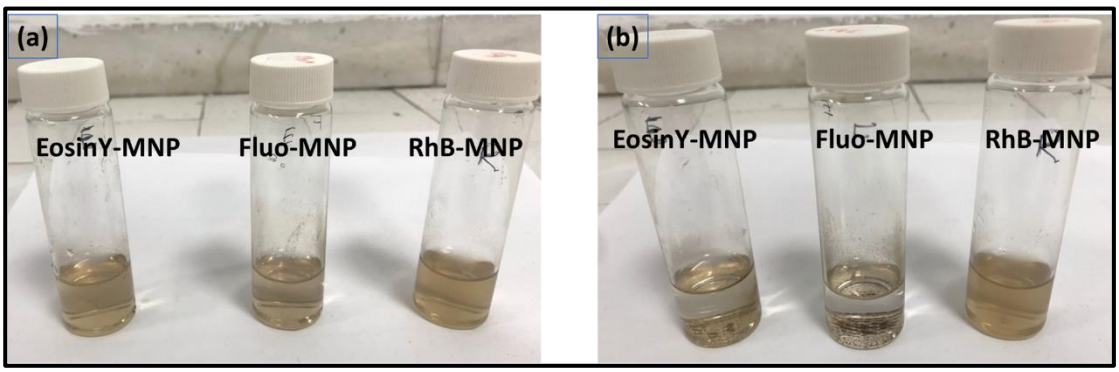

Figure 7. Image of dye-MNPs solution a.) immediately after preparation, b.) 24 hours after preparation.

The images of the dye-MNPs solutions are prepared in Figure 7. a) After preparation are given. In Figure 7. b), the images after keeping the same solutions at room temperature for 24 hours are given. According to Figure 7. b), the stability of Eosin-Y and Fluorecein-MNPs solutions was observed to be quite low. According to Figure 7. b), the Rhodamine B-MNPs particle retains its homogeneous dispersed appearance and is more stable.

Although fluorescein-MNPs gave better results according to emission intensity, Rhodamine B-MNPs gave the best results according to stability observation. For this reason, we continued to work with Rhodamine B-MNPs nanomaterials.

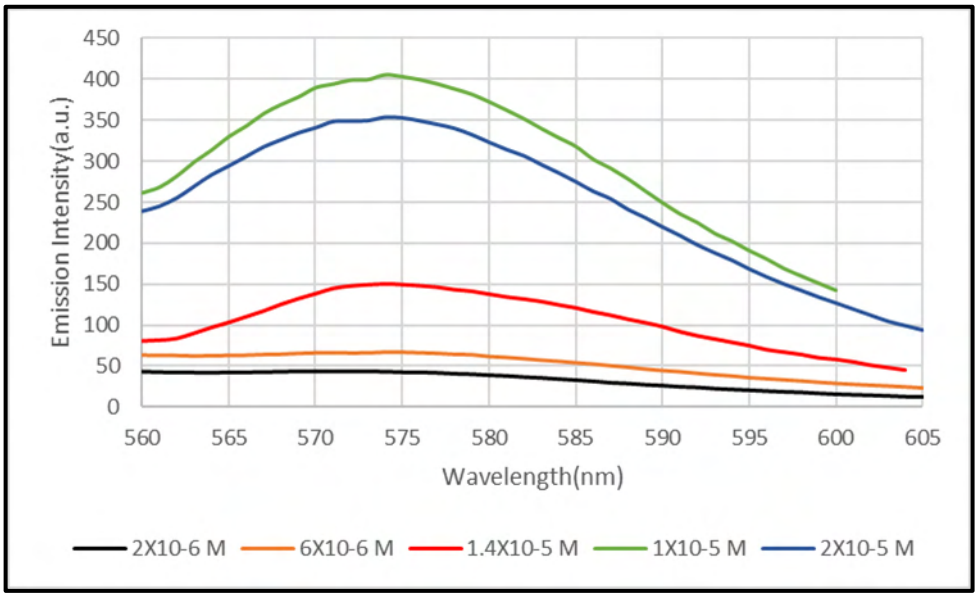

Figure 8. Spectra of rhodamine B-MNPs solution which are prepared by using various Rhodamine B concentrations.

In Experimental part 4, dye attaching procedur was performed using Rhodamine B solution at different concentrations. According to Figure 8, the optimum results were obtained when 1x10-5 M rhodamine B solution was used.

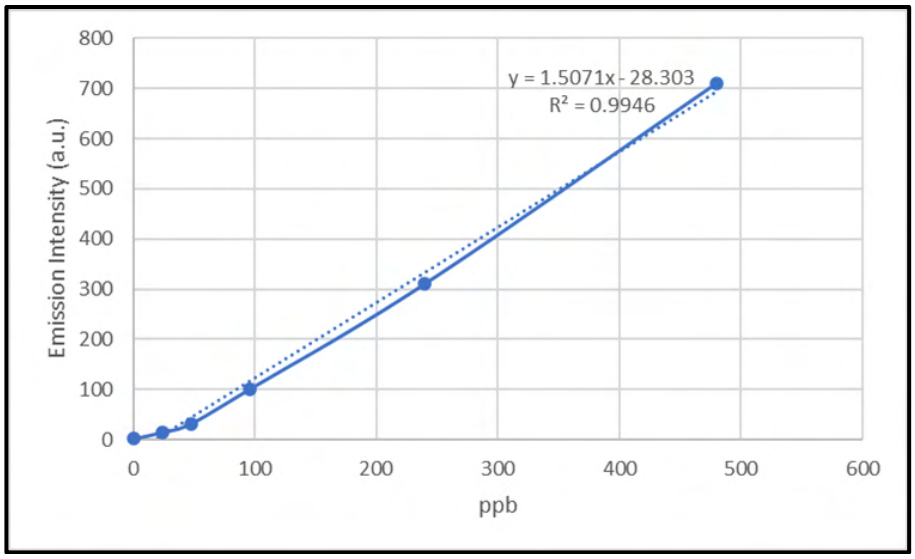

Figure 9. Calibration curve of Rhodamine B solutions

A calibration curve was drawn using a blank and five different concentrations in experimental part 5. In the calibration curve (Figure 9), the amount of dye on the surface of RhB-MNPs prepared under optimum conditions (prepared by adding $1x10^{-5}$ M Rhodamine B) was calculated as approximately 286 ppb.

CONCLUSIONS

In this study, different dye molecules were attached to the magnetic nanoparticle surface coated with chitosan, a biocompatible polymer, by EDC/NHS coupling reaction, and a comparison study was made. Magnetic nanoparticles were given optical properties and nanomaterials were prepared especially for use in intracellular imaging. The optimum value was obtained when Rhodamine B dye was used. This work can be improved by using different dyes and polymeric materials.

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CYTOTOXICITY OF ORANGE PEEL (*Citrus sinensis*) ESSENTIAL OIL NANOEMULSIONS ON THE RAINBOW TROUT GONADAL CELLS

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ABSTRACT

The citrus industry holds a significant position in the agricultural industry. However, it also generates substantial amounts of orange peel (*Citrus sinensis*) wastes. Essential oil production is one of the widely used bio-economical methods for the evaluation of these wastes. Essential oils are evaluated below their potential for use in different sectors due to their volatile nature and low stability against environmental stress conditions, which the nanoemulsion can overcome. Therefore, this study aimed to form and characterize of the nanoemulsion of orange peel essential oil (OPEON) and investigate its cytotoxicity on the rainbow trout gonadal (RTG-2) cells. The OPEON (0.1:0.3:0.6:99 w/w, EO: Tween 80: Ethanol: water) was successfully created using an ultrasonic homogenizer. The OPEON was characterized using TEM (~100 nm), zeta sizer (the ζ-potential value of -12.6 mV, and the polydispersity index of 0.657, conductivity of 0.00547 mS/cm), and FT-IR analysis. Treatments of 125, 250, 500, 1000 ppm of the OPEON have statistically significant toxic effects on the RTG-2 cells after 24 hours of exposure. Based on the study results and considering the toxic effect on cells, there is a potential for effective use of nanoemulsion forms of essential oils, especially in the pesticide industry.

Keywords: Orange peel essential oil, Nanoemulsion, Zeta sizer, Cytotoxicity, Rainbow trout gonadal cells

INTRODUCTION

Citrus fruit, a valuable plant grown worldwide, has a crucial position in fruit consumption, fruit juice, marmalade, jelly, canned food, and essential oil production. In terms of usage area, orange (*Citrus sinensis*) is the most used citrus species (Sandhu et al., 2021). However, up to 60% of the processed fruit weight emerges as solid waste containing highly valuable bioactive compounds (essential oils, flavones, polyphenols, etc.), which creates an economic burden (Omran et al., 2018; Victor et al., 2021). Considering the rich functional components of orange peel, essential oil extraction is one of the widely used bioeconomic methods in the evaluation of these wastes (Gavahian et al., 2019; Siddiqui et al., 2022).

Essential oils are concentrated hydrophobic liquids and complex compounds characterized by a strong odor and composed of various plant metabolites. Essential oils are clear and soluble in lipid/organic (ether, alcohol, fixed oils) solvents and have less density than water (Kar et al., 2018). The biological activities of essential oils vary depending on the chemical composition, which varies according to the plant parts used for extraction, the extraction method, the phenolic stage of the plant, the harvest season, the age of the plant, the nature of the soil and environmental conditions (Said-Al Ahl et al., 2017). Citrus essential oils are widely used in sectors such as beverages, ice cream, cookies, biscuits, cakes, room fresheners, household products, perfumes, pharmaceuticals, aromatherapy, and detergents (Geraci et al., 2017; Hanif et al., 2019). However, there are some drawbacks that limit the high usage potential of essential oils. The high volatility and sensitivity of essential oils to chemical

conversion or degradation reactions such as oxidation, isomerization, polymerization and rearrangement depending on environmental parameters such as temperature, light and atmospheric oxygen limit their potential for use in the field (Pavoni et al., 2020; Oladipupo et al. al., 2022). In addition, essential oils have poor physico-chemical properties, such as fast half-life and low solubility in water. As a way to deal with this, nano formulations are being developed that can retain essential oils without interfering with their bioactivity, provide deeper tissue penetration, increase bioactivity as they allow easier cellular uptake, and achieve the desired slow release (Pavoni et al., 2020; Mustafa and Hussein, 2020).

Nanoemulsions are two-phase dispersion of two immiscible liquids in nanosizes, which are water-in-oil (W/O) or oil-in-water (O/W) formulations and droplets stabilized by amphiphilic surfactants. Nanoemulsions have droplet sizes of 20–200 nm in diameter. The large surface area provided by their nanometric size provides higher loading capacity and improved solubility, resulting in increased bioavailability of poorly soluble compounds. Nanoemulsions are kinetically stable (Feng et al., 2018; Barradas and de Holanda e Silva, 2021; Sharma et al., 2022). Although there are a few studies in which nanoemulsion forms of orange peel essential oil (OPEON) are obtained by different methods and its antifungal, antibacterial and larvicidal activities are shown, there is no study showing toxicity *in vitro* according to our best knowledge (Azmy et al., 2019; Das et al., 2020; Farouk et al., 2022). Based on these observations, this study was modeled to generate data to create and characterize of the OPEON and its cytotoxicity on rainbow trout gonadal (RTG-2) cells.

MATERIAL AND METHOD

The orange peel essential oil was purchased from a local company, BIOMESI Bioagrotechnology R&D, located in Adana, Turkey (Durmuş et al., 2023). The RTG-2 cell line (Registration Number: 95121808) was purchased from Türkiye ŞAP Enstitüsü (Ankara, Turkey) (Çiçek, 2023).

The OPEON was formed using an ultrasonic homogenizer (BANDELIN electronic GmbH & Co. KG, Berlin, Germany) following the method described by Durmuş (2020) with minor changes. 30 µL of essential oil, 90 µL of ethanol, 180 µL of Tween 80 and 29.7 µL of distilled water (0.1:0.3:0.6:99 w/w) were placed in a glass beaker and exposed to an ultrasonic homogenizer. Ultrasonic homogenizer operating conditions were set as 15 min, 70 amplitude, 20 kHz and 500 W, and a titanium probe (2 mm diameter and 1950 mm height (MS72)) was used. In addition, ice was used around the beaker to avoid thermal effects during the process (Durmuş, 2020).

Transmission electron microscope (TEM) (Hitachi High Tech HT7700, Japan), zeta sizer (Malvern Zeta sizer Nano ZSP, Malvern Instruments Pvt Ltd, UK) and Fourier transform infrared spectroscopy (FTIR) (Bruker VERTEX 70v brand, Germany) was used to determinate of surface morphology, zeta potential and molecular structure of the OPEON, respectively (Sogan et al., 2023). These analyzes were carried out with service procurement at the Eastern Anatolia High Technology Application and Research Center (DAYTAM, Erzurum, Turkey).

The RTG-2 cells were cultured 89.5% Eagle's minimal essential medium (EMEM: with L-glutamin medium, ATCC 30-2003) supplemented with 10% fetal bovine serum (Biowest S1810-500) and 0.5% penicillin-streptomysin (Sigma P4333) in 25 cm^2 culture flasks (Isolab 120.11.025) at 23.7 ˚C without CO_2 respiration (Çiçek, 2023).

The OPEON were prepared at different concentrations (125, 250, 500, and 1000 ppm) by dissolving in ethanol:distilled water solution prepared in a 1:1 ratio. Experimental groups were applied on the RTG-2 cells seeded 24 hours ago at a density of $3x10^4$ cells/well. For the control groups, ethanol: distilled water and Tween 80: ethanol were used. Then, a cell viability test was carried out after 24 hours of incubation.

Sulforhodamine B test was performed for cell viability testing. Briefly, after 24 hours of incubation with the experimental groups, 100 µL of cold 10% trichloroacetic acid solution

(CAS No: 76–03–9, Sigma Aldrich, USA) was applied to the RTG-2 cells (4 °C, 1.5 hours). Following washing 5 times with distilled water and air drying, the cells were fixed with 50 µL of 0.4% SRB dye (CAS No: 3520–42-1, Sigma Aldrich, USA) was prepared in 1% acetic acid (CAS No: 64–19–7, Sigma Aldrich, USA) (30 min in the dark). Washing was done 5 times with 5% acetic acid solution and air drying. Then, 150 µL of 10 mM Tris base (CAS No: 77–86–1, Sigma Aldrich, USA, pH 10.5) was added to each well and kept in an orbital shaker (15-20 min, 150 rpm). The absorbance values were read in a micro-plate reader (EpochTM, BioTek, USA) at 564 nm (Vichai and Kirtikara, 2006).

The study data (n= 6 independent experiments) were evaluated using the GraphPad Prism 9.00 Statistical Software (GraphPad Software, Inc., California, USA). Experimental groups were analyzed using One-way analysis of variance (ANOVA) and the statistical significance was accepted at $p \leq 0.05$ level (Çiçek, 2023).

RESULTS AND DISCUSSION

Detailed characterization is necessary to confirm the presence of nanostructures in the production of nanomaterials. Therefore, in this study, TEM, Zetasizer, and FTIR were utilized for the characterization of the OPEON. During the nanoemulsion process, the reaction mixture turned milky white (as observed macroscopically) after ultrasonication process. Eventually, this mixture became translucent and dispersed. TEM images of the OPEON are shown in Figure 1 at different scales. According to TEM images, the OPEON consisted of spherical droplets and was obtained in sizes of 100 nm and above. The Ostwald maturation phenomenon, in which a possible solubility in the aqueous phase of the emulsion system transferred from small droplets to large droplets, may have led to the obtaining of nanoemulsions of different sizes (Farouk et al., 2022). In this study, negative staining (uranyl acetate) was used to remove water from the outer layer of nanoemulsion droplets and visualize it (Klang et al., 2012; Somala et al., 2022). TEM images show that the OPEON has no physical deformation, no agglomeration and a smooth structure.

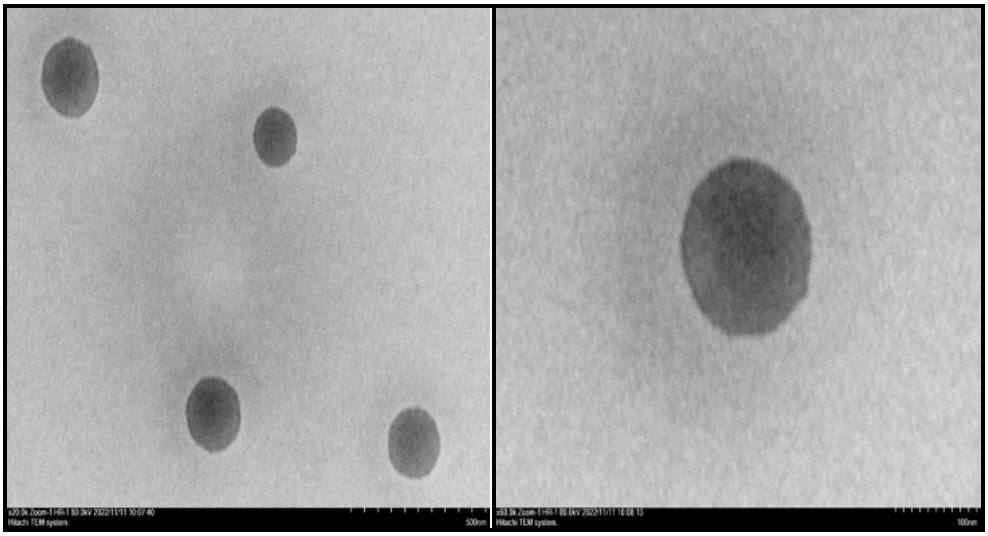


Figure 1. TEM images of the OPEON at 500 nm and 100 nm scales

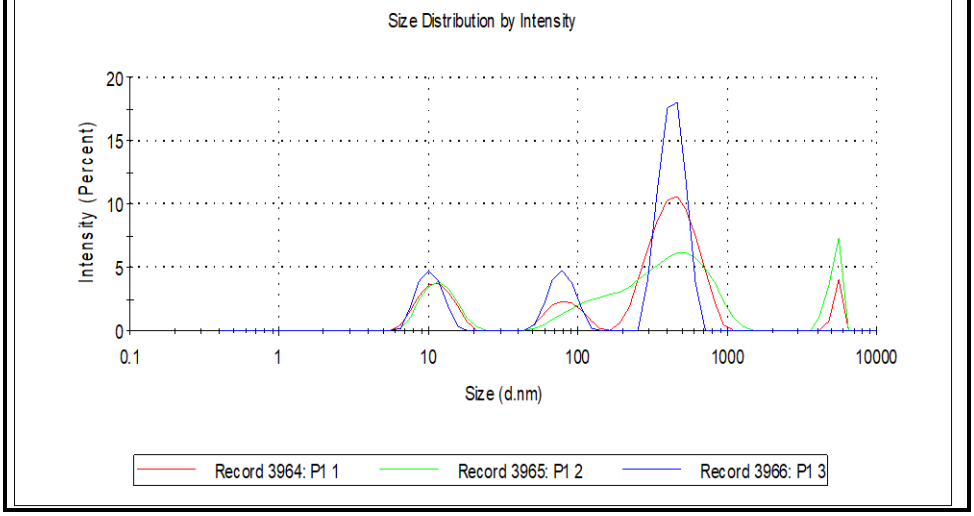
Zetasizer analysis was performed to measure the particle size, zeta potential values, and polydispersity indexes of the OPEON droplets at a constant temperature of 25°C (as shown in Figure 2). The average droplet particle sizes of the OPEON ranged from 405 to 1291 nm, with a 701.2 nm of mean droplet particle. Considering the size distribution graph, it is understood that nanoemulsions with sizes around 10 nm and 100 nm are obtained at the same density percentage, and there are also emulsion forms, with sizes around 1000 nm and above. This explains the visualization of nanoemulsions with sizes around 100 nm in TEM analysis.

Increases in the size of the emulsion structure may depend on the type of surfactant and its chemical composition in the essential oil (Mohammad et al., 2019).

The mean zeta potential (ζ-potential) value of the OPEON was determined as -12.6 mV. High negative and positive ζ-potential values may indicate that repulsive forces are more dominant than attractive forces. In this study, the OPEON was obtained using an ultrasonicator. Mechanical stress occurring during the ultrasonication process can cause the release of free -OH and -COOH groups from the essential oil, leading to an increase in the negative charge on the surface of the nanoemulsion. The ζ-potential value of nanoemulsion, which is considered electrostatically stabilized, is expected to be in the range of ±30 mV (Gurpreet and Singh, 2018). Therefore, based on the mean ζ-potential value, it can be assumed that the OPEON has a suitable shelf life and can be used effectively and with long-term effect in various areas for different purposes (Farouk et al., 2022).

The mean polydispersity index (PDI) of the OPEON was determined as among 0.509-0.943. The PDI shows a narrow distribution of nanoemulsions size if it is below 0.2 or 0.25 (Kaci et al., 2018). In this study, the OPEON was obtained in different sizes, and the dispersions of nanoemulsions between 10 nm and 100 nm are less than the dispersions of nanoemulsions with sizes close to 1000 nm. This explains the high PDI values. If the PDI value is greater than 0.5, the system is called broad size distribution (Golfomitsou et al., 2018). Tween 80 concentration is the most crucial factor affecting the PDI value of nanoemulsions (Pongsumpun et al., 2020). This suggests that the concentration of Tween 80 used in this study should be kept higher in other studies.

Sample	Z-avarage (d.nm)	ZP (mV)	PDI
P11	405	-14.8	0.509
P12	407.5	-13.1	0.519
P13	1291	-10	0.943
Mean	701.2	-12.6	0.657
Std. Dev.	510.8	2.43	0.248



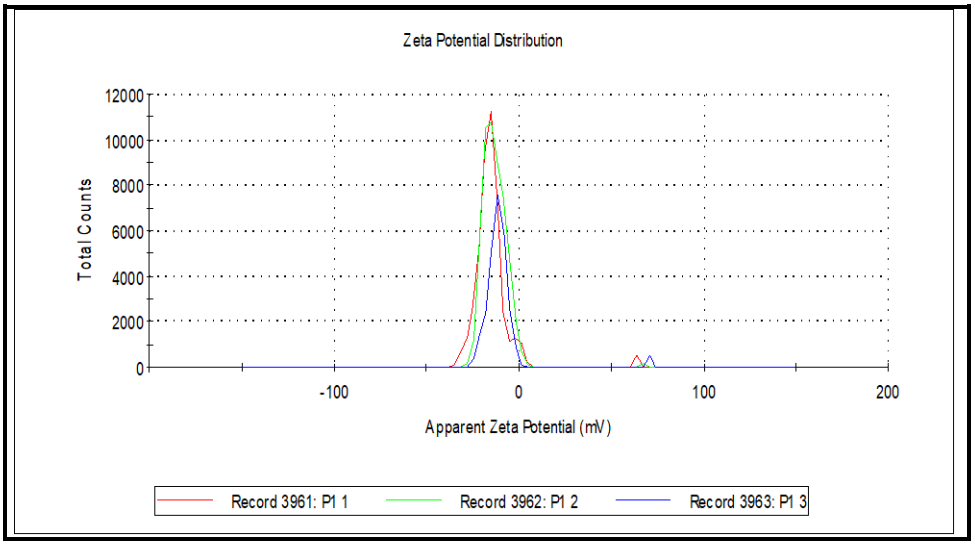


Figure 2. The droplet particle size distribution, zeta potential distribution, polydispersity indexes of the OPEON (P11, P12, P13: three repetitions; ZP: Zeta potential, PDI: Polydispersity index)

FTIR analysis was performed to characterize the molecular structure and functional groups of the OPEON, as shown in Figure 3. The peaks observed at 3753 cm^{-1}, 3496 cm^{-1} and 3481 cm^{-1} corresponds to O-H stretching of alcohol, phenol, and hydroxyl groups, the peak at 2921 cm^{-1} indicates to C-H and O-H stretches of the alkanes, and the peak at 2856 cm^{-1} is -C-H aldehydic stretching and -C-H stretch, as well as carboxylic acid O-H stretch (Opoku et al., 2021; Soni et al., 2022). An absorption band at 2368 cm^{-1} indicates the O=C=O stretching of carbon dioxide. In this study, the C=O ester groups at 1733 cm^{-1} may be associated with the ester groups found in Tween 80 (Osanloo et al., 2022). The peak was observed at 1652 cm^{-1} corresponds to vibratory stretching bonds of groups C=O (Amide type I), the band at 1558 cm^{-1} indicates amide II and N-H bending (Hosseinnia et al., 2017; Al-Hilifi et al., 2022). The peaks observed at 1458 cm^{-1}, 1350 cm^{-1}, and 1297 cm^{-1} are related to CH_2 bending vibration, NO_2 stretch and CH_3 bend, respectively (Zhang et al., 2017; Michelina et al., 2019). The peak at 1247 cm^{-1} represents the C-O-C stretch, while the peak at 1099 cm^{-1} indicates the C–O stretching. The peaks among at 500-950 cm^{-1} refer to C-H and C=C bends (Min et al., 2021).

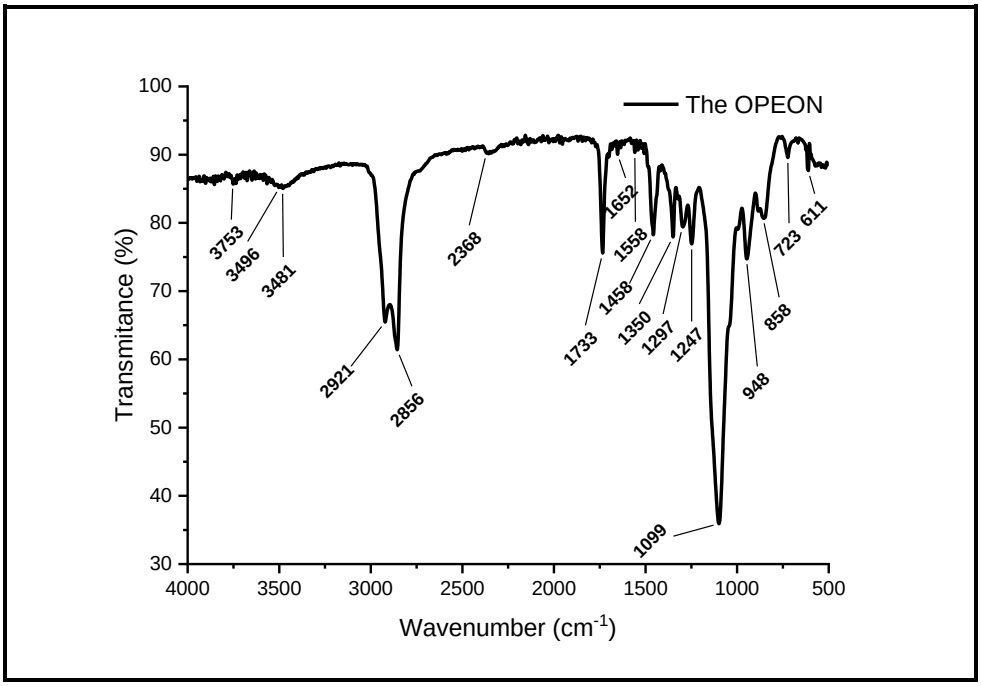


Figure 3. The FTIR spectra of the OPEON

The cytotoxic effect of the OPEON prepared at different concentrations on the RTG-2 cells after 24 hours of exposure is shown in Figure 4. Treatments of the OPEON (125, 250, 500, and 1000 ppm) showed significantly higher cytotoxic effects compared to the control group. Although 1:1 ethanol and Tween 80 treatments showed toxic effects compared to the control group, they did not reduce the RTG-2 cell viability as much as the OPEON treatments. The cytotoxic effects of nanoemulsion forms of essential oils are generally higher than that of free essential oils, depending on surfactant, nano size, electrical properties, chemical composition of the essential oil, dose, and time. In addition, the interaction between the surface charges of nanoemulsions and the charges of cell membranes can increase the cytotoxic effect (Yoon et al., 2018; Marchese et al., 2020).

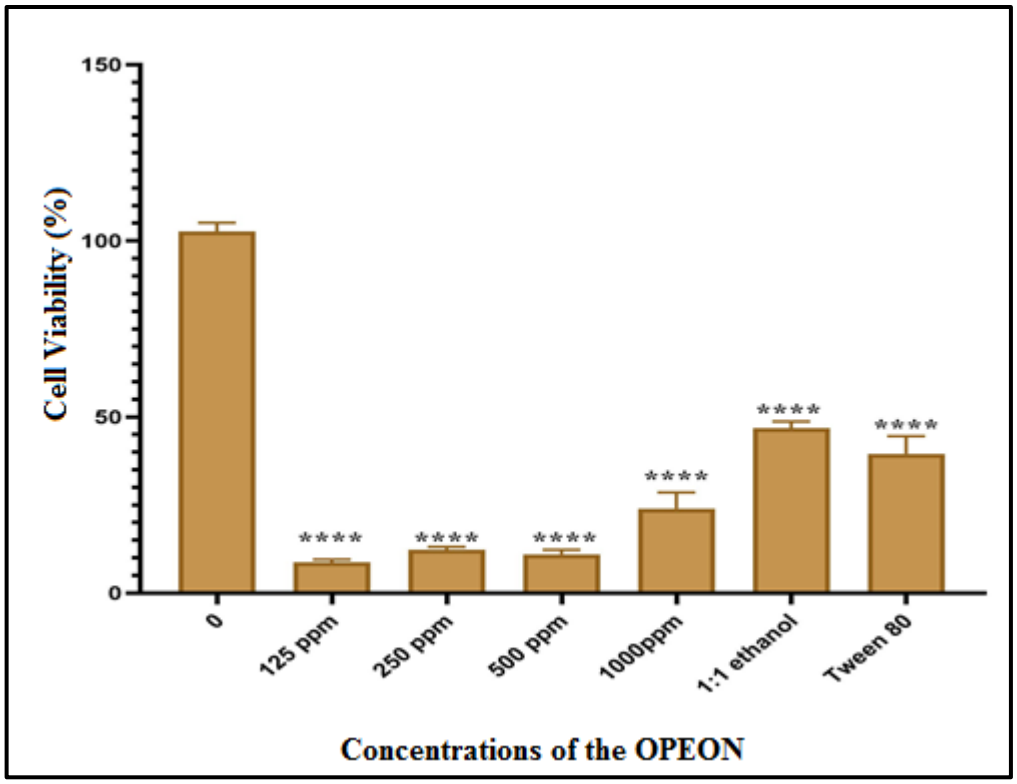


Figure 4. The cytotoxic effects of the OPEON on the RTG-2 cells for 24 hours

CONCLUSIONS

This study demonstrated that the OPEON was successfully obtained at different sizes. However, for long-term stability, it is recommended to change the formulation ratios for subsequent processes. Considering the toxic effect of the OPEON on the RTG-2 cells, future studies may focus on its potential for use in fields such as pesticide production, antibiotic agent.

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SPATIAL-TEMPORAL ANALYSIS OF TEMPERATURE VALUES IN THE THRACE REGION USING INNOVATIVE TREND METHOD

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ABSTRACT

Climate change is one of the most significant environmental challenges the world is facing, and the increasing temperature values serve as one of its most prominent indicators. In this context, climate scientists and researchers are focusing on regional analyses to comprehend the impacts of climate change at a local scale and shape future mitigation strategies. Understanding temperature trends and patterns in the Thrace region, characterized by diverse landscapes and agricultural importance, holds paramount importance for informed decision-making and sustainable development. This study aims to examine the spatial-temporal trends of temperature values occurring between 1982 and 2021 for three provinces (Edirne, Kırklareli and Tekirdağ) situated in the Thrace region of Turkey. For this purpose, an Innovative Trend Analysis (ITA) method is employed to identify how temperature data has changed over time and its regional distribution. The ITA method is utilized to detect low, moderate, and high-density temperature trends. Subsequently, the identified trends from the ITA method are cross-validated using the widely accepted Mann-Kendall (MK) test. Ultimately, this study is expected to contribute to a deeper understanding of climate dynamics in the Thrace region, providing a foundation for evidence-based policies to conserve natural resources and enhance resilience against the ever-changing climate.

Keywords: Climate Change, Mann-Kendall, Temperature, Trend, Innovative Trend Analysis

INTRODUCTION

Climate change represents one of the most pressing global challenges we face today (Newell, 2010). The rapid growth of industry, the consumption of fossil fuels, deforestation, and various human activities have resulted in heightened concentrations of greenhouse gases in the atmosphere, accelerating global warming (Wuebbles & Jain, 2001). Climate change is a critical global issue with far-reaching consequences for the environment, ecosystems, and human societies (Vitousek, 1994).

One prominent consequence of climate change is the significant rise in the global mean temperature, which has led to various impacts and risks. Notably, this has caused shifts in seasons, resulting in changes in their timing and temperature variations (J. Wang vd., 2021). Extreme temperature events have become more frequent and intense, with a projected increase in heatwaves, especially in urban areas (Tsai vd., 2023). The relationship between temperature changes and influencing factors is intricate, involving natural variability, topography, and human activities (Huang vd., 2021).

Elevated temperatures also influence precipitation patterns, leading to alterations in drought occurrences and rainfall variability (Kamal vd., 2021). As temperatures continue to rise, the frequency of cold weather extremes decreases, while heatwaves become more frequent

and severe (Arnell vd., 2021). Extreme heat events carry significant socioeconomic consequences and are a growing concern (P. Wang vd., 2021). In summary, climate change is driving a substantial increase in temperatures, leading to a range of impacts on seasons, extreme temperature events, precipitation patterns, and socioeconomic factors.

It is essential to assess the consequences of climate change at various levels of warming to comprehend the associated risks (Arnell vd., 2021). The distribution of seasonal mean temperature anomalies has shifted towards higher temperatures, leading to an increase in extremely hot outliers. However, discerning long-term climate change can be challenging due to the inherent variability of local weather and climate (Hansen vd., 2012). Temperature and trend analysis are closely related in the study of climate change. Trend analysis is a method used to examine long-term changes in temperature patterns over time. By analyzing temperature data, trends can be identified and used to make predictions about future changes. One study examined the maximum and minimum temperature trends and found evidence of increasing temperature trends (Easterling vd., 1997). The relationship between temperature and trend analysis is a crucial connection in understanding climate change and predicting future climate scenarios (Amjad vd., 2023). Consequently, climate change and the associated rising temperature trends have become fundamental subjects of scientific research.

Trend analysis methods are used to have information about the increase or decrease of meteorological factors. Innovative trend analysis is one of these analysis methods. Innovative trend analysis methods have been used in various studies to analyze temperature and precipitation trends and their implications for climate change. Gobin et al. compared statistical downscaling methods for climate change impact analysis and found that dry day frequency is projected to increase significantly in the summer months, while total precipitation is projected to decrease significantly (Tabari vd., 2021). Agbo et al. compared different trend analysis methods and found that Şen's innovative trend method was useful in identifying trends even for parameters with non-monotonic variations (Agbo vd., 2021). These studies highlight the importance of trend analysis in understanding climate change impacts and informing water resource management and agricultural practices.

The Thrace region, located in the northwestern part of Turkey, holds significant agricultural areas and is economically important. Temperature, being a vital climatic parameter, directly influences agricultural production in this region, making it essential to understand temperature trends and variability.

In this article, the temporal-spatial variation of the temperature values between 1982 and 2021 in the Thrace region using the " Innovative Trend Method" is examined. This method serves as a powerful statistical tool to identify long-term trends and reveal seasonal variations in temperature variables. The study aims to provide valuable insights into temperature changes over the past four decades and offer essential information for future climate scenarios. Understanding the regional impacts of climate change and taking appropriate measures necessitate conducting spatial-temporal analyses. The results of this study will inform policy makers, environmental experts and other stakeholders about the temperature trends in the Thrace region.

MATERIAL AND METHOD

Study Area

The Thrace Region is located in the northwestern part of Turkey. Geographically, it lies between the Marmara Sea and the Aegean Sea, forming the European part of Turkey (“Trakya”, 2023). The region generally experiences a temperate climate, with mild winters and hot summers, influenced by its proximity to the sea. These climatic conditions create a favorable environment for agricultural activities. In the Thrace Region, it has been determined that the average temperature is 13.6°C, with the lowest temperatures occurring in January and the

highest temperatures in June and July. Among the provinces in the Thrace Region, Edirne has the highest temperatures during the summer months, while during the winter months, Kırklareli and Edirne have the lowest temperatures according to long-term average data (Hanedar vd., 2019).

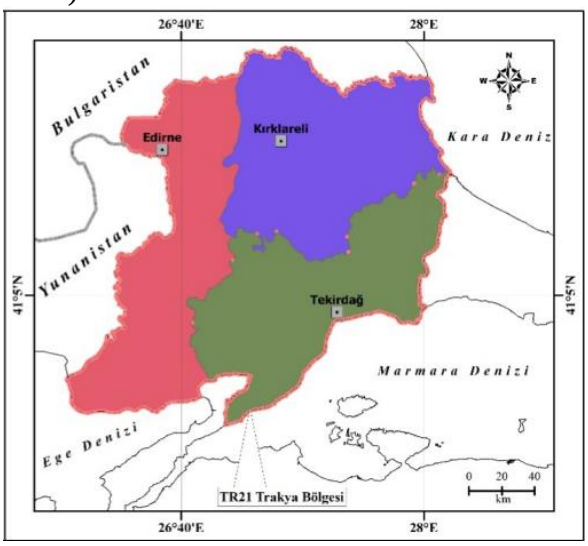

Figure 1. Study Area

Data Collection

Meteorological data used in this study were obtained from the NASA POWER website in the provinces of Edirne, Kırklareli and Tekirdag in the Thrace region (NASA Langley Research Center., t.y.). The primary variable of interest is the temperaturerecord for the period 1982 to 2021.

Data quality checks were performed to ensure the accuracy and consistency of temperatures records. All missing or inconsistent data points were properly resolved and a high level of data integrity is maintained to increase the reliability of the analysis.

Data for monthly average, maximum, and minimum temperatures from three locations (Edirne, Kırklareli, and Tekirdağ) in the Thrace region have been acquired from NASA POWER for the period 1982-2021 (Table 1).

Table 1. Meteorological stations' information.

No	Stations	Elevation (m)	Latitude (N)	Longitude (E)	Period
1	Edirne	199.14	41.6776	26.5559	1982-2021
2	Kırıkkale	175.48	41.7359	27.2247	
3	Tekirdağ	98.23	41.0846	27.3848	

Methodology

In the period between 1982 and 2021, Innovative Trend Analysis was employed to determine temperature trends. Additionally, the results obtained from Innovative Trend Analysis in the study area were compared with the values obtained from the Mann-Kendall and Sen's Slope Estimator analyses to strengthen the findings.

Innovative trend method

The Innovative Trend Analysis method is an approach that allows for the prediction of future trends by statistically analyzing climate data. This method is used to understand the effects of climate change and develop strategies to combat climate change (Esen, 2022). Climate data includes time series of parameters such as precipitation, temperature, relative humidity, evaporation, and sunshine duration, recorded on a monthly basis (Topçu & Karaçor,

2021). These data are analyzed using statistical methods such as regression analysis and correlation analysis. Regression analysis is used to determine changes and long-term averages in time series, while correlation analysis is used to examine the relationship between parameters. As a result of these analyses, trends related to climate change and potential issues like drought or water scarcity in the future can be predicted (Esen, 2022). This Innovative Trend Analysis method can contribute to the development of effective strategies in the fight against climate change.

In the Innovative Trend Analysis, the existing data series is divided into two equal halves. Both sub-series are separately sorted in ascending order. Then, the first sub-series (X_i), arranged according to the Cartesian coordinate system, is placed on the X-axis, and the second sub-series (X_j) is placed on the Y-axis. If the data points are above the 1:1 line, it indicates no trend. If the data points are in the lower triangle area of the 1:1 line, it suggests a downward trend, and if they are in the upper triangle area, it suggests an upward trend (Ceribasi, 2018; Ceribasi & Dogan, 2016; Çeribaşı, 2018; Şen, 2012, 2012; Yıldırım, 2015).

The implementation stages for Innovative Trend Analysis are as follows:

1. The fact that the time data is n pieces is divided into two.

$$(x_1, x_2, \ldots, x_n),\ \{y_{1,n/2}\}=\{x_1, x_2, \ldots, x_{n/2}\} \text{ ve } \{y_{2,n/2}\}=\{x_{n/2+1}, x_{n/2+2}, \ldots, x_n\} \quad (1)$$

2. The resulting data is sorted from large to small or small to large.

$$\{r_1\}=\{\min(y_{1,n/2}),\ldots y_i,,\ldots,\max(y_{1,n/2})\}\ (1 < i < n/2) \quad (2)$$

$$\{r_2\}=\{\min(y_{2,n/2}),\ldots y_j,,\ldots,\max(y_{2,n/2})\}\ (1 < j < n/2) \quad (3)$$

3. To obtain the scatter plot, mark the values in data set $\{r_2\}$ that correspond to the values in data set $\{r_1\}$ on the graph. The graph has the same scale on both the horizontal and vertical axes and includes the minimum value of data set $\{r_1\}$ and the minimum value of data set $\{r_2\}$ [$\min(y_{1,n/2})$, $\min(y_{2,n/2})$), and it includes the maximum value of data set $\{r_1\}$ and the maximum value of data set {r2} [max(y1,n/2), $\max(y_{2,n/2})$]. Here, the $\{r_1\}$ data represents the first half of the series on the horizontal axis, while the $\{r_2\}$ data represents the second half of the series on the vertical axis.

4. In the same scatter chart, place a line with a 45° angle (that is, a 1:1 slope) passing through the origin on the chart.

5. Examine the distribution of values on the graph with respect to the 1:1 line to determine the trend. If most of the data points are clustered above the line, this is considered an upward trend; if most of the data points are clustered below the line, it is interpreted as a downward trend. If the values are evenly distributed along the line, there is no trend.

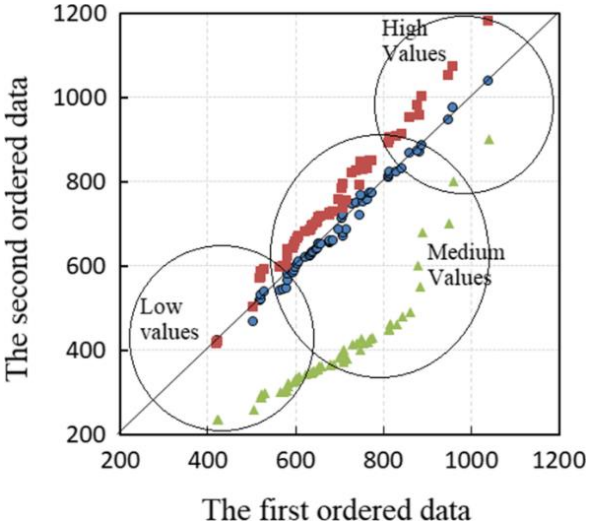

Figure 2. ITA scatter plot illustrating increasing (squares), decreasing (triangles), and trendless time series (circles)

An ITA scatter plot is a graphical representation commonly employed to depict trends within time series data. This plot utilizes distinct shapes or symbols to denote increasing (squares), decreasing (triangles), and trendless or stable (circles) time series. This visual representation serves as a convenient method to swiftly assess data and identify the presence of different trends over time (Figure 2).

Mann-Kendall Test

Mann-Kendall and Sen's slope analysis methods are statistical methods used to determine trends and changes in time series data. The Mann-Kendall test is used to determine the trends of ordered pairs in the data. This test allows for the comparison of ordered data to detect increasing or decreasing trends. Sen's slope analysis method, on the other hand, is used to calculate the slopes in the data. This method is used to determine the rate and direction of change in the data. These methods are used in various fields. For example, Mann-Kendall and Sen's slope analysis methods are used for trend analysis of meteorological parameters (Agbo vd., 2021).

Mann-Kendall and Sen's slope analysis methods have been widely used to analyze temperature trends in various studies. For example, Navatha et al. applied these methods to investigate the trends in temperature in the Jagtial district of Telangana state. The Mann-Kendall test and Sen's slope estimate test were used to identify the existing trend direction and magnitude of change over time. The results of the study showed that the annual average maximum temperature in Jagtial exhibited an increasing trend (Navatha vd., 2021). Similarly, Kasimu et al. conducted a study on the spatial and temporal variation of land surface temperature in an urban agglomeration in Northwest China. Although the study did not explicitly mention the use of Mann-Kendall and Sen's slope analysis methods, it focused on the analysis of temperature trends over time. The study highlighted the spatially heterogeneous response of land surface temperature in the region (Zhang vd., 2022).

These references demonstrate the application of Mann-Kendall and Sen's slope analysis methods in analyzing temperature trends and provide valuable insights into the direction and magnitude of temperature changes over time. In this analysis, the MK test was employed to detect noteworthy trends in precipitation parameters throughout the research period. This test functions by comparing the rankings of data points within the time series to determine the presence of a systematic upward or downward trend. The significance of the trend is assessed using the calculated test statistic and its corresponding p-value. The mathematical formula for the MK test is as follows:

$$S = \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} sgn(x_j - x_i) \quad (4)$$

Here, 'n' represents the total count of data points, 'xi' and 'xj' denote data values in time series 'i' and 'j' (where 'j' > 'i'), respectively. Additionally, 'sgn(xj−xi)' refers to the sign function defined as follows:

$$sgn(x_j - x_i) \begin{cases} +1, \text{if } x_j - x_i > 0 \\ 0, \text{if } x_j - x_i = 0 \\ -1, \text{if } x_j - x_i < 0 \end{cases} \quad (5)$$

The MK test is applicable to time series of elements 'xi' taken from 'i=1,2,⋯,n−1,' and elements 'xj' taken from classes 'j=i+1,2,⋯,n' in such a manner that...

The variance is calculated as:

$$Var(S) = \frac{n(n-1)(2n+5) - \sum_{i=1}^{m} t_i(t_i-1)(2t_i+5)}{18} \quad (6)$$

Here, 'n' represents the total count of data points, 'm' represents the number of connected groups, and 'ti' indicates the number of connections within group 'i.' For situations where the

sample size exceeds 10 (n > 10), the standard normal test statistic is computed using the ZS Equation (7):

$$Z_S \begin{cases} \frac{S-1}{\sqrt{Var(S)}}, \text{if } S > 0 \\ 0, \text{ if } S = 0 \\ \frac{S-1}{\sqrt{Var(S)}}, \text{if } S < 0 \end{cases} \quad (7)$$

A positive value of ZS indicates an increasing trend, while a negative Z_S value suggests a decreasing trend. To assess these trends, a specific level of significance denoted as α is employed. The p-value (probability) is utilized to gauge the statistical significance and the strength of evidence for any differences (Dawson & Trapp, 2004). The MK analysis examines 'k' years of temperature data for a specific location to determine whether trends exist across these years.

The analysis was indeed conducted at the significance level of α=0.05, implying that trends with p-values less than 0.05 were considered statistically significant ($|Z_S|>1.96$). Therefore, this indicates the presence of a significant trend in the precipitation data over the years.

Sen's Slope Estimator Test

While the MK test effectively detects linear trends, it may not capture non-linear trends that could exist in the precipitation data. To overcome this limitation, the SS Estimator test was utilized in this study. The SS test offers a robust and flexible approach for estimating the magnitude and direction of trends, even when non-linear trends are present. By combining the SS test with other statistical methods such as the MK trend test, a comprehensive approach to trend analysis in climate data is achieved (Dwevedi vd., 2022; Jiqin vd., 2023; Toma vd., 2022). These tests assist researchers in identifying and quantifying long-term trends, detecting changes in trends over time, and assessing the significance of these trends. The SS Estimator calculates the median of all possible slopes between data points, providing a resistant estimator that is less affected by outliers (Sen, 1968).

The equation for SS concerning a set of N data sample pairs can be expressed as follows:

$$Q_i = \frac{X_j - X_k}{j-k} \quad (8)$$

When considering 'Xj' and 'Xk' as the data values at times 'j' and 'k' (where 'j' > 'k'), if there is only one data point per time period, the total number of data sample pairs 'N' can be calculated using the formula N = n(n − 1)/2, where 'n' represents the number of time periods. However, if there are multiple observations in one or more time periods, 'N' will be less than n(n − 1)/2. The values of Qi are then arranged in ascending order, and subsequently, the average of the 'n' values or the slope of the SS estimator is determined as follows:

$$Q_{med} = \begin{cases} Q_{[(n+1)/2}, \text{ if n is odd} \\ \frac{Q_{\left[\frac{n}{2}\right]} + Q_{\left[\frac{n+2}{2}\right]}}{2}, \text{if n is even} \end{cases} \quad (9)$$

The symbol Qmed represents the data trend, and its value reflects the magnitude of that trend. To determine whether the median slope significantly deviates from zero, it's necessary to calculate a confidence interval for Qmed with a predetermined probability. The confidence interval for the time slope can be determined using the following method (Gilbert, 1987):

$$C_\alpha = Z_{1-\alpha/2}\sqrt{Var(S)} \quad (10)$$

In this context, Var(S) is defined as specified in Equation (3), and Z1-α/2 is obtained from the standard normal distribution table. Next, we compute two values: M1=(N-Cα)/2 and M2=(N+Cα)/2, where N represents the total number of slope estimates Qi. To determine the lower and upper limits of the confidence interval, denoted as Qmin and Qmax, respectively, we

identify the M1-th largest and (M2+1)-th largest slope estimates among the N ordered slope estimates Qi. In cases where M1 is not an integer, we interpolate to determine the lower limit Qmin accordingly. Similarly, if M2 is not an integer, interpolation is used to find the upper limit Qmax. This rigorous process ensures the derivation of a reliable confidence interval for the time slope estimate.

RESULTS AND DISCUSSION

Analysis of Annual Temperature

For the provinces of Tekirdağ, Edirne, and Kırklareli located in the Thrace region, the annual average air temperature, temperature at 2 meters above ground, and minimum and maximum temperatures are presented in Figure 3. According to this figure, the highest average air temperature in the region is in Tekirdağ (14.899°C), while the lowest value is in Kırklareli (13.365°C). When the values measured at 2 meters above ground level are averaged, the highest value is in Tekirdağ (14.320°C), and the lowest value is again in Kırklareli (13.072°C).

The year with the highest average temperature in the region is observed to be 2019. In this year, the air temperature was measured as 16.11°C in Tekirdağ, 15.18°C in Edirne, and 14.76°C in Kırklareli. For temperatures at 2 meters above ground level in 2019, it was recorded as 15.57°C in Tekirdağ, 14.80°C in Edirne, and 14.48°C in Kırklareli.

When examining the maximum temperatures measured in the Thrace region between 1982 and 2021, it is observed that the highest maximum temperatures in Tekirdağ (40.88°C) and Edirne (45.12°C) were recorded in the year 2000, while in Kırklareli (44.04°C), it was in 2007. On the other hand, for minimum temperatures, the lowest minimum temperatures in Tekirdağ (-13.03°C) and Kırklareli (-19.85°C) were reported in 1985, while in Edirne (-23.42°C), it was in the year 2010.

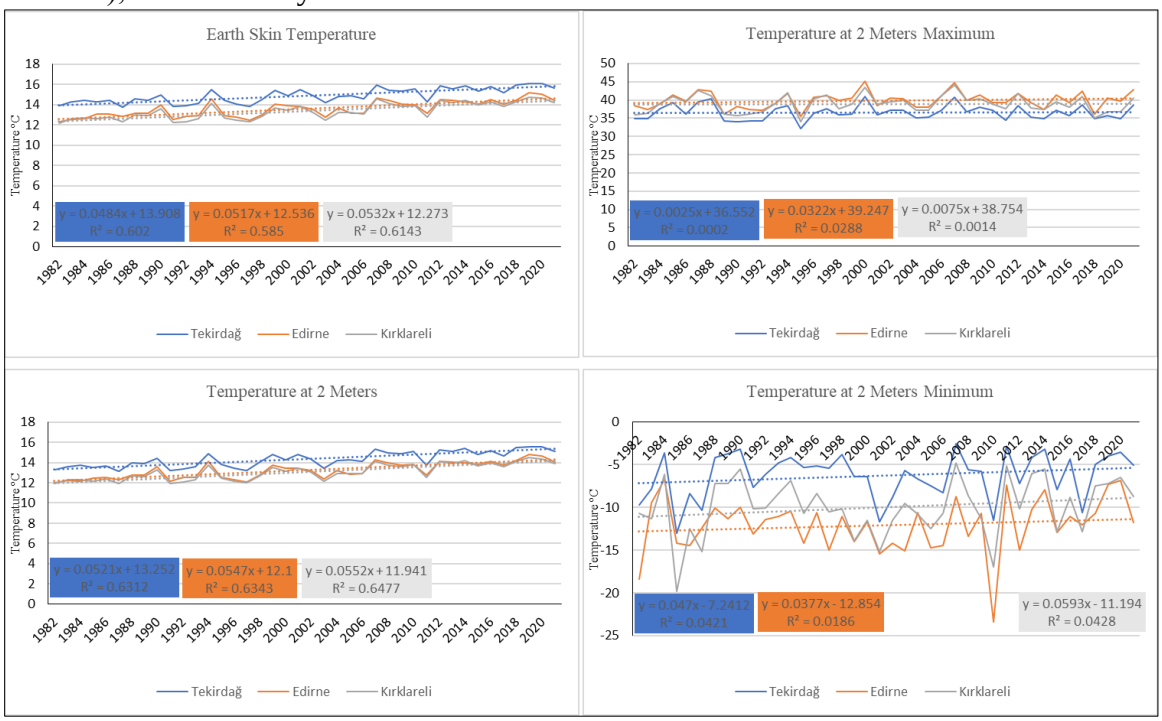

Figure 3. The annual mean, minimum, and maximum temperature values for the provinces by year

The results of the Innovative Trend Method applied to temperature data are presented in Figures 4, 5, 6, 7 and Table 2, respectively.

Applying the Innovative trend analysis method to the annual average temperature data for the years 1982-2021 in the regions of Tekirdağ, Edirne, and Kırklareli, an increasing trend

is observed for low, medium, and high values. Based on these findings, it can be concluded that the annual average temperatures have increased for low, medium, and high levels (Figure 4).

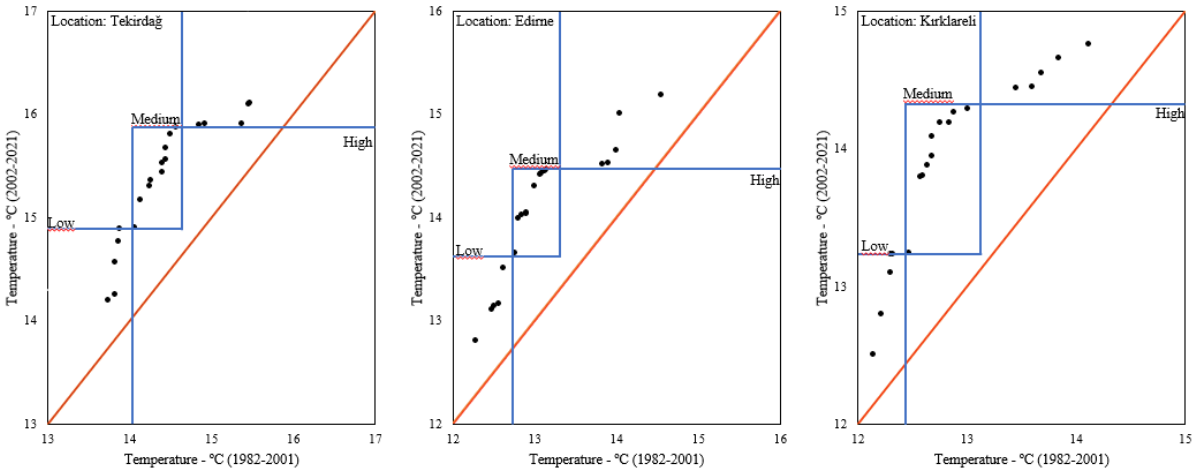

Figure 4. Earth's mean temperature

A trend analysis of the annual average temperature data at a height of 2 meters above the Earth's surface for Tekirdağ, Edirne, and Kırklareli during the same period is presented in Figure 5. According to these data, an increasing trend is observed for low, medium, and high values of annual temperature averages using the Innovative Trend analysis method. Consequently, it can be inferred that the values of annual average temperatures have increased for low, medium, and high categories. These results show similarities with the average temperature data of the Earth's surface, while also indicating a faster trend in the 2-meter height temperature data compared to the surface temperature, especially for Tekirdağ.

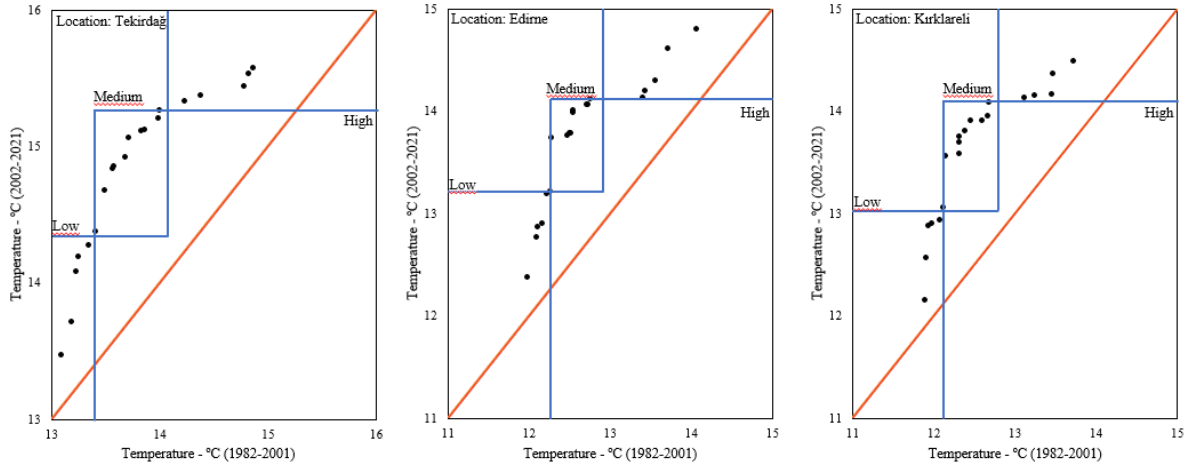

Figure 5. Mean temperature at 2 meters

The trend analysis of maximum temperatures at 2 meters for Tekirdağ, Edirne, and Kırklareli during the same period is presented in Figure 6. According to these data, maximum temperatures over the years have been classified as low, medium, and high values using the Innovative Trend Analysis method. For Tekirdağ, the results indicate an increasing trend in low temperature values, a pattern of initial increase followed by a decrease in medium values, and a decreasing trend in high values. In the case of Edirne, the analysis of maximum temperatures shows an increasing trend in low temperature values, an initial increase followed by a period of no trend in medium values, and then a resurgence in the increasing trend. High values, on the other hand, show no clear trend. In the analysis of maximum temperatures for Kırklareli, there

is an increasing trend in low temperature values, an initial increase followed by a sudden decrease in medium values, and then a return to an increasing trend, followed by a decrease. High values initially exhibit a decreasing trend, but the highest values show an increase.

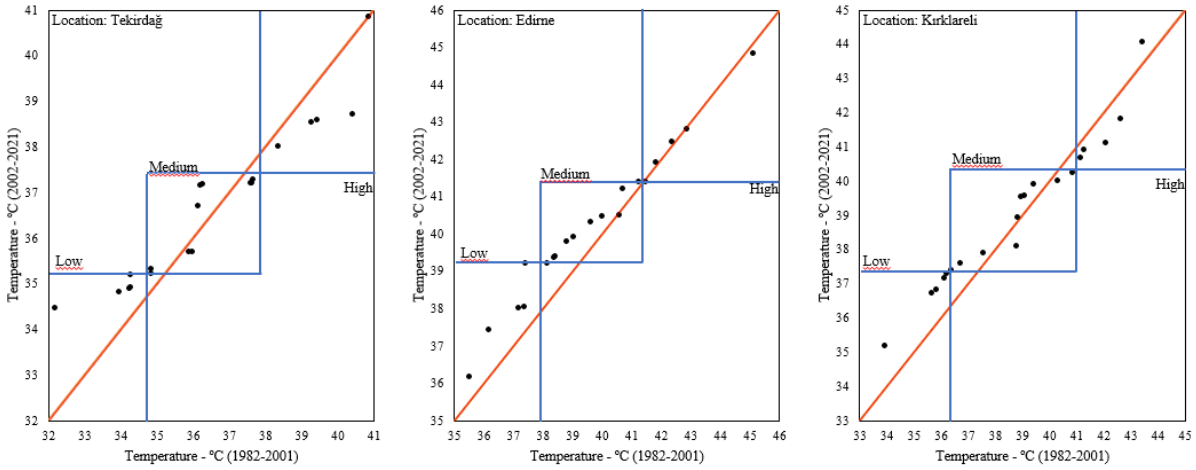

Figure 6. Maximum temperature at 2 meters

The trend analysis of minimum temperatures at 2 meters for Tekirdağ, Edirne, and Kırklareli during the same period is presented in Figure 7. For Tekirdağ, there is an increasing trend in low temperature values, an initial increase followed by a period of no trend in medium values, and then a resurgence in the increasing trend for high values. In the case of Edirne, the analysis of minimum temperatures shows an initial decrease followed by a period of no trend in low temperature values, generally an increasing trend in medium values, and initially an increase followed by a period of no trend in high values. For Kırklareli, the analysis of minimum temperatures reveals an increasing trend in low, medium, and high temperature values.

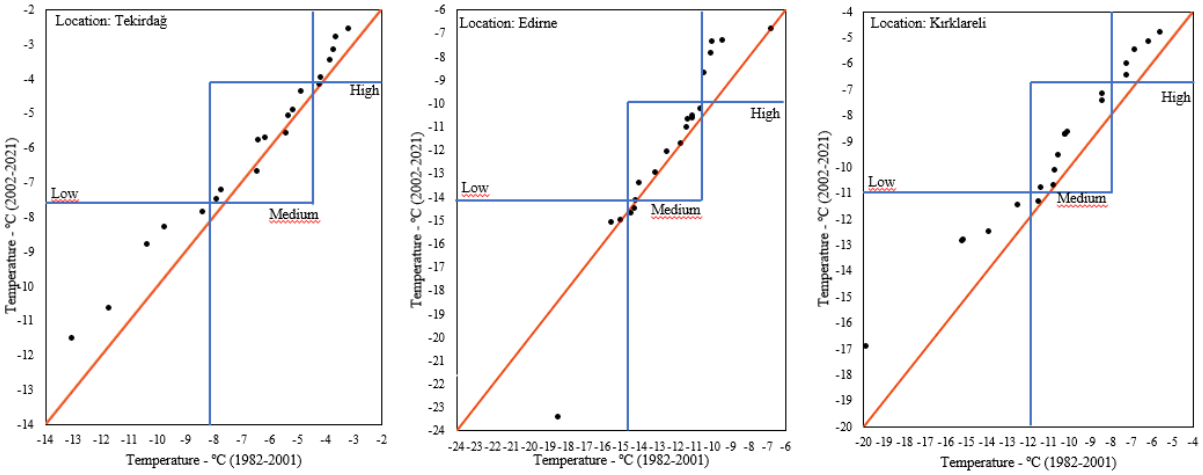

Figure 7. Minimum temperature at 2 meters

When examining the MK and SS analysis results for Tekirdağ, Edirne, and Kırklareli, it has been reported that surface and 2-meter height temperatures show a significant ($p < 0.05$) increasing trend. In contrast, no trend has been observed in maximum and minimum temperatures at 2 meters height ($p > 0.05$). According to Sen's slope analysis, the highest slope values for surface and 2-meter height temperatures are calculated as 0.05418 ^{o}C year^{-1} and 0.05637 ^{o}C year^{-1} in Kırklareli, respectively (Table 2). Additionally, a 0.03634 ^{o}C year^{-1} increase in maximum temperature at 2 meters height has been calculated in Edirne, and a 0.04854 ^{o}C year^{-1} increase in minimum temperature has been calculated in Kırklareli.

Table 2. Comparison of the results of MK and SS analyses with Innovative Trend Analysis

Parameter	Location	Innovative Trend Method					Mann Kendall and Sen's Slope				
		Slope (S)	Upper Cofidence Limit at 95%	Lower Cofidence Limit at 95%	Decision	Direction of Trend	MK (Z)	p	Sen's Slope	Decision	Direction of Trend
Earth Skin Temperature	Tekirdağ	0.04602	0.00525	-0.00525	YES	↗	5.1861	0.000	0.04921	YES	↗
	Edirne	0.04722	0.00585	-0.00585	YES	↗	5.0576	0.000	0.05266	YES	↗
	Kırklareli	0.0505	0.00602	-0.00602	YES	↗	5.2327	0.000	0.05418	YES	↗
Temperature at 2 Meters	Tekirdağ	0.0498	0.0057	-0.0057	YES	↗	5.2899	0.000	0.0531	YES	↗
	Edirne	0.05072	0.00655	-0.00655	YES	↗	5.5004	0.000	0.05537	YES	↗
	Kırklareli	0.05247	0.00659	-0.00659	YES	↗	5.3719	0.000	0.05637	YES	↗
Temperature at 2 Meters Maximum	Tekirdağ	0.00715	0.00914	-0.00914	NO	○	0.2679	0.789	0.00834	NO	○
	Edirne	0.02735	0.00538	-0.00538	YES	↗	0.967	0.333	0.03634	NO	○
	Kırklareli	0.0124	0.00799	-0.00799	YES	↗	0.3146	0.753	0.01445	NO	○
Temperature at 2 Meters Minimum	Tekirdağ	0.02672	0.00613	-0.00613	YES	↗	0.967	0.333	0.04	NO	○
	Edirne	0.01572	0.0147	-0.0147	YES	↗	0.8039	0.421	0.04031	NO	○
	Kırklareli	0.05805	0.00924	-0.00924	YES	↗	0.8622	0.389	0.04854	NO	○

According to Table 2, some interesting results were obtained in the analyzes made in regions such as Edirne, Kırklareli and Tekirdağ based on data from the NASA POWER site between 1982 and 2021. These results show that the average temperature values are increasing, and this increase is confirmed by trend analysis. Innovative Trend Analysis, Mann-Kendall and Sen's Slope analyses show a marked upward trend in the average temperature in these regions.

However, when the same analyses are applied to changes in the maximum and minimum temperature data at a height of 2 meters, different results emerge. In these regions, the Innovative Trend Analysis shows that there is a trend, while the Mann-Kendall and Sen's Slope analyses also reach the conclusion that there is no trend, that is, statistically at maximum and minimum temperature, these two methods of analysis do not find a trend of change that is the same and significant.

These results may indicate that climate change or temperature changes in these regions are affected in different ways at different altitude levels.

CONCLUSIONS

Changes in mean temperature, minimum and maximum temperatures reflect the multifaceted effects of climate change. These impacts can affect natural ecosystems, agriculture, water resources, energy production, and human settlements. Therefore, it is important to understand climate change and adapt to these changes.

As a result, it was concluded that while there is strong data and analysis that average temperatures are increasing in Edirne, Kırklareli and Tekirdağ, this trend does not appear similarly at maximum temperatures at a height of 2 meters. These results suggest that more research needs to be done to examine climate change impacts and understand the differences between regions.

When considering the temperatures between 1982 and 2021, an analysis of the increases was conducted by separating the first 30 years from the last 10 years. It was observed that the highest increase, with 1.245 °C (9.54%) for earth temperatures and 1.264 °C (9.91%) for temperatures at 2 meters above ground level, occurred in Kırklareli.

Furthermore, when the temperature differences between ground-level and temperatures at 2 meters above ground level were compared, it was determined that the highest difference was 0.580 °C in Tekirdağ, while the smallest difference was 0.292 °C in Kırklareli. The widening difference between ground-level temperature and temperature at 2 meters above ground level suggests that Tekirdağ and Edirne have been subjected to more urbanization compared to Kırklareli.

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PHYTOCHEMICAL ANALYSIS AND IDENTIFICATION OF BIOACTIVE COMPOUNDS IN SPINACH LEAVES (*Spinacia oleracea* L.)

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ABSTRACT

Green vegetables contain various phytochemicals in suitable amounts, which are very helpful in preventing and fighting numerous diseases. They also have different types of vitamins and minerals for the effective functioning of the body system. Spinach is a leafy green flowering plant with edible leaves. The bioactive components and phytochemicals, such as flavonoids, polyphenols, carotenoids, and ascorbic acid present in the spinach (*Spinacia oleracea* L.) leaves, are responsible for their nutritional and medicinal properties. The study analyzed the bioactive compounds and phytochemicals present in spinach leaves. Phytochemicals in the spinach, including saponins, steroids, tannins, glycosides, flavonoids, phenols, phlorotannin, and ascorbic acids, were determined and screened according to the standard method using extracts from different solvents like water, ethanol and ethyl acetate. The leaf samples were collected, dried, and ground for extraction. The solvents, water, ethanol and ethyl acetate used for the extracts were incubated for 72 hours, then filtered and centrifuged. The centrifuged extracts were subjected to Gas Chromatography-Mass Spectroscopy (GC-MS) for further analysis of the bioactive compounds present. This analysis was achieved on a GC-MS Shimadzu GC–MSQP2010 Plus system equipped with an RTX-5 M.S. capillary column (0.25 mm x 30 m x 0.25 lm). The result established from the GC-MS analysis of the ethyl acetate extract and the ethanol extract of the spinach leaves was identified from 25 compounds in the chromatogram of each extract. The active compounds' names, compound structures, and molecular weights in the ethyl acetate and ethanol extracts were identified. This GC-MS spectrum proved the similarity percentage of these components as compared to the Wiley online library (WILEY8.LIB), which was the library source. This phytochemical composition's medicinal and nutritional value makes the plant highly essential for good health. However, these plant phytoconstituents have not been lost due to cooking. As a result of these positive effects seen in spinach, it is a green vegetable consumed everywhere in the world.

Keywords: Bioactive compounds, Gas Chromatography-Mass Spectroscopy, Phytochemicals, Spinach

INTRODUCTION

Agriculture is a major sustainable aspect that has immensely contributed to TRNC in terms of production, and commercial revenue. Cyprus is a semiarid island with insufficient rainfall. Out of about 329,890 hectares in northern Cyprus, 56% that is equivalent to 187,068 hectares is adequate for agricultural practices. The climatic conditions and to some extent shortage of water have been the major constraint. However, irrigation of lands is supposedly done for the growing of fruits and vegetables of different varieties. From 1985 to 2001, the land for Agricultural purpose has decreased by 6% and this was as a result of the urban development

that has occurred over time (Agricultural Statistics, 2001). The farmers who grow fruits and vegetables have extensively adopted the consistent use of the modern irrigation systems with the most appropriate method of irrigation (Metochis and Eliades, 2002).

In Africa, western region especially and using Nigeria as a case study, Agriculture is still and will continue to stand as the most important sector that boost the economy of Nigerian. Not less than 65% of Nigerian population in general is evaluated to survive on agricultural products for adequate living, while 35% of the Gross Domestic Product are accompanied with agricultural contribution through the sector of modern agriculture. (FAO, 2006). Agriculture right from time immemorial has pose relevance in the development of Nigeria especially in the areas of economy and commerce. The merits of this has assisted in the availability of food for the vast population, employment for more than half of the entire population with the conversion of raw material for the earnings foreign exchange in the fast growing sector of industry (Adeboye, 1996).

Spinach **(***Spinacia oleracea L.*) is a leafy and at the same time highly nutritious vegetable, which is adequately rich in nutrients and phytochemicals. Vitamins A (from β-carotene), C and K are the micronutrients that are present in spinach, with common minerals like calcium, iron and potassium. The phytochemicals present includes carotenoids, flavonoids, steroids, phlobatannins, saponins and phenolic compounds just to mention but a few of them (Bergquist, 2006). Various researchers have worked on identifying the bioactive compounds that are present not just in vegetables but also in fruits when considering the pre-harvest and postharvest factors.

One of the main reasons why the consumption of spinach is annually increasing is because people are now being conscious and more sensitive with their health than before and spinach which has always remains as one of the healthy vegetables rich in most of these essential nutrients needed for a healthy living. The vegetable is easy to grow with a short life span, which makes it possible for the vegetable to be grown all through the year.

The top five countries in the world that grow spinach include China, which is leading with the production of 27,540,167 tons per year. This is followed by the United States of America with a total production of 435,721 tons annually and followed by Turkey with a production of 229,793 tons. While Japan is the fourth with a production of 226,865 tons and Kenya the fifth and leading in Africa with an annual production of 178,707 tons annually. Nevertheless, Cyprus id quite rich in wild spinach.

Thus, the intention of this research is to investigate the different bioactive compounds and identify the phytochemicals present in the spinach leaves which could have a great impact on our nutrition and health benefits.

MATERIAL AND METHOD

The Bloomsdale variety leaves belong to the savoy species, which is the most popularly grown in Cyprus usually 30cm long and 15cm broad on the average, was selected and collected with plastic zip lock bags from a vegetable farm in northern Cyprus and taken to the laboratory. The spinach leaves were washed four times with water from the tap; this is to remove any debris from the leaves. This is then rinsed with distilled water and leave to be well dried to be suitable for extraction (Olasupo et al., 2018, Tiwari et al., 2005).

The qualitative studies of the bioactive compounds from plant materials depends mostly on the selection of the proper extraction method (Smith, 2003, Sasidharan et al., 2011). This extraction is the initial step taken in any medicinal or nutritional study of plants because it has significance on the result of the research. Sample preparation techniques are also known as the extraction methods.

The collected samples that are already well dried through the use of oven for 24 hours at 100 degree celcius were grinded to powder form. 5 grams of this powdered sample were then

soaked in centrifuge bottles with 50 ml of water, ethanol, and ethyl acetate separately. The whole mixture was then incubated at 4°C for 72 hours. Immediately after this incubation period, the mixtures were carefully filtered with the use of the filter paper and centrifuged at 6,000 rpm at 4°C for a period of 60 minutes. The extracts were then concentrated to dryness in a rotary evaporator (IKA-RV 10 Control) at and were stored at 4°C for further use (Romanik et al., 2007, Moldoveanus and David 2015, Roshanak et al., 2016).

Phytochemical analysis of the test samples were carried out according to standard methods (Trease and Evans, 1989; Harborne, 1998) on test for;

1. Saponin

5ml of each of the plant extracts were added to 20ml of distilled water in a test tube. They were vigorously shaked and then mixed with 3 drops of olive oil. A stable persistent froth forms an emulsion. (Ejikeme et al., 2014).

2. Taninns

1 ml of each extract were boiled in 20 ml of water in a test and then filtered. A few drops of 0.1% ferric chloride were added and once green or a blue-black coloration is observed, it confirms the presence of tannin (Alhakmani et al., 2013).

3. Phenol

5 ml of the extract will be pipetted into a 30 ml test tube, then 10 ml of distilled water will be added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol will also be added and left to react for 30 min. Development of bluish green colour is a positive presence of phenol. (Alhakmani et al., 2013).

4. Flavonoids

3 ml of 1% Aluminium chloride solution was carefully added to 5 ml of each extract. A persistent yellow coloration appeared, indicating the presence of flavonoids. 5 ml of dilute ammonia solution was further added to the above mixture followed by the addition of concentrated H2 SO4. The yellow coloration disappeared. The yellow coloration which disappeared indicates a positive test for flavonoids (Chang et al., 2002),

5. Glycosides

5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution and underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides which confirms a positive presence of cardenolides. A violet-green ring appearing below the brown ring, in the acetic acid layer, indicates that glycoside is present (Rangari 2002, Roshanak et al., 2016).

6. Steroids,

2 ml of acetic anhydride were added to 2 ml extract of each sample followed by careful addition of 2 ml H2 SO4. The color will change from violet to blue or green which confirms the presence of steroids (Rangari 2002).

7. Terpenoids (salkowski test),

5 ml of each extract were mixed with 2 ml of chloroform, and followed by a 3 ml concentrated H2 SO4 carefully added to form a layer. A reddish brown coloration of the interface was formed showing the positive presence of terpenoids (Rangari 2002).

8. Phlobatannins

2 ml of extract of each plant samples was boiled with 1% aqueous hydrochloric acid and a red precipitate showed the presence of phlobatannins (Rangari 2002, Roshanak et al., 2016).

RESULTS AND DISCUSSION

The results of the phytochemical test carried out on each of the extracts were recorded as shown in Table 1. This preliminary screening on each of the extracts show significant quantity of the phytochemical constituents, revealing the presence of saponins, flavonoids, terpenes, phenols, tannins, alkaloids, phlobatanin, glycoside and steroids. However, the phytochemical constituents in the various part of the spinach plant vary as well (Uraku 2016, Altemimi et al., 2017).

This present investigation revealed that the aqueous extract of spinach shows abundant presence of terpenes, and flavonoids, with moderate presence of saponins and less presence of phlobatanin and cardenolides but steroids, glycosides, phenol, glycosides and tannins are absent in the aqueous extract.

For the ethanol extract, tannins and glycosides are moderately present, while steroids and phenol are less present but saponins, flavonoids, terpenes, cardenolides and phlobatanin are absent.

The investigation also revealed much abundant presence of saponins and cardenolides in ethyl acetate extract, with a moderate presence of phenol and less presence of tannins and flavonoids, but showed absence of phlobatanin, terpenes, steroids, glycosides.

Phytochemicals are non-nutritive plant chemicals that have disease preventive properties (Kumar et al., 2009). Different phytochemicals have been found to possess a wide range of activities. The phytochemicals are known to have antimicrobial activity (Ebana, 1995).

Different studies have proved that spinach has a high nutritional value (Maeda et al., 2005). Many researchers also reported that, glycosides play very crucial role in reducing blood pressure. They could also be used in treating heart failure (Nyarko AA & Addy M E 1990).

The spinach leaf extract with their phytoconstituents are reportedly known for anti-inflammatory, antidiarrheal, antimicrobial, antioxidant and insecticidal activities (Chouhan HA & Singh SK, 2011). Steroids are very much important in pharmacy because of their relationship with compounds like sex hormones and can be used for drug production (Okwu DE 2007).

Tannin and flavonoid are thought to be responsible for antidiarrheal activity (Enzo, 2007). Usman and Osuji reported that tannin has been widely used topically to sprains, bruises and superficial wounds as such. Similarly, Elmarie and Johan reported tannin to have antibacterial. Phytochemicals such as terpenoid, flavonoid, tannin, steroid, and alkaloid have antiinflammatory effects (Liu, 2003) Flavanoids show anti allergic, anti-inflammatory, anti-microbialand anti-cancer activity (Yamato and Gayor, 2002).

The result established from the GC-MS analysis that was carried out from the ethyl acetate extract and the ethanol extract of the spinach leaves were identified from 25 compounds from the chromatogram on each of the extract, and this is well summarized in Table 2 and Table 3 respectively. The active compounds name, compound structures and molecular weight in the ethyl acetate and ethanol extract were identified. This GC-MS spectrum confirmed the similarity percentage of these various components when compared to the Wiley online library (WILEY8.LIB) which was the library source.

Table 1. Phytochemical screening of spinach leaf in different solvent

Compounds	Water	Ethanol	Ethyl acetate
Cardenolides	+	-	+++
Flavonoids	+++	-	+

Glycosides	-	++	-
Phenol	-	+	++
Phlobatamin	+	-	-
Saponins	++	-	+++
Steroids	-	+	-
Tannins	-	++	+
Terpenes	+++	-	-

+++ (Abundant); ++ (Moderate); + (Less); - (Absent)

Table 2. GC-MS analysis of the Ethyl acetate extract of spinach leaf

Similarity Percentage	Name of Compound	Molecular Formular	Molecular Weight
1	1,2,2-Trimethylcyclopropylamine	$C_6H_{13}N$	99
2	Ethyl 1-Hexyl-4-Hydroxy-2(1h)-Oxo-3-Quinolinecarboxylate, 4-Hydroxy-3-(2-Oxo-2h-1-Oxa-3-Phenanthryl)-2(1h)-Quinolinone	$C_{22}H_{13}NO_4$	355
3	8,9,9,10,10,11-Hexafluoro-4,4-Dimethyl-3,5-Dioxatetracyclo[5.4.1.0(2,	$C_{12}H_{12}F_6O_2$	302
4	Didecyl1,4-Dihydro-2,6-Dimethyl-3,5-Pyridinedicarboxylate,3,4,5,6,9,10-Hexahydro-9-(4-Hydroxyphenyl)-3,3,6,6-Tetramethylacridine-	$C_{23}H_{27}NO_3$	365
5	2,2-dimethyl-4-(2-propyl)aminobutanone 2-Butanone,3,3-dimethyl-4-[(1-methylethyl)amino]- (CAS)	$C_9H_{19}NO$	157
6	N-(P-Anisidinomethyl)-4-Methylphthalimide 4-Bromo-N-[(6-Methyl-2-Pyridyl)Aminomethyl]Phthalimide	$C_{15}H_{12}BRN_3O_2$	345
7	2-isopropylthio-5-trifluoracetyl-1,3-oxathiolyium-4-olat. 1,3-Oxathiol-1-ium, 4-hydroxy-2-[(1-methylethyl)thio]-5-(trifluoroacetyl)-, hydroxide, inner salt (CAS)	$C_8H_7F_3O_3S_2$	272
8	3,3-Dimethyl-2-Phenyl-2-(1-Oxo-1,2,3,4-Tetrahydronaphthalen-2-Yl)Azirane,1(2h)-Naphthalenone, 2-(3,3-Dimethyl-2-Phenyl-2-Aziridinyl)-3,4-Dihydro- (CAS)	$C_{20}H_{21}NO$	291
9	5-Methoxy-1-Aza-6 Oxabicyclo(3.1.0)Hexane,6-Oxa-1-Azabicyclo[3.1.0]Hexane, 5-Methoxy- (Cas)	$C_5H_9N\ O_2$	115
10	1,2,5-Oxadiazole Furazan (CAS), Azoxazole, 1-Oxa-2,5-diazacyclopentadiene	$C_2H_2N_2O$	70
11	Borane, compd. with carbon monoxide (1:1) (CAS) Borane carbonyl,Borane, carbonyl-	CH_3BO	42

	Carbon monoxide-borane,Borine carbonyl (BH3CO), Borane-carbon monoxide (1:1) Boron, carbonyltrihydro-, (T-4)- BH3CO		
12	Borane, triethyl- (CAS) Triethylborane Triethylboron (C2H5)3B, Triethylborine	$C_6H_{15}10B$	98
13	[10b]-Triethylborane	$C_6H_{15}10B$	98
14	pnz-sar,Glycine,N-[[(4 methoxyphenyl)methoxy]carbonyl]-N-methyl-(CAS)	$C_{12}H_{15}NO_5$	253
15	[1R*,2R*]-1-acetyl-1,2-dihydroxycyclohex-3-ene	$C_8H_{12}O_3$	156
16	4-(Mesyloxy)-3,3-dimethyl-2-butanone	$C_7H_{14}O_4S$	194
17	1,1'-bibicyclo(2.2.2)octyl-4-carboxylic acid [1,1'-Bibicyclo[2.2.2]octane]-4-carboxylic acid (CAS)	$C_{17}H_{26}O_2$	262
18	2-ethylsulfenyl-3,4-dimethoxycarbonyl-5-trifuoroacetyl-furane3,4-Furandicarboxylic acid,2-(ethylthio)-5-(trifluoroacetyl)-, dimethyl ester (CAS)	$C_{12}H_{11}F_3O_6S$	340
19	3-Butyn-1-ol (CAS) 3-Butynol 1-Butyn-4-ol 3-Butyne-1-ol 3-Butynyl alcohol 4-Hydroxy-1-butyne 2-Hydroxyethylacetylene HC..CCH2CH2OH (2-Hydroxyethyl)acetylene 1-Hydroxy-3-butyne	C_4H_6O	70
20	3-chloromethylfuran,Furan,3-(chloromethyl)-(CAS)3-(Chloromethyl)furan,3-Furylmethyl chloride	C_5H_5CLO	116
21	2-[3'-(1''-Hydroxy-1''-methylethyl)-2',2'-dimethylcyclobutyl] ethanal	$C_{11}H_{20}O_2$	184
22	7-hydroxy-5,6,7,8-tetrahydroindoliziaine 7-Indolizinol, 5,6,7,8-tetrahydro- (CAS)	$C_8H_{11}NO$	137
23	1,2,5-Oxadiazole,Furazan(CAS), Azoxazole,1-Oxa-2,5diazacyclopentadiene	$C_2H_2N_2O$	70
24	3-Butyn-1-ol (CAS) 3-Butynol,1-Butyn-4-ol,3-Butyne-1-ol,3-Butynylalcohol,4-Hydroxy-1-butyne2Hydroxyethylacetylene HC.CCH2CH2OH(2Hydroxyethyl)acetylene1-Hydroxy-3-butyne	C_4H_6O	70
25	3-Butyn-1-ol (CAS) 3-Butynol 1-Butyn-4-ol $$ 3-Butyne-1-ol 3-Butynyl alcohol $$ 4-Hydroxy-1-butyne2Hydroxyethylacetylene HC.$.CCH2CH2OH(2Hydroxyethyl)acetylene $$ 1-Hydroxy-3-butyne	C_4H_6O	70

Table 3. GC-MS analysis of the Ethanol extract of spinach leaf

Similarity Percentage	Name of Compound	Molecular Formular	Molecular Weight

1	8,9,9,10,10,11-Hexafluoro-4,4-Dimethyl-3,5-Dioxatetracyclo[5.4.1.0(2,	$C_{12}H_{12}F_6O_2$	302
2	Didecyl1,4-Dihydro-2,6-Dimethyl-3,5-Pyridinedicarboxylate,3,4,5,6,9,10-Hexahydro-9-(4-Hydroxyphenyl)-3,3,6,6-Tetramethylacridine-	$C_{23}H_{27}NO_3$	365
3	Trimer From Isobutyroyl Pyrazine	$C_{24}H_{25}CLN_6O_3$	480
4	N-(P-Anisidinomethyl)-4-Methylphthalimide,4-Bromo-N-[(6-Methyl-2-Pyridyl)Aminomethyl]Phthalimide	$C_{15}H_{12}BRN_3O_2$	345
5	Ethyl 1-Hexyl-4-Hydroxy-2(1h)-Oxo-3-Quinolinecarboxylate,4-Hydroxy-3-(2-Oxo-2h-1-Oxa-3-Phenanthryl)-2(1h)-Quinolinone	$C_{22}H_{13}NO_4$	355
6	Tetradecane,2,6,10-trimethyl-(CAS),2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	240
7	Cyclohexane,1,1'-[1-(2,2-Dimethylbutyl)-1,3-Propanediyl]Bis-(Cas) Heptan, 1,3-Dicyclohexyl-5,5-Dimethyl-	$C_{21}H_{40}$	292
8	1,2,2-Trimethylcyclopropylamine	$C_6H_{13}N$	99
9	Piperidine, 1-nitro- (CAS) N-Nitropiperidine 1-Nitropiperidine	$C_5H_{10}N_2O_2$	130
10	2,4(1H,3H)-Pyrimidinedione, 5-nitro-,5-Nitrouracil,2,4-Dihydroxy-5-nitropyrimidine Uracil, 5-nitro-	$C_4H_3N_3O_4$	157
11	1-(3-Fluorobenzyl)-2(1h)-Imino-3-Methylpyridine Hydrobromide 1,4-Dihydro-4-Imino-1-(4-Phenylbenzoylmethyl)Pyridine Hydrobromide,2-(4-Phenylbenzoylmethylthio)Benzoxazole Hydrobromide 2(3h)-Imino-3-	$C_{19}H_{17}BRN_2O$	368
12	2-Tetradecanol (CAS) sec-Tetradecyl alcohol Tetradecanol-2	$C_{14}H_{30}O$	214
13	2-isopropylthio-5-trifluoracetyl-1,3-oxathiolyium-4-olat. 1,3-Oxathiol-1-ium, 4-hydroxy-2-[(1-methylethyl)thio]-5-(trifluoroacetyl)-, hydroxide, inner salt (CAS)	$C_8H_7F_3O_3S_2$	272
14	1h-Imidazole, 1-(1-Oxooctadecyl)-	$C_{21}H_{38}N_2O$	334
15	2-Hexadecanol (CAS) Hexadecanol-2	$C_{16}H_{34}O$	242
16	Octadecane, 6-Methyl- (Cas) 6-Methyl Octadecane	$C_{19}H_{40}$	268
17	9,12-Octadecadienoic Acid (Z,Z)-, 2,3-Bis(Acetyloxy)Propyl Ester (Cas) 1-Linoleyl-2,3-Diacetin	$C_{25}H_{42}O_6$	438

18	2-(4,5-Dihydro-3-Methyl-5-Oxo-1-Phenyl-4-Pyrazolyl)-5-Nitrobenzoic Acid	$C_{17}H_{13}N_5O_5$	367
19	Nonadecane (CAS) n-Nonadecane	$C_{19}H_{40}$	268
20	Thiophene, 3-methyl-2-pentadecyl-	$C_{20}H_{36}S$	308
21	2-Isononenal (Cas) Branched Chain 2-Nonenal	$C_9H_{16}O$	140
22	Tridecane, 6-methyl- (CAS) 6-Methyltridecane	$C_{14}H_{30}$	198
23	Piperidine, 1-nitro- (CAS) N-Nitropiperidine $$ 1-Nitropiperidine	$C_5H_{10}N_2O_2$	130
24	Octadecane, 3-ethyl-5-(2-ethylbutyl)- (CAS) 3-Ethyl-5-(2'-ethylbutyl)octadecane	$C_{26}H_{54}$	366
25	3-Tert-Butyl-5-Chloro-2-Hydroxybenzophenone	$C_{17}H_{17}CLO_2$	288

CONCLUSIONS

For this present research, the commonly consumed spinach variety in north Cyprus, the Bloomsdale variety leaves which belongs to the savoy species contains adequate quantity of phytochemicals that are helpful in the prevention deadly diseases to mankind.

The GC-MS analysis of these phytochemicals, and the studies of the bio active compounds, indicate positive results for ethyl acetate, ethanol and aqueous extract of the spinach leaves. The phytochemical screening also shows the presence of saponins, terpenoids, phlobatannins, flavonoids, taninns, glycosides, steroids and phenols.

This current research has also explained that the spinach is one of the vegetables in north Cyprus that is consumed on daily basis, not only because it is cheap to get, but it's a green vegetable that is very rich in most of the phytochemicals and bio active compounds present in leafy green vegetables. The medicinal and nutritional values which are of these phytochemical composition, makes the plant to be highly essential for good health and also with its properties that can be useful in combating health challenges. However, there have not been losses of these plant phytoconstituents, as a result of cooking, except for vitamins and minerals. As a result of these positive effects seen in spinach, it is a green vegetable that is consumed everywhere in the world. It is best consumed cooked, except for a very few places where little amounts are added to meals like salad and eaten raw.

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EFFECT OF SALINITY STRESS ON BIOCHEMICAL, GROWTH, AND YIELD CHARACTERISTICS OF WHEAT

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ABSTRACT

Wheat is crucial in providing food and nutritional security, but rapidly increasing soil and water salinity severely threatens its production worldwide. Salinity has a direct impact on soil productivity and limits global yield potential. It also deleteriously impacts wheat growth and development, reducing grain production and quality. Wheat plants use a variety of physiological, biochemical, and molecular mechanisms to adapt to salt stress at the cell, tissue, and whole plant levels to optimize growth and yield while mitigating the harmful impacts of the saline environment. An experiment was conducted at the Institute of Graduate Studies and Research Department of Plant Sciences and Technologies, Cyprus International University, to examine the effect of salinity stress. In the present experiment, wheat varieties V1, V2, and V3 were tested under EC control, 7.5 dS m^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} following a completely randomized design (CRD) with factorial arrangement. The findings revealed that wheat growth and yield characteristics decreased dramatically as saline levels increased. Among V1, V2, and V3, the highest reduction in plant height, SFW, SDW, RFW, RDW, number of tillers, spike length, and grain yield were noted in V3 when treated with 12 dS m^{-1}. Furthermore, V1 performed best as compared to all other varieties.

Keywords: Salinity, Wheat, Antioxidants, Growth, Yield

INTRODUCTION

Earth contains different type of salts. Major portion of earth is covered with water and there is approximately 3% NaCl in that water (Breiman & Graur, 1995; Royo & Abió, 2003; Hajihashemi et al., 2009). However, the total area of the earth that becomes salt-affected is not precisely known according to an estimate about 930Mha of the earth is salt affected and distributed throughout the world (Robin et al., 2016).

Soil salinization is a severe environmental issue worldwide, primarily affecting soil and crop productivity in arid and semi-arid areas due to high temperature and low rainfall (de Sa et al., 2021). Over the previous 45 years, the salt-affected soil area has expanded by 77 million ha, becoming a severe hazard, particularly for irrigated agriculture (Munns, 2002). Salt-affected soils cover an area of around 1060.1 Mha in the world and their area is gradually increasing due to the influence of climate change; soil salinity tends to increase with increase in sea level intrusion and temperature, decrease in precipitation and improper irrigation management (Eswar et al., 2021). It is a major challenge to modern agriculture, impeding and impairing crop growth and development, especially in coastal areas and most arid regions of the world (Zaman et al., 2018).

It has been reported that salt stress has affected 20% of agricultural land. However, this problem is enhancing gradually owing to anthropogenic activities as well as different changes within climatic conditions (Arora, 2019). Due to salinity stress according to an estimate 50% reduction in crop production has been recorded (Acquaah, 2007). While, there is need to

increase food supply up to 70% by 2050 in order to fulfill the demands of continuously increasing population (FAO, 2009).

Salinity has a detrimental effect on the chemical, physical, and biological properties of soil because of the high concentration of salts of basic cations in the soil and yield because of osmotic and ionic stress besides inhibit seed germination, structure and functions of the photosynthetic machinery, resulting in less economical agricultural production and rural poverty (Jahan et al., 2020). However, soil parent material, anthropogenic activities, and arid and semiarid climatic conditions contribute to unfavorable soil conditions like low rainfall which limits leaching of salts, causing accumulation of Ca-minerals that strongly changing soil organic matter turnover and nutrient recycling resultantly crop production decrease (Acquaah ,2007; FAO, 2009.; Wiese, 1977). Improving future agricultural management demands that soil salinity should be taken into consideration (Ennaji et al., 2018).

Variation in climatic conditions, increase in temperature and low rainfall are the red signals indicating that there is need to bring salt affected soils under cultivation to fulfil day by day increasing demands of food (Akhiyarova et al., 2005; Akbari et al., 2007). So, it is important to examine the maximum adverse outcome of excessive salts in the crop growth along with isolate salt tolerant varieties for maximum yield (Huang et al., 2010; Turan et al., 2009; Çelik & Atak 2012).

The growth and production of wheat is reducing throughout the world due to several factors such a decrease in agricultural land, environmental variations, extensive application of chemical fertilizer and less application of organic fertilizers (Masuda, 2016). Many sections of the world are vulnerable to stressors such as salt, which has a harmful effect on plant growth and yield production(Hasanuzzaman et al., 2014a; Hasanuzzaman et al., 2014b; Shabala & Munns 2012) . Around 20% of agricultural fields are saline, and because of the significant issue of global warming, increasingly cultivable lands are becoming salty. Because many stressors, including salinity, result in yield decreases of up to 50% (Munns and Tester, 2008; Munns et al., 2019), while it is important to enhance food production up to 70% by 2050 (FAO, 2009).

Salinity effect plant growth and development via various mechanisms, among them two most important are discussed here (1) the harmful impacts of sodium (Na^+) and chloride (Cl^-) ions, and (2) the osmotic capacity influencing plant physiology. Though, plants can tolerate salinity stress (Rahman et al., 2016; Afzal et al., 2005). Based on their ability to tolerate salinity plants can be tolerant, moderately tolerant, and sensitive. Different researchers classified wheat as a tolerant crop. However, wheat can tolerate salinization to some extent, but its production decrease under heigh level of salinity stress (Poustini & Siosemardeh, 2004; James et al., 2006). Plant synthesis energy via photosynthesis, this energy is utilized in maintenance, plant growth, and to survive under several types of abiotic and biotic hassles during growth stages (Miransari & Smith, 2019).

Under salinity stress plants activate various type of signaling pathways. Salinity stress give rise to artificial drought conditions under such conditions water is present in high amount but due to high concentration of soluble salts plants are unable to absorb water. In response to salinity coupled with drought stress production of reactive oxygen species (ROS) increases. These ROS negatively effects plant metabolism and reduces its ability to cope under stress conditions (Miransari & Smith, 2019).

Keeping in view the above-mentioned adverse impact of salinity on crop yield. This trail aims to define the influence of salt stress on three wheat varieties. Our objectives are;

I. To examine the impact of soil salinity on biochemical parameters of wheat under different salinity levels.
II. To determine salinity level at which maximum yield can be obtained under climatic conditions of Nicosia.

MATERIAL AND METHOD

Experiment

A pot experiment was performed at Institute of Graduate Studies and Research department of Plant sciences and technologies, Cyprus International University. The wheat verities were grown in pots containing 20 kg by weight. Soil for the experiment was taken from the farm. The soil was air-dried and sieved through a 2 mm sieve. Before the start of experiment soil properties, such as ECe, pHs, saturation percentage, SAR, texture and NPK was determined following standard methods.

Development of salinity

After preliminary analysis, four different levels of soil salinity i.e. control (C), S1 = 7.5, S2 = 10 and S3 = 12 dSm^{-1} was prepared by using a calculated amount of NaCl, Na_2SO_4, $CaCl_2$, $CaSO_4$ salts and mixing along with the soil in a mixing tub to achieve maximum homogeneity so that the required salinity levels can be establish in soil samples (Muhammed and Ghafoor, 1992).

Pot filling, fertilization, seed sowing and irrigation

Each pot was filled with 10 kg soil, after developing the required salinity levels, and seeds of wheat shall be sown in each pot. As per requirement thinning was carried out 45 days after planting, leaving 4 plants in each pot. The recommended dose of NPK for wheat was be applied before sowing according to planned treatments. Each pot was treated as per the following treatments.

Treatments layout

Four salinity levels and wheat varieties were used to determine effect of salinity stress on wheat growth, and yield parameters. A completely randomized design with factorial arrangements of 12 treatments each with 3 replications was used.

Varieties = V1, V2, V3 (Perre, Cumhuriyet 75, Gori)

Salinity levels = C, S1, S2, S3

Number of pots = V*S*Replications = 3*4*3 = 36

Plant parameters

Following parameters of crop will be measured after harvesting the wheat crop.

Shoot length (cm)

To measure the length of wheat shoot, meter rod will be used. Later, mean of shoot length will be calculated.

Root length (cm)

Plants root length of all samples will be estimated with meter rod. After those values of mean shall be determined.

Shoot fresh weight (g)

Fresh weight of shoot will be measured using electronic weighing balance.

Shoot dry weight (g)

Weight of dry shoot will be measured after putting shoot samples in an oven at 65± 5°C temperature for 24 hours.

Number of tillers

At maturity after randomly selecting one plant per pot total numbers of tillers of that plant were counted and mean value for number of tillers per plant were calculated.

Spike length (cm)

Spike lengths of three randomly chosen plants were measured by using meter rod. For measuring spike length, length from start point of spike to the upper tip of the spike was noted and it’s mean length was calculated.

Grains per spike

After harvesting the spikes were manually threshed and separately counted the number of grains per spike and then average was calculated.

100 grain weight

Grains were counted by using seed counter, weighted on an electrical balance at the time of harvesting from each replication. At the end mean value for 100 grain weight was estimated.

Grain yield

Grains of each pot were manually harvested, and their weight was recorded by weighing balance.

Straw yield

Plants of each pot were harvested manually, tied into bundled and sun dried for a week. By means of digital balance total biomass of sun-dried samples were recorded.

Harvest index

Harvest index % (HI%) can be calculated by the given formula.

$$\text{HI}(\%) = \frac{\text{Grain Yield(g)}}{\text{Straw yield(g)}} \times 100$$

Biochemical parameters

Total chlorophyll content

The chlorophyll content of fully mature wheat leaf will be determined using Chlorophyll meter (SPAD-value) at three different points (Welburn, 1994) and then averaged.

RESULTS AND DISCUSSION

Shoot length (cm)

Salinity stress adversely effects different physiological along with biochemical developments of plant in addition to reduces its growth along with improvement. ANOVA table 4.1.1 shows the impact of soil salinity on shoot length of three varieties of wheat i.e., V1, V2, and V3. ANOVA table showed that the interaction between salinity stress and plant height is highly significant in all wheat varieties. Furthermore, it can be depicted from the Fig 4.1.1 that with increase in salinity considerable reduction in shoot length was observed. However, highest shoot length (115cm) was observed in V1 under controlled conditions. While lowest shoot length i.e., was observed in V3 treated with 12 dS m^{-1} salt stress.

Moreover, the results of this research demonstrated that salinity stress reduced shoot length by 21.7%, 39%, and 52% in V1 treated with SL1, SL2, and SL3 respectively as compared to control. In V2 a reduction in shoot length was also observed. The results showed 25%, 40%, and 63% reduction in shoot length as associated to control. Lastly the outcomes of this study indicated a reduction of 29%, 44.8%, and 65.4% in shoot length in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. According to our findings V1 performed best as linked to V2 and V3. The outcomes of our research demonstrated that with increasing level of salinity plant height was reduced. Our conclusions are also justified by Nazeer et al. (2021) who stated that salinity negatively effects growth of wheat by decrease in tissue water contents. Elevated absorption of salts in soil solution induces osmotic stress. Osmotic influence reduces activity of meristematic cells in shoot axis thereby cell elongation and consequently plant height (Qiong et al., 2016).

Siddiqui et al. (2021); EL Sabagh et al. (2021) also reported the adverse impact of salinity on plant height. Salinity stress increases the concentration of Na and Cl ions that leads to ionic stress which adversely effects biochemical and metabolic processes in older leaves that resultantly causes decrease in leaf expansion and photosynthetic capacity. Leaves are the production houses of plant and because of impaired functioning of leaves the ability of plant to generate energy decreases that ultimately decreases plant height (Fathalla and El-Mageed, 2020). In another research Kalhoro et al. (2016) reported reduction in plant height, spike size, quantity of spikelets $spike^{-1}$, 1000 grain mass, as well as yield. However, salinity level of 10 dS m-1 has more harmful effect as associated to 2.16, 4.0, 6.0, 8.0 dS m^{-1}. The impact of soil

salinity on shoot arid weight, and wheat yield was observed by Elgharably et al. (2010). In this experiment wheat response to EC level 2.2, 6.7, 9.2 and 11.8 dS m^{-1} and four different concentrations of N and P was recorded. The results showed that EC level 11.8 dS m^{-1} considerably decreased the shoot, and grain yield but application of N and P increased the growth and yield related components.

Root length (cm)

Salinity stress adversely effects different biological as well as bio-chemical methods of plant and reduces its growth and development. ANOVA table 4.2.1 shows the impact of soil salinity on root length of three varieties of wheat i.e., V1, V2, and V3. ANOVA table showed that the interaction between salinity stress and plant height is highly significant in all wheat varieties. Furthermore, it can be depicted from the Fig 4.2.1 that with increase in salinity significant an increase in root length was observed. However, highest root length (40 cm) was observed in V1 treated with 12 dS m^{-1} salinity stress. While lowest root length i.e., 19 was observed in V2 under controlled conditions.

In addition, the findings of this study showed that salinity stress increased root length by 29%, 54%, and 62% in V1 treated with SL1, SL2, and SL3 respectively as compared to control. In V2 an increase in root length was also observed. The results showed 29%, 46%, and 61% increase in root length as compared to control. Lastly the results of this study showed an increase of 22%, 60%, and 72% in root length in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. According to our findings V1 performed best versus V2 and V3.

The outcomes of our study revealed that with increasing level of salinity plant height was reduced. Our outcomes are also justified by Nazeer et al. (2021) who described that salinity negatively effects growth of wheat by decrease in tissue water contents. High intensity of salts in soil mixture stimulates osmotic effect. Osmotic influence reduces activity of meristematic cells in shoot axis thereby cell elongation and consequently plant height (Qiong et al., 2016).

Siddiqui et al. (2021); EL Sabagh et al. (2021) also reported the adverse effect of salinity on plant height. Salinity stress increases the concentration of Na and Cl ions that leads to ionic stress which adversely effects biochemical and metabolic processes in older leaves that resultantly causes decrease in leaf expansion and photosynthetic capacity. Leaves are the production houses of plant and because of impaired functioning of leaves the ability of plant to generate energy decreases that ultimately decreases plant height (Fathalla and El-Mageed, 2020). In another research Kalhoro et al. (2016) reported reduction in plant elevation, spike size, quantity of spikelets $spike^{-1}$, 1000 grain mass, along with yield. However, salinity level of 10 dS m-1 has additional unfavorable effect as associated to 2.16, 4.0, 6.0, 8.0 dS m^{-1}. The impact of soil salt on shoot dry weight, root dry weight and wheat yield was observed by Elgharably et al. (2010). In this experiment wheat response to EC level 2.2, 6.7, 9.2 and 11.8 dS m^{-1} and four different concentrations of N and P was recorded. The results showed that EC level 11.8 dS m^{-1} considerably decreased dry weight of shoot and root and also reduced grain yield, but application of N and P increased the yield related components. So, from the previous studies it can be concluded that salinity reduces root size.

Grains per spike

Salinity stress adversely effects different physiological and biochemical processes of plant and reduces their growth and development. ANOVA table 4.9.1 shows the impact of soil salinity on grains per spike of three varieties of wheat i.e., V1, V2, and V3. ANOVA table showed that the interaction between salinity stress and plant height is highly significant in all wheat varieties. Furthermore, it can be depicted from the Fig 4.9.1 that with increase in salinity significant reduction in grains per spike was observed. However, a highest grain per spike (51)

was observed in V1 under controlled conditions. While a lowest grain per spike i.e., 22 was observed in V2 treated with 12 dS m^{-1} salinity stress.

In addition, the findings of this research showed that salinity stress reduced grains per spike by 8%, 37%, and 45% in V1 treated with SL1, SL2, and SL3 respectively as compared to control. In V2 a reduction in grains per spike was also observed. The outcomes showed 10%, 22%, and 55% reduction in grains per spike as associated to control. Lastly the results of this study showed a reduction of 4%, 25%, and 44% in grains per spike in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. According to our findings V1 performed best as assessed to V2 and V3. The findings of our research demonstrated that with increasing level of salinity plant height was reduced. Our outcomes are also justified by Nazeer et al. (2021) who stated that salinity negatively effects growth of wheat by decrease in tissue water contents. High-level absorption of salts in soil mixture causes osmotic stress. Osmotic impact reduces activity of meristematic cells in shoot axis thereby cell elongation and consequently plant height (Qiong et al., 2016).

Siddiqui et al. (2021); EL Sabagh et al. (2021) also stated the adverse effect of soil salinity on plant height. Salinity stress increases the concentration of Na^+ as well as Cl^- ions that leads to ionic stress which adversely effects biochemical and metabolic processes in older leaves that resultantly causes decrease in leaf expansion and photosynthetic capacity. Leaves are the production houses of plant and because of impaired functioning of leaves the ability of plant to generate energy decreases that ultimately decreases plant height (Fathalla and El-Mageed, 2020). In another research Kalhoro et al. (2016) reported reduction in plant elevation, grains per spike , quantity of spikelets $spike^{-1}$, 1000 grain weight, as well as yield. Though, salinity level of 10 dS m^{-1} has additional unfavorable effect as linked to 2.16, 4.0, 6.0, 8.0 dS m^{-1}. The impact of salt on grains per spike, grains per spike and wheat yield was observed by Elgharably et al. (2010). In this experiment wheat response to EC level 2.2, 6.7, 9.2 and 11.8 dS m^{-1} and four different concentrations of N and P was recorded. The results showed that EC level 11.8 dS m^{-1} significantly reduced the shoot, grains per spike, and grain yield but application of N and P increased the yield related components. So, from the previous studies it can be concluded that salinity reduces grains per spike.

Grain Yield (g/pot)

Salinity stress adversely effects different physiological as well as biochemical processes of plant and reduces their growing along with development. ANOVA table 4.10.1 shows the impact of soil salinity on grain yield of three varieties of wheat i.e., V1, V2, and V3. ANOVA table showed that the interaction between salinity stress and plant height is highly significant in all wheat varieties. Furthermore, it can be depicted from the Fig 4.10.1 that with increase in salinity significant reduction in grain yield was observed. However, highest grain yield (38g) was observed in V1 under controlled conditions. While lowest grain yield i.e., 7g was observed in V3 treated with 12 dS m^{-1} salt stress. Additionally, the consequences of this investigation indicated that salinity stress lowered grain yield by 19%, 25%, and 76% in V1 treated with SL1, SL2, and SL3 respectively as linked to control. In V2 a reduction in grain yield was also observed. The findings indicated 2.7%, 31%, and 76% reduction in grain yield as compared to control. Lastly the results of this study showed a reduction of 6%, 35%, and 78% in grain yield in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. According to our findings V1 performed best as linked to V2 and V3.

The outcomes of our research demonstrated that with increasing level of salinity plant height was reduced. Our results are also explained by Nazeer et al. (2021) who described that salinity negatively effects growth of wheat by decrease in tissue water contents. High-level absorption of salts into soil solution stimulates osmotic stress. Osmotic impact reduces activity of meristematic cells in shoot axis thereby cell elongation and consequently plant height (Qiong

et al., 2016). Siddiqui et al. (2021); EL Sabagh et al. (2021) also informed the unfavorable effect of soil salinity on plant height. Salinity stress increases the concentration of Na along with Cl ions that leads to ionic stress which adversely effects biochemical and metabolic processes in older leaves that resultantly causes decrease in leaf expansion and photosynthetic capacity. Leaves are the production houses of plant and because of impaired functioning of leaves the ability of plant to generate energy decreases that decreases plant height (Fathalla and El-Mageed, 2020). In another research Kalhoro et al. (2016) reported reduction in plant height, grain yield, number of spikelets spike^{-1}, 1000 grain weight, and yield. However, salinity level of 10 dS m^{-1} has additional unfavorable effect as linked to 2.16, 4.0, 6.0, 8.0 dS m^{-1}. The effect of salinity on grain yield, grain yield and wheat yield were observed by Elgharably et al. (2010). In this experiment wheat response to EC level 2.2, 6.7, 9.2 and 11.8 dS m^{-1} and four different concentrations of N and P was recorded. The results showed that EC level 11.8 dS m^{-1} significantly reduced the shoot, grain yield, and grain yield but application of N and P increased the yield related components.

In addition, the findings of this experiment showed that salinity stress reduced grain yield by 19%, 24%, and 76%, in V1 treated with SL1, SL2, and SL3 respectively as compared to control. In V2 a reduction in grain yield was also observed. The results showed 3%, 33%, and 77 % reduction in grain yield as associated to control. Lastly the results of this study demonstrated a reduction of 20.5 %, 25 %, and 76.9 % in grain yield in pots treated with 7.5 dSm^{-1}, 10 dS m^{-1}, and 12 dS m^{-1} correspondingly. In accordance with our findings V1 performed best as compared to V2 and V3.

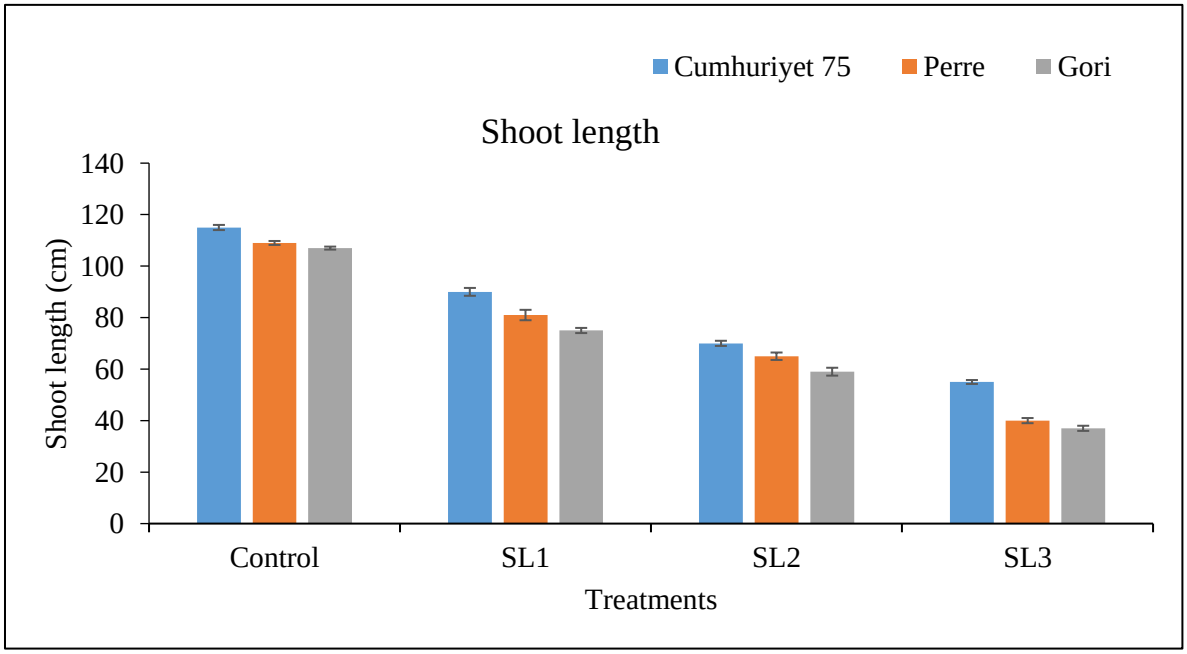

Figure 1. Effect of salinity on shoot length

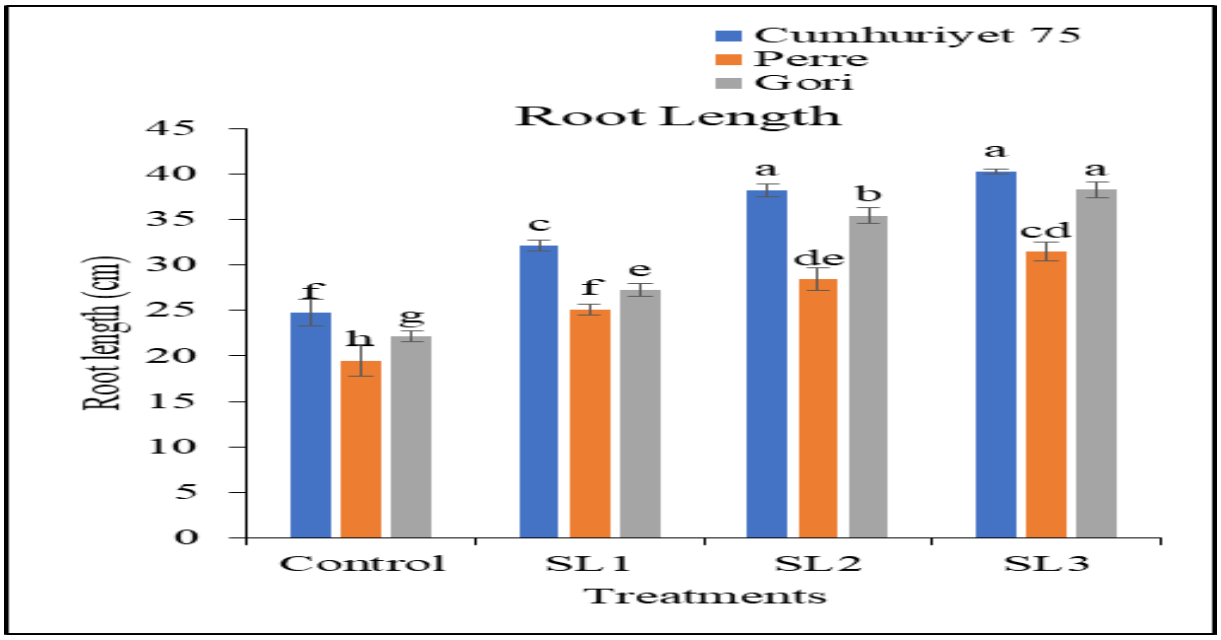

Figure 2. Effect of salinity on root length

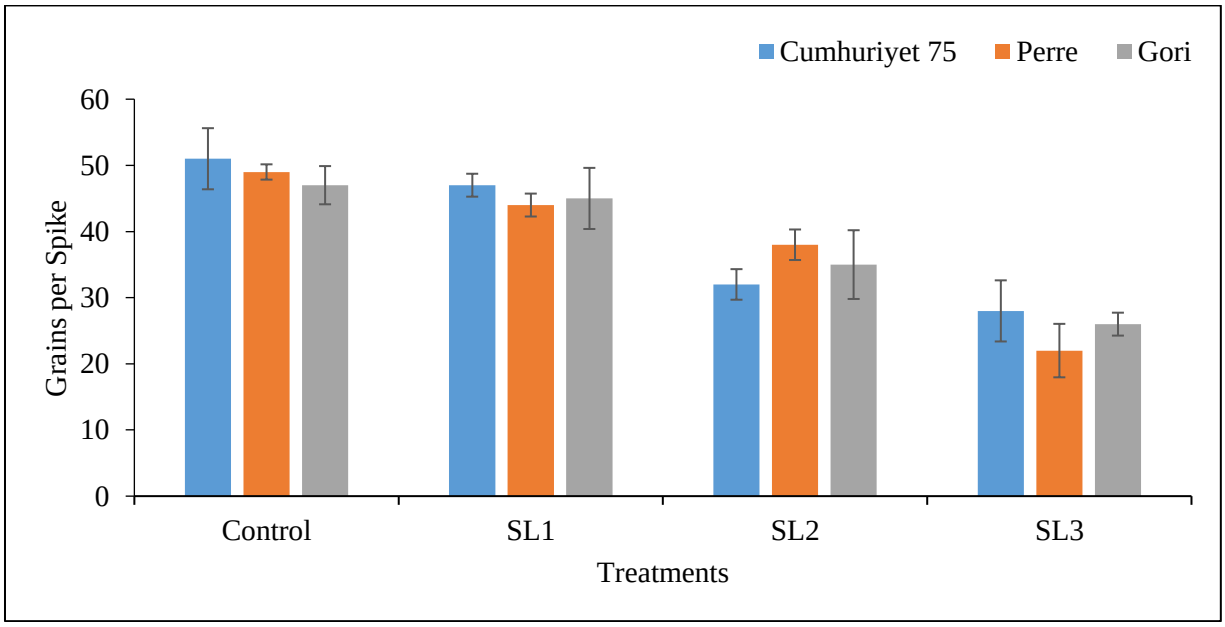

Figure 3. Effect of Salinity on grains per spike

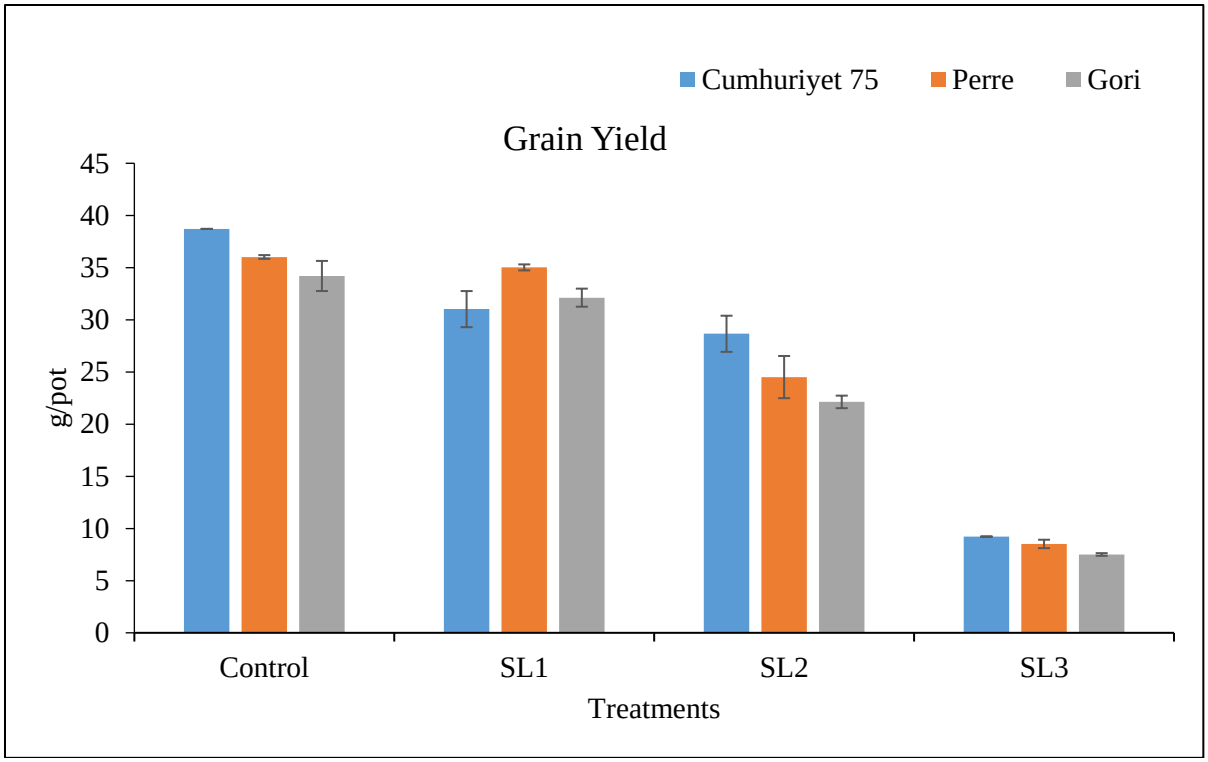

Figure 4. Effect of Salinity on grain yield of wheat

CONCLUSIONS

Keeping under consideration the interactive effect of salinity levels and varieties best results of plant height, spike length, number of tillers, 1000 grain weight, along with grain yield were observed in V1 under control conditions. However, this experiment suggested that improving salt acceptance of wheat varieties can be an effective strategy to increase crop growth and production under saline conditions. While using salt tolerant varieties and applying organic amendments along with salt tolerant microbes can be more effective.

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COMPARATIVE ANALYSIS OF BIOFILM MORPHOTYPES OF TYPE 1 FIMBRIAE N-TERMINAL DOMAIN DISRUPTED MUTANT AND WILD-TYPE STRAIN IN *SALMONELLA* TYPHIMURIUM

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ABSTRACT

Type 1 fimbriae can be found in most *Salmonella* strains and many other members of Type 1 fimbriae are found in most *Salmonella* strains and many other members of the family *Enterobacteriaceae*. Type 1 fimbriae are one of the structures that allow *Salmonella* to attach itself effective onto abiotic surfaces and different host cells. In this study, the aim was to determine the effects of N-terminal domain on biofilm in *S.* Typhimurium ATCC 14028 strain, which was cloned only N-terminal domain of the *fimF* gene. In the *S.* Typhimurium *fimF* mutant, the biofilm formation was found to be statistically significantly reduced compared to the wild-type strain ($p<0.05$), and the biofilm forming capacity of the *fimF* gene N-terminal domain cloned construct increased compared to the *fimF* mutant ($p<0.05$). When the morphotypes of biofilms formed by wild-type and other strains are examined; The wild-type strain displayed the 'rdar' morphotype, while the *fimF* mutant strain and its N-terminal domain cloned construct be detected changed to the 'bdar' morphotype.

Keywords: *Salmonella* Typhimurium, Type 1 fimbriae, N-Terminal Domain, Biofilm formation, Biofilm morphotypes, Cellulose production

INTRODUCTION

Members of the *Salmonella* genus are significant food pathogens that create a major public health problem throughout the world, due to their long-term persistence ability on the surface and tissues of the plants, along with using human and animal's systems as a host. These bacteria, which cause hospital or food related epidemics in developing countries, are described as the members of the family *Enterobacteriaceae*, rod-shaped, Gram-negative and anaerobic (Crump, 2004; Threlfall, 2002; Su, 2007; Roy, 2021).

When *Salmonella* bacteria form a biofilm by attaching onto the surfaces, it causes serious health problems. Biofilms are communities formed by microorganisms, irreversibly adhering to abiotic and biotic surfaces. The formation of biofilm begins with adhesion to biotic and abiotic surfaces. Getting bacteria close enough to a surface is a pre-requisite for biofilm formation. As bacteria get close to a surface, both attractive and repulsive forces are activated. At a distance of approximately 10-20 nm from the surface, the negative charges on the bacterial surface are repelled by the negative charges on most environmental surfaces. The repulsive force can be overcomed by the attractive van der Waals forces between bacterial cells and surface, and the usage of fimbriae and flagella to provide mechanical attachment to the surface (Gonzalez-Escobedo & Gunn 2013; Rabin et al. 2015).

Some bacterial pathogens are equipped with multiple adhesive structures (Rehman et al., 2019). Adhesion of bacteria to mucosal surfaces plays an important role in the pathogenesis of most bacterial infectious diseases in animals and humans (Beachey, 1981). The characterization of fimbrial operons *fim, lpf* and *pef* found in *Salmonella* Typhimurium display that fimbrial adhesins perform multiple functions during the initial phase of an infection. Adhesins mediate first contact with epithelial cells. This event demonstrates a role for fimbrial adhesins in the elicitation of invasion and inflammatory response. Therefore, fimbriae are important virulence factors for *S.* Typhimurium (Baumler et al., 1997).

Type 1 fimbriae characteristically consist of fibrils of 1 μm in length and 6 nm in diameter (Korhonen et al., 1980; Wagner & Hensel 2011). Type 1 fimbriae are enterocytes specific adhesins (Althouse et al., 2003). Another characteristic feature of *S.* Typhimurium type 1 fimbriae is their ability to mannose-sensitive hemagglutination. It was found that this fimbriae type is combined with the Chaperon-Usher pathway. Biochemical and immunological tests carried out has shown that type 1 fimbriae of *S. enterica* serovar Typhimurium direct the relationship between pathogen and the host by mediating binding to mannose-containing glycoconjugates on eukaryotic cell surfaces (Boddicker et al., 2002).

Type 1 fimbrial proteins in *S.* Typhimurium are encoded by the *fim* gene cluster (*fimAICDHFZYW*). FimAICDHF is expressed as a single transcriptional unit. *Fim* gene cluster consists of six structural genes, three regulatory genes and a tRNA specific to rare arginine codons (AGA and AGG). *FimA, fimI, fimC, fimD, fimH* and *fimF* structural genes are all expressed as a single transcript from the P_{fimA} promoter. *FimZ, fimY* and *fimW* regulators are all expressed by independent promoters. The structural components of fimbriae are FimA (main subunit), FimI, FimH (adhesin) and FimF (adapter) (Zeiner et al., 2012).

FimF gene and therefore the FimF protein it encodes, act as adapters for insertion of terminal adhesins into the fimbrial structure. It is thought that in absence of this gene and protein, adhesin binding activation cannot be achieved. On the other hand, in the study conducted with type 1 fimbriae adapter proteins of many pathogens, including *E. coli*, it was suggested that only the N-terminal ends of the adapter subunits act as adapters for the addition of fimbrial subunits (Kloppsteck et al., 2016).

N-terminal region of a protein regulates the function of that protein and ensures that it functions correctly and also determines the role of the protein in question in biological processes. In this study, it was aimed to clarify whether the function of *S.* Typhimurium type 1 fimbrial protein, FimF, which causes it to act as an adapter protein, originates from the N-terminal region, the role of the *fimF* gene encoding this protein in biofilm formation.

MATERIAL & METHODS

Bacterial Strains and Culture Conditions

S. Typhimurium ATCC 14028 wild-type strain and it's *fimF* gene mutant were obtained from the culture collection created by the Prokaryotic Genetics working group (Ankara University, Faculty of Science, Department of Biology). *S.* Typhimurium ATCC 14028 wild-type strain, *fimF* gene mutant (Δ*fimF*) and the N-terminal domain cloned strain (Δ*fimF*pBAD$fimF^{N}$) were incubated in Luria Bertani (LB) medium at 37 °C in a 200 rpm rotating incubator for 18 hours. *S.* Typhimurium ATCC 14028 *fimF* gene mutant strain, which underwent deletion using

homologous region recombination technique, was grown in medium containing chloramphenicol (20 μg/mL^{-1}) and *S.* Typhimurium ATCC 14028 strain, in which only the N-terminal domain of the *fimF* gene was cloned, was grown in medium containing ampicillin (100 μg/mL^{-1}) and arabinose (0.01%).

Determination of Biofilm Formation Amounts and Biofilm Morphotypes on Polystyrene Surfaces

The biofilm forming properties of bacteria were quantitatively determined in systems with 96 well polystyrene surfaces. *Salmonella* strains were grown in NaCl-free LB broth (LB^{-NaCl}) at 37 ºC in a 200 rpm shaking incubator for 18 hours. 30 μL of bacterial cultures prepared at a concentration of 1×10^9 CFU/mL (colony forming unit) were taken and transferred to 96 well polystyrene microplate wells containing 100 μL of LB^{-NaCl} broth. Microplates were incubated at 20 °C for 72 hours under static conditions. The supernatants were discarded at the end of the incubation, after that the wells were washed thrice with phosphate-buffered saline (PBS, pH 7,0±2,0). After the washing process, 140 μL of 95% methanol was added to fixation of the biofilm structures attached to the wells and kept at room temperature for 20 minutes. Biofilm structures were dyed for 15 minutes using 1% crystal violet. The plates were washed with sterile distilled water and the microplates were dried at room temperature after removing the dye that did not adhere to the biofilm structures. In order to dissolve the dye penetrating into the produced biofilm 140 μL (33%) of glacial acetic acid was added to the medium, and these plates were kept at 24 °C for 30 minutes. The dye adhered to the biofilm was determined at OD_{595} nm using an ELISA reader (Biorad, USA). The study was conducted in three parallels and two repetitions (Stepanovic et al., 2004; Vestby et al., 2009).

To determine biofilm morphotypes, active bacterial cultures were inoculated on LB^{-NaCl} agar containing Congo red (40 mg/L, Sigma-Aldrich, Germany). Petri plates were incubated at 20 ºC for 8 days. Colonies formed on the petri plate surface were visualized using a stereo microscope (Leica DMS1000, Germany). The study was conducted in six parallels and three repetitions (Römling & Rohde, 1999).

The pellicle forming properties of *S.* Typhimurium ATCC 14028

Wild-type and mutant strains of *S.* Typhimurium ATCC 14028 were inspected for their pellicle forming properties in the liquid-air interface. *Salmonella* strains were developed at 37 ºC for 18 hours at 200 rpm. 500 μL of active cultures were taken and inoculated into test tubes containing 4500 μL of salt-free LB medium. Tubes were photographed after 8 days of incubation in a 20 ºC static incubator. The results were evaluated according to the pellicle formation in the liquid-air surface and the amount of the decomposition of this pellicle. The pellicle was classified as fragile if it broke very easily, and as rigid if it did not break up or broke slightly (Römling et al. 2000; Solano et al. 2002).

Statistical Analysis

GraphPad Prism 8 Software was used to perform the necessary statistical analyzes for the data obtained from the study. In determining the differences between the experimental groups, the 'F' value was taken as the basis and one-way ANOVA test was applied. Variations between groups were evaluated using the Turkey accuracy test.

RESULTS

Biofilm production characteristics on a polystyrene surface

When the biofilm formation abilities of *S*. Typhimurium ATCC 14028 wild-type and mutant strains on polystyrene surfaces at 24., 48. and 72. hours were examined; Compared to the wild-type strain, at the rates of % 93,1, % 55,3 and % 68,6 respectively in *S*. Typhimurium ATCC 14028 Δ*fimF* strain and also at the rates of % 80,2, % 4,86 and % 13,6 in *S*. Typhimurium ATCC 14028 Δ*fimF(pBADfimF*^{N}*)* strain decreases were observed in these time periods (Figure 1).

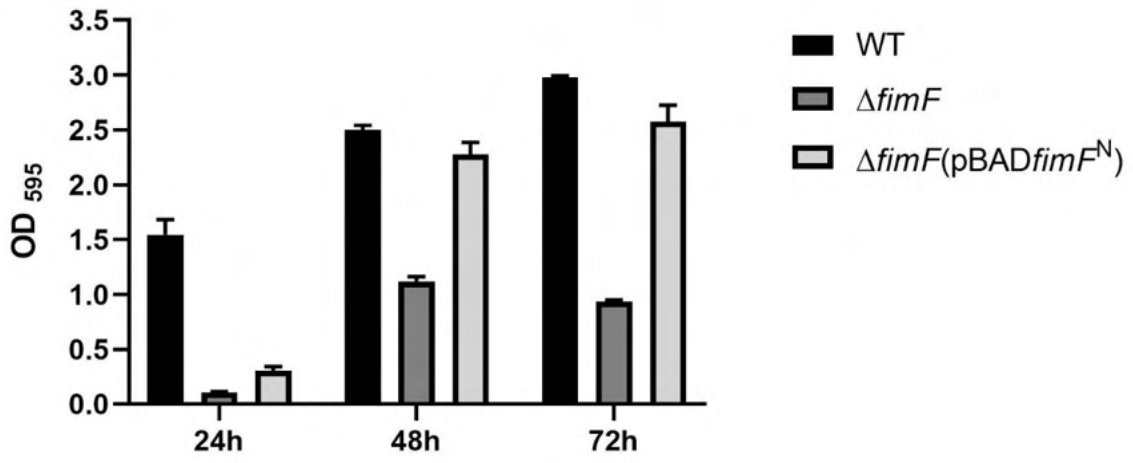


Figure 1. Biofilm production characteristics of wild-type and mutant strains of S. Typhimurium ATCC 14028 on polystyrene substrates.

Biofilm morphotypes produced on solid media with Congo Red

Biofilm morphotypes produced by *S*. Typhimurium wild-type and mutant strains on solid medium with Congo Red; biofilm EPS 'rdar' (red, dry and rough) with thin aggregative fimbriae (curli fimbriae) and cellulose, 'bdar' (brown, dry and rough) containing only curli fimbriae, 'pdar' (pink, dry and rough) containing only cellulose, 'saw' (smooth and wet) with neither curli fimbriae nor cellulose. The biofilm morphotype of *S*. Typhimurium wild-type strain was determined as 'rdar', *S*. Typhimurium Δ*fimF* 'bdar' and *S*. Typhimurium Δ*fimF*(pBAD*fimF*^{N}) 'bdar' (Figure 2).

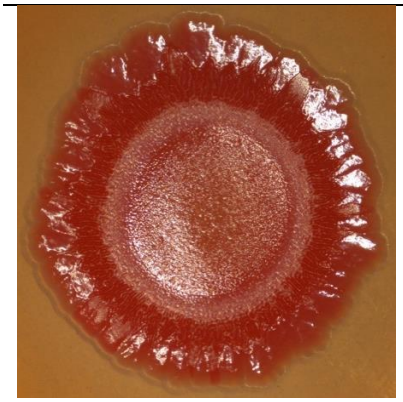
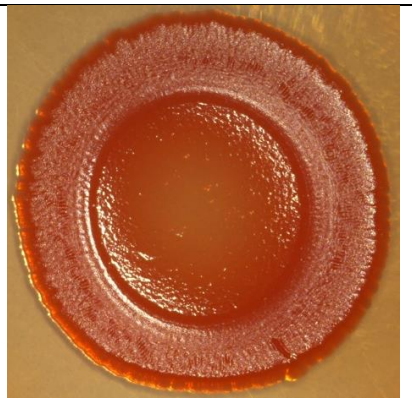
WT	Δ*fimF*	Δ*fimF*(pBAD*fimF*^{N})
		

Figure 2. Stereo microscope image of biofilm morphotypes of S. Typhimurium ATCC 14028 wild-type and mutant strains formed on Congo Red agar surface

Amount of pellicle formation and characteristics of pellicle structures

S. Typhimurium wild-type and mutant strains were tested on salt-free LB medium with Congo red. As a result, the wild-type strain with the 'rdar' morphotype had a rigid pellicle structure, while Δ*fimF*(pBAD*fimF*^{N}) strain with the 'bdar' morphotype had a durable pellicle structure, although not as much as the wild-type strain; The Δ*fimF* strain with the 'bdar' morphotype was found to be vulnerable to physical factors such as pellicle structure, shaking and mixing (Figure 3).

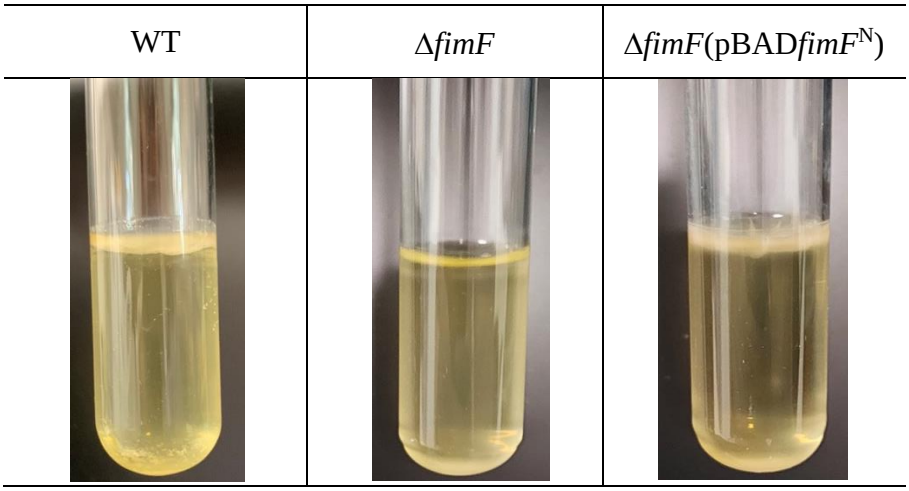


Figure 3. Amounts of pellicle formation of S. Typhimurium ATCC 14028 wild-type and mutant strains in salt-free LB medium

The observations made according to the turbitide change in the medium, the formation of pellicle and ring structure in the liquid-air interface, and the durability of the pellicle structure as a result of shaking and mixing are given in Table 1.

Table 1. Characteristics of pellicle structures formed by S. Typhimurium ATCC 14028 wild-type and mutant strains

Bacterial Cultures	Turbitide in the medium (turbidity)	The pellicle structure formed in the liquid-air interface	The ring structure formed in the liquid-air interface	Durability of the pellicle structure
Wild-type	More	Rigid	More	More (+)
Δ*fimF*	Less	Fragile	Less	Less
Δ*fimF*(pBAD*fimF*^{N})	Less	Rigid	More	More

DISCUSSION

Type 1 fimbriae characterized by mannose-sensitive hemagglutination in *S.* Typhimurium are important for attachment to eukaryotic host cells. The type 1 fimbriae *fim* gene cluster

(*fimAICDHFZYW*) consists of six structural genes (*fimA, fimI, fimC, fimD, fimH* and *fimF*) and three regulatory genes (*fimZ, fimY* and *fimW*). The first condition for the biofilm formation process is the attachment of the microorganism to a suitable surface. *FimF*, a type 1 fimbriae gene, acts as an adapter in cell adhesion. FimF, which is one of the elements involved in the adhesion of planktonic cells to the surface in *S*. Typhimurium, has an important role in biofilm formation.

According to the results of our study, a statistically significant ($p<0.05$) decrease was determined in the biofilm forming capacity of the mutant strain in terms of the *fimF* gene compared to the wild-type strain. As cellulose production in microorganisms increases, the biofilm structures (pellicle) formed in the liquid-air interface acquire robust physical property characteristics, which makes the pellicle structures stable against physical disintegrating forces. In Δ*fimF* and the N-terminal domain of the *fimF* gene showing the 'bdar' morphotype, while it was determined that cellulose production of Δ*fimF* strain was low, the pellicle structure of this strain was not resistant to physical dispersant, while a rigid pellicle structure was determined in the mutant strain obtained by cloning the N-terminal domain of the *fimF* gene, although not as much as in the wild-type.

In the lights of all these results, the N-terminal domain of the *S*. Typhimurium *fimF* gene was restored by cloning into the pBAD vector and re-expressed the biofilm phenotype at a statistically significant level ($p<0.05$), although not as much as in the wild-type strain; This proves that the N-terminal domain of the gene in question is the region of the protein that carries the adapter function, thus proving that the *fimF* gene is a fimbrial gene that has an important role in biofilm formation in *S*. Typhimurium.

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SYNTHETIC SOIL CONDITIONERS USED IN SOIL REMEDIATION: A REVIEW

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ABSTRACT

Soil conditioning in agriculture refers to the formation and stabilization of aggregates that allow proper aeration and drainage in the root zone and can therefore increase crop yields. Soil conditioners are beneficial as they make the soil more functional as an ecosystem and more productive as a support for crop production.Soil conditioners create a suitable environment for the proliferation and survival of beneficial microorganisms and earthworms in the soil. Soil conditioners can be classified into four categories as organic, inorganic, synthetic and other soil conditioners according to their origin and composition. The new generation soil conditioners are highly cross-linked polyacrylamides in which 40% of the amides are hydrolyzed to carboxylic groups. The roots of the plant grow through the matrix of these hydrogelled particles and draw water from them as needed. Polysaccharides and polyacrylamides, which are among the synthetic soil conditioners, are generally used to improve aggregate stability and maintain productivity.

Key words: Soil, remediation, synthetic soil conditioner

INTRODUCTION

In our age, agricultural areas are getting narrower due to reasons such as wrong land cultivation, non-purpose land use, barrenness, not using the land in accordance with the land use capability class, erosion, soil pollution, as well as rapid industrialization and urbanization. Scarcity of water resources, desertification of soil and excessive use of fertilizers are the main factors that lead to the degradation of cultivated areas (Thakur et al., 2015). Soil conditioning in agriculture refers to the formation and stabilization of aggregates that allow proper aeration and drainage in the root zone and can therefore increase crop yields. Soil conditioners create a suitable environment for the proliferation and survival of beneficial microorganisms and earthworms in the soil. They also add nutrients to the soil, allowing plants to grow healthier, stronger and more productive. The new generation soil conditioners are highly cross-linked polyacrylamides in which 40% of the amides are hydrolyzed to carboxylic groups. These polymers do not interact directly with soil matrices, but form aqueous gels and act as water reservoirs for the plant-soil system. The roots of the plant grow through the matrix of these hydrogelled particles and draw water from them as needed (Quchi et al., 1989, Bouranis et al., 1995). Polysaccharides and polyacrylamides, which are among the synthetic soil conditioners, are generally used to improve aggregate stability and maintain productivity (De Boodt, 1975, Azzam, 1980, Wallace and Nelson, 1986). Polysaccharides (PSD), polyacrylamides (PAM), polyvinyl coride (PVC), polyphenol hydrochloride (PPH), hydrolyzed polyacrylontrile (HPAN), Polyvinyl alcohol (PVA) and Vinyl acetate-maleic acid (VAMA) copolymers are used as synthetic soil conditioners.

EFFICIENCY OF SYNTHETIC SOIL CONDITIONERS ON SOIL AMELIRATION PROCESSES

Soil hydrogels consist of either natural sources containing the most common and degradable components such as polysaccharides (PS) and polypeptides (PP) or synthetic material containing petrochemical-based acrylic acid (AA), its salts and acrylamide (AM) (Zhou et al.,2018). Over the last two decades, synthetic hydrogels and the combination of natural and synthetic hydrogels have become increasingly important and have replaced traditional natural hydrogels (Behera and Mahanwar, 2020). Different soil hydrogels have varying degrees of these properties depending on the nature of the monomers used and the polymerization process. All soil hydrogels basically exhibit the following three properties: swelling, water absorbency and nutrient release (Palmqvist, 2017). The swelling property of superabsorbent hydrogels overcomes worldwide problems related to water consumption in agriculture, helps improve soil water retention and reduce plant wilting rate (Cheng et al., 2018). The swelling behavior of the superabsorbent hydrogel facilitates water absorption in sandy soils with low water retention capacity and allows the water content to settle in the plant slowly and for a long time (Ogieglo et al., 2015). Another marvelous property of the hydrogel, besides its swelling, is its water retention or water holding capacity, which is different in each soil type (Akhter et al., 2004).

Over the past few decades, various types of polysaccharide-based superabsorbent hydrogels have been proposed for agricultural applications due to their excellent hydrophilic properties (high swelling capacity and high swelling rate), excellent biocompatibility and biodegradability (Bhattacharyya, et al., 2013, Hemvichian, et al., 2014). The high water absorption of these materials is attributed to interconnected superporous structures of several hundred microns in diameter, forming open channels that allow capillary action (Kuang et al, (2011). The properties of superabsorbent hydrogels vary depending on the nature of their components, the polymerization process (grafting or cross-linking) and other parameters. The use of superabsorbent hydrogels in agriculture comparatively increases the swelling rate up to 60-80%, provides maximum water retention and provides gradual release of nutrients to plants for a longer period of time (Rizvan et al., 2021). Abd El-Rehim,et al., (2004) reported that the polyacrylamide (PAAm),and polyacrylate (PAAcK) hydrogels improve sandy soil properties because they often absorb and keep water one thousand times more than their own weight, reduce watering frequency of the plants, and enhance water retention in soil. On the other hand, when superabsorbent hydrogel is added to soil, it reduces irrigation water consumption and improves the physical properties of the soil (Hemvichian, et al., 2014, Özdemir et al., 2014). It was reported that polyvinyl alcohol (PVA), polyacrylamide (PAM)a applications decreased the plasticity index and increased plastic limit values in surface soils samples with three different textures as clay, loam and sandy loam. Additionally these synthetic materials increased aggregate stability and decreased the dispersion ratio (Kassım and Özdemir, 2022, Özdemir and Civelek, 2023).

Yangyuoru et al. (2006) reported that water retention ability improved of the soils amended with the polymeric absorbents over the control. Superabsorbent hydrogel (SH) acts as a water reservoir and releases water into the soil or directly into the rhizosphere in a controlled manner. Therefore, the use of superabsorbent hydrogel in agriculture reduces the death rate of plants and increases crop production in arid zones (Zhong et al., 2012). According to Demitri et al. (2013), the main advantages of cellulose-based SH applied in arid and desert areas are that it can control the release of stored water as the soil dries, maintains soil moisture for a relatively long time, and provides better oxygen supply to plant roots by increasing the porosity of the soil. Parvathy and Jyothi (2014) investigated the effect of a superabsorbent hydrogel based on saponified cassava starch-g-poly(acrylamide) on the physical-chemical and biological properties of soil.

As a result, they stated that the amount of moisture retained in the soil depends on the concentration of superabsorbent matrices, which provide better control of the release of

adsorbed water. They also reported that these SHs are potential candidates to be applied as alternatives in combating global climate change, as they can improve soil properties in cases where moisture availability decreases. Many researchers (Mitchell 1986, Lentz and Sojka 1994, Sojka and Lentz 1996), have reported that adding high molecular weight anionic PAM to irrigation water at a very low concentration (at 2-10 g m^{-3} or 2-10 ppm) reduces soil runoff and increases water infiltration during the first few hours. Superabsorbent hydrogel plays an amazing role in preventing nutrient loss during intense runoff of rainwater from the upper surface of the soil because these superabsorbent hydrogels (SAHs) absorb water and swell to retain water for longer periods of time. SAHs facilitate the growth of plants with limited use of water and fertilizer. It also improves the health of the soil and makes it fertile in horticulture and drought areas. The major quantity of applied fertilizers, containing phosphates and nitrates, is lost during rainfall that definitely pollutes the environment and sea water (Sarkar al., 2015). Soil hydrogels increase the availability of nutrients for plants, as they have a controlled slow release of nutrients at the required rate (Kaur and Purewal., 2019). Çağlar and Demir (2021) reported that the applications of polyacrylamide and polyvinylalcohol had a positive effect on the nitrogen, phosphorus and potassium uptake of the canola and jute plant species.

CONCLUCION

Soil hydrogels are widely used in agriculture as soil conditioners and plant growth promoters. Hydrogels are biodegradable, environmentally friendly, cost-effective, and increase nutrient availability through slow release of fertilizer. Due to their low cost, abundance, and environmentally friendly properties, polysaccharides have been shown to be able to replace petroleum derivatives in the preparation of superabsorbent hydrogels.

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CHANGES IN THE SOIL PROPERTIES OF AGRICULTURAL LANDS AROUND ORGANIZED INDUSTRIAL ZONE CAUSED BY INDUSTRY IN VAN, TÜRKIYE

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ABSTRACT

In this study, it was aimed to investigate some properties of the soils around the Van organized industrial zone. Different six sampling points within each direction were determined in the north, south, and east directions of industrial zone. The total set of 54 soil samples were taking from 0-20 cm depth in different three positions as 0.2 km, 1.0 km and 2.0 km far away to pollutant source. GPS readings were recorded for each sampling points. Generally, electrical conductivity (EC) and lime ($CaCO_3$) content means decreased while the distance to industrial zone increased in soils. In soil samples the highest pH, EC and lime content means as 9.19, 326.70 **µ**mhos cm^{-1} and 42.34 % in 0.2 km far away to industrial zone; the lowest means of these parameters in 2.0 km far away to pollutant source were obtained. Van Organized Industrial Zone operates in the paint production, tile adhesive and joint filler, packaging cardboard and bag manufacturing, vehicle plate manufacturing, PVC and drilling pipes, styrofoam and thermal insulation materials manufacturing/foam packaging manufacturing, food, electricity, marble processing, facade cladding, textile clothing, detergent and cosmetic products, construction, petroleum products, paper and napkin production, furniture, metal, automotive, cable and plastic production sectors. It is thought that the waste materials released to the environment from these industrial activities cause changes in the soil properties studied.

Key words: Industry, soil reaction, soil electrical condactivity, lime content

INTRODUCTION

After the Industrial Revolution, depletion of natural resources, carbon emissions, pollution and human health problems have become threats worldwide. The developing world generally appears to have high levels of polluting activities in the industrial sector. Nowadays, problems related to industrialization such as increasing greenhouse gas emissions, air and water pollution, increasing waste volumes, desertification and soil chemical or heavy metal pollution are increasing. Diffuse pollution is a significant threat to soil conservation, and this is much more pronounced in urban communities with multiple emissions sources (Biasoli and Ajmone-Marsan, 2007). Depending on the properties of the soil, chemicals emitted can either react with other soil factors, be absorbed by soil substances, or mix directly with groundwater, causing other types of pollution. Due to the behavior of these chemicals in the soil, it is very difficult to determine their fate in the soil. With the onset of widespread chemical degradation, some soil functions are inhibited. The most important of these functions are the buffering, filtering and transformation capacity of the soil (EEA, 2014).With the loss or inhibition of these functions, the soil loses its capacity to remove harmful chemicals or reduce their impact on crop growth and yield (Halm and Grathwohl, 2005).

MATERIAL AND METHOD

The research was carried out in a location with the coordinates of 38° 33' north and longitude 43° 17' east (Figure 1). The research area is located within the continental climate zone (Anonymous, 2019). Van Organized Industrial Zone operates in the paint production, tile adhesive and joint filler, packaging cardboard and bag manufacturing, vehicle plate manufacturing, PVC and drilling pipes, styrofoam and thermal insulation materials manufacturing/foam packaging manufacturing, food, electricity, marble processing, facade cladding, textile clothing, detergent and cosmetic products, construction, petroleum products, paper and napkin production, furniture, metal, automotive, cable and plastic production sectors.

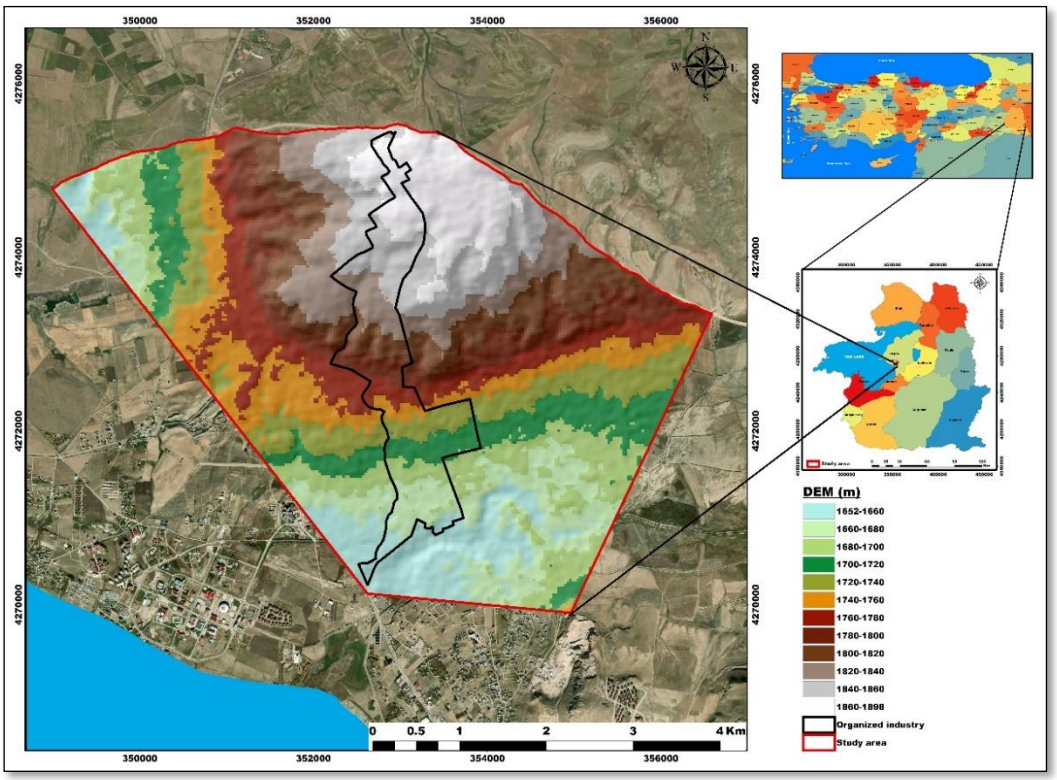

Figure 1. The study area.

Different six sampling points within each direction were determined in north, south and east directions of industrial zone. The total set of 54 soil samples were taking from 0-20 cm depth in different three positions as 0.2 km, 1.0 km and 2.0 km far away to pollutant source. GPS readings were recorded for each sampling points (Figure 2)

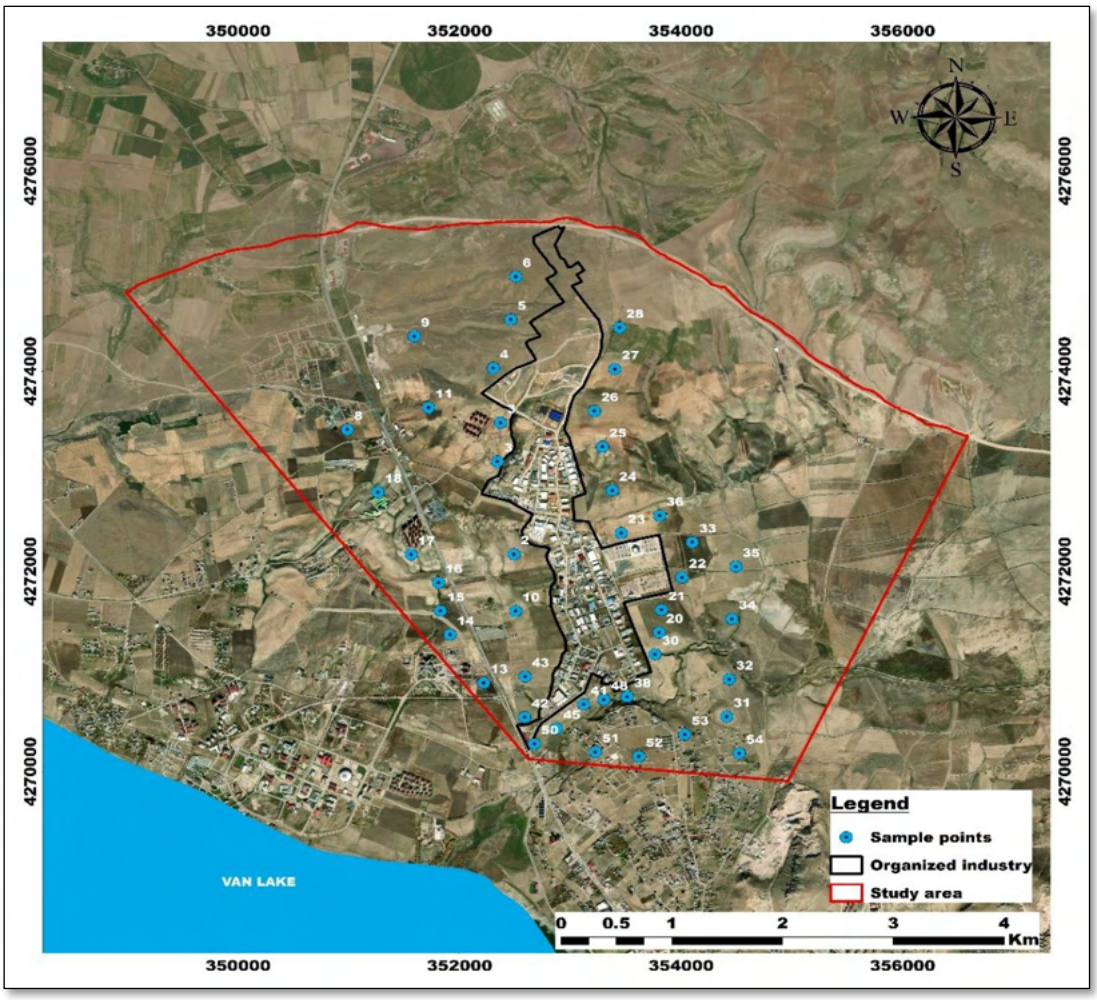

Figure 2. Locations where soil samples were taken in the study area.

Some chemical properties of soil samples taken from 0-20 cm depth were determined as follows; soil reaction in 1:2.5 (W:V) soil:water suspension by meter and soil salinity by EC meter, in the same suspension (Black,1965), lime content by Scheibler calcimeter (Goh et al.,1993). SAS package program was used for statistical analysis (SAS, 1998).

RESULTS AND DISCUSSION

According to variance analyzes results, effects of distance to pollutant souce were found significant for electrical condactivity (EC) means and lime ($CaCO_3$) contents at 1% significance level in east direction of organized industrial zone. The lime contents were also significantly (5%) affected by distances to the pollutant source in south direction of organized industrial zone. The effect of distance on all of investigated soil chemical pro perties was non significant in west direction of organized industrial zone. The changes in pH means were not found significant in all directions statistically. (Table1).

Table1. The results of variance analyses results of some chemical properties of soil samples taken from different distance and direction to organized industrial zone.

Directions		$CaCO_3$	EC	pH
	Df	2	2	2
East	F	23.54**	10.35**	1.21 ns
West	F	2.18 ns	1.06 ns	1.33 ns
South	F	4.44*	0.37 ns	0.33 ns

Generally, electrical condactivity and lime content means decreased while the distance to industrial zone increased in soils. As that seen in Figure 3,4,5 the highest EC and $CaCO_3$ means were obtained in 0.2 km far away from pollutant source as 8.47, 138.34 µmhos cm^{-1} and 26.90 % respectively for east direction. The lowest means of these parameters were 8.37, 106.91 µmhos cm^{-1} and 8.06 % respectively in 2.0 km far away from pollutant source. For south direction the highest pH, EC and $CaCO_3$ means were obtained as 8.51, 151.55 µmhos cm^{-1} and 17.67 % in 0.2 km far away from pollutant source while the lowest means of, EC and $CaCO_3$ were 8.44, 131.24 µmhos cm^{-1} and 8.07 % in 2.0 km far away from pollutant source (Figure 3,4,5). In soil samples taken from the west direction, as the distance to the industrial zone increased, the decreases in the pH, EC and $CaCO_3$ means occurred in a very narrow range as following 8.53-8.31, 177.55 µmhos cm^{-1} – 202.120 µmhos $cm^{-1}$and 13.42 % -16.67 %.

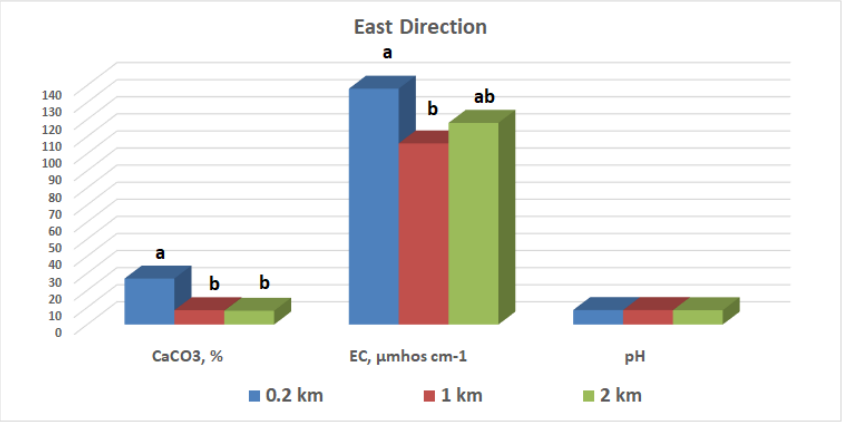

Figure 3.The changes in pH, EC and $CaCO_3$ means depending on distance in the east direction.

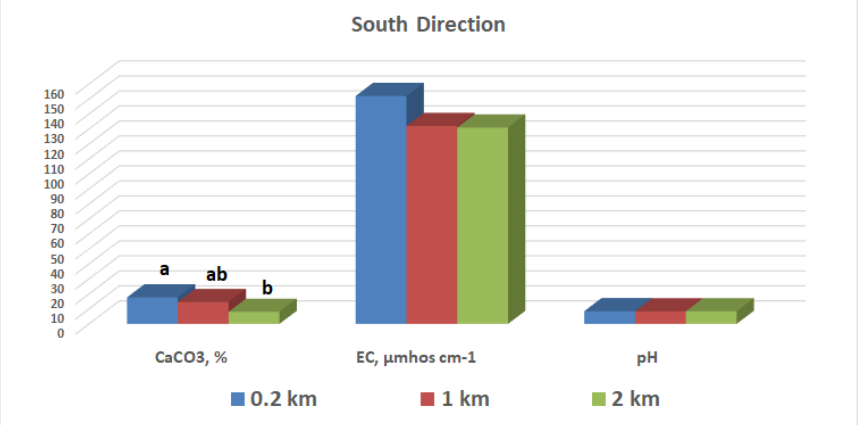

Figure 4.The changes in pH, EC and $CaCO_3$ means depending on distance in the south direction.

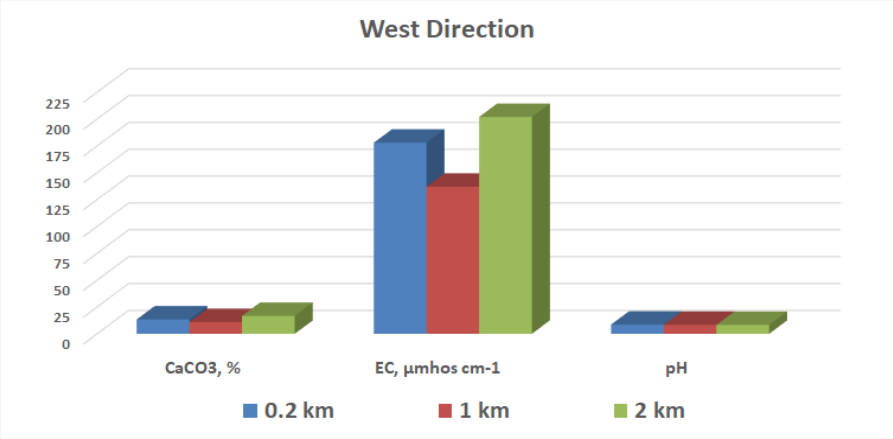

Figure 5. The changes in pH, EC and $CaCO_3$ means depending on distance in the west direction.

When taking into account analyses results soil itwas determined that pH, EC and $CaCO_3$ means of soil samples taken from away to 0.2 km were higher than those taking from more far away distances to organized industrial zone. Although the pH means (for east, south and west directions: 8.47, 8.51 and 8.53) were found to be higher at a distance of 0.2 km from the zone, they were considered slightly alkaline according to the reported limit value. Similarly, higher EC means (for east, south and west directions: 138 µmhos cm^{-1}, 151 µmhos cm^{-1} and 202 µmhos cm^{-1}) closer to the source were also considered nonsaline according to the reported limit values (Müftüoğlu et al., 2014). In tis study $CaCO_3$ means (for east, south and west directions: 26.90%, 17.67% and 16.67%) of soil samples taken from away to 0.2 km to organized industrial zone were found in high level according to reported limit values.

In this study, it is thought that the very high $CaCO_3$ values found in the agricultural lands close to the organized industrial zone are caused by industrial activities such as marble processing, construction, food detergent and cosmetic products. It is known that high $CaCO_3$ in soil reduces the availability of plant nutrients (Marschner, 1995).

It was reported that since many agricultural sustainability issues are related to soil quality, its assessment is very important. Thus, its assessment and the direction of change with time is a primary indice of whether agriculture is sustainable. (Karlen et al., 1997; Masto et al., 2007; Glanz, 1995). Rojas (2018) reported that pH, EC and $CaCO_3$ parameters are among the chemical soil quality indexes. A further increase in the lime level in the research area of the Van Lake basin soil, which is reported as calcareous (Gülser and Karaçal, 1992), will create problems in terms of the sustainability of plant production.

CONCLUSIONS

In our study, it was concluded that wastes should be disposed of in a controlled manner to ensure sustainability in the lands around the industrial zone and to protect soil health and quality. In addition, the results of this research are considered as stimulating and guiding for the industrial branches in the research area to operate within the framework of solid waste regulations that can prevent environmental pollution.

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EFFECTS OF CYCOCEL (CCC) DOSES AND APPLICATION STAGES ON SEED YIELD AND YIELD COMPONENTS OF MUNG BEAN (*Vigna radia*ta (L.) Wilczek)

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ABSTRACT

The aim of this study was to reveal the effects of different cycocel doses and application stages on seed yield and yield components in mung beans. The field experiment was conducted in Adana, Turkey during summer season of 2020 and 2021. Experimental design was split plots based on randomized complete blocks with three replication. The main plots were application stages (seedling and beginning of flowering) and sub-plots were cycocel doses (0, 500, 750, 1000 ppm). In present study, KPS1 excotic genotype was used as a research material. As mean of the years the highest pods per plant, branches per plant, seed weight per plant were observed at 1000 ppm CCC. Cycocel application reduced the plant height and the first podding height. According to combined analysis, greatest seed yield was produced by cycocel application of 1000 ppm with 2530 kg ha^{-1} at seedling stage while minimum seed yield was found in control dose (no cycocel) at seedling stage with 1944 kg ha^{-1}.

Keywords: Application Stages, Cycocel, Mung bean, Seed yield, Yield components

INTRODUCTION

Mung bean (*Vigna radiata* L.) Wilczek) can be grown arid and semi-arid region at the world. Its seeds contain high protein, carbonhydrates and vitamins and its cultivation is widely in Avustralia, Asia, Africa and America (Li et al., 2010; Singh et al., 2013; Dahiya et al., 2015; Abdul Rahman, 2018). Seeds of mung bean can be evaluated as a feed for livestocks and food for human and green manure in the world (Azadi et al., 2013; Nair et al., 2013). Consumption of mungbean is gradually increasing at the world. Mungbean cultivation is not spreading in Turkey, but of landraces of mungbean genotypes are grown in some regions of Turkey (Akdağ, 1995; Dalkılıç, 2010) and it can be succesfully produced for seed in Turkey (Pekşen et al., 2015; Karaman, 2019; Ton, 2021).

Plant growth regulators are natural or synhetic compounds and they are important for increasing seed yield and quality in legumes as in other crops (Kumar, 2021) Cycocel is retarding the plant growth and it is used to prevent lodging in cereals (Kumlay and Eryiğit, 2011). The effect of cycocel application was investigated in some crops, but there are limited studies on the use of growth retards in legumes. It is reported that maximum dry matter in chickpea was obtained from cycocel application of 2000 ppm (Verma et al., 2018). Some studies on various legume crops showed that cycocel application increased some morphological and agronomical traits compared to control in pea (Alan, 1990), in faba bean (Beşer and Adak, 1999), in chickpea (Hajyzadeh, 2008**)** and in mung bean (Kshirsagar et al., 2008). Güler (2010) reported that the greatest seed yield in chickpea was obtained from 1000 ppm cycocel dose applicated in the beginning of flowering. Bora and Sarma (2006) reported that cycocel increased the seed yield and protein content in pea. The studies on mung bean showed that

application of cycocel and some plant growth retards improved seed yield and yield components in comparison to control. (Bhadane et al., 2020). Some plant growth regulators and cycocel inhibites flower shedding, so it can be increased yield in mung bean (Khwairakpam and Kumar, 2019a).

The aim of this study was to reveal the effects of different cycocel doses and applications stages on seed yield and yield components.

MATERIALS AND METHODS

The field experiment was conducted at the research area of Field Crops Department, Faculty of Agriculture, University of Çukurova, Adana, Turkey during summer season of 2020 and 2021. Some meteorological values of Adana for experimental years are present in Table1.

Table 1. Some metorological values in the experimental years

Meterological Parameters	Min Temperature (^{O}C)		Max Temperature (^{O}C)		Mean Temperature (^{O}C)		Relatively Humidity (%)		Total Rainfall (mm)	
Years / Months	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
May	12.9	14.9	40.3	34.0	23.3	23.9	61.0	64.9	66.6	4.1
June	16.4	18.9	39.5	37.7	25.0	25.9	70.9	67.2	38.2	0.4
July	22.9	23.8	37.6	41.0	29.4	30.0	74.3	68.0	0.0	15.8
August	21.7	22.7	41.3	39.8	29.7	30.5	65.9	64.1	1.2	1.2

The texture of experiment soil was siltly-clay loam. The values of pH, salt content, lime and organic matter were 7.25, 0.25 mmhos cm^{-1}, 36.8%, 1.19% respectively. In present study KPS1 exotic genotype provided from Field Crop Department, Faculty of Agriculture, University of Ondokuz Mayıs was used as a research material. This variety showed a good adaptation in the previous experiments conducted in Çukurova conditions. The study was organized in split plots experimental design over randomized complete blocks (RCBD) with three replications. The main plots were application stages (seedling and beginning of flowering) and sub-plots were cycocel (chlormequat chloride 460 g/lt) doses (0, 500, 750, 1000 ppm). The experiment was established on 2th of June in 2020 and 24 th of May in 2021. Each plot was sown in 5 rows of 4m lenght with an inter row spacing of 45 cm and intra row spacing of 5 cm. Fertilizer was applied at a rate of 40 kg N ha^{-1}, 100 kg P_20_5 ha^{-1} before sowing. Rhizobia inoculant was not applied in the experiment. Insufficient nodule formation was observed in the root, hence addition nitrogen fertilizer (Ammonium sulphate, 21% N) was also applied 80 kg N ha^{-1} to plots at seedling stage of plant. The plots was irrigated 4 times during growing period in germination, before flowering and pod stages in both of the years. Harvest was applied in the middle of three rows after eliminating the border rows on September 12, 2020 and August 31, 2021. Net plot area was 3 m x1.35 m=4.05 m^2. Data were recorded from five randomly selected plant in each plot. Plant height (cm), number of main branches per plant, number of pods per plant, number of seed per pod, 100-seed weight (g), seed weight per plant,(g), seed yield (kg ha^{-1}) were observed.

Data were analyzed according to the split plots experiment design for combined years by using the MSTAT-C a computer software package. Comparisions among the means were made by using LSD (5%).

RESULTS AND DISCUSSION

Plant height

As shown in Table 2, as mean of the years, differences among the application stages were not significant, but plant height was significantly affected by cycocel doses. Plant height varied from 62.4 (1000 ppm) to 74.8 cm (no cycocel). Increasing cycocel doses led to decrease in plant height. Similarly, previous studies recorded that cycocel application reduced plant height in mung bean (Kshirsagar et al., 2008) in chickpea (Güler, 2010). Cycocel application of 500 ppm in faba bean and 300 ppm in chickpea at three leaves stage reduced plant height (Beşer and Adak, 1999; Haiyzadeh, 2008)

Statiscial analysis revealed that year x application stage and year x cycocel dose interactions were no significant effect on plant height. Plant height was also not affected by interaction between cycocel dose and application stage. Plant height was significantly higher in 2020 (73.6 cm) due to greater precipation and relatively humidity and lower temperature in the vegetative stage (June) compared to 2021 (64.1 cm).

Table 2. The effects of cycocel doses and application stages on plant height first podding height and branches per plant in mung bean

Treatments	Plant Height (cm)			First Podding Height (cm)			Main Branches per plant		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Stages (S)									
1	74.9	63.9	69.4	41.6	24.7	33.2	3.2	2.7	2.9
2	72.3	64.3	68.3	35.0	28.6	31.9	3.2	2.7	2.9
LSD 5%	YXS: N.S.		N.S	YXS: N.S.		N.S	YXS: N.S		N.S
ppm	Doses (D)								
0	78.6	71.1	74.8a	42.5	30.0	36.3a	3.1	2.5	2.8b
500	77.0	64.5	70.8ab	38.8	27.7	33.3ab	2.9	2.8	2.9b
750	73.2	61.5	67.3b	36.1	23.2	29.6c	2.9	2.5	2.7b
1000	65.6	59.3	62.4c	35.8	25.8	30.8bc	3.8	3.1	3.4a
Mean	73.6A	64.1B	68.8	38.3A	26.7B	32.5	3.2A	2.7B	2.9
LSD 5%	YXD:N.S.		D:4.85	YxD:N.S.		D:3.29	YXD:N.S.		0.38
CV %	8.3			12			15		

1: Seedling 2: Beginning of flowering

First Podding height

According to mean of the years, application stages didn't affected the first podding height (Table 2). However, effect of cycocel doses was significant on this trait. Increasing cycocel doses reduced the first podding height due to decreasing in plant height. The highest value was obtained from control dose (no cycocel) with 36.3 cm whereas the lowest value was found at cycocel dose of 750 ppm with 29.6 cm These results are in line with report of Beşer and Adak (1999) who recorded that 500 ppm cycocel dose at pods formation reduced first podding height in faba bean. Statiscial analysis exhibited that there are no significant interactions of year x stages and year x doses. First podding height was higher in the first experimental year (38.3 cm) due to plant height than in the second experimental year (26.7 cm).

Number of main branches per plant

Combined mean showed that branches per plant was not significantly influenced by application stages, but there were significant differences among the cycocel doses in the branches per plant (Table 2). According to mean of the years, branches per plant ranged between 2.8 (0 ppm)-3.4 (1000 ppm) in different cycocel doses. There were no differences among the cycocel doses up to 750 ppm. However, main branches number were improved by application of 1000 ppm cycocel. It is also reported that branches per plant in pea increased with cycocel application (Bora and Sarma, 2006). On the other hand interactions of year x application stages, year x cycocel doses and of application stages x cycocel doses were no-significant for this trait. Main branches per plant in the first year was greater than in interaction was non-significant the second year as in plant height.

Number of pods per plant

As mean of the years, differences among the applications stages were non-significant for pods per plant, but the pods per plant were affected by interaction of year and application stages (Table 3). Pods per plant obtained from cycocel applications at flowering stage were greater in 2020 (34.2) than 2021 (21.8). In mean of years pods per plant was affected by cycocel doses and increase in cycocel doses significantly increased pods per plant and the highest value was achieved by 1000 ppm doses with 33.6 while the lowest value was at control doses with 22.6. Interaction between year and cycocel doses was also significant for pods per plant. The highest value was obtained from 1000 ppm (40.2) in 2020 while the lowest value was found at doses of 0 ppm in 2021 (21.9). Similar to our findings some studies reported that cycocel application increased pods per plant compared to control in mung bean (Bhadane et al., 2020; Khwairakpam and Kumar, 2019b). However, contrary to our findings Güler (2010) reported that the highest pods per plant was obtained from at beginning of flowering in no cycocel application in chickpea. Pods per plant in the first year which is lower temperature and rainfall during the flowering period (July) was greater than in the second year. It was also reported by Warrag and Hall (1984) who more pods per plant was obtained in cowpea at 27/19 ^{0}C than at 33/19 ^{0}C day/ night air temperature.

Number of seeds per pod

Seeds per pod were not affected by application stages in mean of years (Table 3). However year x stage interaction were significant found for this trait. The highest value was obtained from seedling stage in 2021 while the lowest value was observed in both of applications stages in 2020. Effects of cycocel doses on seeds per pod were significant in mean of the years. Control application (no cycocel) produced minumum seeds per pod (9.1) while maximum value was found in 750 ppm dose (9.8). Nevertheless differences among the other cycocel doses except in control were non-significant for seeds per pod. Interaction of year x cycocel dose was not significant. Seeds per pod in the second year (10.5) were significantly greater compared to the first year (8.6). Increase in pods per plant led to decreasing seeds per pod in the first year. Razzaque et al. (2015) reported that increasing pods per plant decreased the seeds per pod because assimilation used during seed filling is limited.

Seed weight per plant

According to mean of years, significant differences among the stage applications were not observed in seed weight per plant (Table 3). Interaction between year and application stage was significant for this trait. The highest value was achieved by cycocel application at beginning of flowering stage with 14.7 g in 2020. Seed weight per plant was significantly affected by year x dose interaction. Increase in cycocel doses increased the seed weight per plant in 2020 and the highest value was obtained from doses of 1000 ppm. In 2021, the seed weight per plant increased up to 500 ppm and then decreased in higher cycocel doses. As mean of the years, 1000 ppm cycocel dose produced maximum seed weight per plant (15.9 g) while lowest value

was obtained from control dose. Similarly, Khwairakpam and Kumar et al. (2019b) recorded that cycocel application improved seed yield per plant in mung bean. Similarly, Bhadane et al. (2020) reported that cycocel application improved seed yield per plant in mung bean. Application stage x dose interaction and differences among the years were not significant.

Table 3.The effects of cycocel doses and application stages on pods per plant, seeds per pod and seed weight per plant in mung bean

Treatments	Pods Per Plant			Seeds Per Pod			Seed Weight Per Plant (g)		
	2020	2021	Mean	2020	2021	Mean	2020	2021	Mean
Stages (S)									
1	26.3b	26.6b	26.4	8.5c	10.7a	9.6	12.1b	14.5a	13.3
2	34.2a	21.8b	27.9	8.7c	10.3b	9.5	14.7a	13.7ab	14.2
LSD 5%	YXS: 6.82		N.S.	YXS: 0.29		N.S.	YXS: 1.89		N.S.
(D)ppm	Doses (D)								
0	23.2cd	21.9d	22.6c	8.1	10.1	9.1b	10.6d	13.7bc	12.2b
500	27.1bc	24.1cd	25.6bc	8.6	10.6	9.6a	11.8cd	15.2b	13.5b
750	30.4b	23.8cd	27.1b	8.7	10.7	9.8a	12.7bcd	14.2bc	13.5b
1000	40.2a	26.9bc	33.6a	8.9	10.4	9.7a	18.4a	13.4bcd	15.9a
Mean	30.2A	24.2B	27.2	8.6B	10.5A	9.5	13.3	14.1	13.8
LSD 5%	YXD:4.53		3.21	YXD:N.S.		0.5	YXD:2.93		2.1
CV %	13					6.5	17.8		

1: Seedling 2: Beginning of flowering

100-seed weight

Application stages had no influence on 100-seed weight in mean of years. Effect of year x application stage interaction was also not significant (Table 4). The effect of cycocel doses on 100-seed weight was significant. The highest value was observed in 500 ppm (7.22 g) and thereafter decreased in increased dose. However, differences betweeen control and cycocel doses up to 1000 ppm were non-significant. The lowest value was obtained from 1000 ppm of cycocel. Bhadane et al. (2020) reported that cycocel application improved 100 seed weight in mung bean. Combined analysis showed that year x dose and stage x dose interactions were found insignificant. 100-seed weight in the first year (7.35 g) was greater than the second year (6.48 g). The increase in 100 seed weight in the first year may be due to the decrease of in the seeds per pod.

Seed yield

Application stages and cycocel doses had insignificant effect on seed yield mean of the years (Table 4). Year x stage interaction was also no significant. Mean of the years showed seed yield varied from 2096 (no cycocel) to 2330 kg ha (750 ppm). Seed yield in the first year (1912 kg ha^{-1}) which is higher plant height, lower seeds per pod and later maturation was significantly less compared to second year (2511 kg ha^{-1}).

Table 4. The effects of cycocel doses and application stages on 100-seed weight and seed yield in mung bean

Treatments	100-Seed Weight (g)			Seed Yield (kg ha^{-1})		
	2020	2021	Mean	2020	2021	Mean
Stages (S)						
1	7.45	6.36	6.91	1949	2599	2274
2	7.26	6.59	6.92	1875	2422	2148
LSD 5%	YXS: N.S.		N.S.	YXS: N.S.		N.S.
ppm	Doses (D)					
0	7.29	6.66	6.97ab	1783	2409	2096
500	7.76	6.68	7.22a	1797	2520	2159
750	7.39	6.44	6.91ab	2085	2575	2330
1000	6.99	6.13	6.56b	1983	2538	2261
Mean	7.35A	6.48B	6.92	1912B	2511A	2211
LSD 5%	YXD:0.44		0.43	YxD:N.S.		N.S.
CV %	7.4			10.0		

1: Seedling stage 2: Beginning of flowering

According to combined analysis over the years interaction of application stage x cycocel dose was significant for seed yield (Table 5). The greatest seed yield was produced by cycocel application of 1000 ppm with 2530 kg ha^{-1} at seedling stage application. However, the seed yield obtained from cycocel application of 1000 ppm at seedling stage was similar to cycocel applications of 500 and 750 ppm at seedling stage and dose of 750 ppm at beginning of flowering. The minimum seed yield was found in no cycocel at seedling stage. These results were close agreement with the finding of some studies which cycocel application may improve seed yield in various crops such as mung bean (Bhadane et al., 2020) pea (Bora and Sarma, 2006), chickpea (Güler, 2010) and rape (Pourmohammad et al., 2013).

Table 5. Interaction between application stage and cycocel doses for seed yield in combined mean over the years.

Treatments	Seed Yield (kg ha^{-1})			
Stage/ Doses	0 ppm	500 ppm	750 ppm	1000 ppm
1	1944d	2323ab	2301ab	2530a
2	2248bc	1995cd	2360ab	1992cd
LSD 5%	SXD: 266			

1: Seedling 2:Beginning of flowering

CONCLUSIONS

As mean of the years, the highest pods per plant, branches per plant, seed weight per plant was observed in 1000 ppm CCC. All of the traits were not affected by application stages according to mean of years. Increase in cycocel doses reduced the plant height and the first podding height. Seeds per pod were significant lower in control doses compared to other cycocel applications. Seed yield was affected by cycocel doses x application stages interaction. Greatest seed yield was produced by cycocel application of 1000 ppm at seedling stage. However, the seed yield obtained from cycocel application of 1000 ppm in seedling stage was similar to doses of 500, 750 ppm in seedling stage and doses of 750 ppm in flowering stage.

Other traits were not affected application stage x cycocel dose interaction. Present study revealed that cycocel application in mung bean may improve the seed yield.

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DETERMINATION OF THE HARVEST TIME OF SILAGE CORN IN HIGH ALTITUDE REGIONS

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ABSTRACT

Animal feeding with silage has become an indispensable technique all over the world. However, the cultivation of maize, which is the most important crop of silage in high altitude regions, is risky. For this reason, it is necessary to determine the varieties to be grown according to the altitude and their harvest times. This research is about the harvesting of corn varieties (SZE TC-513, Prestige and OSSK-644) with 3 different maturation periods on 3 different dates (1 September, 10 September and 20 September) in Erzurum, which has an altitude of 1860 m. The research was carried out in the experimental area of Atatürk University Plant Production and Research Center in 2012 and 2103. The field trial was set up as 3 replications according to randomized complete blocks experimental design, and the mean scores found to be significant were lettered according to the 5% probability level with the LSD multiple comparison test. Silage yield, some plant characteristics and silage quality characteristics were investigated during two years. According to the two-year average results; under current ecological conditions, Prestige and OSSK-644 varieties have higher silage yields (78.6 and 75.6 t/ha respectively). Between the harvest dates, September 20 (81.2 t/ha) was determined to give higher yields. According to the results of the research, it can be recommended to use mid-early varieties in high altitude regions and similar ecologies and to harvest them at the end of September.

Keywords: Silage maize, high altitude, variety, harvest time

INTRODUCTION

Corn (*Zea mays* L.) is the plant most used in making silage all over the world, due to its high yield, rich in soluble carbohydrates and dry matter, and easy cultivation. However, maize is a tropical grass, and it is productive in warm climates with a long development period and sufficient rainfall or irrigation. A frost-free growing period of at least 3 months is needed to grow corn safely. For this reason, the last frosts of spring and the first frosts of autumn in high altitude regions shorten the corn growing season and put corn agriculture at risk. For this reason, it is important to choose the appropriate variety and determine the harvest time for corn cultivation in high altitude regions.

For the silage corn harvest, the plants must form the cob, set the grain and reach the milk-dough stage. In the sources related to silage, it is reported that 50% of the total silage yield and 70% of the feeding value come from the cob (Açıkgöz, 2002). For this reason, varieties that do

not form sufficient cobs are deficient in terms of both yield and quality. It is also a known fact that the yields of very early varieties are low (Güney *et al*., 2010). For this reason, it should be determined which corn varieties with which growing period are suitable in a region. Güney *et al.* (2010) found that among the corn varieties they examined under Erzurum conditions, those with an FAO value between 400 and 500 reached silage maturity early, but their yield was low. They found that those with an FAO value between 500 and 600 are more suitable for this region, while those with an FAO value above 600 give good results for some years, and for some years they cannot reach the desired maturity.

Adjusting the harvest time in silage corn is important for both the yield of the plant and the quality of the silage. When the plants are harvested early, they are rich in water and low in carbohydrates; when it is harvested late, a very hard grained and dried forage is obtained. If the grains become too hard, their evaluation by animals becomes difficult, and the silage gains a straw appearance (Kılıç, 1986). If the water content of the plant is high, the risk of leakage increases. In the appropriate harvest period, the cobs should be mature enough. Demarguilly (1973) stated that if the corn to be ensiled is harvested earlier than the dough formation period, the amount of dry matter produced per hectare decreases and the nutrient losses increase by leaching during ensiling. Kılıç and Gül (2007) determined that the most suitable harvest time for obtaining high dry matter and silage quality in Diyarbakır is the hard dough stage. However, this maturation takes place as warm weather permits. As a matter of fact, in studies conducted at high altitudes in the Eastern Anatolia Region, there are reports that silage corn is damaged by cold in the first week of September in some years (Güney, 2005). This shows that it will not be advantageous to wait longer in similar ecologies. For this reason, it is also necessary to determine the harvest dates in high altitude regions.

This study deals with the harvest dates of corn varieties with different developmental periods at different times in high altitude regions, which are risky for silage maize cultivation. In the study, it was tried to determine the appropriate ripening value and harvest dates for maize in a region with a high altitude and continental climate.

MATERIALS AND METHODS

The research was carried out in the irrigated trial area of Atatürk University Faculty of Agriculture in 2012 and 2013. In the study, 3 different varieties of corn (*Zea mays* L.) (SZE TC-513, Prestige and OSSK-644) and 3 different harvest dates (1 September, 10 September and 20 September) were used. The varieties used were selected from materials with different FAO values (early-FAO: 500, mid-early-FAO: 550 and mid-late-FAO: 640). The research was established in the randomized complete blocks experimental design with 3 blocks according to the factorial arrangement.

Sowing was done in a pre-prepared seed bed with 70 cm row spacing and 15 cm row spacing. There were 4 rows in the parcels, the width of the parcel was 2,8 m, the length of the parcel was 3 m and the area was 8,4 m^2. As fertilizer, 150 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1} were applied. All of the phosphorus fertilizer was mixed by sprinkling on the plots during the seed bed preparation, and the nitrogen fertilizer was divided into two parts, half of which was applied during planting and the other half when the plants were 40-50 cm tall. After the planted plants completed the emergence, the first weed control was carried out in the form of hoeing at a height of approximately 20-25 cm. In this hoeing, the plants were diluted to be 15 cm above the row. The second hoe was made in the form of throat filling when the plants were about half a meter tall. The second part of the nitrogen fertilizer was given before this application. Taking

into account the rainfall and the morphological structures of the plants, flood irrigation was done according to the need (Tan, 2018).

In the research, plant height and ear ratio were determined by cutting 5 plants from the root collar of the middle rows during harvest. In the harvests, one row at the edges of the parcel and 0,5 m from the heads were discarded as the edge effect, and the remaining area (2,8 m^2) was harvested. After the harvested plants were weighed as wet, they were first dried in the open air for a week and then dried in a drying oven set at 60 °C for 48 hours, and dry matter ratio and dry matter yield were determined. The methods followed by Güney (2005) and Geren *et al.* (2003) were used to determine the morphological and agricultural characteristics. Crude protein ratios were determined by Mikro Kjeldahl method (Kacar, 1984), ADF (Acid Detergent Fiber) and NDF (Neutral Detergent Fiber) ratios were determined with the help of ANKOM Fiber Analyzer according to the principles stated by Van Soest (1963). The relative feed value (RFV) is Rohweder *et al*. (1978), dry matter digestion and dry matter consumption were determined by calculation.

The two-year data obtained in the research were subjected to variance analysis according to the randomized complete blocks experimental design. Analyzes were made with the help of MSTAT-C package program. The differences between the means were compared and grouped at the 5% probability level according to the LSD Multiple Comparison Test.

Erzurum province, where the research was conducted, has an altitude of 1869 m and is located on 39° 51' north latitude and 41° 61 ' east longitude. The continental climate prevails in the province, with cold and snowy winters and cool and dry summers. Autumn and spring, which are the transitional seasons, are short, and the winter period is long. Some climate data of Erzurum province for the years 2012 and 2013 and the long-term average are shown in Table 1. In the first year of the experiment (2012), the total precipitation amount (313,4 mm) was below the long-term average, the monthly average temperature (5,6 °C) was at the same level as the long-term average. In the second year of the experiment (2013), precipitation values were lower than both 2012 and the long-term average. However, the monthly average temperatures in the second year of the experiment are close to both 2012 and long-term averages. In May-August, when plants are actively growing, temperatures were close to each other in both years, except for June 2012, it was more rainy.

Table 1. Some climatic data of Erzurum province for 2012, 2013 and the long-term average (LTA)[1]

Months	Total Precipitation (mm)			Mean Temperature (°C)		
	2012	2013	LTA	2012	2013	LTA
January	6,7	28,7	19,6	-8,8	-9,5	-9,3
February	22,2	28,5	23,1	-14,6	-7,4	-7,9
March	8,4	30,9	32,0	-6,7	-0,8	-2,3
April	37,2	36,3	51,5	7,2	7,2	5,5
May	73,0	32,3	70,3	11,0	11,5	10,6
June	7,0	25,1	46,7	15,7	15,0	14,9
July	19,8	7,8	25,8	19,0	19,4	19,3

August	22,8	5,2	16,5	22,0	19,5	19,4
September	11,0	11,5	22,5	15,0	13,6	14,6
October	41,7	17,2	46,8	9,4	6,0	8,0
November	34,2	19,6	30,7	3,8	2,3	0,7
December	29,4	8,3	20,5	-5,9	-13,4	-6,1
Total/Mean	313,4	251,4	406,0	5,6	5,3	5,6

1 It was taken from the data of Erzurum Meteorology Regional Directorate.

The texture class of the soils of the study is clay-loam. According to the EC and % salt values of the soil, it is seen that there is no salinity problem and it is in the salt-free class. It has a pH value of 7,56 and is slightly alkaline, with a lime rate of 1,14% and a slightly calcareous structure. P_2O_5 and K_2O values suitable for plants in the soil are 44.1 kg ha^{-1} and 1710 kg ha^{-1}, respectively, phosphorus amount is low and potassium amount is sufficient. The organic matter content in the soil is insufficient (1,01%; Anonymous, 2019).

RESULTS AND DISCUSSIONS

In the second year of the study, silage maize plants were found to be taller, cob ratios and dry matter were higher. Accordingly, their silage yields are higher (Table 2). Differences in climatic characteristics between years can lead to significant differences in characteristics such as plant height (Öztürk *et al*., 2008). This may be due to the fact that precipitation was higher in 2013, especially in the months in which the experiment was conducted.

In the study, variety selection significantly affected plant height, cob ratio, dry matter ratio and silage yield of silage mass Sorting, cob ratio and dry matter content are genetic characteristics of plants and emerge when environmental conditions allow. In this study, the earliest cultivar, TC-513, was shorter (179,0 cm), while the cultivar with the highest ear rate (42,13%) and dry matter rate (24,83%). Later maturing varieties have longer plant heights, but lower ear and dry matter ratios. Many researchers working with different corn varieties pointed to similar results (Kim *et al*., 2001; Kılıç and Gül, 2007; Güney *et al.*, 2010; Kaya and Kuşaksız 2012; Guyader *et al.,* 2018).

Since harvesting at different dates affected the development times of the plants, it led to an increase in length, an increase in the cob and dry matter ratio, and an increase in silage yield (Table 2). The lowest yield was determined at the harvest on September 1 with 68,9 t ha^{-1}, while the highest yield was obtained from the last harvest date with 81,2 t ha^{-1} With the delay of the harvest date; Kaya and Kuşaksız (2012) reported that plant height, Rabelo *et al.* (2015) determined that the ear rate and Çağrı (2020) determined silage yield increased.

Table 2. Silage yield and some characteristics of corn varieties harvested on different dates*

Applications	Plant Height (cm)	Ear Ratio (%)	Dry Matter Ratio (%)	Silage Yield (t ha^{-1})
Variety				
SZE TC-513	179,0 C	42,13 A	24,83 A	70,0 B

Prestige	195,7 B	31,91 B	24,53 AB	78,6 A
OSSK-644	211,0 A	30,05 C	24,04 B	75,6 A
Harvest Date				
1 September	186,9 B	30,19 C	21,69 C	68,9 C
10 September	194,2 B	35,12 B	24,77 B	74,0 B
20 September	204,6 A	38,77 A	26,94 A	81,2 A
Year				
2012	182,5 B	31,74 B	22,78 B	73,7
2013	207,9 A	37,66 A	26,16 A	75,8
Mean	195,2	34,70	24,47	74,7
Variety x H. Date	ns	0.05	ns	0.05

*Means marked with the same letter are statistically similar. Statistically significant at the 5% level, ns: non-significant

In the study, harvest dates had the greatest effect on the quality parameters of silage maize, and the effect of cultivars was found to be insignificant (Table 3). In 2012, the first year of the study, crude protein ratio and RFV value were found to be higher than the other year. This may be related to the lower content of ADF and NDF in silage material in 2012.

The effects of cultivars used in the study on silage quality parameters were not found significant. Depending on the cultivars, crude protein ratio was 9,58-9,83%, NDF ratio was 39,83-40,24%, ADF ratio was 34,00-34,21% and RFV value showed insignificant changes between 144,4-145,9%.

Delayed harvest dates significantly affected feed quality in silage maize. As the harvest time was delayed, crude protein ratio decreased, irregular changes were observed in ADF and NDF ratios, and RFV value increased. These irregular changes may have occurred due to the increase in the cob ratio in the forage, although the structural materials increased with over time. Horst *et a.l* (2021) also determined that crude protein ratio decreases with maturation.

Table 3. Some nutritional value characteristics of silage maize varieties harvested on different dates*

Applications	Crude Protein (%)	NDF (%)	ADF (%)	RFV
Variety				
SZE TC-513	9,58	39,83	34,11	145,9
Prestige	9,83	40,24	34,21	144,4
OSSK-644	9,63	40,24	34,00	144,7

Harvest Date				
1 September	9,98 A	40,40 A	34,45 A	143,3 B
10 September	9,82 A	40,83 A	33,72 B	142,9 B
20 September	9,23 B	39,07 B	34,16 AB	148,7 A
Year				
2012	10,08 A	39,89	33,48 B	146,9 A
2013	9,29 B	40,32	34,73 A	143,0 B
Mean	9,68	40,10	34,30	145,0
Variety x H. Date	ns	ns	ns	ns

*Means marked with the same letter are statistically similar. Statistically significant at the 5% level, ns: non-significant

CONCLUSION

It has been revealed in many studies that the variety and harvest time have an effect on the yield and feed quality of silage maize. This research focused on the determination of the corn varieties to be grown for silage and the harvesting times in high altitude regions such as Erzurum. According to the results of the research, harvesting at a later date resulted in a higher yield as it provided a longer development period for the plants. It also led to an increase in the cob ratio and dry matter ratio, which are of great importance for silage. Filya (2002) states that the dry matter ratio in silage corn should be more than 20%, and even around 35% gives better results. Harvests done at the wrong time cause high losses with leakages after silage, or decrease silage quality (Hunt *et al*., 1989). In this study, since the low temperature that causes freezing did not occur in September in the years in which the research was carried out, it was revealed that September 20 was more suitable for harvesting. Prestige and OSSK-644 varieties with longer growth times were found to be more productive because an early autumn low temperature did not occur.

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DETERMINATION OF LOW TEMPERATURE RESISTANCE IN *LOLIUM PERENNE* L. GENOTYPES COLLECTED FROM HIGH ALTITUDE REGION

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ABSTRACT

Perennial ryegrass (*Lolium perenne* L.) is an important species both as a forage plant and as a turfgrass. One of the most important reasons limiting the use of the plant is low temperatures. This research is planned to provide material for the breeding program to be started for low temperature resistance in perennial ryegrass. For this purpose, approximately 1000 clones of genotypes were collected from the high altitude regions of the Eastern Anatolia Region (Erzurum, Erzincan, Kars, Bayburt, Ardahan, Ağrı) in 2019. These clones were planted in Atatürk University Plant Production and Research Center experimental field and evaluated in terms of turfgrass and forage plant parameters for two years (2020 and 2021). Considering the two-year results, 46 turfgrass type and 62 forage type plants were selected from these genotypes. These selected plants were cloned and grown in viols in 2022 and subjected to cold resistance tests together with control cultivars. As a result of the test carried out in the growth chamber, the deaths of the genotypes from low temperature were between -1 ^{o}C and -15 ^{o}C. Forage type control variety (Lipresso) was completely damaged by cold at -9 ^{o}C, and 43 lines more resistant than this variety were determined. On the other hand, the control variety (Esquine), which is a turfgrass type, was frozen at -13 ^{o}C, and 5 lines were determined that were based on a lower temperature than the control variety. Studies should continue to develop new varieties related to these lines that are more resistant to low temperature than control varieties.

Keywords: Perennial ryegrass, genotypes, cold resistance

INTRODUCTION

Native to Europe, Asia, and North Africa, the perennial ryegrass (*Lolium perenne* L.) is a worldwide cultivated perennial cool season forage crop that has been cultivated and bred for over 100 years (Sun et al., 2020; Zhang et al., 2020). Perennial ryegrass, also known as English ryegrass, is known as the most used turfgrass type in green areas (Yılmaz and Kısakürek, 2018). It is used for green or dry hay production or silage material in humid and regular rainfall areas.

It is a suitable roughage because it is easy to grow, resistant to form and grazing, fast growth and development, and its grass is delicious and nutritious. It is a suitable roughage because it is easy to grow, resistant to mowing and grazing, fast growing, and its grass is delicious and nutritious. (McGrath, 2008; Açıkgöz 1991; Herridge et al., 2021). Türkiye is in the natural spreading areas of perennial ryegrass, it is not cultivated in agricultural lands and is only found in natural pasture areas as a forage crop (Elçi, 2005).

It has been reported that the genetic diversity of perennial ryegrass is high in natural vegetations because it has cross-pollination (Bolaric et al., 2005; Humphreys et al., 2003). **Elçi** (2005), stated that perennial ryegrass grows naturally in every region of Türkiye, Bolaric et al. (2005) also stated that the limits of the genetic diversity of this plant are not known and that it is an interesting plant for breeding programs. Drought, high salinity and extreme temperatures are the main limiting factors frequently encountered in the production of perennial ryegrasses, as with many plants. Among these stresses, which have become more complex with the effect of climate change, it is estimated that the incidence of abnormal temperatures will be higher in the future (Miao et al., 2022).

One of the most important problems of perennial ryegrass culture is that it is not resistant to low temperatures. It is known that perennial grass has low tolerance to cold stress compared to other cool season grasses (Tan, 2018). However, some sources reported that more resistant perennial ryegrass genotypes were found than species such as red fescue and bentgrass (Jones, 1984). In a study investigating the cold resistance of perennial grass cultivars, it was stated that there was a close relationship between the maturity of the cultivars and their cold resistance and survival rates (Fuller and Eagles, 1978). Perennial ryegrass genotypes collected in Romania were subjected to artificial freezing tests and their survival temperatures (LT50) were found to be in line with the average temperature in the coldest months of the place where they were collected (Tcacenco et al., 1989). Therefore, local genotypes need to be evaluated in order to develop new low temperature tolerant varieties. Especially natural genotypes grown at high altitudes are materials that can be used for this purpose. This research was planned to determine the low temperature tolerance of genotypes collected from high altitude regions in order to develop low temperature tolerant cultivars in perennial ryegrass.

MATERIAL AND METHODS

The plants that make up the material of the research were collected from the provinces in the Northeast Anatolian Region (Erzurum, Ağrı, Ardahan, Bayburt, Erzincan, Kars and Iğdır) in 2019. Material collection was done in the form of removing the plants with soil. Random sampling method was used in these collection studies (Tan and Taşkın, 2018; Şehirali and Özgen, 2012). At least 10 plants were removed by looking at the phenotype of the plants at each stop where the collection was made. During the collection, location information, altitude and coordinates were recorded. As a result of the collection studies carried out from 100 points, 1000 genotypes were included in the study. The plants, which were removed from the field with soil, were brought to Atatürk University Herbal Production Application and Research Center greenhouses and transferred to pots without wasting time. The shoots of the plants were cut with the help of scissors and planted in separate pots. The rooted plants were transferred to Atatürk University Herbal Production and Application Center experimental areas in April 2020.

In the field study established in 2020, individual plant investigations were carried out for two years (2020 and 2021) and phenological and morphological observations and measurements were carried out. According to the data obtained, the result of forage and turf type plants were determined. These studies were carried out according to the principles of the UPOV and Certification Center Directorate of the Ministry of Food, Agriculture and Livestock (Anonymous, 2002According to the data obtained, 62 forage and 46 turf type plants were selected. Clones were taken from these selected plants, grown in viols and subjected to cold resistance tests together with control varieties. The cold resistance tests, which were taken as the basis for the determination of crown measurements, were carried out in the growth cabinet of Atatürk University Faculty of Agriculture, Department of Field Crops. Each plant was cloned

and placed in 10 plant growing cabinets. When the plants, which were initially kept at 15-25 ºC for 1 month, grew 10-15 cm, the cabin temperature was gradually reduced to 5 ºC and acclimation was provided to the low temperature. Then, the temperature was decreased by 2 ºC every 24 hours and it was determined to what temperature the genotypes could survive (Humphreys and Eagles, 1988; Tcacenco *et al*., 1989; Crosatti *et al*., 2008). The simple statistical values of the obtained data were calculated and lettered at the 5% probability level according to the Duncan multiple comparison test.

RESULTS AND DISCUSSIONS

Field conditions are not only a widely used method to determine the cold resistance of the plant, but also very easy and important in terms of being the main place of the plant (Fowler et al., 1981). However, definite results cannot be obtained in determining the temperature range in which plants are resistant to cold (Fowler and Gusta, 1979). Because the concept of enduring winter is a very complex subject. For this reason, cold tests carried out under controlled conditions are more suitable for determining the cold tolerance of plants. In addition, the detection of cold resistance differences between plants and obtaining results in a shorter time than field conditions are other advantages (Roberts and Grant, 1968; Fowler et al., 1981, Pomeroy and Fowler, 1973). It is known that cold tolerance studies in which field conditions and controlled laboratory conditions are carried out together, as in this study, give safer results.

In November 2021, 10 pieces of each of the selected plants were cloned and transferred to large-size viols. Plants kept under controlled conditions for 1 month in Atatürk University Herbal Production, Research and Application Center greenhouses were ensured to root and sprout. Plants reaching a plant height of 10-15 cm were then gradually reduced from 15 °C to 5 °C in the growth cabinet in the Department of Field Crops of the Faculty of Agriculture, and acclimatization to low temperature was provided first. Then, the temperature was decreased by 2 oC every 24 hours and it was determined to what temperature the genotypes could survive (Humphreys and Eagles, 1988; Tcacenco *et al*., 1989; Crosatti *et al.*, 2008). In order to test the resistance of plants to low temperature with control varieties, control varieties planned in the project were also grown in greenhouses under pot conditions. At the same time, clones of these cultivars were taken and included in the cold resistance tests. The results revealed that both forage and turf type genotypes showed variation in cold tolerance (Table 1).

Table 1. Some simple statistical values of selected forage and turf type genotypes in perennial grass (Lolium perenne L.)

Genotypes	Minimum	Maximum	Mean	Standart Deviation	Coefficient of Variation
Turf Types	-15	-1	-11	3.751	35.09
Forage Types	-15	-1	-12	3.724	35.40

The results of the turf type genotypes of perennial grass (*Lolium perenne* L.) and the temperatures at which crown deaths occur are shared in Table 2. The degrees of death from low temperature of the genotypes varied. It is an expected result that the death rates of plant material with different properties and collected from different places are different. The death rates of the genotypes varied between -1 ºC and -15 ºC. University, Kuşçu-1, Gökdağ, Gümüşsu, Yoğurtlu and Güzeltepe are the genotypes that die at -15 ºC with higher tolerance to cold than the others.

Çayırköprü, Yamaçlı and Pişkidağ genotypes, on the other hand, have the lowest cold tolerance with a death temperature of -1 ^{o}C. Perennial grass is known to be more sensitive to low temperature than many cool season grasses in the same category (Tan, 2018). In this study, the presence of genotypes that can survive down to -15 ^{o}C is promising for the results of the research. The control variety lost its viability at -13 ^{o}C. Six of the local genotypes withstood lower temperature than the control. It has also been reported by other researchers that low temperature resistance among local genotypes is high (Küçüközdemir, 2016). Researchers have reported that the frost tolerance of wild forms of perennial ryegrass plants in natural vegetation has a significant genetic variation (Wilkins 1991; Hulke *et al.*, 2007). In another study, it has been reported that the cold acclimation process or exposure to low non-lethal temperatures in perennial ryegrass significantly increases frost tolerance (Ebdon *et al.,* 2002).

Table 2. Low temperature death rates of selected turf type genotypes in perennial ryegrass (Lolium perenne L.)

No	Genotypes	Degree of Death (°C)	No	Genotypes	Degree of Death (°C)
1	University	-15 I	25	Maden	-11 F
2	Kalor	-13 GH	26	Gez	-13 GH
3	Arıbahçe-1	-13 GH	27	Eğerti-2	-9 E
4	Arıbahçe-2	-11 F	28	Büyükgeçit	-11 F
5	Uzunyayla-1	-13 GH	29	Alaca	-7 D
6	Uzunyayla-2	-11 F	30	Gümüşsu	-15 I
7	Kuşcu-1	-15 HI	31	Dadaşkent	-9 E
8	Kuşcu-2	-13 F G	32	Yamaçlı	-1 A
9	Kahramanlar	-9 E	33	Pişkidağ	-1 A
10	Çiftlikköy-1	-13 GH	34	Bayırbağ	-9 E
11	Çayırlar-2	-13 GH	35	Altınbaşak	-13 GH
12	Çayırca	-13 GH	36	Yoğurtlu	-15 I
13	Uluköy-1	-3 B	37	Kömürköy	-7 D
14	Uluköy-2	-5 C	38	Akdağ	-11 F
15	Demirbağ	-9 E	39	Ağıl	-13 GH
16	Halitpaşa	-13 GH	40	Ahmetli	-11 F
17	Beyler	-9 E	41	Eymür	-11 F
18	Gökdağ	-15 I	42	Bayrampaşa	-13 GH
19	Aksu	-11 F	43	Göldere	-13 GH
20	Güllük	-11 F	44	Söğütlü	-13 GH
21	Aşağıkırzı	-13 GH	45	Güzeltepe	-15 I
22	Çayırköprü	-1 A	46	Posof-2	-7 D
23	Taşkesen	-9 E	Control	Esquine	-13 GH
24	Alıççık	-11 F	Mean		-10,69

Means marked with the same letter are indistinguishable at the 0.05 level.

The results of the death rates of the forage genotypes of the perennial ryegrass (*Lolium perenne* L.) plant are given in Table 3. Similarly, the temperatures at which the crown death of the genotypes occurred varied between -1 ^{o}C and -15 ^{o}C. The number of damaged genotypes increased with decreasing temperature. University, Kuşçu-1, Gümüşsu, Saztepe, Yoğurtlu and Güzeltepe are the genotypes that die at -15 ^{o}C with higher tolerance to cold than the others.

Yarbaşı, Çayırköprü, Yamaçlı and Pişkidağ genotypes are the ones with the lowest cold tolerance, dying at -1 ^{o}C. It is known that the cold tolerance of plants differs even in the species and variety of the same plant, depending on the region where they are grown, growing conditions, age and crown structure (Alnuaimi, 2019). As a matter of fact, in our study, the cold tolerance thresholds of the plants collected from different growing environments were determined to be different, and there are genotypes that come to the fore by surpassing the commercial varieties (Table 3). Control variety Lipresso lost its viability at -9 ^{o}C. However, many genotypes collected from the region showed greater resistance than the control.

Table 3. Low temperature death rates of selected forage genotypes in perennial ryegrass (*Lolium perenne* L.)

No	Genotypes	Degree of Death (°C)	No	Genotypes	Degree of Death (°C)
1	Üniversite	-15 H	33	Eskipolat	-11 F
2	Kuşcu-1	-15 H	34	Eğerti-1	-13 G
3	Kuşcu-2	-13 G	35	Eğerti-2	-9 E
4	Kahramanlar	-9 E	36	Büyükgeçit	-11 F
5	Çiftlikköy-1	-13 G	37	Alaca	-7 D
6	Çiftlikköy-2	-11 F	38	Kandilli	-11 F
7	Dadaşköy-1	-13 G	39	Çayköyü	-13 G
8	Dadaşköy-2	-9 E	40	Gümüşsu	-15 H
9	Çayırlar-1	-13 G	41	Dadaşkent	-9 E
10	Çayırlar-2	-13 G	42	Yarımca	-13 G
11	Beypınarı-1	-11 F	43	Yamaçlı	-1 A
12	Beypınarı-2	-7 D	44	Pişkidağ	-1 A
13	Çayırca	-13 G	45	Saztepe	-15 H
14	Ilıca	-13 G	46	Akyazı	-13 G
15	Gürlevik-1	-7 D	47	Beşiktaş	-11 F
16	Gürlevik-2	-11 F	48	Yoğurtlu	-15 H
17	Çağlayan-1	-13 G	49	Ulalar	-13 G
18	Uluköy-1	-3 B	50	Kömürköy	-7 D
19	Uluköy-2	-5 C	51	Akdağ	-11 F
20	Yarbaşı	-1 A	52	Tütenli	-5 C
21	Arpalı	-13 G	53	Kınalıtaş	-13 G
22	Nişantaşı	-11 F	54	Gökçedere	-13 G
23	Aşağıkırzı	-13 G	55	Bayrampaşa	-13 G
24	Çayırköprü	-1 A	56	Kitre	-11 F
25	Taşkesen	-9 E	57	Çayıryolu	-13 G
26	Alıççık	-11 F	58	Göldere	-13 G
27	Maden	-11 F	59	Söğütlü	-13 G
28	Gez	-13 G	60	Güzeltepe	-15 H
29	Masat	-11 F	61	Posof-1	-11 F
30	Sındıran	-9 E	62	Posof-2	-7 D
31	Başçakmak	-7 D	Control	Lipresso	-9 E
32	Gelinkaya	-11 F	Mean		- 10,52

Means marked with the same letter are indistinguishable at the 0.05 level.

CONCLUSION

Perennial ryegrass genotypes collected from the natural vegetation of the Northeast Anatolian Region showed a high variation in mortality from low temperature. This is an expected and desirable situation in order to develop low temperature tolerant varieties. 6 genotypes in turf type and 42 genotypes in forage type showed a more resistant performance to cold stress than commercial (control) varieties. In this research, which includes field and laboratory studies, genotypes with high cold tolerance can be used as materials for the development of new cold-resistant varieties.

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THE EFFECTIVENESS OF TWO DIFFERENT GNRH ANALOGUES WITH OR WITHOUT BETA CAROTENE + VITAMIN-E USED IN OVULATION SYNCHRONIZATION IN HOLSTEIN HEIFERS

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ABSTRACT

In this study, it was aimed to investigate the effects of Ovsynch protocol using two different GnRH analogues with or without β-carotene + Vitamin E on pregnancy rates and ovulation time. For this purpose, 80 Holstein breed heifers aged at least 15 months and 350 kg weight were used. All animals were divided into four groups and subjected to the same Ovsynch® procedure (Groups 1, 2, 3, and 4). As the GnRH agent for the Ovsynch protocol, Buserelin acetate in Groups 1 and 2, and Lesireline acetate in Groups 3 and 4 were used. In addition, a single injection of β-Carotene+Vitamin-E was implemented in the heifers in Groups 2 and 4 on 7 days before the initiation of Ovsynch. All heifers were inseminated 20 hours after the last GnRH administration. In the study, the transrectal ultrasonographic examination was performed on heifers during the Ovsynch protocol, 20, 36, and 48 hours after the second GnRH injection, and 30 days after insemination. On the specified days, blood samples were also taken for the evaluation of β-Carotene, vitamin E, and progesterone (P4) levels. The highest pregnancy rate was detected in Group 4 (60%), and pregnancy rates in Groups 1, 2, and 3 were obtained at 40%, 50%, and 50% respectively ($P > 0.05$). While β -carotene and vitamin E levels were found significant ($P < 0.05$) between non-pregnant and pregnant heifers in all groups, no significant changes in serum progesterone levels were observed ($P > 0.05$). However, the difference between all groups was statistically significant when ovulation rates were evaluated ($P<0.05$). In conclusion, it was detected that the long-action GnRH analogues and the combination of β-carotene and Vitamin E used in the Ovsynch protocol increased the pregnancy rates in heifers. The combinations are thought to can be used especially as an effective and inexpensive method for getting pregnant in a short time after puberty in heifers.

Keywords: β-carotene, GnRH, Heifer, Synchronization of Ovulation, Vitamin E

INTRODUCTION

Fertility programs for dairy cows and heifers are essential to raise dairy herd profitability. (William et al., 2020). Generally, a calving interval of about one year is considered to be an optimal indicator of the fertility and profitability of dairy herds (Temesgen et al., 2022). Holstein heifers achieve mating maturity when they reach 21.2-24.8 months of age and at least 340-363 kg body weight. Puberty in heifers is dependent on many factors including, but not limited to, breed, age, and body weight (Stevenson and Ahmadzadeh, 2011). There is considerable variability between farms in the way that heifer fertility is managed (Wathes et al., 2014). One of the important problems in dairy heifers is their inability to become pregnant on time after reaching mating maturity. If cows cannot become pregnant after their first artificial insemination, it leads to further economic losses and expenses. These included expenses for replacement heifers, the added cost of extra feed provided for additional days, increased labor expenses involved in managing the animals, additional breeding costs, and the financial implications of calf loss and reduced milk production due to the extended open period. (Tadesse et al., 2022). Due to these factors timely artificial insemination of heifers is economically very

important. For this purpose, numerous oestrus synchronization protocols have been developed in recent years (Bisinotto et al., 2014). Fertile timed insemination is achieved through the strategic administration of gonadotropin releasing hormone (GnRH), Prostaglandin (PG) F2α, and Progesterone (P4) (William et al., 2020). The success rates of pregnancies achieved through these protocols in heifers vary (Akçay et al., 2022). To increase the pregnancy rate in heifers, it is important to synchronize ovulation as well as estrus. In recent years, different ovulation synchronization methods have been used extensively to increase fertility.

In the presented study, the effect of the Ovsynch procedure performed by applying two different GnRH analogues and β carotene vitamin E in heifers on ovulation time and pregnancy rate was investigated.

MATERIAL AND METHOD

Animals

The study was conducted on a farm located in Güneşli town of Kayseri province. Eighty Holstein heifers, aged between 15-24 months, and with no reproductive problems according to the records, were used in this study. The heifers were housed in free-roaming paddocks and had access to water ad libitum. They were fed twice a day with a ration that included alfalfa and corn silage. Furthermore, all animals were negative for diseases such as IBR-IPV, BVD-MD, Brucella, Tuberculosis, and Leptospirosis. Ethical approval was obtained from the Erciyes University Local Ethics Committee for Animal Experiments (2008/12).

Synchronization Procedure

The following substances were used in this study for synchronization: Dalmavital (15 mg β-Carotene and 20 mg dl-α-Tocopherol acetate/ml, Vetaş, Turkey), which contains β-Carotene and vitamin E; Dalmarelin (Lesirelin acetate, Vetaş Turkey) and Receptal (0.004 mg Buserelin acetate/ml, Intervet, Turkey), both of which are GnRH analogues; and Dalmazin (0.075 mg D-Cloprostenol/ml, Vetaş Turkey), a prostaglandin.

Eighty heifers included in the study were divided into four groups and the Ovsynch procedure was applied to all animals (Groups 1, 2, 3, and 4).

Fifteen milliliters Dalmavital was intramuscularly (im) administered to the heifers in Groups 2 and 4 on seven days before the application of GnRH. On day 0, 2.5 ml Dalmarelin (62.5 mg lesirelin acetate) and 2.5 ml Receptal (0.0105 mg buserelin acetate) were administered im to the heifers in groups 3-4 and groups 1-2, respectively. Seven days after the first GnRH administration, 2 ml Dalmazin (0.150 mg D-cloprostenol) was administered im to animals in all groups.

48 h after the injection of Prostaglandin F2α, 2.5 ml of Dalmarelin was administered im to the heifers in Groups 3 and 4 while 2.5 ml of Receptal was administered to the heifers in Groups 1 and 2. All animals in the groups were inseminated once via the rectovaginal route, 20 hours after the last GnRH application. The sperms were thawed at 35°C for 45 seconds.

Ultrasonographic Examination

Transrectal ultrasonographic examination was performed to determine ovarian structures, follicle dynamics, and ovulation times at -7, 0, 7, 9, and 10-12 days and to check pregnancy on the 40th day on all heifers.

Blood Samples

Blood samples were collected from animals in order to determine and compare the hormonal and biochemical parameters in Dalmavital administration, in the first and second administration of GnRH, at the stage of PGF2α injection and at 24-hour intervals until 48 hours after the second GnRH injection. The obtained serum samples were stored at -20°C for later use.

Analysis of Serum β-Carotene, Vitamin E, and P4

Serum β-Carotene and vitamin E levels were determined according to the methods described by Suzuki and Katoh, (1990) and Martinek (1964), respectively. Serum progesterone analyzes were performed using the microtitration plate enzyme immunoassay method reported by Prakash et al. (1987).

Statistical Analysis

Pregnancy rates, CL and follicle findings, and ovulation rates obtained in the study were analyzed using the chi-square method. Variance (ANOVA) analysis and t-test methods were used to compare β-carotene and vitamin E levels within and between groups.

The Kruskal-Wallis test and Mann-Whitney test were utilized to compare progesterone levels between the groups, and evaluate them within themselves, respectively. These tests were performed with the SPSS 15.0 statistical program. Friedman two-way analysis of variance was used in the SigmaStat program to evaluate the β-carotene, vitamin E, and progesterone values between days.

RESULTS AND DISCUSSION

The mean age of the heifers (n=80) included in the study groups was 17.45±2.49 months, and their live weight was 400.97±60.28 kg. The pregnancy rates in Groups 1, 2, 3, and 4 were determined as 40% (8/20), 50% (10/20), 50% (10/20), and 60% (12/20), respectively. In the statistical evaluation of the pregnancy rates obtained, although there was a numerical increase observed between the groups, it was detected that this increase was not statistically significant ($P>0.05$).

In the ultrasonography examinations, no statistical difference was found in the numbers of animals with and without CL on the -7, 0, 7, and 9 days of the study when comparing groups ($P>0.05$). Also, there was no statistically significant difference observed in the numbers of animals with and without follicles in the groups, at the 9th day of the study, at the time of insemination, and 36 and 48 hours after the second GnRH injection ($P>0.05$).

The study groups were compared in terms of the numbers of ovulations determined in ultrasonographic examinations performed at 20, 36, and 48 h after the second GnRH injection, a statistically significant difference was observed between the groups ($P<0.05$) (Table 1). The ovulation occurred between 20-36 hours in Group 4, where the highest pregnancy rate was obtained, while it occurred between 36-48 hours in the other groups (Groups 1, 2, and 3).

Table 1. Ovulation findings determined in ultrasonography examinations performed 20, 36, and 48 h after the second GnRH injection in the study groups.

Groups	0-20. h	20-36. h	36-48. h
Group 1	1	2	10
Group 2	2	2	12
Group 3	3	2	9
Group 4	3	10	1
		P<0.05	

While there was no statistical difference between the β-carotene levels on day -7 in serum samples taken from heifers that conceived and those that did not conceive, significant differences were observed between the β-carotene levels on days 0, 7, 9, 10, and 11.

When the β-carotene levels of the heifers that were pregnant and did not conceive within the study groups at -7, 0, 7, 9, 10, and 11 days were compared, it was determined that there was no difference in Groups 1 and 2 (P>0.05). A statistical difference was found between β-carotene levels in Groups 3 and 4, on day 7 and day 0, respectively, (P<0.05).

In heifers that conceived or did not conceive, no difference was detected in β-carotene levels on days -7, 0, 7, 9, 10, and 11 between Groups 1 and 3 (P>0.05), while a significant difference was found in Groups 2 and 4 (P>0.001) (Table 2). A significant statistical difference was found in the vitamin E levels of the animals that conceived and did not conceive in Groups 2 and 4 on days -7, 0, 7, 9, 10, and 11 (P<0.05). There was a statistically significant difference between the vitamin E levels of the nonpregnant and pregnant heifers in groups 1 and 3 on the specified days (P<0.05) (Table 3). It was found that there was no statistical difference between the progesterone levels at -7, 0, 7, 9, 10, and 11 days in pregnant and nonpregnant animals in Groups 1 and 4. However, a significant statistical difference in the progesterone levels was detected on the -7th and 7th days in Groups 2 and 3 (Table 4).

Table 2. Comparison of β-carotene values of pregnant and non-pregnant animals in the study groups (µg/dl) (Mean±SD)

		-7. Day	0. Day	7. Day	9. Day	10. Day	11. Day
Group 1	**Pregnant**	66.26±11.53	67.49±12.35	75.87±19.97	73.26±18.53	71.08±13.27	74.81±18.17
	Non-Pregnant	75.13±16.55	72.67±20.72	75.75±14.78	77.39±24.38	75.71±18.69	77.42±17.30
				P>0.05			
Group 2	**Pregnant**	76.82±26.99	139.53±29.45	113.95±22.88	113.18±16.10	109.46±18.75	100.97±27.70
	Non-Pregnant	81.92±15.10	139.03±21.11	115.08±17.86	111.98±10.89	100.93±16.01	91.47±14.92
				P>0.05			
Group 3	**Pregnant**	68.64±17.21	69.42±21.24	62.83±9.74	67.71±10.80	68.18±11.66	70.27±10.95
	Non-Pregnant	71.78±17.57	79.30±25.98	73.64±26.03	67.10±18.50	72.87±22.42	71.20±11.90
		P>0.05		P<0.05		P>0.05	
Group 4	**Pregnant**	73.29±11.64	144.70±25.64	126.23±17.46	119.25±18.22	124.94±15.58	117.86±17.70
	Non-Pregnant	68.60±10.60	125.44±14.45	131.59±20.18	117.68±17.77	117.59±16.06	108.50±13.51
		P>0.05	P<0.05		P>0.05		

Table 3. Comparison of Vitamin E values of pregnant and non-pregnant animals in the study groups (mg/dl) (Mean±SD)

		-7.Day	**0. Day**	**7.Day**	**9.Day**	**10.Day**	**11.Day**
Group 1	**Pregnant**	0.33±0.09	0.33±0.09	0.45±0.16	0.44±0.09	0.45±0.16	0.50±0.13
	Non-Pregnant	0.34±0.076	0.36±0.10	0.42±0.10	0.44±0.11	0.45±0.15	0.48±0.14
		P>0.05					
Group 2	**Pregnant**	0.35±0.07	0.57±0.11	0.60±0.11	0.55±0.18	0.64±0.11	0.53±.007
	Non-Pregnant	0.36±0.14	0.61±0.13	0.52±0.09	0.52±0.07	0.54±0.11	0.57±0.07
		P<0.05	P>0.05		P<0.05	P>0.05	
Group 3	**Pregnant**	0.31±0.08	0.31±0.08	0.35±0.12	0.43±0.12	0.37±0.06	0.35±0.06
	Non-Pregnant	0.37±0.18	0.37±0.19	0.39±0.11	0.33±0.09	0.41±0.18	0.37±0.12
		P>0.05			P<0.05	P>0.05	P<0.05
Group 4	**Pregnant**	0.49±0.11	0.74±0.10	0.72±0.15	0.58±0.08	0.66±0.10	0.59±0.12
	Non-Pregnant	0.52±0.12	0.76±0.15	0.70±0.15	0.57±0.09	0.59±0.12	0.59±0.10
		P>0.05					

Table 4. Comparison of progesterone levels of pregnant and non-pregnant animals in the study groups (ng/ml) (Median (25-75%))

		-7. Day	**0. Day**	**7. Day**	**9. Day**	**10. Day**	**11. Gün**
Group 1	**Pregnant**	2.04 (0.40-3.40)	2.04 (0.32-3.86)	3.41 (0.30-4.01)	0.12 (0.08-0.18)	0.13 (0.09-0.18)	0.08 (0.05-0.14)
	Non-Pregnant	2.59 (1.23-4.01)	2.76 (1.23-4.61)	0.91 (0.23-3.43)	0.09 (0.05-0.36)	0.18 (0.08-0.23)	0.14 (0.11-0.24)
		P>0.05					
Group 2	**Pregnant**	0.13 (0.04-0.48)	1.47 (0.14-2.90)	2.93 (1.99-4.27)	0.15 (0.07-0.26)	0.03 (0.02-0.15)	0.06 (0.03-0.13)
	Non-Pregnant	1.65 (0.10-4.47)	0.70 (0.05-1.75)	1.46 (0.19-3.60)	0.12 (0.09-0.16)	0.08 (0.05-0.20)	0.08 (0.05-0.17)
		P<0.05		P>0.05			
Group 3	**Pregnant**	3.37 (2.49-4.01)	4.02 (1.49-5.06)	3.47 (1.46-8.01)	0.20 (0.08-0.35)	0.10 (0.04-0.21)	0.09 (0.03-0.20)
	Non-Pregnant	0.42 (0.08-3.10)	0.17 (0.07-3.15)	0.31 (0.17-0.71)	0.17 (0.12-0.29)	0.16 (0.10-0.43)	0.19 (0.09-0.91)
		P>0.05		P<0.05		P>0.05	
Group 4	**Pregnant**	1.46 (0.11-3.57)	1.11 (0.20-1.66)	2.12 (0.46-5.12)	0.08 (0.05-0.15)	0.07 (0.04-0.13)	0.14 (0.06-0.18)
	Non-Pregnant	1.79 (0.62-2.73)	0.24 (0.15-1.27)	0.37 (0.06-1.89)	0.16 (0.08-0.21)	0.13 (0.04-0.26)	0.25 (0.06-0.49)
		P>0.05					

The aim of treatments to control the estrous cycle is to provide optimal pregnancy rates after estrus or ovulation synchronization (Bo et al., 1995; Burke et al., 2000). Although classical estrus synchronization programs in which prostaglandins or progestagens are used alone can produce an adequate estrus response, they cannot sufficiently provide an ovulation synchronization to allow fixed-time insemination (Bo et al., 1995; Burke et al., 2000; DeJarnette et al., 2001; Macmillan et al., 1996; Pursley et al., 1995; Stevenson et al., 1999). The variable responsiveness of the ovaries to synchronization programs is the most limiting factor in the application of new and effective reproductive technologies in cattle (Bo et al.,

1995). Control of the estrous cycle has a wide range of applications, including lengthening or shortening the luteal phase and altering the follicular wave design using GnRH or estradiol. At the end of the synchronization programs, it is necessary to have a healthy dominant follicle in the growth phase to effectively control the development of the follicular wave (Rivera et al., 1998).

It is stated that under normal conditions, 66% of non-pregnant cows in the farm will be in the diestrus period of the cycle (Cartmill et al., 2001). Cirit (2002) reported that on the first day of hormone administration, an average of 41.70% of the cows in the groups were in the luteal phase. In the present study, CL was detected in 15 (75%), 13 (65%), 13 (65%), and 11 (55%) heifers in groups 1, 2, 3, and 4, respectively, on the 7th day before the study. On the day of the first GnRH injection (day 0), presence of the CL was detected in 15 (75%), 14 (70%), 13 (65%) and 16 (80%) heifers in the groups, respectively. It was determined that these results are consistent with results of previous studies.

Many researchers reported that the first GnRH administered in protocols based on GnRH-PGF2α increased the rate of having an active CL at the time of PGF2α injection, by causing ovulation or luteinization Stevenson et al., 2000; Peters et al., 1999; Stevenson et al., 1999). Pursley et al. (1997) stated that the presence of CL in PGF2α injection affects the success of ovulation synchronization. Similarly, Demiral et al. (2006) support this theory in their study. In current study, an increase was observed in the number of heifers with CL in groups 1, 2, 3, while a decrease was detected in group 4 compared to the day of the first GnRH administration on the day of PGF2α injection (7th day). It is difficult to attribute this situation to a reason with the data obtained in the study.

In many studies using the Ovsynch protocol, the success of ovulation synchronization is determined by considering the rate of animals that ovulate after the GnRH injection between 24 to 48 hours. (Vasconcelos et al., 1999; Fricke et al., 1998; Cordoba and Fricke, 2001). Cirit (2002) reported that the rate of ovulation varied between 73.7% and 87.0% after the last PGF2α injection, between the 48-96h. These reported data are consistent with the data found in the presented study.

Pursley et al. (1995) reported that ovulation occurred within 24–32 h after the last GnRH injection in 100% of lactating dairy cows. Demiral et al. (2006) reported that ovulation (99.7%) was distributed to the until 42nd hour and the highest ovulation rate (%35) occurred the between 24 and 30 h in the heifers which they applied the cosynch protocol, in the examinations performed at 6-hour intervals after the last GnRH injection. In the present study, the highest ovulation rate was found in Groups 1 (76.92%), 2 (75%), and 3 (64.29%) between the 36-48 h after the second GnRH injection. But it was determined in Group 4 (71.43%) after the second GnRH injection between the 20-36 h. It was concluded that the difference between the reported results and the findings of the present study could be attributed to variations in the care, nutrition, age of the heifers, and differences in the application dosages of the preparations used.

Yıldız et al. (2005) reported that the difference between the mean Vitamin E and β-carotene levels in pregnant and non-pregnant cows was statistically significant in their study on pregnant and non-pregnant cows after mating. In the present study, the statistical difference was found to be significant in the comparison of vitamin E and β-carotene levels between the days of indicated in the study the pregnant and non-pregnant heifers in all groups. However, no difference was found in vitamin E and β-carotene levels in heifers that pregnant and nonpregnant after insemination.

Parmigiani et al. (2003) reported that calving-first estrus and calving-conception intervals were shortened, and pregnancy rates increased in cows in their study, where they used the preparation containing β-carotene and vitamin E used in the present study. Data obtained in the present study is consistent with the results reported by Parmigiani.et al. (2003).

Graves Hoagland et al. (1989) and Schweigert and Zucker (1988) reported that a positive relationship was found between the levels of vitamin E and β-carotene and the levels of progesterone in their studies. Naziroglu et al. (1997) and Yıldız et al. (2005) reported that there was a negative relationship between vitamin E and β-carotene levels and progesterone levels in pregnant cows, but there was no relationship in non-pregnant cows. In the present study, no correlation was found between vitamin E and β-carotene levels and progesterone levels in all groups. It was thought that the difference between the data obtained in the present study and the data reported in the studies could be attributed to the difference in the methods applied in the studies and the maintenance nutritional status of the subjects.

Pursley et al. (1995) reported a pregnancy rate of 55% in cows treated with Ovsynch, Schmitt et al. (1996) reported a pregnancy rate of 53% in the group in which they used hCG instead of the second GnRH injection, and 45.5% in the ovsynch group in which GnRH was used. In the present study, pregnancy rates in Groups 1, 2, 3, and 4 were determined as 40%, 50%, 50%, and 60%, respectively. It was thought that the results obtained in Groups 2, 3, and 4 agreed with the reported studies, and also the low pregnancy rate obtained in Group 1 might be because the present study was conducted in heifers.

Demiral et al. (2006) reported 41% and 51% pregnancy rates in cows and heifers with the co-synch program. In the present study, the pregnancy rate in heifers in Groups 1 and 3 in which the ovsynch protocol was applied was 40% and 50%, respectively; and the pregnancy rate in the heifers in Groups 2 and 4 in which the ovsynch protocol was applied in combination with β-carotene and vitamin E was 50% and 60%, respectively. The pregnancy rates obtained in the present study was consistent with results of pregnancy rates reported by Demiral et al. (2006).

Kırbaş et al. (2007) and Öztürk (2007) reported that pregnancy rates ranged from 23.1% to 41.9% in the ovsynch protocol in which they used lesirelin acetate as a GnRH analogue. In the present study, pregnancy rates were determined as 50% and 60%, respectively, in Groups 3 and 4 where lesirelin acetate was used. It was thought that the high rates obtained in the presented study may be due to the differences in the geographical regions where the studies were conducted, the dose of the preparation used, the care and feeding conditions of the animals, their age, and their live weight.

CONCLUSION

As a result, it was concluded that β-carotene+Vitamin E applications before the Ovsynch procedure with long-acting GnRH analogues in heifers can play a positive role in increasing pregnancy rates and especially in the conception of heifers reaching mating maturity in a short time.

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EFFECTS OF DIFFERENT ETHANOL CONCENTRATIONS IN PEPPER (*Capsicum annuum*) EXPOSED TO SALINITY

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Plants are exposed to various stress factors such as drought, salinity, heat or chemical compounds in agricultural fields. These stress factors adversely affect plant growth and development, reduce the yield and quality of plant products, and cause economic losses. Plants can cope with stress conditions by using various defense and acclimation mechanisms. Besides, exogenous application of various compounds is known to increase the stress tolerance of plants. In this study, the effects of different ethanol concentrations on stress tolerance in pepper seedlings exposed to salt stress were investigated. For this purpose, 0 and 150 mM NaCl were applied to pepper seedlings pre-treated with 20 and 40 mM ethanol. Plants were harvested 10 days after NaCl treatment. Some physiological and morphological parameters were examined in the harvested plants. According to our findings, especially 20 mM ethanol pre-treatment changed plant growth parameters such as plant height, leaf number, fresh weights of root and stem as well as carotenoid contents in plants exposed to salt stress.

Keywords: Pepper, NaCl, Ethanol, Plant Growth Parameters, Pigment, Total Phenolic Compounds

INTRODUCTION

Salinity is one of the most important abiotic stress factors affecting plant growth. Salinity negatively affects plant growth and development by causing osmotic stress and ion stress. Salt stress affects many metabolic processes in plants, including photosynthesis. (Çulha and Çakırlar, 2011). In addition, salt stress reduces plant height, fresh and dry weight of plants and the number of leaves, as well as adversely affects seed yield and root development (Munns, 2003; Ashraf et al., 2004; Kaya and İnan, 2017). The negative effects of salinity on plants reduce the yield and quality of plant products and ultimately lead to economic losses.

Stress tolerance of plants is essential for their survival in extreme environmental conditions. Plants have developed various defense responses at physiological, biochemical and gene levels to cope with the negative effects of environmental stresses. Besides, exogenous application of various compounds is known to increase the stress tolerance of plants (Kaya and Doganlar 2019; Das et al. 2022; Kaya, 2023).

The aim of this study is to determine the effects of different ethanol (EtOH) concentrations on stress tolerance in plants exposed to salt stress. For this purpose, various morphological and physiological parameters were investigated in pepper seedlings which are treated with ethanol and salt stress.

MATERIAL AND METHOD

This study was conducted at research field Alanya Alaaddin Keykubat University Gazipaşa Vocational School. In this study, *Capsicum annuum* sp. (Üç Burun Cv.) seedlings were used as plant material. Seedlings were grown in pots containing a 3:1 mixture of peat:perlite (v/v) under natural

conditions having an average temperature of 30 °C and an average humidity of 65%. At the about 6 th week of growth, 20 and 40 mM EtOH were applied as foliar for some plants (every day for a week). EtOH concentrations were determined according to the literature (Das et al. 2022; Rahman et al. 2022). One day after last EtOH treatment, both ethanol pre-treated and non-treated plants were irrigated with 150 mM NaCl, every three days for 10 days. Control plants were irrigated with distilled water. (Table 1). At the 10th day of NaCl application, plants were harvested. Some of the plants were used to determine growth parameters (plant height, root length, number of leaves as well as leaf, shoot and root fresh weight /dry weight). Others were used to determine contents of chlorophyll, carotenoid (De Kok and Graham, 1980; Lichtenthaler and Welburn, 1983) and total phenolics (Singleton et al 1999).

Groups	**Treatments**
1	Distilled water (Control)
2	20 mM EtOH
3	40 mM EtOH
4	150 mM NaCl
5	150 mM NaCl+20 mM EtOH
6	150 mM NaCl+40 mM EtOH

Table 1. Treatment groups

Experiments were repeated three times and statistical analyses were performed with SPSS software 20.0. The differences between the treatment groups were determined according to the Tukey test ($p < 0.05$).

Figure 1. Pepper seedlings treated with EtOH and NaCl

RESULTS AND DISCUSSION

A. Morphological parameters

Table 2 shows the effects of EtOH and NaCl treatments for plant height, root length and number of leaves. According to our findings, salt stress decreased plant height, root length and number of leaves in all treatment groups, regardless of EtOH application. However, 20 mM EtOH pre-treatment increased plant height and number of leaves ($p < 0.05$). However, EtOH pre-treatment did not show a significant effect on root length.

Treatments	Plant Height (cm)	Root Length (cm)	Number of Leaves (cm)
Control	30,16±0,44a	26,00±0,57a	20,67±1,52a
20 mM EtOH	24,83±0,52b	21,33±1,66b	18,33±0,57ab
40 mM EtOH	30,33±1,45a	23,83±1,16ab	21,67±2,51a
150 mM NaCl	24,33±0,88b	23,83±0,72ab	17,67±0,57b
150 mM NaCl+20 mM EtOH	26,50±0,28ab	21,83±0,60ab	18,67±1,51ab
150 mM NaCl+40 mM EtOH	22,33±1,30b	21,50±0,28ab	18,33±0,57ab

Table 2. The effects of EtOH and NaCl treatments on plant height, root length and number of leaves in pepper seedlings. The different lowercase letters are significantly different from each other ($P<0.05$) among different treatment groups according to Tukey test.

Table 3 shows the effects of EtOH and NaCl treatments on fresh weights (FW) of root, shoot and leaves. 150 mM NaCl treatment decreased FW of root, shoot and leaves in all plants compare to the control. However, FW of root and shoot in plants treated with 20 mM EtOH + 150 mM NaCl are found to be higher compared to plants treated with 150 mM NaCl ($p < 0.05$).

Treatments	Leaf FW (g)	Shoot FW (g)	Root FW (g)
Control	13,14±1,36a	10,91±0,64a	11,95±0,36ab
20 mM EtOH	10,41±0,38b	8,96±0,48ab	9,90±0,80bc
40 mM EtOH	11,73±1,54ab	11,36±1,13a	13,23±1,41a
150 mM NaCl	10,24±0,98b	8,31±1,31b	10,45±1,10b
150 mM NaCl+20 mM EtOH	9,65±0,86c	8,54±0,47ab	11,10±0,61ab
150 mM NaCl+40 mM EtOH	8,10±1,03c	7,74±1,73b	8,14±1,48c

Table 3. Effects of EtOH and NaCl treatments on FW of root, shoot and leaves in pepper seedlings. The different lowercase letters are significantly different from each other ($P<0.05$) among different treatment groups according to Tukey test.

NaCl treatment generally reduced dry weights (DW) of root, shoot and leaves of pepper seedlings. While the combined application of 150 mM NaCl + 40 mM EtOH decreased DW of the shoot and leaf compared to the 150 mM NaCl application alone, 150 mM NaCl + 20 mM EtOH treatment did not cause any significant change. However, the combined application of 150 mM NaCl and 20 mM EtOH increased the root dry weight ($p < 0.05$) (Table 4).

Treatments	**Leaf DW (g)**	**Shoot DW (g)**	**Root DW (g)**
Control	1,87±0,15a	1,58±0,07a	1,14±0,09ab
20 mM EtOH	1,36±0,04ab	1,26±0,03ab	0,81±0,05ab
40 mM EtOH	1,81±0,18a	1,69±0,13a	1,30±0,19a
150 mM NaCl	1,46±0,12ab	1,36±0,18ab	0,69±0,20b
150 mM NaCl+20 mM EtOH	1,46±0,08ab	1,41±0,06ab	1,10±0,07ab
150 mM NaCl+40 mM EtOH	1,05±0,13b	1,00±0,08b	0,72±0,05b

Table 4. Effects of EtOH and NaCl treatments on DW of root, shoot and leaves in pepper seedlings. The different lowercase letters are significantly different from each other (P<0.05) among different treatment groups according to Tukey test.

B. Physiological parameters

Both combined and separate EtOH and NaCl treatments increased the contents of Chl a, total Chl and carotenoids and ratio of Chl a/b compared to the control and decreased the Chl b content. (Table 5 and 6). 150 mM NaCl treatment did not cause any significant change on total phenolic contents. However, 20 and 40 mM EtOH treatments decreased total phenolic content in plants exposed to salt stress ($p < 0.05$) (Table 5).

Treatments	**Chl a (µg/g)**	**Chl b (µg/g)**	**Chl a/b**
Control	4,53±0,12c	5,33±0,68a	0,90±0,16d
20 mM EtOH	11,72±0,28a	2,00±0,05bc	5,85±0,28c
40 mM EtOH	10,93±0,53b	2,15±0,10b	5,11±0,49c
150 mM NaCl	12,87±0,67a	1,83±0,09c	7,09±0,76a
150 mM NaCl+20 mM EtOH	11,96±0,17a	1,96±0,04c	6,09±0,18b
150 mM NaCl+40 mM EtOH	10,87±0,70b	2,17±0,15b	5,07±0,63c

Table 5. Effects of EtOH and NaCl treatments on contents of Chl a and Chl b and ratio of Chl a/b in pepper seedlings. The different lowercase letters are significantly different from each other (P<0.05) among different treatment groups according to Tukey test.

Treatments	Total Chl (µg/g)	Carotenoids (µg/g)	Total Phenolic (mg/g)
Control	9,87±0,19b	1,58±0,19c	17,73±0,15ab
20 mM EtOH	13,72±0,28a	5,52±0,42ab	16,38±0,20c
40 mM EtOH	13,09±0,49a	7,23±0,64a	18,07±0,13a
150 mM NaCl	14,70±0,76a	2,97±0,32bc	17,55±0,20ab
150 mM NaCl+20 mM EtOH	13,93±0,18a	5,35±0,70ab	16,24±0,18c
150 mM NaCl+40 mM EtOH	13,05±0,63a	2,48±0,43bc	16,86±0,24bc

Table 6. Effects of EtOH and NaCl treatments on contents of Total Chl, Carotenoids and Total Phenolic in pepper seedlings. The different lowercase letters are significantly different from each other ($P<0.05$) among different treatment groups according to Tukey test.

CONCLUSIONS

According to the literature, EtOH pre-application increases plant stress tolerance in plants exposed to different stresses (Das et al. 2022; Rahman et al. 2022). Similar to that, salt stress decreased growth in pepper seedlings by negatively affecting all growth parameters. 20 mM EtOH pre-treatment had a positive effect on plant length, number of leaves, fresh and dry weights of root and shoot in salt-stressed plants. However, detailed studies are needed to elucidate the effects of ethanol on stress tolerance.

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THE DYNAMIC DUO: EXPLORING THE SYNERGISTIC EFFECTS OF SOIL INVERTASE ACTIVITY AND BIOCHAR

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ABSTRACT

Soil enzymes have been recognized as crucial components of ecosystems since their initial report over a century ago. While enzymes in soil systems were initially used as descriptive parameters, they are now appreciated for their various properties in soil processes, microbial activities, and ecosystem responses to changes in management and climate. Invertase, an enzyme that plays a key role in the hydrolysis of sucrose into glucose and fructose, is present in microorganisms, plants, and animals. Biochar, a carbon-rich organic material obtained by carbonizing biomass such as manure, wood, or leaves at high temperatures between 300°C and 1000°C, has been applied for centuries to enhance agricultural soils, potentially leading to more sustainable plant production and reduced greenhouse gas emissions such as CO_2 or CH_4. Biochar can benefit soil microorganisms in numerous ways, including nutrient provision and protection from predators by adsorption in soil surfaces and pores. While the agricultural, economic, and practical applications of biochar have been extensively discussed in published books and book chapters, little information is available regarding the effects of biochar addition on soil invertase activity. The aim of this study was to investigate the impact of different biochar derived from various materials on invertase activity in soil based on the existing literature.

Keywords: Microbial activities, Biochar, Soil enzymes, Invertase, Soil microorganisms

INTRODUCTION

Soil enzymatic activities are one of the biological parameters for assessing soil fertility. The activity of a soil enzyme can serve as an indicator for various biological processes taking place in the soil. However, there is limited information available regarding the functions of soil enzymes in plant-soil systems and their responses to soil amendments (Antonious, 2003). Soil enzyme activities are of great interest to soil biologists and scientists as they provide valuable insights into the biogeochemical processes occurring in the soil. Moreover, they are useful for understanding the effects of anthropogenic management, such as agriculture and forestry, as well as pollution on soils. Additionally, these analyses are generally accurate and cost-effective to perform (Nannipieri et al., 2018).

Soil, as a living ecosystem, is a dynamic and complex entity that can be influenced by various factors, affecting its health and quality (Zhang et al., 2014). Soil enzymes and microorganisms are commonly used as biomarkers to assess the environmental quality of soils (Karlen et al., 1997; Sukul, 2006). Soil enzymes play active and crucial roles in catalyzing biochemical reactions, facilitating the mineralization and immobilization of organic matter, regulating nutrient cycling, removing pollutants, and providing energy for microorganisms and plants (Kizilkaya et al., 2004). Natural disturbances, climate change, and human activities often lead to changes or modifications in soil enzymatic activities (Gianfreda & Rao, 2008; Zhu et al., 2010) (Fig. 1). Additionally, soil microorganisms play a critical role in carbon (Cenkseven et al., 2017), nitrogen (Aka & Darici, 2005), and phosphorus (Barea & Richardson, 2015)

cycling, as well as the mineralization of organic residues (Kocak & Darici, 2016). It is evident that microorganisms residing in the soil ecosystem are highly susceptible to changes in the soil environment and are regarded as early warning indicators for monitoring soil health (Kocak & Cenkseven, 2021; Kocak & Darici, 2022; Nielsen et al., 2002).

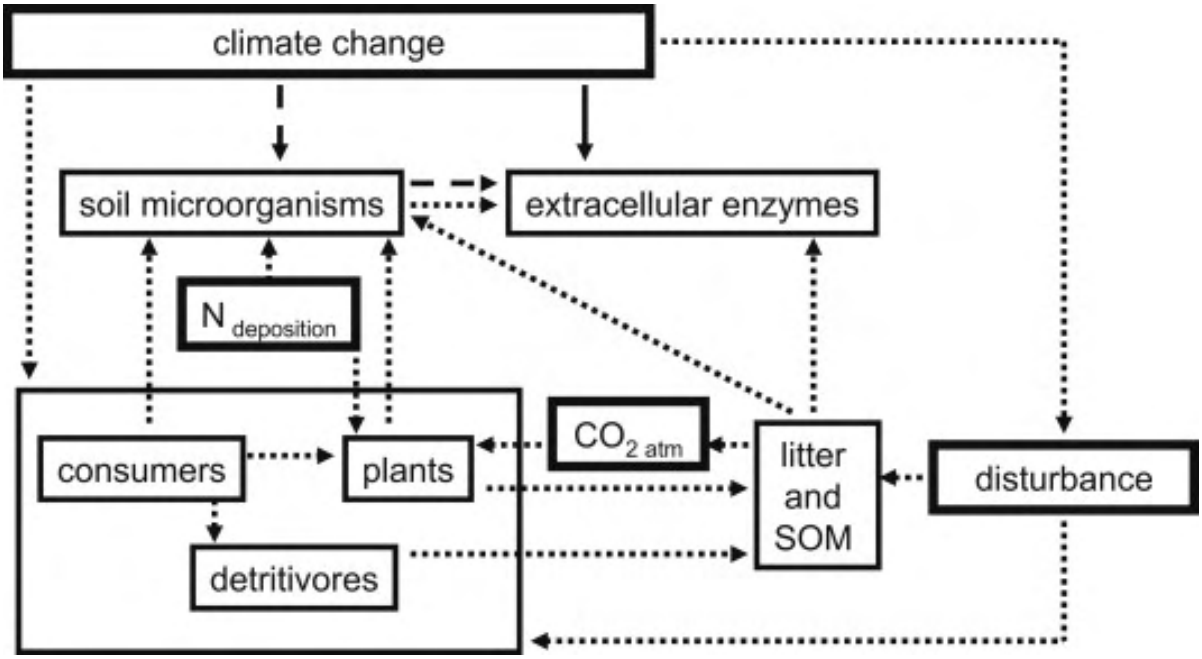

Figure 1. Effects of climate change on microorganisms and enzymatic activities in the soil ecosystems (Henry, 2012)

Biochar is a high-carbon product (Fig. 2) derived from various sources such as wood products (e.g., bark), industrial or organic residues (e.g., manure, purification sludge), and agricultural products (e.g., seeds, bark, leaves, stems). It is produced through pyrolysis, a process conducted under low or oxygen-depleted conditions (Razzaghi et al., 2020). When applied to soil, biochar can enhance the soil's organic carbon content, exhibiting long-term stability and reducing carbon release from the soil (Cross & Sohi, 2011). The high carbon content of biochar results in a negative charge, enabling it to sequester soil organic matter. As a consequence, it supports the accumulation of organic carbon in the soil, making biochar a sustainable energy source (Lehmann, 2007; Zhang et al., 2019). Additionally, biochar's ability to accumulate carbon in the soil helps mitigate CO2 emissions into the atmosphere and contributes to mitigating the adverse effects of global warming due to its resistance to decomposition (Kocak & Ortas, 2021; Lehmann et al., 2021).

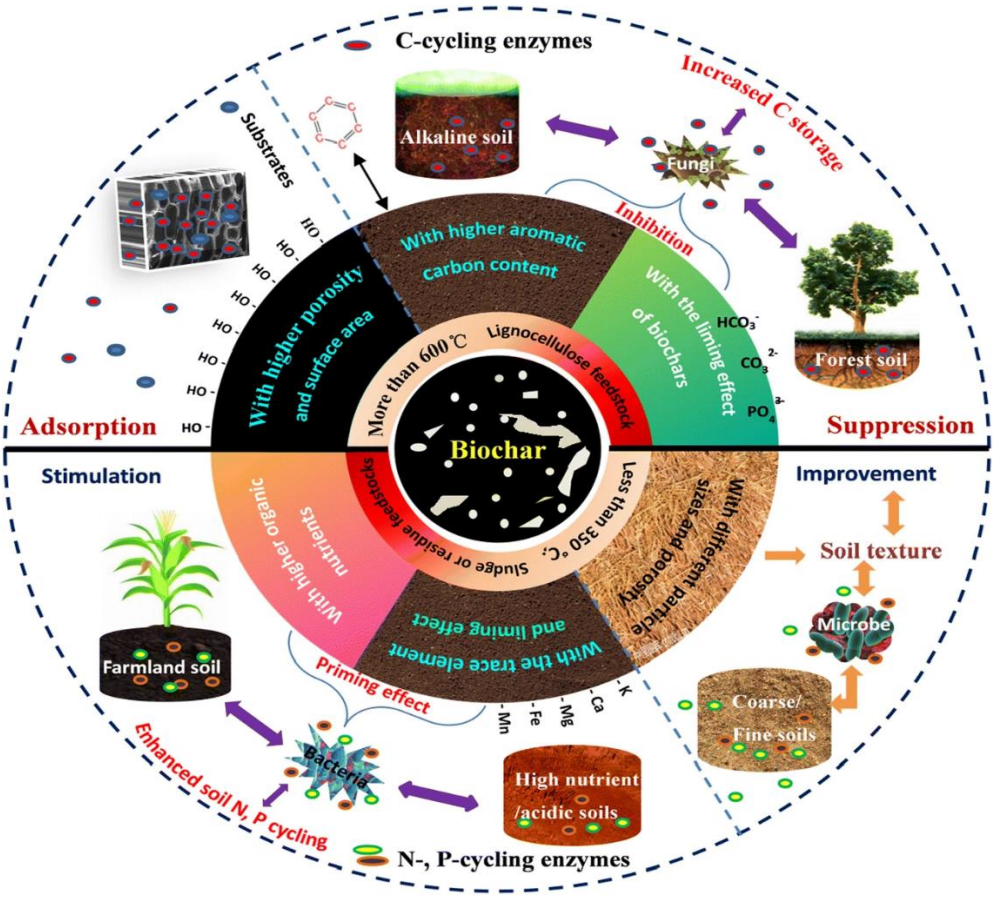

Figure 2. Effects of biochar on soil nutrient cycles and enzymatic activities (Zhang et al., 2019)

In this mini review, soil invertase activity, which is a considered as an important enzymatic activity in carbon metabolism, and effects of biochar on this activity were evaluated and discussed.

EXPLORING THE EFFECTS OF DIFFERENT ORGANIC SOURCES ON SOIL INVERTASE ACTIVITY

Invertase is a unique enzyme that catalyzes the conversion of sucrose into glucose and fructose. This enzyme is present in plants, animals, and microorganisms (Naga Raju et al., 2017). Sucrose is a crucial carbohydrate in plants, and therefore, invertase is among the soil enzymes that actively participate in litter decomposition (Ge et al., 2017).

In general, it has been observed that the introduction of organic sources into the soil stimulates soil invertase activity. Zi et al. (2022) reported that the addition of N and P (0, 10, 20, and 30 g m^{-2} $year^{-1}$) increased soil urease activity in an alpine meadow in Hongyuan County, China. Zhu et al. (2022) indicated that the addition of Bacillus biofertilizer stimulated invertase activity in Cd-stressed soil in Xinjiang Province, China. Shen et al. (2022) found that three different forms of nitrogen [$(NH2)_2$)CO, $NaNO_3$, and NH_4Cl] stimulated soil invertase activity in an acidic soil in the Jiaodong Peninsula of China. Xie et al. (2021) observed that chicken manure and combinations of chicken manure with polyacrylamide, straw mulching, buried straw, and bio-organic manure significantly increased soil invertase activity compared to the control group with no treatment in an oat field in Jiangsu Province, eastern China. In another study, Xu et al. (2022) investigated the effect of exogenous indole-3-acetic acid (IAA) on the growth and development of ryegrass in cadmium (Cd)-contaminated soil and its impact on soil physiology, biochemistry, and microbial activity. The study found that Cd pollution increased

soil basal respiration and invertase and catalase activity, while decreasing fluorescein diacetate (FDA) hydrolase activity. The addition of exogenous IAA reduced soil basal respiration and increased FDA hydrolase activity, thus enhancing the survival of soil microorganisms. Xu et al. (2022) also found a negative correlation between soil invertase activity and soil FDA hydrolase activity. Furthermore, Iqbal et al. (2022) investigated the effect of adding cattle or poultry manure to chemical fertilizers on soil invertase activity. The study revealed that the addition of manure significantly increased soil enzymatic activities, including soil invertase, compared to solely applying chemical fertilizer. Overall, the addition of organic sources to soils generally stimulates soil invertase activity.

EFFECTS OF BIOCHAR ON SOIL INVERTASE ACTIVITY

In a subtropical Moso bamboo forest, Zhang et al. (2023) found that urea applications at rates of 100 and 300 kg N ha^{-1} increased soil invertase activity, while biochar-based urea applications at the same rates significantly decreased this enzyme activity. In the study, soil invertase activity showed correlation with CO_2 emissions and was associated with N_2O emissions ($P<0.05$), without considering the urea and biochar-based urea treatments (Zhang et al., 2023).

In another study on Cd-contaminated soils, Zhu et al. (2022) discovered that the addition of cotton straw biochar at a rate of 3% (w/w) significantly increased invertase activity by 17.51% ($P<0.05$) compared to the control. The authors suggested that cotton straw biochar may have created a beneficial soil environment for the development of soil microorganisms (Zhu et al., 2022).

Furthermore, Zhou et al. (2022) investigated the combined effects of bacterivorous nematodes and organic materials on microbial activities in petroleum-contaminated soils. They reported that the addition of 1% biochar and nematodes stimulated soil invertase activity by 12.4% compared to the control group with no treatment (Zhou et al., 2022).

In an orchard experiment conducted in Zhejiang Province, China, Song et al. (2022) found that a combination of rice straw biochar and an organic-inorganic fertilizer (4 kg biochar + 1.7 kg organic-inorganic mixed fertilizer per plant) increased soil invertase activity by 41% compared to the control treatment without biochar and fertilizer.

In another study, Sial et al. (2022) obtained walnut shells biochar at three different temperatures [300 °C (WSB-300), 450 °C (WSB-450), or 600 °C (WSB-600)] and incorporated them into soil incubation for 120 days with a constant treatment of 1.5% (w/w). The percentage increase in invertase activity, compared to the control treatment, followed the order: WSB-300 (9.7%) < WSB-450 (19.0%) < WSB-600 (29.4%).

Furthermore, Mei et al. (2022) investigated the combined effects of rice straw biochar and *Bacillus cereus* RC-1 on soil urease activity in a 120-day incubation experiment on a Cd-contaminated paddy soil. The study revealed that all treatments (control, sole biochar, sole *Bacillus cereus* RC-1, and combination of biochar and soil microorganism) increased invertase activity in the soil by 21.13% to 31.20%.

In a winter wheat field experiment conducted in Shannxi province, China, Li et al. (2022) found that straw biochar, pyrolyzed at temperatures ranging from 350 °C to 550 °C and applied at a rate of 4000 kg ha^{-1} resulted in the highest soil invertase activity compared to the control and other non-pyrolyzed straw incorporations. The authors of the study additionally claimed that the incorporation of biochar significantly increased soil enzymatic activities at different growing stages.

In a 90-day incubation experiment, Khan et al. (2022) investigated the effects of pristine and Mg-modified rice-straw biochar (RBC and MRBC), pyrolyzed at 350 °C, at different application rates (0%, 1%, and 2.5%). They observed that MRBC2.5 > RBC2.5 > MRBC1 >

RBC1 > Control (0%) in terms of the increase in soil invertase activity. The authors suggested that the addition of both RBC and MRBC increased soil pH, which was beneficial for soil enzymatic activities.

Furthermore, Zheng et al. (2021) found that different straw biochar amendments, pyrolyzed at 500 °C and added at rates of 0, 2, 10, and 50 g/kg dry soil, increased soil invertase activities in a tobacco pot experiment compared to the control. The authors suggested that the addition of biochar may have increased soil enzymatic activity and affected bacterial community populations by increasing the levels of soil organic carbon and nitrogen.

In another study conducted in a subtropical Moso bamboo plantation, Zhang et al. (2021) investigated the effects of chemical fertilizer (CF), biochar-based fertilizer (BF) derived from the same chemical fertilizer (pyrolyzed at 500 °C), and a mixture of these fertilizers (BCF) on soil invertase activity. They reported that CF and BCF treatments significantly increased soil invertase activity by 15% and 9.5%, respectively, compared to the control with no treatments. In contrast, BF application significantly reduced soil invertase activity by 8.2% in the same study (Zhang et al., 2021).

CONCLUSIONS

Microorganisms play a vital role in the soil by driving nutrient cycles, enabling plants to utilize essential macro and micro elements. Without their activity, plants would be unable to access these nutrients. Conversely, plants are responsible for supplying carbon and energy to the soil. Without their presence, soil would consist solely of mineral particles resulting from the weathering of parent material and rocks. Additionally, soils serve not only as a substrate for plants but also as a habitat for microorganisms, as well as micro, meso, and macrofauna.

Soil enzymes are crucial for the decomposition of organic matter and the breakdown of toxic substances within soil ecosystems. In this study, it was observed that biochar derived from organic sources generally stimulated soil invertase activity. Overall, when incorporated into the soil, biochar has the ability to regulate the physical, chemical, and biological properties of the soil. As a result, it has positive effects on soil bacterial and fungal communities, promoting soil invertase activities.

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WHEAT BIOFORTIFICATION IN TURKEY: CURRENT STATUS, CHALLENGES, AND PROMISING OPPORTUNITIES

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ABSTRACT

Wheat biofortification is a promising strategy to address nutrient deficiencies and improve nutrition security in Turkey. This comprehensive review analyzes wheat biofortification's current status, challenges, and opportunities in Turkey. The findings demonstrate the potential of biofortified wheat varieties in delivering essential micronutrients to vulnerable populations, thereby improving public health outcomes. Interdisciplinary collaborations among researchers, breeders, policymakers, and farmers are crucial to developing and disseminating locally tailored biofortified wheat varieties. Optimization of breeding strategies ensures high nutritional quality, yield potential, and agronomic suitability across diverse regions of Turkey. Farmer engagement, capacity building, and knowledge dissemination through extension services are essential to promote awareness and acceptance of biofortified wheat. Active involvement of farmers in the development and evaluation process, along with training programs and knowledge exchange, facilitates widespread adoption. Integrating biofortification with sustainable agricultural practices and crop management techniques enhances wheat production systems' nutritional value and climate resilience. Overall, this review emphasizes the significance of wheat biofortification as a solution to nutrient deficiencies, highlighting the importance of interdisciplinary collaborations, farmer engagement, and sustainable approaches.

Keywords: Wheat biofortification, Nutrient deficiencies, Nutrition security, Interdisciplinary collaborations, Farmer engagement

INTRODUCTION

Biofortification is a strategy to improve the nutritional quality of staple crops by increasing their concentration of essential micronutrients, such as iron (Fe), zinc (Zn), and selenium (Se) (Szerement et al., 2021). Biofortification can be achieved through agronomic practices, conventional breeding, or genetic engineering. Biofortification can potentially reduce the prevalence of micronutrient deficiencies, especially in developing countries where cereal-based diets are predominant and dietary diversification is limited (Gupta et al., 2020).

Wheat is one of the most important cereal crops and a major source of calories and protein for millions of people worldwide. Turkey is the seventh-largest wheat producer and the fourth-largest wheat exporter globally (Xu et al., 2023). Wheat accounts for about 40% of the total cultivated area and 20% of Turkey's agricultural gross domestic product (Ozkan et al., 2004). However, wheat production and consumption in Turkey face several challenges, such as climate change, soil degradation, pests and diseases, low productivity, and poor quality. Moreover, wheat grains grown in Turkey have low Fe, Zn, and Se levels, contributing to the high prevalence of micronutrient malnutrition among the Turkish population, especially children and women (Hincal, 2007).

Biofortification of wheat in Turkey faces specific challenges and opportunities. Evaluating the current status and identifying strategies to overcome barriers for successful implementation is essential. Challenges may include selecting appropriate biofortified wheat varieties suitable for local conditions, ensuring farmer adoption and acceptance, and establishing effective delivery mechanisms to reach the target populations. Moreover, it is important to consider the socio-economic and cultural factors influencing the acceptance and utilization of biofortified wheat. This review paper will provide a comprehensive analysis of the current state of wheat biofortification in Turkey, identify the challenges involved, and explore promising opportunities for scaling up biofortification efforts to improve wheat's nutritional value and effectively address micronutrient deficiencies.

WHEAT PRODUCTION AND CONSUMPTION IN TURKEY

Wheat is a staple crop in Turkey, as it is the main ingredient of bread consumed daily by most of the population. Wheat also has a cultural and historical significance, as it was one of the first crops cultivated in Anatolia, the Asian part of Turkey. Wheat accounts for about 60% of the total cereal production in Turkey and is grown in almost every region of the country. According to the US Department of Agriculture (USDA), Turkey is expected to produce 17 million tonnes of wheat in 2022-23, up from 16 million tonnes in 2021-22. This increase is due to improved weather conditions expected to boost yields. However, wheat production is still below domestic consumption, forecast at 21 million tonnes in 2022-23, up from 20.6 million tonnes in 2021-22. The rising consumption is driven by increasing household demand for wheat-based products, such as bread, pasta, biscuits and cakes.

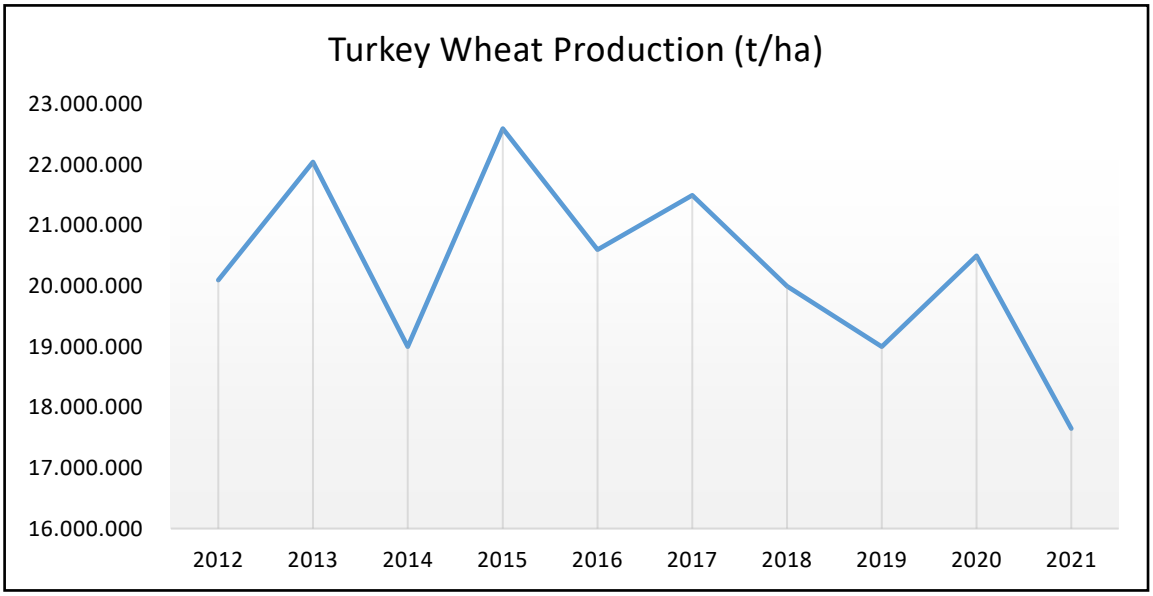

Figure 25. Turkey wheat production 2012-2021 (t/ha) (FAOSTAT, 2023)

The role of wheat in Turkish diets is equally noteworthy. Turkey has a per capita wheat consumption rate of approximately 190 kilograms per year, demonstrating the profound reliance on this grain in daily nutrition (Pekcan & Karaagaoglu, 2000). Wheat-based products such as bread, pasta, and traditional dishes like "pide" and "lahmacun" are dietary staples people of all ages and backgrounds enjoy. This heavy reliance on wheat places it at the core of the Turkish culinary heritage, emphasizing the importance of addressing any nutritional deficiencies that may arise from the wheat-centric diet.

Turkey imports wheat from various countries, mainly Russia and Ukraine, to meet the domestic demand. In 2022-23, wheat imports are projected to reach 11 million tonnes, up from 10 million tonnes in 2021-22 (Oxford, 2022). Turkey also re-exports some imported wheat as

processed products, such as flour and pasta. Turkey is the world's largest flour exporter, with Iraq being the leading destination. In 2022-23, wheat exports are expected to reach 6.65 million tonnes, up from 6.55 million tonnes in 2021-22 (Reidy, 2022). Wheat has a nutritional significance in the Turkish diet, providing carbohydrates, protein, fiber, vitamins, and minerals. Wheat also has health benefits, such as lowering cholesterol, blood pressure, and blood sugar levels and preventing constipation and obesity. However, excessive wheat consumption may cause some adverse effects, such as gluten intolerance, inflammation, and weight gain.

MICRONUTRIENT DEFICIENCIES AND BIOFORTIFICATION STRATEGIES IN TURKEY

Micronutrient deficiencies are a major public health problem in many developing countries, affecting millions of people's growth, development, and well-being. One of the most prevalent micronutrient deficiencies is zinc (Zn) deficiency, which impairs the immune system, increases the risk of infections and contributes to stunting and mortality in children (Kiran et al., 2022). Wheat is a staple food crop in Turkey, providing more than 50% of the daily energy intake and 40% of the protein intake for the population. However, wheat grown in Turkey is often low in Zn concentration due to the country's widespread occurrence of Zn-deficient soils (Cakmak & Kutman, 2018). According to a survey conducted by (Cakmak, 2008), about 70% of the arable land in Turkey has less than 0.4 mg kg^{-1} of DTPA-extractable Zn, which is considered the critical level for wheat production. Zn deficiency in wheat reduces crop yield and quality and affects the Zn status of consumers, especially those who rely on wheat as their primary source of Zn (Rehman et al., 2017).

The health implications and socioeconomic costs associated with Zn deficiency are significant. Zn deficiency is estimated to cause about 800,000 deaths per year worldwide, mostly among children under five years old (Ackland & Michalczyk, 2016). In Turkey, Zn deficiency is associated with an increased incidence of diarrhoea, pneumonia, malaria, and other infectious diseases and impaired cognitive development and learning outcomes in children (Cakmak, 2008). Zn deficiency also reduces the productivity and income of farmers and workers, leading to economic losses and poverty. A study by (Cakmak, 2008) estimated that the annual economic benefits from Zn wheat fertilization in Turkey are about US$100 million, based on the increased grain yield and reduced seeding rate.

Biofortification is a promising strategy to increase the micronutrient content of staple food crops, such as wheat, and to improve the micronutrient status of consumers. Biofortification can be achieved by different approaches, including agronomic, genetic, and breeding methods. Agronomic biofortification involves the application of micronutrient fertilizers or foliar sprays to enhance the uptake and accumulation of micronutrients in the edible parts of plants (Szerement et al., 2021). Genetic biofortification exploits the natural variation in micronutrient concentration among different genotypes or species of plants and selects or introduces those with higher micronutrient levels (Kumar et al., 2017). Breeding biofortification combines agronomic and genetic methods to develop new varieties of crops with improved micronutrient traits.

The selection of appropriate biofortification methods for wheat in Turkey depends on several factors, such as the availability and cost of micronutrient fertilizers, the adoption rate and preference of farmers and consumers, the environmental conditions and soil characteristics, and the genetic potential and diversity of wheat germplasm (Saltzman et al., 2017). Agronomic biofortification with Zn fertilizers is effective and profitable in increasing wheat grain yield and Zn concentration in Turkey (Cakmak, 2008). However, this method requires continuous application of fertilizers and may have negative environmental impacts due to leaching or runoff of excess nutrients. Genetic biofortification with Zn-efficient or Zn-enriched wheat varieties may offer a more sustainable and long-term solution to Zn deficiency (Jaiswal et al.,

2022). However, this method requires more research and development efforts to identify or create suitable genotypes that perform well under different agroecological conditions and consumer preferences. Breeding biofortification may combine the advantages of both agronomic and genetic methods by using Zn fertilizers as a selection tool to enhance the expression of desirable micronutrient traits in wheat plants.

CURRENT STATUS OF WHEAT BIOFORTIFICATION IN TURKEY

In Turkey, both genetic and agronomic biofortification strategies have been implemented for wheat in recent years, with promising results. For genetic biofortification, several research projects have been conducted by national and international institutions, such as Sabanci University, International Center for Agricultural Research in the Dry Areas (ICARDA), International Maize and Wheat Improvement Center (CIMMYT), HarvestPlus Program, and Ministry of Agriculture and Forestry. These projects have identified wheat genotypes with high zinc and iron concentrations in their grains, using screening methods based on atomic absorption spectrometry (AAS), X-ray fluorescence (XRF), or near-infrared reflectance spectroscopy (NIRS). Some of these genotypes have been released as new varieties or used as parents in breeding programs. For example, 'Zinco', 'Demir2000', 'Demir99', 'Sahin', 'Kiziltan', 'Karatopak', 'Gerek79', and 'Seri82' are some of the wheat varieties that have been developed or registered for high zinc and/or iron content in Turkey.

According to a recent review by (Mishra et al., 2022), Turkey has participated in several international projects on wheat biofortification, such as HarvestPlus and AgroSalud, which aim to develop and disseminate zinc-enriched wheat varieties using conventional and molecular breeding approaches. Moreover, Turkey has conducted extensive field trials and experiments to evaluate zinc fertilization's effects on wheat yield, quality, and human health outcomes (Cakmak, 2009; Cakmak et al., 2010, 2017; Rehman et al., 2017). For example, (Niyigaba et al., 2019) reported that foliar zinc application significantly increased grain zinc concentration from 27 mg kg^{-1} to 48-49 mg kg^{-1} across seven countries, including Turkey. Furthermore, Turkey has established a national biofortification program, which involves collaboration among various stakeholders, such as farmers, extension agents, seed companies, policymakers, and consumers, to promote the adoption and consuming biofortified wheat varieties.

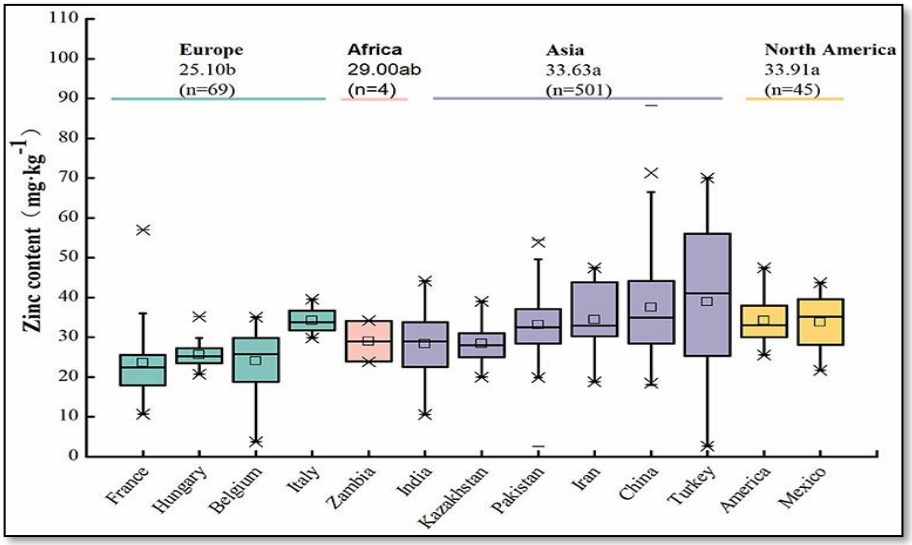

Figure 26. Zinc content of wheat among different countries and continents (Wang et al., 2020)

The findings on the levels of key micronutrients in wheat varieties cultivated in Turkey are promising but variable. (Wang et al., 2020) summarized the results of several studies that measured the iron and zinc concentrations in different wheat genotypes grown in Turkey under different soil and climatic conditions. They found that the average iron concentration ranged from 28 to 46 mg kg^{-1}, while the average zinc concentration ranged from 24 to 40 mg kg^{-1}. These values are higher than the global averages of 20 mg kg^{-1} for iron and 17 mg kg^{-1} for zinc (Cakmak, 2008). However, they also noted a large variation among genotypes, locations and years, indicating that genetic and environmental factors influence the micronutrient content of wheat grains.

CHALLENGES IN WHEAT BIOFORTIFICATION IN TURKEY

Wheat biofortification is a strategy to increase the micronutrient content of wheat grains, especially iron (Fe) and zinc (Zn), to improve the population's nutritional status. Wheat is one of the major staple crops in Turkey, where micronutrient deficiency is a public health problem affecting millions of people, especially children and women. However, wheat biofortification faces several challenges in Turkey, such as:

Soil quality: The availability and uptake of Fe and Zn by wheat plants depend on the soil properties, such as pH, organic matter, and cation exchange capacity (Dhaliwal et al., 2019). Turkey has diverse soil types, ranging from calcareous to acidic, affecting micronutrients' mobility and solubility (Cetin, 2016). Therefore, wheat biofortification requires soil-specific management practices to optimize plant micronutrient supply.

Crop management: The agronomic practices, such as fertilization, irrigation, and pest control, also influence the micronutrient content of wheat grains (Verma et al., 2023). For example, spraying Zn fertilizer at the grain development stage improved grain Zn concentration by 68% (Wu et al., 2020). However, farmers' adoption of these practices may be limited by the availability, affordability, and accessibility of inputs and technologies. Moreover, crop management should also consider the trade-offs between yield and quality, as some practices may increase micronutrients but reduce grain size or protein content.

Awareness among farmers and consumers: The success of wheat biofortification depends on farmers' and consumers' acceptance and demand for biofortified wheat varieties. Farmers need to know the benefits of growing biofortified wheat, such as improved crop performance, higher market value, and lower production costs. Consumers must be aware of the health benefits of consuming biofortified wheat products, such as reduced risk of anemia, stunting, and infections. However, awareness and knowledge about wheat biofortification are still low among both farmers and consumers in Turkey. Therefore, effective communication and extension strategies are needed to promote wheat biofortification and increase its adoption and consumption.

PROMISING OPPORTUNITIES AND INNOVATIONS

Wheat biofortification is a strategy to improve the nutritional quality of wheat grains by increasing the concentration of micronutrients such as iron (Fe) and zinc (Zn), which are essential for human health and development. Wheat is a major staple crop and food source for many people in Turkey and other developing countries, where micronutrient deficiency and hidden hunger are widespread problems affecting children, women, and vulnerable groups. Recent research and innovations in wheat biofortification techniques include exploiting natural genetic variation among wheat varieties and wild relatives, using conventional breeding and transgenic technology, applying agronomic practices such as fertilization and irrigation, and enhancing post-harvest processing and storage methods.

Potential collaborations between research institutions, government agencies, and agricultural stakeholders are needed to strengthen the value chain of biofortified wheat, increase the adoption and dissemination of biofortified wheat varieties, evaluate the impact of biofortification on nutritional outcomes and health benefits, and raise awareness and demand among consumers and policymakers. According to a recent study, genetic biofortification has more potential than agronomic biofortification in increasing wheat grains' Fe and Zn contents in Turkey, with an average increase of 74% and 79%, respectively (Cakmak, 2008, 2009; Cakmak et al., 2010, 2017; Cakmak & Kutman, 2018). Another study reported that durum wheat (Triticum durum), widely used for pasta production in Turkey, can be biofortified with Fe and Zn using natural genetic diversity or transgenic approaches (Hocaoğlu et al., 2020).

FUTURE DIRECTIONS AND POLICY IMPLICATIONS

Wheat biofortification is a promising strategy to improve the nutritional status of millions of people who suffer from micronutrient deficiencies, especially iron and zinc. Wheat is one of the major staple crops in Turkey, where about 20% of children under five years old and 30% of women of reproductive age are anemic. Therefore, increasing wheat grains' iron and zinc content through agronomic or genetic approaches can have significant health and economic benefits for the Turkish population.

However, wheat biofortification faces several challenges that need to be addressed by future research and policy measures. Some of these challenges are: (a) identifying and developing wheat varieties with high micronutrient density and agronomic performance, (b) evaluating the bioavailability and bioefficacy of the micronutrients in biofortified wheat products, (c) assessing the consumer acceptance and willingness to pay for biofortified wheat products, (d) scaling up the production and distribution of biofortified wheat seeds and products, and (e) monitoring and evaluating the impact of wheat biofortification on nutritional outcomes and food security.

To overcome these challenges, wheat biofortification requires a multidisciplinary and multi-stakeholder approach that involves researchers, breeders, farmers, processors, consumers, policymakers, and extension agents. Government policies, incentives, and regulatory frameworks are crucial in supporting biofortification efforts. Some of the policy actions that can facilitate wheat biofortification in Turkey are: (a) providing subsidies or tax exemptions for biofortified wheat seeds and products, (b) creating awareness and demand for biofortified wheat products through public education and promotion campaigns, (c) establishing quality standards and certification systems for biofortified wheat products, (d) integrating biofortified wheat products into public food distribution programs such as school feeding or social safety nets, and (e) strengthening the institutional capacity and coordination among different actors involved in biofortification.

CONCLUSION

Wheat biofortification presents a promising strategy to address nutrient deficiencies and improve nutrition security in Turkey. The key findings highlight the significance of biofortified wheat varieties in delivering essential micronutrients to vulnerable populations, combating hidden hunger, and improving public health outcomes. To ensure successful adoption and dissemination of biofortified wheat, collaboration among researchers, breeders, policymakers, and stakeholders is essential. Optimizing breeding strategies, ensuring agronomic suitability, and addressing technical challenges are crucial areas that require interdisciplinary collaboration. Policy support, incentives, and well-defined regulatory frameworks are needed to create an enabling environment for biofortification. Integrating biofortification into agricultural and health policies, providing incentives, and strengthening market linkages can

promote widespread adoption and sustained production of biofortified wheat. Further research is necessary to advance the field of wheat biofortification. Optimizing breeding strategies, conducting long-term sustainability assessments, and evaluating the impact on human health outcomes are important areas of focus. Biofortification has the potential to contribute to sustainable development goals related to nutrition security, food production, and health in Turkey. By addressing nutrition security, enhancing climate resilience, and promoting sustainable agriculture, biofortification can improve the overall well-being and resilience of the population.

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INNOVATIVE IRRIGATION MANAGEMENT IN AEROBIC RICE CULTIVATION: A COMPREHENSIVE REVIEW OF TECHNOLOGIES AND PRACTICES

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ABSTRACT

Aerobic rice cultivation has gained recognition as a promising approach to sustainable agriculture. This review paper comprehensively assesses innovative irrigation management in aerobic rice cultivation, focusing on technologies and practices that optimize water use efficiency, enhance nutrient management, and promote sustainable crop production. The paper examines various aspects of aerobic rice systems, including water-saving strategies, integrated nutrient management, and the challenges in adopting these practices. The review highlights the significant role of innovative irrigation technologies in water conservation. Techniques such as drip irrigation, alternate wetting and drying (AWD), and precision irrigation have shown substantial water savings compared to traditional flooding. Studies have reported reductions in water consumption from 30% to 50% through adopting these technologies while maintaining or improving crop yields. Furthermore, innovative irrigation management practices improve nutrient availability, uptake, and utilization. Proper irrigation scheduling based on crop water requirements and soil moisture monitoring optimizes nutrient uptake efficiency, enhancing nitrogen, phosphorus, and potassium utilization. Despite the potential benefits, adopting innovative irrigation management practices faces challenges. Technical barriers, economic limitations, and policy-related constraints hinder widespread adoption. Farmers may lack knowledge and training in these technologies, and the initial investment and operational costs may pose financial challenges. Inadequate policy support and limited access to extension services further impede adoption. To overcome these challenges, the review suggests the need for capacity-building programs, supportive policies, and collaborations among stakeholders.
Keywords: Aerobic rice, Innovative irrigation, Water use efficiency, Nutrient management, Sustainable agriculture

INTRODUCTION

In recent years, more people are beginning to understand the need for environmentally friendly and resource-conserving farming methods. Aerobic rice cultivation is a method that integrates cutting-edge technology with environmentally responsible water use (Datta et al., 2017; Matloob et al., 2022; Sandhu et al., 2021). Drip and subsurface irrigation are two of the most innovative irrigation methods used in aerobic rice production because they allow water to be delivered directly to the plant's roots, maximizing water use efficiency and minimizing water loss through evaporation and runoff (Mallareddy et al., 2023). Compared to conventional flooded rice systems, these cutting-edge irrigation methods can cut water use by as much as half. As a result, water is saved, energy spent on water pumping is reduced, and agricultural output becomes more environmentally friendly.

Using cutting-edge sensor-based technologies has further transformed aerobic rice cultivation irrigation management. Using remote sensing devices, weather-based controllers, and soil moisture sensors, water is only used when and where necessary (Keswani et al., 2019). These tools let farmers make more informed decisions about their irrigation systems, leading to less water being wasted and more efficiently irrigated crops. Improved crop yields, water savings, and resource conservation have all been shown to emerge from sensor-based irrigation management in aerobic rice system (Mallareddy et al., 2023).

Aerobic rice cultivation has widely implemented water-saving methods, including alternating wetting and drying (AWD) and other creative approaches. Instead of keeping the ground permanently flooded, AWD requires letting it dry out at regular intervals and then soaking it again. Soil health is improved, methane emissions are reduced, and more nutrients are available thanks to this water conservation method. Water savings of up to 30% are possible with AWD compared to continuous flooding, and yields are kept constant or even increased (Lampayan et al., 2015). The vast potential of innovation and technology in revolutionizing irrigation management in aerobic rice farming is displayed in the convergence of precision irrigation, sensor-based technologies, and water-saving strategies like AWD. In this review, we hope to shed light on how innovation and technology have contributed to the development of sustainable agricultural production, especially in aerobic rice farming.

Importance of Efficient Irrigation Management in Aerobic Rice Cultivation

Effective irrigation management is of paramount importance in the growth of aerobic rice, as it optimizes water usage efficiency, minimizes water loss, and enhances crop output. The presence of limited water supplies and the escalating competition for water resources underscore the need to implement suitable water management strategies within the agricultural sector. The utilization of precision irrigation techniques, such as drip irrigation and subsurface irrigation, has been observed to enhance water use efficiency in aerobic rice systems. Research findings indicate that implementing new irrigation technologies has demonstrated substantial water conservation benefits, with potential savings ranging from 30% to 50% compared to conventional flooded rice systems (Champness et al., 2023; Satyanarayana et al., 2007). By implementing methods that transport water directly to the roots of plants, these strategies effectively reduce water loss caused by evaporation and runoff, optimizing water utilization.

Furthermore, implementing efficient irrigation management strategies in aerobic rice production enhances water use efficiency and increases crop productivity (Wang et al., 2020). Sufficient provision of water, in appropriate quantities and at optimal timings, is necessary for the cultivation and maturation of crops. Sensor-based technologies and precision irrigation systems have emerged as significant tools (Canaj et al., 2021). These technologies facilitate accurate irrigation scheduling by enabling real-time soil moisture and crop water demand monitoring. Previous research has indicated that the implementation of sensor-based irrigation management techniques can enhance crop yields and optimize water production in the context of aerobic rice cultivation (Adeyemi et al., 2017; Gonçalves et al., 2022; Mallareddy et al., 2023). The performance of effective irrigation management strategies contributes to improving nutrient absorption, mitigating plant stress, and, ultimately, facilitating crop growth and output (Abioye et al., 2020; Dhaliwal et al., 2022).

Sustainable farming methods benefit from careful irrigation management since they reduce water waste. Water conservation, lower energy needs for water pumping, and less water-related emissions are all benefits of aerobic rice production, which uses less water than conventional methods. The negative impacts of overwatering can be lessened by using modern irrigation methods, such as subsurface irrigation, which minimizes water loss due to evaporation and runoff (Abioye et al., 2020). Subsurface irrigation has been shown to significantly reduce water waste compared to more conventional surface irrigation techniques.

By minimizing the amount of water used in irrigation and the related carbon emissions, as well as by preserving water quality, these methods contribute to environmental sustainability.

Exploring Innovative Irrigation Technologies for Enhancing Water Use Efficiency in Aerobic Rice Cultivation

To achieve sustainable crop production, the cultivation of aerobic rice necessitates the implementation of new irrigation technology and techniques designed to enhance water use efficiency. An example of a method employed in this context is alternate wetting and drying (AWD), when the soil is intentionally subjected to a partial drying phase before being re-flooded. Implementing alternate wetting and drying (AWD) systems in vehicles creates aerobic conditions, decreasing water consumption and mitigating methane emissions. Several studies have documented substantial reductions in water usage, up to 30%, in aerobic rice systems that employ alternate wetting and drying (AWD) techniques while maintaining rice yields at satisfactory levels (LaHue et al., 2016; Lampayan et al., 2015). This is in contrast to the conventional practice of continuous flooding. This methodology not only facilitates water conservation but also aids in mitigating climate change by reducing greenhouse gas emissions.

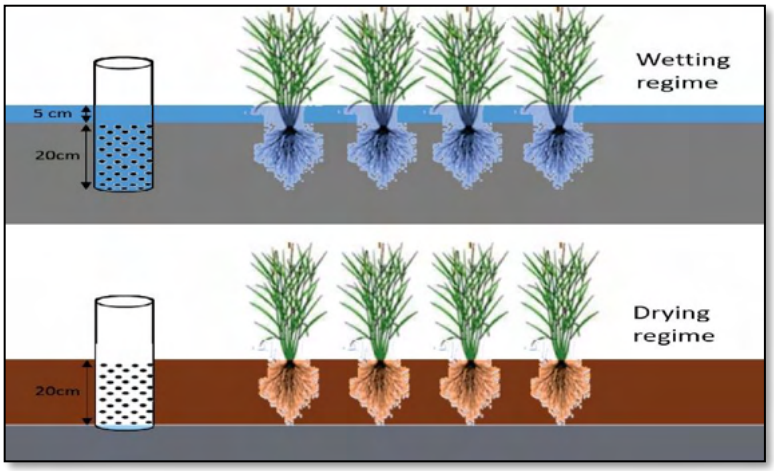

Figure 27. Illustration of management of alternate drying and wetting (AWD) (Riaz et al., 2018).

Drip irrigation is a prevalent novel method employed in aerobic rice production. The process entails the accurate distribution of water precisely to the area surrounding the roots via a system of tubes or emitters. Drip irrigation is a water application method that allows precise control, reducing water loss due to evaporation and runoff (Kisekka et al., 2017). Numerous empirical studies have substantiated the assertion that drip irrigation can curtail water usage by as much as 50% compared to the typical flooding method (Rizwan et al., 2018). In addition, it promotes increased efficiency in water utilization and nutrient absorption, resulting in enhanced agricultural productivity and the preservation of valuable resources. Drip irrigation facilitates the accurate delivery of fertilizers, diminishing nutrient losses and mitigating environmental repercussions.

Figure 28. Rice cultivation through drip irrigation (Mallareddy et al., 2023)

Precision irrigation systems provide cutting-edge technology that enables the optimization of water utilization in the growing of aerobic rice. These systems use real-time monitoring of soil moisture levels, meteorological conditions, and crop water demands to deliver precise irrigation scheduling. Precision irrigation is a method that aims to optimize water distribution by providing water at the exact time and location it is required. This approach effectively reduces water wastage and enhances overall water use efficiency (Abioye et al., 2020; Daccache et al., 2015). Sensor-based technology, including soil moisture sensors and weather-based controllers, is critical in implementing precision irrigation strategies. According a study conducted by(Adeyemi et al., 2017), it has been demonstrated that implementing precision irrigation techniques can lead to a notable reduction in water usage, reaching up to 40%. Furthermore, this approach has been found to sustain or even enhance rice yields compared to conventional irrigation methods. This technology empowers farmers to make well-informed decisions about irrigation, optimizing water resource utilization and strengthening overall output.

Specific Water-Saving Strategies for Aerobic Rice Cultivation

The issue of water scarcity and the imperative for sustainable water management has spurred the investigation of targeted water conservation measures in the context of aerobic rice growing. A productive strategy involves using an irrigation schedule tailored to meet the specific water demands of crops. The proposed approach entails evaluating the crop's water requirements at various growth phases and administering irrigation to those needs. Numerous studies have demonstrated that the judicious timing and appropriate application of irrigation by crops' water needs can deliver substantial reductions in water usage without compromising and, in some cases, enhancing agricultural productivity.

Table 3. Water use efficiency and water productivity of aerobic rice systems.

Location	Water Use Efficiency (WUE), Water Productivity (WP) or % Water Saving	Reference
IRRI, Philippines	Using the alternate flooding strategy resulted in 0.54–0.66 kg grain m^{-3} water productivity for aerobic rice. Aerobic plots utilized 27% less water than the alternative flooding irrigation approach.	(Grassi et al., 2009)
University of Tokyo and Kyoto	The water productivity of aerobically grown rice ranged from 1.4% to 37.5% higher than that of rice that had been transplanted, or 0.75 to 0.96 kg grain m^{-3}. There was no discernible difference	(Kato et al., 2009)

University, Japan	in grain output between aerobic and transplanted rice grown in Japanese clay loam soils.	
IARI, New Delhi	Water productivity ranged from 3.52 to 3.07 kg ha^{-1} mm^{-1} for the aerobic rice system, 3.07 to 2.28 kg ha^{-1} mm^{-1} for the SRI method, and 2.28 to 2.28 kg ha^{-1} mm^{-1} for transplanted rice. Compared to traditionally transplanted rice, the aerobic rice system reduced water usage by 50.8%.	(Shahane et al., 2019)
Hyderabad, India	The water yield of aerobic rice (0.70 kg grain m^{-3}) is higher than transplanted rice (0.55 kg grain m^{-3}). Aerobic management saved almost 50% more water than traditional rice-growing methods in sandy clay soils.	(Ramulu et al., 2020)
UAS, Banglore	Aerobic rice systems use water more efficiently (3.84 q $acre^{-1}$ inch) than conventional fields (1.64 q $acre^{-1}$ inch). Also, the economic WUE of the aerobic rice system was greater (₹ 1643.54 acre $inch^{-1}$) than conventional farms (₹ 269.41 acre $inch^{-1}$).	(Thejaswi Kumar et al., 2021)

Soil moisture monitoring is a practical water conservation approach employed in the growth of aerobic rice. Through the constant monitoring of moisture content in the root zone, growers can precisely ascertain the optimal timing and volume of irrigation. Soil moisture monitoring systems offer instantaneous data regarding soil moisture levels, facilitating accurate irrigation scheduling and mitigating the risks associated with excessive or insufficient irrigation. (Champness et al., 2023) conducted a study that demonstrated the effectiveness of soil moisture-based irrigation scheduling in aerobic rice systems. The findings revealed that this approach led to significant water savings of up to 35% while maintaining satisfactory crop yields. This approach guarantees water's application solely when required, mitigating water wastage and enhancing water use efficiency.

Remote sensing technologies, including satellite-based photography and unmanned aerial vehicles (UAVs), offer a broader scope for evaluating agricultural water stress and vegetation indices across extensive regions (Olson & Anderson, 2021). These technologies facilitate the identification of fluctuations in water availability throughout the agricultural field, allowing farmers to make appropriate adjustments to irrigation practices. Research indicates that moisture sensors and remote sensing technologies can reduce water usage to range from 20% to 40% during anaerobic rice production (Mallareddy et al., 2023). This novel methodology optimizes water utilization efficiency and mitigates water wastage, thereby contributing to the sustainable management of water resources in aerobic rice cultivation systems.

Integrated Nutrient Management and Irrigation Practices in Aerobic Rice Cultivation

The optimization of nutrient availability and utilization in aerobic rice production is highly dependent on the combination of nutrient management strategies with irrigation management. The effective management of irrigation is of considerable importance in the context of nutrient dynamics since it exerts influence over critical factors such as soil moisture levels, oxygen availability, and the transportation of nutrients (Nair, 2019). When irrigation is effectively controlled, it has the potential to optimize fertilizer uptake, minimize nutrient losses caused by leaching, and promote overall nutrient usage efficiency. According to (Midya et al., 2021), research has indicated that implementing controlled irrigation methods can enhance fertilizer availability and reduce the potential for nutrient leakage by promoting aerobic soil conditions. Hence, embracing a comprehensive strategy combining nutrient management and

irrigation is imperative to achieve sustainable and effective usage of nutrients in aerobic rice systems.

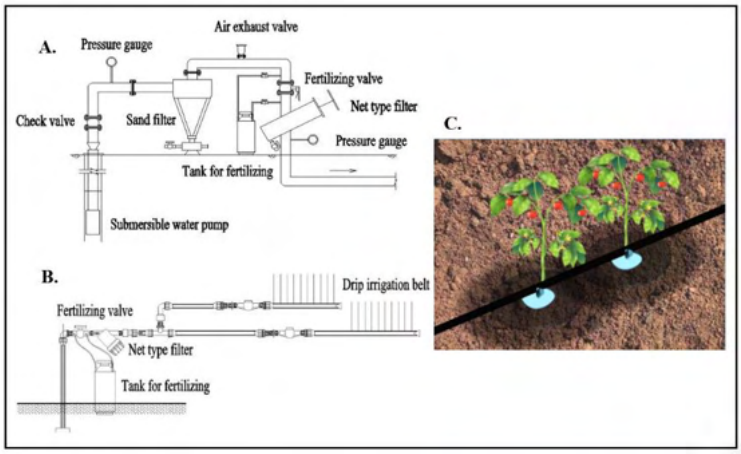

Figure 29. Layout of drip irrigation and fertilization system (Yang et al., 2023)

One of the primary advantages associated with effective irrigation management in aerobic rice farming is the enhancement of nutrient accessibility for plant growth. Insufficient water availability or extreme flooding can reduce oxygen levels within the root zone, constraining plants' ability to absorb nutrients. Efficient nutrient uptake is facilitated by maintaining aerobic soil conditions through well-managed irrigation practices, which helps sustain optimal oxygen levels in the root zone. This practice enhances plant development, optimizes nutrient consumption, and augments agricultural output. The literature has provided evidence that effective irrigation management is crucial in improving nutrient uptake efficiency in aerobic rice production. This leads to notable improvements in the plants' uptake of nitrogen, phosphorus, and potassium (Sarkar et al., 2020; Whetton et al., 2022). By implementing a strategic irrigation schedule, it is possible to coordinate nutrient availability with the various growth stages of the crop. This synchronization facilitates the effective uptake and utilization of nutrients.

Implementing efficient irrigation practices in aerobic rice production can reduce nutrient losses caused by leaching, mitigating the environmental consequences of nutrient runoff (Chen et al., 2021; Zinkernagel et al., 2020). The leaching of nutrients, namely nitrogen, below the root zone can occur due to excessive irrigation or inadequate water management measures. This phenomenon can contribute to water contamination and the inefficient utilization of fertilizers. The implementation of precision irrigation methods, such as drip irrigation or controlled deficit irrigation, enables the regulated application of water, aligning with the specific water needs of the crop and reducing excessive leaching (Zinkernagel et al., 2020). The reduction of nutrient losses not only enhances the efficiency of nutrient utilization but also aids in promoting environmental sustainability through the mitigation of water pollution and the prevention of water body eutrophication.

Challenges and Constraints in Adopting Innovative Irrigation Management in Aerobic Rice Cultivation

Various hurdles and limits hinder the broad implementation of new irrigation management strategies in aerobic rice production despite their potential benefits. Technical impediments encompass a range of challenges, such as farmers' restricted knowledge and comprehension of modern technologies, insufficient training opportunities, and the complexities associated with adopting novel methods. For example, adopting precision irrigation systems may necessitate the utilization of specialized machinery, technical

proficiency, and ongoing surveillance, hence presenting obstacles for farmers with low resources. Given the substantial upfront investment and ongoing operational expenses associated with implementing novel irrigation technology, economic limitations pose a significant barrier. Farmers may encounter challenges in obtaining financial resources and resist adopting technologies without comprehending the enduring economic advantages. Moreover, the accessibility and cost-effectiveness of irrigation infrastructure and resources, including dependable electrical provision and water supplies, can constrain the implementation of novel methodologies in some geographical regions.

Policy-related hurdles are a crucial factor that hinders the extensive implementation of new irrigation management strategies in aerobic rice growing. Insufficient policy support, characterized by the lack of suitable rules and incentives, may deter farmers from embracing these methods. The lack of comprehensive extension services and outreach programs explicitly targeting novel irrigation technologies presents a significant obstacle to effectively transferring knowledge and capacity development in this field. The lack of enough financing for research and development in these technologies and the failure to address difficulties specific to different regions present further limitations. To address these difficulties, it is imperative to establish supportive policies that incentivize farmers to embrace novel irrigation practices. Policymakers must allocate resources toward research and development, extension services, and capacity-building programs to augment farmers' knowledge and skills. Furthermore, establishing partnerships among governmental entities, academic institutions, and industry participants can effectively facilitate the exchange of technology and expertise. Moreover, public-private collaborations can serve as a means to tackle economic limitations by implementing inventive financing mechanisms and sharing costs.

CONCLUSION

In summary, this comprehensive analysis underscores the possibility of employing innovative irrigation management techniques in the growth of aerobic rice to promote sustainable agriculture. The results emphasize the efficacy of technologies such as drip irrigation, alternate wetting and drying (AWD), and precision irrigation in enhancing water use efficiency, nutrient administration, and ecological sustainability. However, additional study, the distribution of knowledge, and joint endeavors are necessary to surmount obstacles and facilitate the general adoption. Through the progression of scientific inquiry, dissemination of knowledge, and cultivation of cooperative relationships, it is possible to establish enduring and adaptable food production systems within the context of aerobic rice agriculture, promoting sustainability and resilience.

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ASSESSMENT OF AGRICULTURAL USE OF SLUDGE IN THE CONTEXT OF CIRCULAR ECONOMY (CE) APPROACH

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ABSTRACT

The objectives of the study are to investigate the land use potential of agro-industry wastewater treatment plants (WWTPs) sludge for agricultural purposes and assess it in the context of circular economy (CE). Because WWTPs sludge is generally disposed off in sanitary landfills as waste material. Its use in land again for plant production is a substantial action in terms of circularity and contributes the economy of agriculture and industry as well as environmental sustainability. The important factors in this attempt are (i) the characteristics of the sludge, (ii) the amount of the sludge applied to the land (sludge loadings) and (iii) the behavior (reaction) of plants to the sludge application. In this framework, presented research was conducted by the sludge samples obtained from wastewater treatment plants of two different agro-industry WWTPs, namely, vegetable processing and vegetable oil manufacturing factories. The pH, salinity, organic matter, nitrogen, phosphorus, potassium, iron, magnesium, sodium, calcium as well as heavy metals were analyzed in order to find out the physical and chemical properties of the sludge samples. The sludge samples were amended with soil mixture and applied at various rates to promote the growth of lettuce plants. Plants growth were assessed by counting plants and leaves. In addition, heavy metal uptake by plants were investigated by measuring the metal concentrations in leaves and roots. The results stated that plant growth was affected from sludge loading, plant nutrients such as nitrogen, phosphorus and potassium, and salt content. High nutrient content of the vegetable processing sludge resulted in enhanced plant growth. Additionally, insignificant metal accumulations were measured in plants due to lower metal contents of the raw sludge. However, all those findings reveal that agro-industry WWTPs sludge are potential asset for farming and may be a typical examples of circular economy activities in sludge management.

Keywords: Sludge, Recycling, Land use, Biosolid, Circular Economy

INTRODUCTION

Treatment of wastewater produces sludge that must be disposed off properly. The old approach so far was to digest and dewater the sludge and then store it in sanitary landfills as waste material. However emerging attempts showed that reuse and recycling of sludge in agricultural land or soil is economically attractive and environmental friendly disposal alternative. Because it contains nitrogen (N), phosphorus (P), potassium (K), and organic materials, thereby it can be used as potential substitutes for conventional fertilizers in agricultural production (Dolgen et. al. 2011). Therefore, agricultural use of wastewater treatment plant sludge is promoted as a part of a solution for resource conservation and also strongly encouraged as a practice in transition to circular economy (CE). One of the key elements in transition towards CE is to ensure more sustainable waste management practices. As sludge contain high mineral contents, recycling of sludge via land application can be

accepted as one of the sustainable sludge management strategies in CE approach (Dolgen and Alpaslan, 2023).

However, there are also concerns that must be addressed to ensure a safe, economical, and environmentally sound approach to apply sludge to the soil. The application of sludges in agriculture may lead to a risk for humans and the environment as a result of heavy metals and toxic organic compounds accumulating to levels high enough to cause damage (Dolgen et. al. 2007). In order to prevent buildup of these compounds to unhealthy levels in soils and plants, extensive scientific research has been conducted to understand the potential risk. In addition, environmental hazards caused by the potentially toxic compounds have been controlled by setting limits on the amounts of such compounds in the sludge as well as in the soils. However, extensive scientific researches on sludge use on cropland are still needed because of their effects, e.g. organic matter enrichment and the possible accumulation of toxic elements in soil evolve slowly and are difficult to predict.

The objective of this study is to investigate the land use potential of the sludge generated from the wastewater treatment plants (WWTPs) of agro-industries. In this framework, sludge samples from WWTPs of vegetable processing and vegetable oil factories were used. In the study, sludge samples were taken from two different dewatering units of the treatment plant, namely, drying beds (SL-I) and filter press (SL-II) for vegetable processing and vegetable oil industries, respectively. Characterization study was carried out and parameters limiting reuse potential were determined, as first. Then, the sludge samples were amended with soil mixture (SM-I and SM-II) and applied at various rates to promote the growth of lettuce plants and to investigate the metal accumulation.

MATERIAL AND METHOD

Characterization Study

The pH, salinity, organic matter, nitrogen, phosphorus, potassium, iron, magnesium, sodium, as well as heavy metals (Cu, Zn, Cd, Cr, Pb, Ni) were analyzed in order to find out the physical and chemical properties of the sludge samples. The analyses of the sludge were performed according to the APHA-AWWAWEF (1992). Dry matter was measured gravimetrically (Method 505A) using furnace. Total nitrogen and phosphorus in the form of phosphate (PO4-P), were analyzed calorimetrically using a Nova 60 spectrophotometer (Merck, Darmstand, Germany); Salinity (Method 2520) and conductivity (Method 2510) were measured using a DC 144 69 DR conductivity meter (HACH, Iowa, USA); and pH was measured using an NEL 890 pH meter (NEL, Ankara, Turkey). Total extractable heavy metals (Ni, Zn, Cu, Pb, Cd, Cr, Mn, and Fe) were measured by means of the direct air-acetylene flame method (Method 3111 B) by means of atomic absorption spectrophotometry (AAS) using a UNICAM 9229 spectrophotometer. The Method 3111 B was used to measure K, Mg, and Na contents. The EDTA titrimetric method was used (Method 3500-Ca D) to measure Ca contents.

Plant Growth Experiments

In the experiments, five sludge treatments (Sets 1 to 5, including a control unit) were used with five replications in a greenhouse. The sludge was applied to the pots at various rates (Set 1: 0%, Set 2: 25%, Set 3: 50%, Set 4: 75%, and Set 5: 100%) before cultivation of the plants. For the control (Set 1), only soil mixture created from a mixture of soil, sand, and manure was used. The characteristics of the soil mixture was also tested before the experiments. No fertilization was provided before or during the study. Three lettuce plants (aysberg) were transplanted in each pot for SL-I whereas two lettuce plants (hugard) were transplanted in each pot for SL-II. The pots were watered to field capacity at the same time whenever the soil mixtures in the pots became dry. The seedlings were grown under controlled conditions for 2 months (between November and February), with constant temperature (21^{o}C) and humidity (60%). During this period, no additional sludge or soil mixture were supplied, but measures to control insects and pathogens were taken after emergence of the plants and 20 days later. At

harvesting, the plants were carefully removed without damaging their roots. The number of live plants and the number of leaves were counted. Then, the leaves and roots were washed with de-ionized water to remove any attached particles, and then dried them at 60^{o}C for 1 week. Afterward, the dry weight of green parts and roots were measured to determine the effect of various sludge loadings on growth of the lettuce plants.

RESULTS AND DISCUSSION

As stated before, three factors, (i) characteristics of sludge, (ii) sludge loadings to the plants, and (iii) behavior (reaction) of plants to sludge should be examined prior sludge application to the land. In the following, those factors are explained from the view of the selected industry sludge characteristics, application ratios and selected plant behavior.

The physical and chemical properties of the sludge samples (SL-I and SL-II) used in the experiments are presented in Table 1 and Table 2. The characteristics of the soil mixtures used in pots are also presented in Table 1 (SM-I and SM-II). The results obtained from characterization study was shown that organic matter contents of SL-I and SL-II were high (Table 1), however nitrogen content of SL-II is relatively low.

Table 1. The characteristics of the dewatered sludge samples and soil mixtures

Parameter	SL-I	SM-I	SL-II	SM–II
pH	6.7	7.5	11.2	7.6
Salinity (‰)	0.2	0.1	0.1	0.1
EC (µmhos)	1500	500	2750	620
Organic Matter (%)	25.2	17.4	38.3	10.8
T. Nitrogen (mg/L)	862.9	2402.1	16.8	60.63
T. Phosphorus (mg/L)	4199.3	709.2	64.83	121.7
Magnesium (mg/L)	5841.5	4673.6	289.2	582.8
Potassium (mg/L)	5273.4	2157.3	120.85	1528.4
Sodium (mg/L)	13902.6	3595.5	1217.7	2501.3

Nutrients in sludge such as phosphorus, magnesium and potassium are important because they support growth of plants and increases the potential for agricultural application. Therefore, it is thought that the low nitrogen content of the SL-II obtained from the vegetable oil factory may adversely affect the plant growth. The observations that turning the color of the leaves from green to yellow in SL-II supported this situation. In addition, due to use of caustic in the production process, the sodium concentration in vegetable processing sludge (SL-I) was found to be high. Heavy metals are one of the most important parameters in terms of agricultural applications. It is seen that the heavy metal contents of both the sludge and the soil used in the experiments are low. This situation presents a significant advantage for the selected industries.

The growth of the lettuce plants was evaluated by counting the numbers of live plants and leaves. For vegetable processing, after the growing period (i.e. 2 months), treatments Set 1 (control), Set 3 (50%), Set 4 (75%), Set 5 (100%) yielded more plants per pot than the other treatments. Even though, treatments Set 2 (25%) yielded less plants per pot than the other treatments, the differences were not significant. Therefore, one may conclude that none of the sludge loadings decreased seed germination or early seedling survival. The high levels of nitrogen, phosphorus and potassium made positive effect on plant growth, in general. The leave number was significantly high in Set 4 (75%). The mean leave number was counted as 16.8 leave/pot. The lowest mean number of leaves per pot was found both in the Set 2 (25%) and Set 5 (100%) treatments, and this represented a significant decrease in the number of leaves compared with the other treatments.

Table 2. Heavy metal concentrations in the dewatered sludge samples and soil mixtures

Heavy Metals as mg/kg SM	SL-I	SM-I	SL-II	SM – II
Nickel (Ni)	40	4	14,74	34,2
Zinc (Zn)	62	5	3,85	134
Copper (Cu)	42	nd	7,08	72
Lead (Pb)	Nd	nd	0,19	57.8
Cadmium (Cd)	0.7	nd	2,15	1.27
Chromium (Cr)	30	nd		
Manganese (Mg)	36	63	39.2	nd
Iron (Fe)	367	189	1.27	1.10

For vegetable oil production sludge (SL-II), treatments Set 2 (25%) and Set 5 (100%) yielded more plants per pot than the other treatments. Similar to SL-I, although other treatments yielded less plants per pot than the other treatments, the differences were not significant (between 2.4-2.8 plants/pot). The high levels of nitrogen, phosphorus and potassium made positive effect on plant growth, in general. The maximum leave numbers were obtained for control (0%) set (i.e. 30.8 leaves/pot). The leave number was significantly high in Set 4 and Set 5 (i.e. 18 and 20 leaves/pot)). The lowest mean number of leaves per pot was found both in the Set 2 (25%) and Set 3 (50%) treatments, and this represented a significant decrease in the number of leaves compared with the other treatments.

Uptake of heavy metals was also determined by the lettuce plants in the green parts and roots of the plants for the 75% and 100% sludge loadings. The results are shown in Table 3 and 4. The measured concentrations are evaluated using the standards given in Table 5. Since specific standards for lettuce have not been included yet in the Codex Standards except lead and cadmium, maximum limits for other metals are adopted from recent literature. Maximum limits of iron and manganese were stated as 425.5 and 500 mg/kg, respectively (Ewers, 1991; Pendias and Pendias, 1992). The limits of chromium, copper, nickel, and zinc are taken from the Weigert (1991). According to Wiegert, maximum limits of chromium, copper, nickel, and zinc are set as 2.3, 73.3, 67.9, and 99.4 mg/kg DS, respectively. In addition, according to the results of another study, limits of nickel, zinc and copper are revealed as 50, 300 and 150 mg/kg for lettuce plants (http://soilslab.cfr.washington.edu/esc311-507/1997).

Table 3. Metal concentrations measured at leaves and roots of SL-I

Vegetable Processing Sludge (SL-I)					
Parameter	**Unit**	**Leave**		**Root**	
		75%	**100%**	**75%**	**100%**
Nickel (Ni)	mg/kg DS	Nd	nd	Nd	0,8
Zinc (Zn)	mg/kg DS	146	188	96	110
Copper (Cu)	mg/kg DS	19,4	18	76	58
Lead (Pb)	mg/kg DS	Nd	nd	Nd	nd
Cadmium (Cd)	mg/kg DS	Nd	nd	Nd	nd
Chromium (Cr)	mg/kg DS	88	80	108	92
Manganese (Mn)	mg/kg DS	142	264	152	110
Iron (Fe)	mg/kg DS	5200	1092	3028	6254

The sludge did not significantly increase the amount of nickel, zinc, and cadmium. This is explained with high pH values of both sludge and soil mixtures (Dolgen et. al. 2004). Studies to determine the metal accumulation in the leaves of lettuce at high sludge rates showed that there was no significant increase in nickel, lead, cadmium and manganese concentrations, but the permissible concentrations for chromium, iron, copper and zinc were exceeded. Although zinc, copper and manganese elements are essential nutrients for plant growth, it should not be ignored if they are taken in large amounts, they may adversely affect plant growth (phytotoxicity).

Table 4. Metal concentrations measured at leaves and roots of SL-II

Vegetable Oil Processing Sludge (SL-II)					
Parameter	**Unit**	**Leave**		**Root**	
		75%	**100%**	**75%**	**100%**
Nickel (Ni)	mg/kg DS	2,14	5,91	0,64	3,77
Zinc (Zn)	mg/kg DS	19,27	25	0,42	29,54
Copper (Cu)	mg/kg DS	5,73	7,05	2,25	7,23
Lead (Pb)	mg/kg DS	0,68	1,59	0,23	2,54
Cadmium (Cd)	mg/kg DS	0,21	0,23	0,08	0,15
Chromium (Cr)	mg/kg DS	0,52	0,46	0,12	0,43
Manganese (Mn)	mg/kg DS	10,70	4,43	10,15	4,46
Iron (Fe)	mg/kg DS	4,74	21,59	17,71	45,39

Table 5. The permissible heavy metal concentrations for leafy vegetables

Parameter	**Unit**	**Standard**	**References**
Nickel	mg/kg	67.9; 50	Weigert (1991); http://soilslab.cfr.washington.edu
Zinc	mg/kg	99.4; 300	Weigert (1991); http://soilslab.cfr.washington.edu
Copper	mg/kg	73.3; 150	Weigert (1991); http://soilslab.cfr.washington.edu
Lead	mg/kg	0.3	FAO/WHO (CODEX) (2001)
Cadmium	mg/kg	0.2	FAO/WHO (CODEX) (2001)
Chromium	mg/kg	2.3	Weigert (1991)
Manganese	mg/kg	500	Ewers (1991); Pendias ve Pendias (1992)
Iron	mg/kg	425.5	Ewers (1991) ; Pendias ve Pendias (1992)

The experiments carried out using SL-II stated that the values exceeding the limit values were not measured in the concentrations of nickel, zinc, copper, chromium, manganese and iron in lettuce plants. However, the excess cadmium and lead elements in the raw sludge caused higher uptake by the plant. In particular, the lead concentration exceeded the limit values in both leaves and roots in 75% and 100% sludge applications. Besides that, for all metals except manganese, there was an increase in concentration in leaves at high sludge ratios.

CONCLUSIONS

The conducted study reveals that the wastewater treatment plant sludge of the vegetable processing and vegetable oil manufacturing industries have a potential for land applications. The results emphasized the significance of the sludge characteristics, sludge loading rate, and appropriate plant species for such applications. Sludge characterization studies demonstrated that the sludge used in this study was poor in heavy metals but rich in organic matter, macronutrients, and micronutrients thus it can be used as a partial substitute of chemical fertilizer and as a soil conditioner. Accumulation of heavy metals are related to plant spices and their tolerance, and the loading rate. Therefore, extensive scientific researches on sludge use on land are still needed. Considering all discussions, one may state that the sludges of agro-industry WWTPs have high potential for agricultural use in the context of circular economy (CE) approach.

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INVESTIGATION OF HUMAN ACTIVITIES IN THE COASTAL AREA AND ITS IMPACT ON BEACH LITTER IN THE SOUTHEAST BLACK SEA

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ABSTRACT

The main objective of the present paper is to investigate the impact of human activities on beach marine litter pollution along the Southeast Black Sea coast of Turkey. Two sandy beaches, Balıklı and Yeniay beaches (designated as T1 and T2 stations), known for attracting local visitors for recreational purposes, were selected as representative pilot study locations to assess the level of beach litter pollution in the region. During the summer of 2022, beach marine litter, specifically macro litter (human-made litter larger than 2.5 cm), was collected and cleaned from these sites. The collected litter was counted to gauge the extent of beach marine litter pollution and determine the Coastal Clean Index (CCI). As a result, a total of 287 items were gathered and removed from the beaches, with plastic emerging as the predominant material, followed by paper and various other types of litter. Commonly found items at the stations included single-use items such as cigarette butts, beverage containers, and plastic fragments. The results pointed to a high level of pollution along the Southeast Black Sea coast, with Balıklı and Yeniay beaches exhibiting litter densities of 1.42 and 1.45 items per square meter, respectively. According to the Coastal Clean Index (CCI), both beaches fell under the category of "extremely dirty," surpassing an index value of 20. The outcomes of this study emphasize that human activities on the beaches play a significant role in the accumulation of marine litter, revealing a lack of awareness among beachgoers regarding the environmental impact of beach marine litter.

Keywords: Beach litter, marine litter, pollution, anthropogenic, Black Sea

INTRODUCTION

A variety of valuable resources that are necessary for human habitation and subsistence are provided by the coastal region in significant quantities (Yılmaz & Terzi, 2018). These resources cover a wide range of purposes, from scenic natural areas and leisure activities to vital industries like fishing, agriculture, and tourism (Salas-Leiton et al., 2021). Coastal areas often function as crucial transit hubs, allowing both local and international trade and business (Citra & Nugraha, 2021; Konstantinus & Woxenius, 2022). The coastal environment also supports diverse sectors like mariculture and aquaculture that have significant beneficial effects on the regional economy and the world's food supply (Mehvar et al., 2018). Additionally, coastal areas support rich biodiversity, also the scenic beauty and recreational opportunities of the coastal region draw visitors, promoting tourism as a significant economic engine for coastal communities (Barbier et al., 2011; Christie et al., 2015). Because of the diverse resources they provide, coastal areas are therefore essential for sustaining both human life and economic prosperity (Lakshmi, 2021).

Coastal regions are frequently confronted with the challenge of waste generation stemming from human activities. The dynamic blend of urban development, thriving tourism, fishing industries, and agricultural practices along the coast collectively contribute to the substantial amount of solid waste produced in these areas (Lotze et al., 2006). Urban development projects create more consumer goods, construction materials, and packaging waste, while tourism brings an influx of visitors, often leading to a higher volume of disposable items (Qiang et al., 2020; Student et al., 2020; Tsai et al., 2021). Additionally, fishing and agriculture activities generate organic waste and packaging materials. As populations grow and coastal areas become popular destinations for both residents and visitors, the demand for resources and services increases, leading to a surge in waste generation.

Increasing global population, rising consumption rates, and depletion of natural resources, the industrial sector is under increasing pressure to maximize manufacturing efficiency and cost-effectiveness. Industry has introduced products like plastics, which are versatile and economical to produce, to address these issues (Geyer et al., 2017). Due to their adaptability, plastics have become commonplace in a wide range of human-made products (North & Halden, 2013). However, a serious issue is raised by the fact that the rate of plastic degradation cannot keep up with their quick production, which causes an accumulation of litter in our environment (Bergmann et al., 2015; Geyer et al., 2017). In order to lessen the negative effects of plastic pollution on the environment, ecosystems, and marine life, effective waste management strategies, recycling programs, and sustainable alternatives are urgently needed.

The allure of the coastal areas has led to a rise in human-generated waste, driven by the region's popularity as a sought-after tourist destination and a hub for fishing, mariculture, agriculture, and various other human activities (Berkun et al., 2005; Kibria et al., 2023). Consequently, the proliferation of beach litter has become a multifaceted environmental challenge that demands urgent action and effective mitigation strategies to safeguard the region's natural beauty and ecological balance. Addressing this pressing issue requires a collaborative approach from all stakeholders to implement immediate and sustainable solutions for waste management and conservation in the coastal areas. For sustainable coastal management and the preservation of the area's distinctive biodiversity, it is essential to comprehend how human activities affect beach litter pollution. Tourism-related garbage, incorrect waste disposal, and recreational activities are just a few anthropogenic elements that significantly contribute to the degradation of beach habitats. Further endangering the delicate coastal ecosystems are the surge in single-use plastic use and the improper management of medical and sanitary waste (Brouwer et al., 2017; Dahms et al., 2019).

This study attempts to explore the link between human activity and beach litter pollution in Turkey's Southeast Black Sea region. This study aims to provide insight on the causes of beach litter pollution and its implications for the marine ecosystem and nearby communities by investigating the composition, distribution, and density of beach litter at several coastal sites. In the end, the results of this research will aid in the development of efficient conservation methods and policy interventions that can aid in the prevention of beach litter pollution and the preservation of the ecological health of the coastal areas along the Southeast Black Sea. In order to ensure the long-term prosperity and health of these coastal regions for future generations, the effective execution of such policies would be essential. To ensure the long-term sustainability of areas and minimize the effects of human generated waste it is vital to implement waste management techniques and raise awareness through campaigns.

MATERIAL AND METHOD

Study area

The study was conducted in the coastal area of Sürmene district, which is situated within Trabzon Province in the Southeast Black Sea Region of Turkey. Specifically, this research focused on the Sürmene districts, which are positioned along the coastline of Trabzon Province (Figure 1). These districts were deliberately chosen as the primary research sites due to their strategic location among the nine districts bordering the coastal areas of Trabzon Province. This selection makes them highly relevant and significant locations for investigating human activity's impact on beach marine litter pollution in the region.

The level of human activity is quite intense along Turkey's southeast Black Sea coast. The region has experienced ongoing coastal development projects that involve land reclamation, which has significantly altered the region's original coastal landscape. Additionally, a sizeable portion of the local population lives close to the riverbanks and coastal areas. The population of Sürmene was estimated at 25,950 according to statistics from 2022 (TUİK, 2022). But it's crucial to understand that there are seasonal variations in the population in this area, with lower numbers in the winter and a noticeable increase in the summer because of the influx of both domestic and foreign tourists attracted to the rural tourism destinations in the area (Efe et al., 2022). The main industries that characterize the region's economy are fishing, mariculture, agriculture, and tourism.

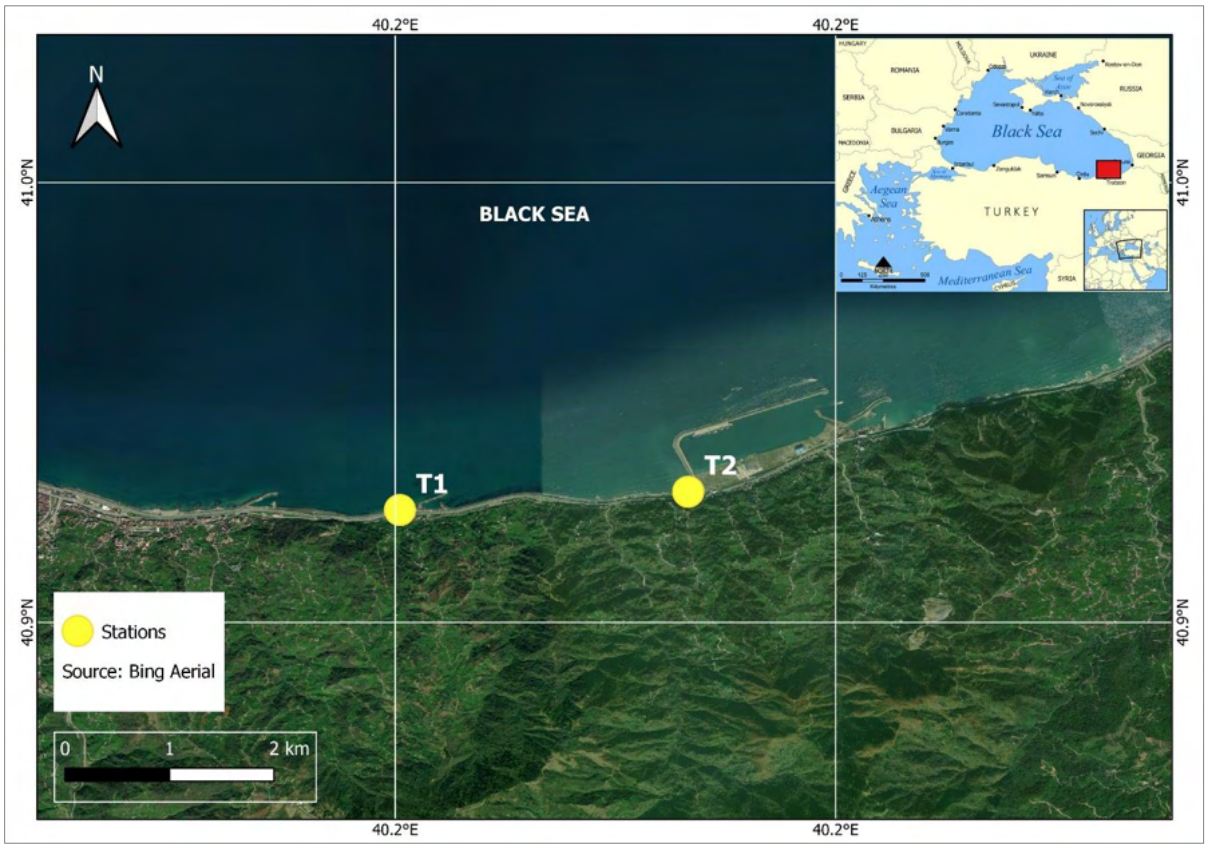

Figure 1. Map of study area includes two recreational beaches (T1 and T2) in the Southeast Black Sea coasts of Turkiye

Marine litter sampling was conducted at two data stations located along the Surmene and Of coasts. The collection of beach litter samples was carried out during the summer season of 2022. Summer attracts a significant surge in human activities in the coastal area, as people engage in various outdoor activities, including visiting beaches for swimming, sunbathing,

picnics, and camping. The selection of data stations considered several factors, such as their designation as recreational beaches.

Litter sampling

All human-generated litter categorized as macro litter with size larger than 2.5 cm found within the designated transect areas of 100 square meters was meticulously gathered and recorded at each data station. However, it should be highlighted that natural debris, such as seaweed, animal bones, and untreated wood, was intentionally excluded from the litter collection process. The collected litter was categorized into 42 subcategories and nine primary categories (plastic, rubber, fabric, wood, metal, glass, sanitary waste, and medical waste) following the OSPAR classification system (Aytan et al., 2019; Terzi et al., 2020; Wenneker et al., 2010). The collected items were counted meticulously for the purpose of estimating the litter density in terms of quantity (numbers). This comprehensive approach allowed valuable insights to be gained into the types and quantities of litter present within the study area.

Litter analysis

An analysis was undertaken to assess the extent of litter pollution in the study area, with a focus on the litter composition, distribution, and density at all stations throughout the study period. To ascertain the litter composition, the percentages of each litter category were calculated in relation to the overall collected litter. The measurement was expressed as items per square meter (items/m2). The litter density was derived using the provided equation (Eq. 1). The density of litter items (D) was calculated by considering the total number of litter items collected from the transect (N), the width of the transect (w), and the length of the transect (l), which were measured in meters (Terzi et al., 2020; Terzi & Seyhan, 2017).

$$D = N/(w * l) \quad (1)$$

The evaluation of the cleanliness status of the beaches was accomplished using the Clean Coast Index (CCI) formulated by (Alkalay et al., 2007). The CCI, represented by Eq (2):

$$CCI = \left(\frac{Total\ litter\ on\ transect}{Total\ area\ of\ transect}\right) * K \quad (2)$$

The CCI scale consists of four categories, ranging from "very clean" (0–2), "clean" (2–5), "moderately clean" (5–10), to "dirty" (10–20), and "extremely dirty" (>20). To prevent values from falling between 0 and 1, a coefficient of 20, denoted as K, was utilized as a multiplier (Akarsu et al., 2022; Alkalay et al., 2007; Chen et al., 2020).

RESULTS AND DISCUSSIONS

In the summer of 2022, two distinct beach stations within the study area located in the Sürmene district of Trabzon Province, situated in the Southeast Black Sea region of Turkey, underwent a comprehensive collection of beach marine litter as part of this research investigation. Every item that falls within the designated transect underwent meticulous registration and counting to facilitate its subsequent classification into 42 subcategories and 9 major litter categories in accordance with the OSPAR classification system. Once this detailed categorization process was completed, the entire collection of litter was promptly removed from the beaches and relocated to the garbage bins, as an important step to reduce litter pollution in the area and protect the coastal and marine environment.

The study focused on assessing the abundance of beach marine litter items found within the designated study area. The results of the investigation revealed that a total of 287 items of beach marine

litter from two stations were collected during the study period. The analysis further demonstrated that plastic materials constituted the most prevalent material, accounting for a significant majority of the litter composition in both study locations when assessed based on the number of items (quantity). Subsequently, other materials with paper being the most prominent, constituted the remaining portion of the beach marine litter found at the study sites. Specifically, the total beach marine litter, based on item count, 75,6% plastics, 20.2% paper, 1.4% metals, 0.7% glass, 0.7% cloth, 0.7% medical waste, and 0.7% sanitary waste (Figure 2).

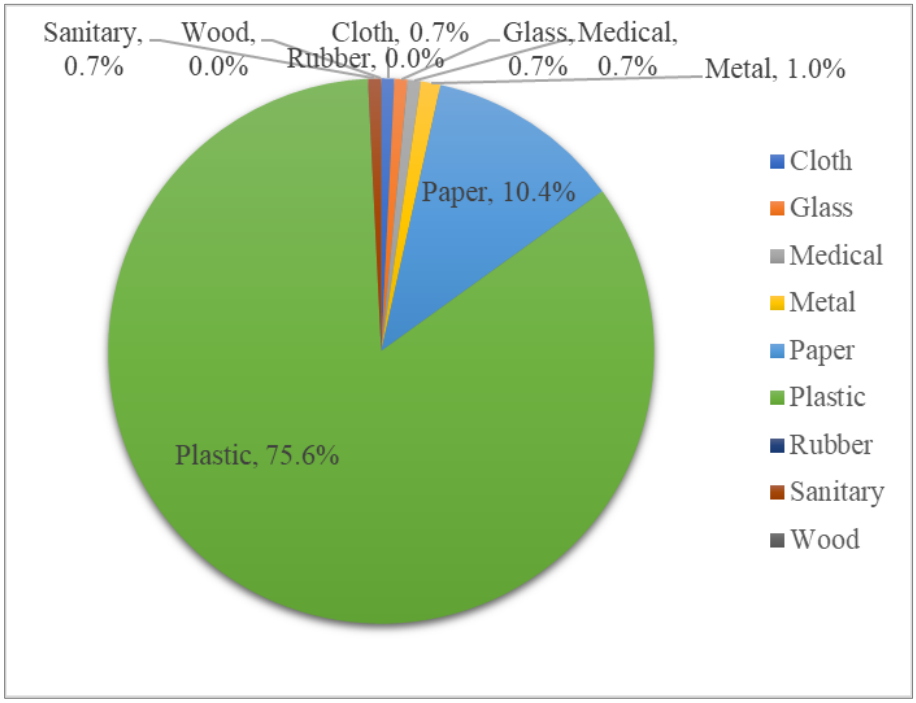

Figure 2. Aggregate beach litter composition in both station

Despite the relatively lower composition of plastic materials in the Balıklı (T1) station compared to the Yeniay (T2) station, plastic remains the predominant litter type in both locations (Figure 3). These study findings are in line with previous research conducted on beach marine litter at both regional and global levels, which consistently highlighted plastic materials as the dominant component in marine litter (Aytan et al., 2019; Bergmann et al., 2015; Galgani, 2014; Terzi et al., 2020; Terzi & Seyhan, 2017). However, it is worth noting that the proportion of plastic materials in marine litter observed in the current study area is relatively lower when compared to more recent investigations on beach marine litter at both regional and global scales. In certain studies, plastics have been reported to account for as much as 85% of the total beach marine litter.

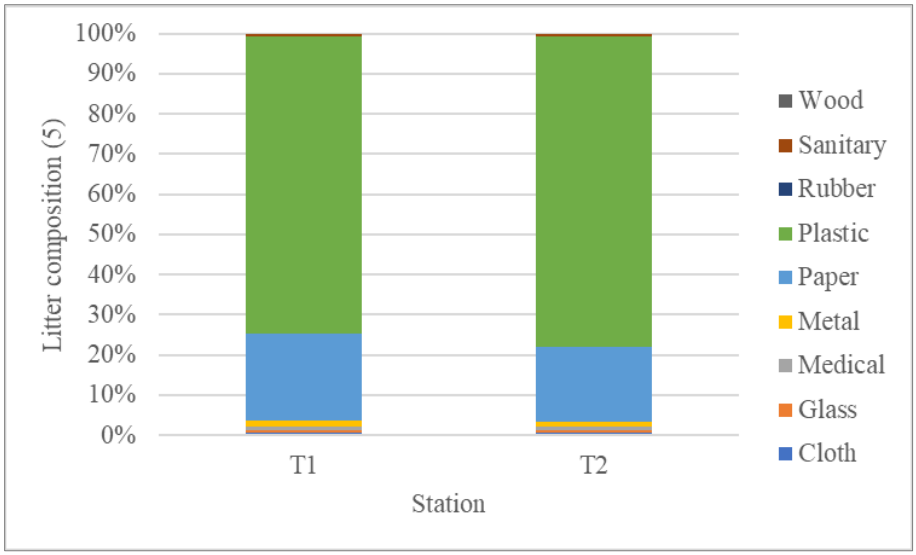

Figure 3. Beach litter composition in Balıklı (T1) and Yeniay (T2)

The examination of marine litter composition clearly demonstrated that plastic is the most predominant material in the study areas. The top twenty commonly found litter categories in both stations were dominated by plastic and plastic-coating items (Figure 4). Notably, plastic pieces exhibited a higher density in the study areas compared to other materials. Additionally, there was a significant abundance of single-use plastic litter, including cups, caps/lids, plastic bottles, cigarette butts, and beverage containers, indicating a substantial utilization of single-use plastic items that contribute significantly to beach litter pollution. The presence of commonly found items such as cigarette butts, beverage containers, plastic bottles, bags, and packages underscores the impact of leisure activities and waste generated by beachgoers because of personal consumption on the beach environment (Portman & Brennan, 2017).

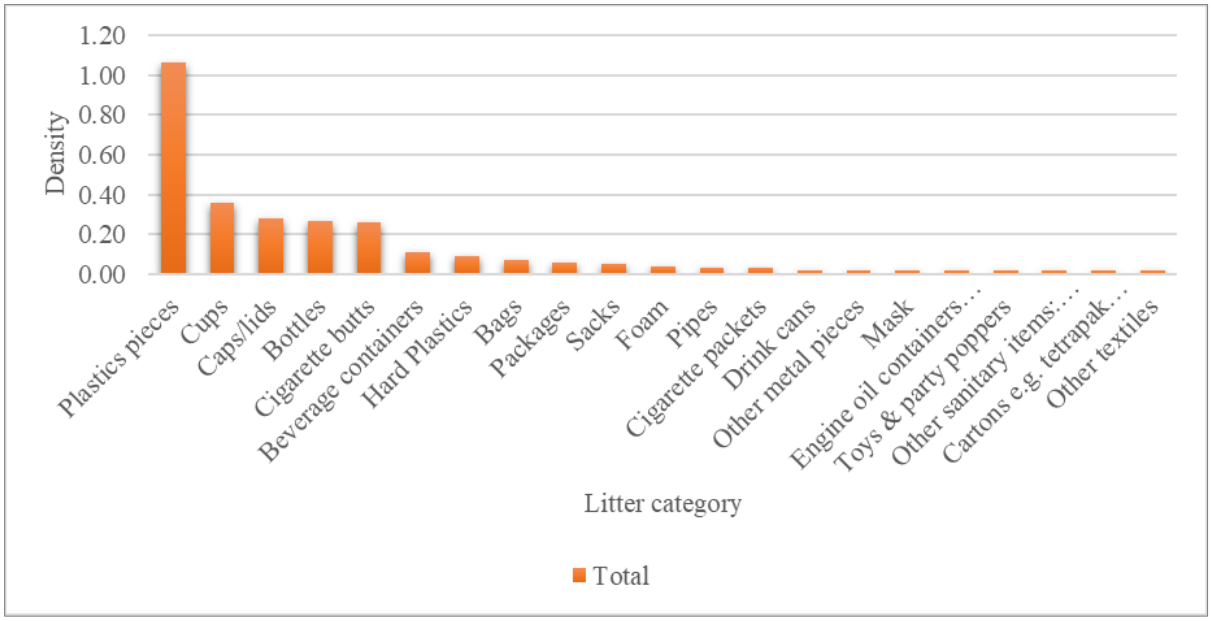

Figure 4. Top twenty most abundant litter categories

The assessment of litter density, quantified as items per square meter (items/m²), revealed a concerning level of beach litter pollution along the Southeast Black Sea coast of Turkey. Both Balıklı

(T1) and Yeniay (T2) beaches exhibited high litter densities of 1.42 and 1.45 items per square meter, respectively, classifying them under the category of "extremely dirty" based on the Coastal Clean Index (CCI) (Figure 5). In comparison, the Black Sea coasts generally have litter densities ranging from 0 to 5 items per square meter (Aytan et al., 2019; Terzi et al., 2020; Terzi & Seyhan, 2017). These findings highlight the severity of the litter pollution issue in the study area, indicating an urgent need for effective waste management and pollution mitigation measures to preserve the coastal environment's integrity and protect marine ecosystems. Implementing strategies to reduce litter and promoting responsible waste disposal practices will be essential to address this pressing environmental challenge and ensure the long-term sustainability of the Southeast Black Sea region.

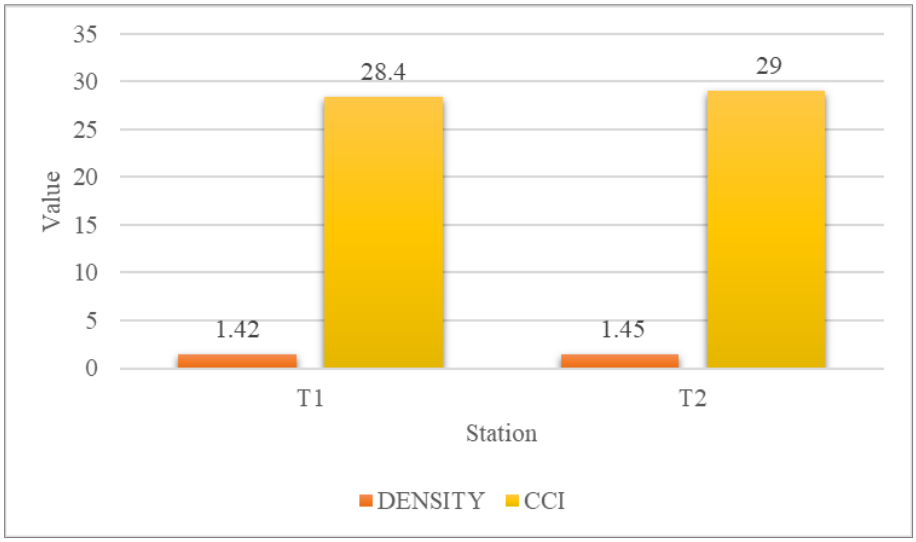

Figure 5. Litter density and Coastal Clean Index (CCI)

The investigation into beach marine litter pollution along the Southeast Black Sea coast has unveiled significant impacts of human activities on the coastal environment, evident by the abundance of human-generated waste in the study area. This discovery highlights the substantial visitation and public consumption of food and beverages, even though the beach is categorized as an underdeveloped recreational area. The research findings underscore the crucial role that human activities play in the buildup of marine litter. Notably, the prevalence of single-use items like cigarette butts, beverage containers, and plastic fragments emphasizes the importance of raising awareness among beachgoers about the consequences of beach marine litter. The lack of awareness regarding marine litter pollution issues contributes to the continuous accumulation of litter on the beaches, posing negative implications for the coastal environment and marine life.

In an effort to curb the usage of single-use plastic bags, Turkey has implemented regulations in 2019 to charge for plastic bags (Çevre ve Şehircilik Bakanlığı, 2019; Dursun, 2020). Similar practices in other parts of the world have shown promising results, leading to a reduction of up to 85% in plastic bag consumption (Cabrera et al., 2021). However, despite these regulations, the cost of plastic bags in Turkey remains relatively low, and some vendors even continue to offer them for free. This practice could undermine the intended purpose of the regulations and potentially encourage unrestrained and excessive use of plastic bags. As a consequence, the persistent issue of plastic waste in the country may be exacerbated by the continued availability and affordability of single-use plastic bags. To achieve the desired reduction in plastic bag usage and address the plastic waste problem effectively, it will be crucial to enforce the regulations more strictly and create awareness among consumers about the importance of reducing plastic usage and embracing reusable alternatives.

These results emphasize the significance of human activities in the accumulation of marine litter and underscore the necessity for increased awareness and responsible waste management practices among beachgoers. The findings shed light on the urgent need to address overconsumption and improper disposal of single-use plastic products to mitigate the growing marine litter problem and safeguard the coastal environment. Effectively tackling beach marine litter requires collective efforts from local communities, authorities, and stakeholders to promote sustainable behaviors and reduce reliance on single-use plastic items. Implementing effective waste management strategies and raising awareness about the environmental consequences of beach litter pollution are essential in preserving the pristine coastal environment of the Southeast Black Sea region for future generations. By raising awareness and enforcing responsible waste management practices, we can combat beach marine litter pollution and contribute to the long-term ecological health and sustainability of the region's coastal areas.

CONCLUSIONS

The findings revealed a concerning state of beach litter pollution, with a total of 389 items collected and removed from the beaches. Plastic emerged as the most prevalent material, followed by paper and various other types of litter. Disturbingly, single-use items like cigarette butts, beverage containers, and plastic pieces were frequently encountered at the study stations. The litter density measurements, using the Coastal Clean Index (CCI), exposed a high level of pollution at both Çavuşlu and Yeniay beaches, exceeding an index value of 20. These results classify both beaches as "extremely dirty," underscoring the severity of the litter pollution issue. Overall, the research highlights the substantial role human activities play in contributing to beach marine litter pollution. It also emphasizes the importance of raising awareness among beachgoers regarding the detrimental environmental consequences of beach litter. Sustainable waste management practices, coupled with education and public outreach, are essential to mitigate the growing problem of beach litter pollution in the Southeast Black Sea region of Turkey. Implementing effective strategies will not only preserve the natural beauty of these coastal areas but also safeguard the well-being of marine life and ecosystems for generations to come.

RECOMMENDATIONS

Based on the study's findings, a number of important suggestions can be made to address the problem of increasing beach litter pollution in the Southeast Black Sea region, particularly in the Balkl and Yeniay beaches. First of all, there is a critical need for focused public awareness campaigns and educational initiatives that target both visitors and local populations. The main goal of these initiatives should be to draw attention to the harmful environmental effects of marine litter, especially that caused by single-use items like cigarette butts, drink cans, and plastic fragments. People can be empowered to actively contribute to the reduction of beach litter pollution by increasing awareness and promoting ethical waste management practices. In order to effectively combat the issue of beach marine litter, it is imperative to implement sustainable waste management techniques. In order to create and implement rules that limit the use of single-use plastics and non-recyclable materials in coastal areas, local authorities and stakeholders should collaborate. Additionally, encouraging the use of environmentally friendly substitutes and providing incentives for recycling will significantly help to reduce litter pollution in the marine ecosystem. Furthermore, community involvement should be encouraged in regular, organized beach clean-up efforts. Along with assisting in the removal of existing litter, involving locals and volunteers in these initiatives fosters a sense of ownership and responsibility for protecting the coastal environment. In addition, ongoing research and monitoring are necessary to evaluate the success of adopted strategies and spot new difficulties. In order to effectively combat beach litter pollution, regular assessments of litter composition, distribution, and density will offer insightful policymaking and adaptive measures. These

suggestions can help the Southeast Black Sea region significantly reduce beach litter pollution and protect the health of the marine ecosystem for the benefit of current and future generations. In order to maintain the natural beauty and ecological integrity of the region's coastal areas, everyone must put forth an effort and make a commitment to sustainable practices.

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GASTROINTESTINAL HELMINTS OF CATTLE IN SEMI INTENSIVE BREEDING AT BELGRADE AREA

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ABSTRACT

The spread area of Belgrade has extremely favorable conditions for modern agricultural production (climate, agricultural land, watercourses, developed processing industry). This economic branch is of strategic importance for supplying Belgrade with food products, along with the resources that abound in the wider environment (Vojvodina and Šumadija). There are numerous villages here, where households keep cattle in small herds or mini-farm, usually in semi-intensive breeding. During our study performed on 2018 we examined faeces of 190 cattle from 42 herds and 29 cattle by post-mortem examination. Determination of eggs and adult parasites performed on their morphological characteristics. The coprological examination established the presence of gastrointestinal helminth eggs in 39.6% of samples. The majority of cattle were infected with two and fewer number with three or four parasite species. At post-mortem examination of cattle we found *Haemonchus contortus* we occured in 57,53%, *Ostertagia ostertagi* in 55,63%, *Trichonstrongylus axeis* in 49.37%, *Cooperia oncophora* in 32.57%, *Ostertagia trifurcata* in 29.79%, *Oesophagostomum radiatum* 21.22%, *Toxocara vitulorum*17.52%, *Dicocelium dendriticum* 15.26%, *Paramphistomum ichikawai* in 14.21%, *Strongyloides papillosus* 11.51%, *Moniezia benedeni* in 9.47% and *Trichuris discolor* in 6.52%.

Keywords: gastrointestinal helmints, cattle, Belgrade, Serbia

INTRODUCTION

Gastrointestinal helminthiasis is a parasitic infection caused by a group of helminth parasites, which affect the gastrointestinal tract (GIT), associated organs, and whose eggs are excreted to the environment through animals' faeces. GIT helminths are ubiquitous parasitic agents of livestock especially ruminants at and are known to limit cattle production in many areas and countries worlvide. The prevalence variations of specific GIT across different regions suggest the influence of several epidemiological factors on the magnitude of the infection. Of many epidemiological factors, husbandry management in general one of the main conditions for the spread and prevalence of infections. Pasture borne gastrointestinal nematode (GIN) parasites are very common in grazing cattle and thereby represent a significant economic and welfare burden to the global ruminant livestock industry (Spence *et al*.,1996; Stancampiano *et al*., 2007) Mortality of cattle due to parasitic diseases not be alarming at times but their indirect effects on livestock productivity like reduction in productive potential such as decreased growth rate and milking or weight loss are considerably greater (Eckstein *et al*.2015; Forbes *et al*.2004; Spriinger *et al*.2021)

In Serbia, in the past period, cattle breeding was mostly done by agro-industrial companies on large farms with thousands of animals. The cattle breeding in rural communities are within backyard or at small farms with agro-pastoral feeding, and is considered an important economic sector of the food industry

Research on the parasitofauna of cattle in Serbia has not been done sporadicly and that was the reason why we renewed these examination (Babić,1965; Aleksić,1976; Vučković,1976; Toplica, 1987; Marusić, 1988; Stankovic, 2007; Pavlovic *et al*.2022a,b).. In our paper we presented results of examination performed in semı ıntensıve breedıng at Belgrade area

MATERIAL AND METODS

The study of GIT infection performed during 2018 we were carried out in herds of cattle originated from from 6 Belgrade districs Mladenovac, Lazarevac, Obrenovac, Grocka, and Vozdovac (from the village Mladenovac, Vlaska, Mala Krsna, Velika Krsna,Medjuluzje, Senjak, Velika Ivanca, Orašac, Mala Vrbica, Rajkovac, Dubona, Šepšin, Resnik, Ritopek, Vrčin, Vinča, Leštane, Pinosava, Grocka, Velike Granice, Granice, Koracica, Jagnjilo, Markovac, Lazarevac, Arapovac, Junkovac, Leskovac, Sokolovo, Rabrovac, Vrbovno, Zvecka, Krtinska and Stepojevac).

During our study we examined faeces of 190 cattle from 42 herds and 29 cattle by post-mortem examination. During study we collected fecal samples and exaitinatiolls perfonned using standard coprological technique with saturated NaCl solution and seditrentatiotr (Euzebry,1981, Pavlovic and Rogozarski,2017), Determination of eggs and adult parasites performed on their morphological characteristics. Examinations we performed with Carl Zeiss AxioLab A1 microscope with the Axiocam 105 Color microscope camera and Zen Lite software.

RESULTS AND DISCUSION

The coprological examination established the presence of gastrointestinal helminth eggs in 39.47% of samples. The majority of cattle were infected with two and fewer number with three or four parasite species. Our examination showed a high overall prevalence of Nematodes infection (39.47%) than Trematodes (15.26%) and Cestodes (9.74%).

During post-mortem examination we found twelve helminth species. In rumen we occured *Paramphistomum ichikawai,* in abomasus we found *Ostertagia ostertagi*, *O.trifurcata* and *Trichostrongylus axei* (which are also found in the small intestines), in small intestine we found *Moniezia benedeni, Toxocara vitulorum, Strongyloides papillosus, Cooperia oncophora* and *Trichuris discolor, in* large intestine we found *Haemonchus contortus* and *Oesophagostomum radiatum* and in bile ducts and in the gall bladder *Dicocelium dendriticum.*

The prevalence of established parasites was as follows: *Haemonchus contortus* we occured in 57,53%, *Ostertagia ostertagi* in 55,63%, *Trichonstrongylus axeis* in 49.37%, *Cooperia oncophora* in 32.57%, *Ostertagia trifurcata* in 29.79%, *Oesophagostomum radiatum* 21.22%, *Toxocara vitulorum*17.52%, *Dicocelium dendriticum* 15.26%, *Paramphistomum ichikawai* in 14.21%, *Strongyloides papillosus* 11.51%, *Moniezia benedeni* in 9.47% and *Trichuris discolor* in 6.52%.

In the current study, high rate of infection were closely associated with animals in poor body condition. Study showed that in older animals both the prevalence and number of GIT were higher than in younger ones. Family Trichostrongylidae contains most of the important gastro-intestinal nematodes of cattle around the world (Harding and Threlfall, 1989; Nwosu *et al*.,2007; Surbu *et al.*,2020). The life cycle of the trichostrongyles is direct. For most species first-stage larvae develop in, and hatch from, the eggs passed in faeces. These larvae moult twice to the ensheathed third-stage, which are infective and are ingested by the cattle. Under ideal environmental conditions this translation takes approximately one week, but the rate of development is temperature-dependent. The infective larvae continue their development in the mucosa of that part of the gut in which the adults live, then emerge into the lumen and become adults. Most of found helminths are pathogenic to their hosts leading, besides other disorders, to anemia, gastroenteritis and depressed growth rates and mortality (Forbes *et al.*2004; Stancampiano *et al*., 2007; Högberg *et al*.2019; Spriinger *et al*.2021).

The disease is related to the grazing diet and the biological cycle of the parasite, which takes place without transitional hosts. The developmental cycle of the parasite is straightforward. From these reason presence of tapeworm and fluke was only at hilly-mountain region of Belgrade were good condition to development of intermediate hosts of this parasites species. The seasonal dynamics of certain types of parasites, the degree of infection and the occurrence of diseases vary not only in different areas but also in the same area during the year.

At the beginning of the grazing season pastures are essentially parasite-free, except for *Nematodirus*. Any free-living stages of the other trichostrongyles remaining on the pasture at the end of the previous summer have died over the winter or during the early spring, before the cattle are put out to graze (Forbes *et al.*,2004; Hesterberg et al.,2007). Where there is essentially no overwinter survival of the parasites on pasture, it is the eggs in the feces of the cows, and other older animals, that introduce trichostrongyle infection to the pastures in the spring. The rate of development and hatching of these eggs increases with seasonal warming of the environmental temperatures, and when the calves begin to graze significantly the pasture contains plentiful infective larvae. These larvae establish infections in the calves, which are more susceptible to infection than are older animals, sometimes at high levels (Larson *et al*.,2007) The adult parasites in the calves then produce more eggs which, if environmental temperatures are sufficiently warm, develop into a second generation of infective larvae (Chihai 2006; Yevstafieva *et al.*,2020). As temperatures cool in the fall, egg hatching and larval development on the pasture slow then stop. Same was happened with intermediate hosts of tapeworm and fluke (Irie *et al.,* 2013; Ayalew et al.,2016; Pavlovic et al.2022a,b

The parasite-host relationship is complex: physiological state and general condition, method of cultivation and nutrition, time of calving, configuration and macroclimate of the soil. In natural conditions, every animal is infected - constant contamination of the pasture. This is contributed by the increased susceptibility of the already infected herd, the introduction of

susceptible animals into the infected herd and the increase in the intensity of the infection in the already infected herd (Forbes *et al.*,2004; Szyszka, and Kyriazakis,2013).. Immunity develops through continuous infections, and then there is the elimination of the present parasites (self cure mechanism), complete or partial inhibition of the development of newly introduced larvae (spring rise) and complete or partial inhibition of the reproductive abilities of female parasites.

CONCLUSION

The result of our study shows a moderate prevalence of gastrointestinal helminth infection. Temporary breeding on pastures in the presence of cattle of all age categories creates favorite conditions for the development and survival of preparasitic forms and their intermediate hosts outdoors, which enables the infection of calves with gastrointestinal strongyles, flukes and tapeworm. This indicates the need to continue these researches in order to control parasitic infections in cattle.

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EFFECT OF MUSHROOM EXTRACTS ON COLOR CHANGE AND SOME CHEMICAL PROPERTIES OF DEHYDRATED SOUPS

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ABSTRACT

The aim of this research was to produce lyophilized water extract from several mushroom species (*Suillus granulatus, Coriolus versicolor, Fuscoporia torulosa*) and to determine its influence on some chemical properties as well as on the color changes of the industrially produced Bio Soups, without the addition of monosodium glutamate. The realisation of the planned research was carried out by designing four variants of dehydrated soup. The content of mineral matters was significantly ($p<0.05$) higher in all soup variants enriched with lyophilized extracts. Moreover, the moisture content in all analyzed soup variants is in accordance with the regulations. From the aspect of the instrumental color value, constancy was established for all parameters in all tested variants from the 0th to the 90th day after production, i.e. all soups had a stable color during storage, which means that there were no changes in other chemical or biological parameters that would lead to a change in the color of the product. Therefore, statistically significant ($p<0.05$) differences were observed only between the control variant in relation to the other variants, i.e. the darker shade in the analyzed variants enriched with lyophilized extracts is a consequence of the applied extracts. In this way, a new product is obtained that does not contain chemical additives, which further increases its value.

Key words: Mushroom extracts, Color, Moisture, Dehydrated soups.

INTRODUCTION

Functional food is food that, in addition to its important nutrients, has a beneficial effect on human health. Interest in functional foods is increasing, and edible mushrooms (fresh or dried) or their extracts can be consumed as functional foods (Niva, 2007).

Dehydrated soups belong to the category of products that are prepared very quickly, and on the other hand, they are safe in terms of microbiological contamination, primarily due to their low water content. In addition to stability during storage, dehydrated soups have beneficial nutritional properties, and often therapeutic ones. Due to these characteristics, soups are an acceptable product for all types of consumers. Generally, commercial dehydrated soups are prepared from various types of vegetables, poultry and beef, mushrooms and the like (Stojanova et al., 2023).

Mushroom soups are a traditional food in China. They have been consumed since ancient times due to their nutritional value, excellent taste and functional properties (Tharshika et al., 2016). Such soups contain important nutrients, such as amino acids, monosaccharides, dietary

fiber and numerous other bioactive components isolated from mushrooms. Dehydrated soups with the addition of mushrooms or mushroom extracts are characterized by anti-cancer effects and have anti-atherogenic and immunomodulatory properties (Prameela and Prameela, 2020).

Vegetable soup, as a functional food, is rich in bioactive compounds such as antioxidants, dietary fibers, essential amino acids and oligosaccharides. It is believed that vegetables and mushrooms included in soups can prevent many chronic conditions of the body (Chandramouli et al., 2012).

The presence of a wide range of dehydrated soups on the market largely replaces the use of homemade soups. On the other hand, the industrial production of dehydrated soups, in addition to other ingredients, includes the use of synthetic additives, which are part of the composition of soups. However, the increased interest and greater need for safe food that does not contain chemical additives, but retains the desired sensory characteristics, implies the use of alternative compounds (Amal et al., 2014).

The science of color (colorimetry) was developed due to the need for objective assessment of color characteristics, which cannot be achieved only by human perception of color, i.e. due to the need to quantify color and express it in numerical values. The color of the object does not depend only on the characteristics of the object itself, but also on the light with which the object is illuminated, as well as on the state in which the observer is, because a tired eye has a reduced sensitivity to color (Đurišić et al., 2007). There are several definitions of color, and according to the SRPS ISO standard (SRPS EN ISO 5492:2012), color is a sensation caused by stimulation of the retina with light rays of different wavelengths. MacDougall (2002) defines color as a combination of visually perceived information contained in light reflected from a sample.

From the aspect of full realization of the mentioned potential, research on the influence of mushrooms and their extracts on the quality of food products is of great importance. Application of mushroom extracts in the food industry can reduce or replace many chemical additives, which contributes to the creation of new and attractive products of increased biological value with reduced content of synthetic additives.

The aim of this research was to produce lyophilized water extract from several mushroom species (*Suillus granulatus, Coriolus versicolor, Fuscoporia torulosa*) and to determine its influence on some chemical properties as well as on the color changes of the industrially produced Bio Soups, without the addition of monosodium glutamate.

MATERIAL AND METHODS

Mushroom collection

In this research, as a work material three Variants of mushrooms collected from the territory of the Republic of North Macedonia were used, as follows: *Suillus granulatus* (L.) Roussel, edible mushroom (collected from Bistra Mountain near the village Sretkovo at an altitude of 1100 m, in a pine forest (Pinus), on a soil substrate); *Coriolus versicolor* (L.) Lloyd, medicinal mushroom (collected from Maleshevska Mountain– Klepalo, at an altitude of 1340 m, in beech forest (Fagetum), on a substrate of Fagus sylvestris trunk); and *Fuscoporia torulosa* (Pers.) T. Wagner & M. Fisch, medicinal mushroom (collected from Ganustiana, near the river Bregalnica, at an altitude of 182 m, on acacia stump [*Robinia pseudoacacia*]).

Preparation of water extract

The water extract was prepared according to the methods of Slawinska et al. (2013) and Ribeiro et al. (2015). Measured mass of dried and finely powdered mushroom sample (10 g) was poured with approximately 200 mL of distilled water and then extracted in a boiling water bath for 1 h.

Bio-soup production

Dehydrated soups were produced in the industry in North Macedonia. The control variant, that is the soup that is conventionally available on the market, was produced according to the following recipe: mixed dried vegetables (carrot, parsnip, onion and parsley); salt; sugar; oil; monosodium glutamate and pasta. The recipe was modified so that instead of monosodium glutamate, lyophilised water extracts were added, separately. Due to the characteristic taste, compensating for the absence of monosodium glutamate and improving the sensory properties of the final product, dried and ground boletus (*Boletus edulis*) commercially available was added. After production, the soups were packed in 45 g bags and stored at room temperature until further analysis.

The realisation of the planned research was carried out by designing four variants of dehydrated soup:

- Variant 1 – control variant;
- Variant 2 – dehydrated vegetable soup without monosodium glutamate, enriched with lyophilised water extract of *S. granulatus*;
- Variant 3 – dehydrated vegetable soup without monosodium glutamate, enriched with lyophilised water extract of *C. versiccolor*;
- Variant 4 – dehydrated vegetable soup without monosodium glutamate, enriched with lyophilised water extract of *F. torulosa*;

Energetic value

The energy value of the soup was calculated based on the content of carbohydrates, proteins and fats according to the following formula:

$$\text{Energy (kJ)} = (\%\ \text{protein} \cdot 17) + (\%\ \text{fat} \cdot 37) + (\%\ \text{carbohydrates} \cdot 17)$$

Content of moisture and mineral substances (ash)

The content of moisture and mineral matter (ash) in dehydrated soups was determined on three randomly taken samples of each variety separately, according to the methodology of Stojanova (2017).

Instrumental color analysis

In order to measure the instrumental color parameter, 5 soup bags from each variety were randomly selected. To determine this parameter, a Dr. Lange colorimeter, spectro color (d/8° portable, The Netherlands) was used.

Before each series of measurements, the instrument was calibrated using a CR-A43 white calibration plate, according to standard operating instructions. Color characteristics are expressed according to CIE L* a* b* (CIE, 1976), which is based on three coordinates that define the color of the samples: L* (lightness of color), a* (proportion of red color (+a*) or green color (-a*)) and b* (proportion of yellow color (+b*) or blue color (-b*)).

The measured values L* a* b* are directly read on the colorimeter, and based on these three values with the help of appropriate mathematical relations, the following color parameters are calculated:

Total color change (ΔE):

The total color change (ΔE) is calculated in relation to the standard sample, which determines the influence of the factor (in this research, the influence of mushroom extracts) on the characteristics and quality of the color.

$$\Delta E = \sqrt{(L0^* - L^*)2 + (a0^* - a^*)2 + (b0^* - b^*)^2}$$

where: L0*, a0* and b0* – parameters of the standard (control variant 1 was taken as the reference value in this research)
L*, a* and b* – sample parameters

Color saturation (C*):
Color saturation (C*) is a measure of the degree of color purity. In the center of the coordinate system it is 0 and increases with the distance of the color from the center to the peripheral parts. It is calculated based on parameters a* and b*.

$C^* = \sqrt{a^{*2} + b^{*2}}$

Color shade (h):
The shade of color (h) is calculated on the basis of parameters a* and b*, and the value of the angle at which the corresponding color is located (points A, B, C) is determined, calculated in relation to the +a* axis of the coordinate system.

$h = \tan^{-1} (b^*/a^*)$

The obtained results were statistically processed using ANOVA test in SPSS 20 package program.

RESULTS AND DISCUSSION

The composition of the soup depends on the starting material from which it is prepared. All types of soups have a high water content after cooking; meat soups contain more protein and minerals such as zinc, calcium, iron and electrolytes, and vegetable soups are rich in dietary fiber, vitamins and phytochemicals (Buren et al., 2019). The great advantage of soup is that, as an independent meal, it simultaneously provides the necessary liquid and nutritional value in the form in which the body absorbs them most easily and is therefore the easiest to use in order to maintain health.

According to the data for the chemical composition of the soups (Table 1), it can be noted that the moisture content in all analyzed variants is in accordance with the regulations according to the Regulations on the quality of soups (Official Gazette of the Republic of Macedonia, 95/2012; Official Gazette of the SC, 56/2003 and 4/2004–other regulations), i.e. <10% and no statistically significant ($p<0.05$) difference was found in any of the analyzed variants. The content of mineral substances was statistically significant ($p<0.05$) higher in all variants enriched with lyophilized extracts (≈5.45% in soup variant 2 to ≈5.59% in soup variant 3), compared to soup from the control variant (≈5.05%). Soup variant 4 (≈1122.49 kJ) were characterized by the statistically significantly ($p<0.05$) highest energy value compared to the control and other analyzed variants.

In general, all soup variants enriched with lyophilized extracts showed a statistically significant ($p<0.05$) higher energy value compared to the control variant, which is expected due to the higher content of the total nutritional composition (carbohydrates, proteins and fats). From the data for the chemical analysis of dehydrated soups, it can be noted that similar results were obtained during all test days after production (minimal differences in values are within the so-called laboratory error), which is why it can be concluded that the obtained soups of all tested variants are characterized by stability and consistency in terms of chemical composition. Higher values for the investigated parameters in the soups in which lyophilized mushroom extracts were applied compared to the control variant, are the result of the favorable chemical

and nutritional composition of the applied extracts, which only supplement the basic composition of the conventionally produced soup.

Table 1: Chemical properties of dehydrated soups on different days after production

Parameter	n	Day of production	Variant 1 (control)	Variant 2	Variant 3	Variant 4
			$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD
Moisture (% d.m.)	3	0	4,92 ± 0,03^{aA}	5,03 ± 0,03^{bA}	4,98 ± 0,05^{aA}	4,85 ± 0,01^{aA}
	3	15	4,90 ± 0,05^{aA}	5,06 ± 0,03^{bA}	4,93 ± 0,03^{aA}	4,89 ± 0,01^{aA}
	3	30	4,95 ± 0,02^{aA}	5,01 ± 0,01^{bA}	4,99 ± 0,01^{aA}	4,83 ± 0,04^{aA}
	3	45	4,93 ± 0,02^{aA}	5,05 ± 0,05^{bA}	4,92 ± 0,01^{aA}	4,87 ± 0,02^{aA}
	3	60	4,95 ± 0,01^{aA}	5,02 ± 0,03^{bA}	4,98 ± 0,02^{aA}	4,90 ± 0,02^{aA}
	3	90	4,94 ± 0,02^{aA}	5,03 ± 0,03^{bA}	4,96 ± 0,07^{aA}	4,92 ± 0,03^{aA}
Mineral matters (% d.m.)	3	0	5,05 ± 0,01^{aA}	5,50 ± 0,01^{bA}	5,59 ± 0,06^{bA}	5,55 ± 0,02^{bA}
	3	15	5,06 ± 0,03^{aA}	5,45 ± 0,02^{bA}	5,52 ± 0,07^{bA}	5,50 ± 0,00^{bA}
	3	30	5,07 ± 0,05^{aA}	5,48 ± 0,02^{bA}	5,51 ± 0,02^{bA}	5,52 ± 0,01^{bA}
	3	45	5,05 ± 0,03^{aA}	5,47 ± 0,03^{bA}	5,53 ± 0,02^{bA}	5,51 ± 0,01^{bA}
	3	60	5,06 ± 0,01^{aA}	5,45 ± 0,02^{bA}	5,55 ± 0,01^{bA}	5,50 ± 0,04^{bA}
	3	90	5,05 ± 0,02^{aA}	5,42 ± 0,02^{bA}	5,54 ± 0,05^{bA}	5,51 ± 0,02^{bA}
Energy value (kJ)	3	0	1067,86 ± 0,05^{aA}	1116,20 ± 0,02^{bA}	1101,28 ± 0,03^{cA}	1122,49 ± 0,07^{bA}
	3	15	1069,11 ± 0,03^{aA}	1116,84 ± 0,01^{bA}	1101,50 ± 0,03^{cA}	1121,00 ± 0,05^{bA}
	3	30	1068,32 ± 0,02^{aA}	1113,28 ± 0,07^{bA}	1100,71 ± 0,05^{cA}	1121,60 ± 0,01^{bA}
	3	45	1067,81 ± 0,03^{aA}	1114,95 ± 0,03^{bA}	1100,77 ± 0,04^{cA}	1121,19 ± 0,01^{bA}
	3	60	1067,72 ± 0,01^{aA}	1114,16 ± 0,07^{bA}	1101,51 ± 0,06^{cA}	1120,68 ± 0,09^{bA}
	3	90	1068,46 ± 0,01^{aA}	1112,86 ± 0,02^{bA}	1100,86 ± 0,03^{cA}	1121,14 ± 0,06^{bA}

^{a,b,c} - values on the same day of different soup variants marked with different letters are statistically significantly different ($p<0.05$), ANOVA, post hoc Tukey's test.
^{A,B} - values of the same variant on different days marked with different letters are statistically significantly different ($p<0.05$), ANOVA, post hoc Tukey's test.

Table 2: Average values for the instrumental color analysis on different days after production

Parameter	n	Day of production	Variant 1 (control)	Variant 2	Variant 3	Variant 4
			$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD
L*	5	0	79,65 ± 0,11^{aA}	77,53 ± 0,09^{bA}	77,61 ± 0,07^{bA}	77,68 ± 0,09^{bA}
	5	15	79,59 ± 0,06^{aA}	77,56 ± 0,05^{bA}	77,63 ± 0,10^{bA}	77,50 ± 0,17^{bA}
	5	30	79,61 ± 0,13^{aA}	77,47 ± 0,09^{bA}	77,60 ± 0,10^{bA}	77,71 ± 0,06^{bA}
	5	45	79,68 ± 0,05^{aA}	77,52 ± 0,09^{bA}	77,64 ± 0,09^{bA}	77,63 ± 0,12^{bA}
	5	60	79,67 ± 0,10^{aA}	77,53 ± 0,08^{bA}	77,65 ± 0,03^{bA}	77,61 ± 0,15^{bA}
	5	90	79,65 ± 0,05^{aA}	77,50 ± 0,1^{bA}	77,61 ± 0,07^{bA}	77,60 ± 0,09^{bA}
a*	5	0	2,35 ± 0,09^{aA}	2,39 ± 0,15^{aA}	2,25 ± 0,31^{bA}	2,31 ± 0,14^{aA}
	5	15	2,37 ± 0,09^{aA}	2,36 ± 0,05^{aA}	2,29 ± 0,03^{bA}	2,35 ± 0,11^{aA}
	5	30	2,35 ± 0,05^{aA}	2,31 ± 0,19^{aA}	2,27 ± 0,15^{bA}	2,37 ± 0,27^{aA}
	5	45	2,31 ± 0,09^{aA}	2,37 ± 0,08^{aA}	2,22 ± 0,04^{bA}	2,35 ± 0,16^{aA}
	5	60	2,30 ± 0,15^{aA}	2,35 ± 0,17^{aA}	2,25 ± 0,14^{bA}	2,33 ± 0,26^{aA}
	5	90	2,33 ± 0,21^{aA}	2,38 ± 0,06^{aA}	2,27 ± 0,30^{bA}	2,36 ± 0,09^{aA}
b*	5	0	42,14 ± 0,06^{aA}	32,17 ± 0,21^{bA}	32,26 ± 0,13^{bA}	32,08 ± 0,35^{bA}
	5	15	42,17 ± 0,05^{aA}	32,14 ± 0,25^{bA}	32,30 ± 0,16^{bA}	32,11 ± 0,04^{bA}
	5	30	42,11 ±0,11^{aA}	32,19 ± 0,31^{bA}	32,31 ± 0,19^{bA}	32,15 ± 0,14^{bA}
	5	45	42,13 ± 0,02^{aA}	32,18 ± 0,10^{bA}	32,38 ± 0,03^{bA}	32,09 ± 0,19^{bA}
	5	60	42,15 ± 0,11^{aA}	32,17 ± 0,14^{bA}	32,37 ± 0,26^{bA}	32,12 ± 0,17^{bA}
	5	90	42,17 ± 0,09 ^{aA}	32,18 ± 0,23^{bA}	32,39 ± 0,11^{bA}	32,10 ± 0,10^{bA}

^{a,b,c} - values on the same day of different soup variants marked with different letters are statistically significantly different ($p<0.05$), ANOVA, post hoc Tukey's test.

^{A,B} - values of the same variant on different days marked with different letters are statistically significantly different ($p<0.05$), ANOVA, post hoc Tukey's test.

Table 3: Average values for the instrumental color analysis on different days after production

Parameter	n	Day of production	Variant 1 (control)	Variant 2	Variant 3	Variant 4
			$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
ΔE	5	0	Ref.*	10,19 ± 0,02^{aA}	10,08 ± 0,10^{aA}	10,25 ± 0,01^{aA}
	5	15	Ref.	10,23 ± 0,10^{aA}	10,06 ± 0,06^{aA}	10,27 ± 0,01^{aA}
	5	30	Ref.	10,15 ± 0,11^{aA}	10,00 ± 0,09^{bA}	10,14 ± 0,01^{aA}
	5	45	Ref.	10,18 ± 0,19^{aA}	9,96 ± 0,16^{bA}	10,25 ± 0,09^{cA}
	5	60	Ref.	10,21 ± 0,13^{aA}	9,99 ± 0,17^{bA}	10,23 ± 0,22^{cA}
	5	90	Ref.	10,22 ± 0,11^{aA}	10,19 ± 0,10^{bA}	10,28 ± 0,12^{cA}
C	5	0	42,21 ± 0,05^{aA}	32,26 ± 0,12^{bA}	32,34 ± 0,15^{bA}	32,16 ± 0,02^{bA}
	5	15	42,23 ± 0,05^{aA}	32,22 ± 0,05 ^{bA}	32,38 ± 0,23^{bA}	32,19 ± 0,11^{bA}
	5	30	42,18 ± 0,03^{aA}	32,27 ± 0,03^{bA}	32,39 ± 0,1^{bA}	32,24 ± 0,02^{bA}
	5	45	42,19 ± 0,08^{aA}	32,27 ± 0,05^{bA}	32,45 ± 0,15^{bA}	32,18 ± 0,07^{bA}
	5	60	42,21 ± 0,07^{aA}	32,26 ± 0,09^{bA}	32,45 ± 0,21^{bA}	32,15 ± 0,14^{bA}
	5	90	42,23 ± 0,15^{aA}	32,27 ± 0,06^{bA}	32,47 ± 0,09 ^{bA}	32,19 ± 0,27^{bA}
h	5	0	86,81 ± 0,03^{aA}	85,75 ± 0,03^{bA}	86,01 ± 0,04^{aA}	85,88 ± 0,03^{bA}
	5	15	86,78 ± 0,02^{aA}	85,80 ± 0,13^{bA}	85,94 ± 0,17^{bA}	85,81 ± 0,09^{bA}
	5	30	86,81 ± 0,07^{aA}	85,89 ± 0,09^{bA}	85,98 ± 0,04^{bA}	85,78 ± 0,05^{bA}
	5	45	86,86 ± 0,05^{aA}	85,78 ± 0,05^{bA}	86,07 ± 0,07^{aA}	85,81 ± 0,09^{bA}
	5	60	86,87 ± 0,09^{aA}	85,82 ± 0,06^{bA}	86,02 ± 0,20^{aA}	85,84 ± 0,14^{bA}
	5	90	86,84 ± 0,12^{aA}	85,77 ± 0,16^{bA}	85,99 ± 0,09^{aA}	85,79 ± 0,26^{bA}

^{a,b,c} - values on the same day of different soup variants marked with different letters are statistically significantly different ($p<0.05$), ANOVA, post hoc Tukey's test.

^{A,B} - values of the same variant on different days marked with different letters are statistically significantly different ($p<0.05$), ANOVA, post hoc Tukey's test.

Stability of the quality and nutritional properties of soups during storage occurs as a result of the addition of mushroom extracts (Mohamed et al., 2020), and lyophilization of the extracts only reduces the water content, which can lead to the concentration of some of the parameters (Fissore and Pisano, 2015). On the other hand, the low moisture content contributes to even greater stability of the final product from the microbiological, chemical and antioxidant aspects, which is in agreement with the results of this research. Higher moisture content increases the possibility of physico-chemical and microbiological changes of the product during its storage (Mohamed et al., 2020).

Kumar (2015) investigated the shelf life of dehydrated soups with the addition of *A. bisporus* mushroom. Several variants were prepared with different concentrations of added ingredients. The author states that the fat content ranged from 5.68 to 5.84%, mineral content from 2.89 to 3.64%, fiber from 0.88 to 1.42% of dry matter of all formulations. The moisture content increased in proportion to the storage period, reaching the highest value of 4.37% in the 12th month, and the author concludes that this is the optimal storage period for the tested dehydrated soups.

According to the data presented in Table 2 and Table 3 related to the instrumental values for the color of the soups, constancy can be observed for all color parameters in all tested variants from 0 to 90 days after production. This indicates the fact that all analyzed soups had a stable color during storage, which means that there were no changes in other chemical or biological parameters that would lead to a change in the color of the product. Namely, the soups from control variant were characterized by the lightest color ($L^* \approx 79.65$). All other variants were characterized by statistically significant ($p<0.05$) lower values for the L* parameter compared to the control variant. Regarding the proportion of red color, no statistically significant differences were observed between the variants in relation to the control variant, nor between the tested variants. With a statistically significant ($p<0.05$) highest share of yellow color ($b^* \approx 42.14$) was characterized control variant compared to other variants.

According to the obtained data, it can be noted that the values for the parameters L* (lightness of color), a* (proportion of red/green color) and b* (proportion of yellow/blue color) were similar in all tested variants of soup enriched with lyophilized extracts mushroom. These results are due to the fact that the lyophilized water extracts of the examined mushroom had the same color, and on the other hand, the base to which they were added (dehydrated soup) was the same for all product variants.

Therefore, statistically significant ($p<0.05$) differences were observed only between the control variant in relation to the other variants, i.e. the darker shade in the analyzed variants enriched with lyophilized extracts is a consequence of the applied extracts and added porcini mushrooms.

Based on the instrumentally measured values for color brightness, the proportion of red and yellow colors, the values for total color change, color saturation and color hue are calculated. Namely, from the data in Table 3, it can be noted that the color change in the variants after the addition of extracts compared to the control amounted to $\Delta E \approx 9.96$ to 10.28. There were no statistically significant differences between the analyzed variants. Statistically ($p<0.05$) highest value of color saturation was found in control variant ($C \approx 42.21$) compared to the other variants. From the aspect of color shade, minimal differences were observed between all tested variants. The values for ΔE, C and h are correlated with the values for L*, a* and b*, so that no significant differences were observed either among the variants enriched with different types of extracts, or during the storage of soups.

The values of the color parameters are proportional to the share of the various components of the dehydrated soups. Each of them has a different color that affects the overall color of the final product. Therefore, the color differences between the analyzed soups are minimal probably due to the minimal variability of the vegetables.

In general, the color brightness L* depends on the moisture content of the product, i.e. its value increases with increasing moisture content (Sun and Muthukumrappan, 2002). A darker color usually develops during sugar caramelization (the Maillard reaction) as well as with increasing temperature (Takahashi et al., 2005).

In a study by Amal et al. (2014) dehydrated soups with the addition of potatoes, lentils, peas and chickpeas were produced, where the pea soup was characterized by the lightest color (L*=82.44, a*=2.39, b*=30.55, C= 30.64, h=85.53), and from the aspect of sensory characteristics, the soup with the addition of lentils (9.10) received the highest score for taste.

CONCLUSION

According to the obtained results, it can be concluded that the wild edible and medicinal mushrooms *Suillus granulatus*, *Coriolus versicolor* and *Fuscoporia torulosa* are is favorable for obtaining water extract, while lyophilized extracts are advantageous for application in dehydrated vegetable soup, produced in industrial conditions. The content of mineral matters was significantly ($p<0.05$) higher in all soup variants enriched with lyophilized extracts. Moreover, the moisture content in all analyzed soup variants is in accordance with the regulations.

From the aspect of the instrumental color value, constancy was established for all parameters in all tested variants from the 0th to the 90th day after production, i.e. all soups had a stable color during storage, which means that there were no changes in other chemical or biological parameters that would lead to a change in the color of the product.

In this way, a new product is obtained that does not contain chemical additives, which further increases its value.

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A REVIEW OF THE RELATIONSHIP BETWEEN CANCER CASES-ENVIRONMENTAL CARCINOGENS AND POLLUTING AGENTS IN TURKISH THRACE REGION

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ABSTRACT

Cancer occurs as a result of the combination of various factors (environmental, genetic). Factors brought by personal or social life change the working system of cells over time. All factors that cause a person to have cancer are called carcinogens (cancer-causing). When environmental factors are examined in cancer cases, obesity, viruses and bacteria, UV rays, alcohol and chemicals, especially smoking, have been identified as the most important carcinogens. When genetic factors were examined in cancer cases, oncogenes, tumor suppressor genes, DNA repair genes and other carcinogenic gene concepts were determined.
As a result of the statistical studies conducted by the WHO (World Health Organization), approximately 19.2 million new cancer cases were seen in the world in 2021. It has been determined that 9.9 million deaths occurred due to cancer. According to the Ministry of Health HSGM (General Directorate of Public Health) Cancer Department Turkey Cancer Control Program 2021 Report, it was determined that between the years 2009-2021, an average of 200,000 to 240,000 people were diagnosed with cancer annually in Turkey. With the increase in the world population, this number is expected to increase gradually in the coming years. The significant increase in cancer cases we have seen in recent years, especially in Turkish Thrace Region, is one of the most important factors in our handling of this issue. In this review, the relationship between cancer cases-environmental carcinogens and polluting agents in Turkish Thrace Region is discussed by scanning the existing literature. The high rate of elderly population in Turkish Thrace Region, the uncontrolled dumping of industrial wastes into the Ergene River in this region, the effects in the region after the Chernobyl disaster and the excessive use of pesticides (pesticides) in agriculture in the Thrace Region, which is a grain warehouse, completely affect the serious increase in cancer cases. From this point of view, it is important to examine Turkish Thrace Region.

Keywords: environmental carcinogens, Turkish Thrace, oncogenes, carcinogens, cancer

INTRODUCTION

The disease that results in abnormal and rapid increase of cells due to damage to DNA is called cancer. Cancer occurs as a result of the combination of various factors (environmental, genetic) (Yokuş and Çakır 2012). All factors that cause a person to get cancer are called carcinogens (Baran et al., 2021). Considering the increase in cancer cases worldwide, it has been determined that it has become the most important disease burden. Cancer, which is one of the biggest causes of death in recent years, is seen as an important problem in terms of shortening or eliminating life expectancy. As a result of statistical studies conducted by WHO (World Health Organization) and IARC (International Agency for Research on Cancer), approximately 19.2 million new cancer cases were seen in the world in 2021. It has been determined that 9.9 million deaths occur due to cancer (WHO and IARC, 2021). According to the Ministry of Health HSGM (General Directorate of Public Health) Cancer Department Turkey Cancer Control Program 2021 Report, it was determined that an average of 200,000 to

240,000 people were diagnosed with cancer annually in Turkey between 2009 and 2021. With the increase in the world population, this number is expected to increase gradually in the coming years (Figure 1) (HSGM Cancer Department, 2021). One of the most important reasons for our work on this subject is the serious increase in cancer cases that we have seen in recent years, especially in the Thrace Region, and the fact that the region has been heavily influenced by environmental carcinogens and pollutants in recent years.

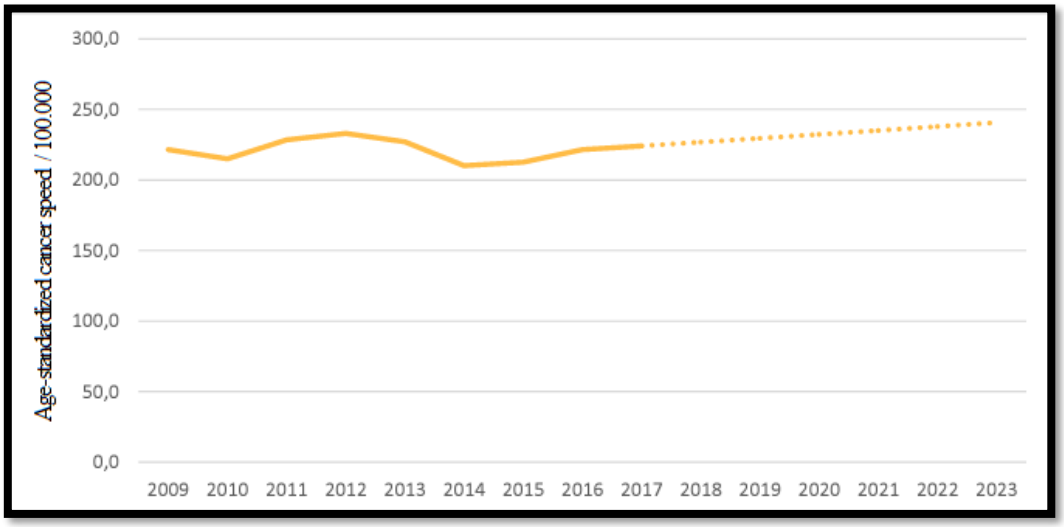

Figure 1: Cancer Incidence in Turkey 2017-2023 (HSGM Cancer Department 2021).

1. Environmental Carcinogens

It is of great importance to minimize the risk of cancer in individuals and societies by recognizing environmental carcinogens and taking the necessary precautions. Smoking, one of the environmental carcinogens, is an important factor as it is 30% effective in the occurrence of all cancers. The most important environmental carcinogen after smoking is obesity. Following these, some bacteria and viruses such as HPV (Human Papilloma Virus), EBV (Epstein-Barr virus) and Hepatitis viruses, H. pylori (Heliobacter pylori), unhealthy eating habits, alcohol consumption and ultraviolet rays (sun, rhodone), Other carcinogens such as air-water-soil pollution are also important factors in the formation of cancer (Bayık, 1989, Özdoğan, 2021a).

1.1.Smoking

The most important factor among the environmental causes of cancer is cigarette consumption. Many types of cancer, especially lung cancer, occur in individuals who smoke. The effect of cigarette consumption on cancer formation was first revealed as a result of an observational study conducted in the middle of the last century (Figure 2). In this observational study, it was noted that most of the patients diagnosed with lung cancer smoked. According to these first observational studies, the fact that 50-60% of the population smoked and that 90% of the patients diagnosed with lung cancer in those years were smokers was seen as an important indicator. The behavior of lung cancer patients, both smokers and non-smokers, has been the subject of many scientific studies in the following years. According to the results of the research, it was determined that the risk of developing lung cancer decreased significantly over time in individuals who stopped smoking (Doll and Hill, 1950). In another study, it was found that patients diagnosed with cancer consumed more cigarettes than patients without a diagnosis of cancer (Doll et al. 1994).

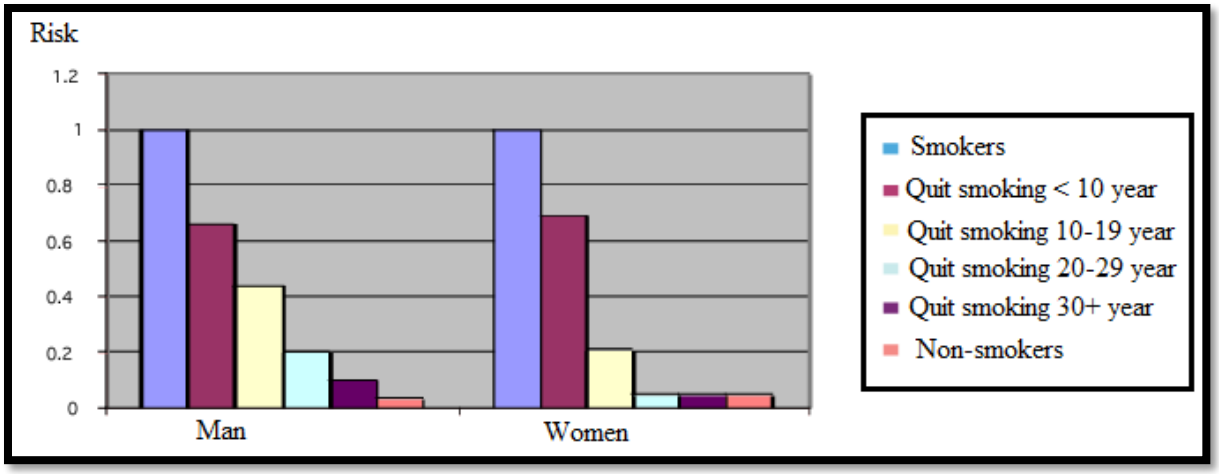

Figure 2: Decrease in lung cancer risk after quitting smoking (Bilir, 2008).

1.2. Obesity

Abnormally high amounts of fat in adipose tissue lead to deterioration in the health of individuals. This is how obesity is defined by WHO (Dönder and Önalan, 2018).

As a result of research on obesity, it has been seen that approximately 1.1 billion people in the world are dealing with obesity or overweight problems. At the same time, the irregular increase in body weight and fat tissue in obesity patients also affects the hormonal metabolism in individuals. This interaction also causes many chronic diseases and various types of cancer in the individual (Urhan and Akbulut, 2017).

Insulin signals produced in beta cells in the pancreas secrete insulin in response to the amount of glucose in the blood. IGF-1 (Insulin Growth Factor-1) is secreted from the liver in response to growth hormones. It is known that IGF-1 and insulin increase the risk of many cancers in case of obesity. They are especially important factors in breast, pancreas, kidney, endometrium and colon cancer. Hyperinsulinemia has been revealed to affect breast cancer and cancer metastases in many mouse model studies. Obesity is associated with these types of cancer, increasing the crude death rate and decreasing response to cancer treatment. Obesity causes changes in the functions of adipose tissue in the body. These changes in adipose tissue activate the mechanisms that cause cancer over time. Obesity causes adipose tissue dysfunction, insulin resistance, and excessive production of adipokines and cytokinins. At the same time, obesity causes cancer cell development by increasing leptin level and decreasing adiponectin level. It is known that the change in the levels of these two adipokine (leptin and adiponectin) molecules is effective in the relationship between obesity and cancer. Adrenal steroid, estrogen, progesterone and androgen hormones also support this relationship. Estrogen hormone signals increase cancer cell growth (Yiğit et al. 2019).

1.3. Virus and Bacteria

In order to survive in a cell, viruses attach their genetic structure to the nucleus of the cell. Viruses continue their existence as obligate intracellular parasites. During this struggle of viruses to survive, they cause the increase of some oncogenes that are harmful to living things. The increase in oncogenes results in cancer of the cell and then of the tissue, resulting in the death of the organism. At the same time, the virus adds these oncogenes to its genetic material and then releases the oncogenes in the tissues and organs it reaches, causing cancer in that area (metastasis). Oncogenes are genes that are necessary for the proliferation and growth of that cell when present in a certain proportion in the cell. When the cell is attacked by microorganisms such as viruses, the number of oncogenes reaches high levels within the cell, which cannot help the cell to multiply and grow in a healthy way, causing tumor formation.

These interactions of microorganisms on cells explain tumor formation (Table 1) (Özdoğan, 2021b).

Carcinogenic microorganisms	**Types of cancer they cause**
Human Papilloma virus (HPV)	Cervical cancer
Helicobacter pylori	Stomach cancer, MALT lymphoma, Oral cancer
Epstein-Barr virus	Nasopharynx (nasal) cancer
Merkel Cell Polyomavirus	Merkel cell cancer
Hepatitis B and C viruses	Liver (Hepocellular) cancer
Human cytomegalovirus	Glioblastoma multiforme (Brain cancer)
Simian virus 40	Brain cancer, Malignant, Lung cancer
Streptococcus anginosus	Esophageal cancer, Head and neck cancer
Salmonella typhi	Gallbladder cancer, Cholangiocarcinoma
Tropheryma whippelli	Extraintestinal lymphoma, stomach cancer
Herpes virus	Kaposi sarcoma (vascular cancer)
Mycoplasma penetrans, M. tuberculosis	Kaposi sarcoma
Chlamydia trachomatis	Ovarian cancer
Chlamydia pneumoniae	Lung cancer
Chlamydia psittaci	Ocular lymphoma

Table 1: Carcinogenic microorganisms and the types of cancer they cause (Banerjee et al., 2015; Özdoğan, 2021b).

1.4. UV Rays

When an individual is exposed to ultraviolet rays, these rays first reach DNA, the genetic material in the cell. DNA is definitely damaged after exposure to ultraviolet. Some DNA repair enzymes are activated to repair this damage. However, if the damage is not repaired, cell death (apoptosis) occurs in order to eliminate the cancerous cell. If cell death does not occur, genes in the unregulated genetic material mutate. When ultraviolet rays reach the individual, they disrupt the immune system in the skin, thereby directly damaging the genetic material and enabling the formation and growth of cancer cells. UV-C rays cause cancer by causing damage to genetic material (Figure 3) (Herring, 2010, Özdoğan, 2017).

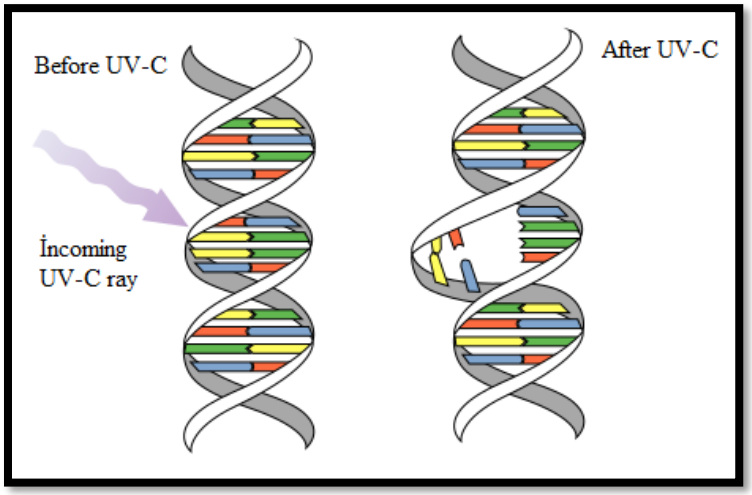

Figure 3: Effect of UV rays on DNA (Herring, 2010)

Ultraviolet rays are divided into 3 categories: UV-A, UV-B and UV-C. UV-A rays, which are the least dangerous, have low energy and the longest wavelength. UV-A rays are divided into two as UVA-1 and UVA-2 according to their energy levels. UVA-2, which has higher energy, is closer to UV-B. It is known that UVA-2 causes premature skin aging (Krutmann et al. 2017). Skin cancer is defined by the Centers for Disease Control and Prevention (CDC) as 'a disease caused by the uncontrolled and spread of cells in the skin'.

The wavelength of UV-A rays is 320-400 nm. These rays can pass through the atmosphere and reach the earth. UV-A rays penetrate the skin and cause skin wrinkles, premature skin aging and skin cancer (Perincek et al. 2007). When exposed to UV-A rays for a long time, keratinocytes in the epidermis layer of the skin are damaged and this increases the risk of skin cancer. At the same time, UV-A rays increase the risk of visual impairment and eye cataracts (Altun, 2019).

UV-B rays create effects at the biochemical level. UV-B rays cause our skin to change color by staying in the sun for too long (Krutmann et al. 2017). The wavelength of UV-B rays is 280-300 nm. UV-B rays are in the middle of the UV band and are 1000 times stronger than UV-A rays. The most important effects of UV-B rays on human health include weakening of the immune system, vision problems and skin cancer. When exposed to UV-B rays, changes in the skin structure occur, as well as tumor formation and cancer in older ages (Perincek et al. 2007). When exposed to UV-B rays for a long time, the epidermis layer of the skin is damaged. This damage causes premature aging of the skin and loss of elasticity. It is also known for certain that UV-B rays are an environmental carcinogen (Narayanan et al. 2010).

UV-C, known as the riskiest ultraviolet rays, has the highest energy and the shortest wavelength (Krutmann et al. 2017). The wavelength of UV-C rays is 200-280 nm. UV-C rays from solar sources cannot pass through the ozone layer or are trapped by gases in the atmosphere. Serious health problems related to vision and skin cancer occur as a result of direct exposure to these rays without taking any precautions (Perincek et al. 2007).

1.5. Alcohol

The factor that increases the risk of cancer in alcohol consumption is not the type of alcoholic beverage consumed, but how long and in what quantity it is consumed. Research shows that the factor that increases the risk of cancer in alcohol consumption is the ethanol contained in alcoholic beverages. While the risk of developing cancer as a result of alcohol consumption is 10% for men, this rate is 3% for women. These rates were revealed as a result of a scientific research conducted by Schutze and his colleagues on 350,000 individuals in 8 countries (Schutze et al. 2011). After alcohol consumption, alcohol molecules are converted into acetalaldehyde, a toxic molecule, by enzymes within the cell. Acetaldehyde creates replication errors in oncogenes or tumor suppressor genes. It also causes functional and morphological changes by binding to proteins in the cell (Jelski and Szmitkowski 2008, Alpertunga and Yıldız 2010). This molecule creates free radicals, increasing lipid peroxidation and hepatic collagen synthesis, causing damage to chromosomes and DNA. This situation causes toxicity in the liver (Lee et al. 2001, Alpertunga and Yıldız 2010).

Continuous occurrence of this damage to DNA causes the mechanism within the cell to malfunction and the cell to proliferate uncontrollably. Acetaldehyde is converted into acetate molecule in the body. Acetate plays a role in energy production. Scientific research has found that acetate is mutated in some individuals (Garaycoechea et al. 2018). This mutation increases cancer as a result of the increase in acetaldehyde (Figure 4). Individuals who consume excessive alcohol for many years are likely to develop esophageal cancer. If we look at the damage of alcohol on DNA, some people have high DNA repair capacity, while some people have low DNA repair capacity. Excessive breaks in the DNA structure and genetic material damage have been detected in people with low DNA repair capacity due to

continuous alcohol consumption. This evidence was revealed as a result of scientific experiments conducted on mice. In this experiment, mice were given alcohol at regular intervals. When the results were observed, excessive DNA breaks were detected in the genes of mice with low DNA repair capacity, and blood production stopped for 7-10 days. The result obtained from this experiment is that people with low system repair capacity in DNA repair and restoration due to alcohol consumption have a high risk of developing cancer (Özdoğan 2023, Garaycoechea et al. 2012).

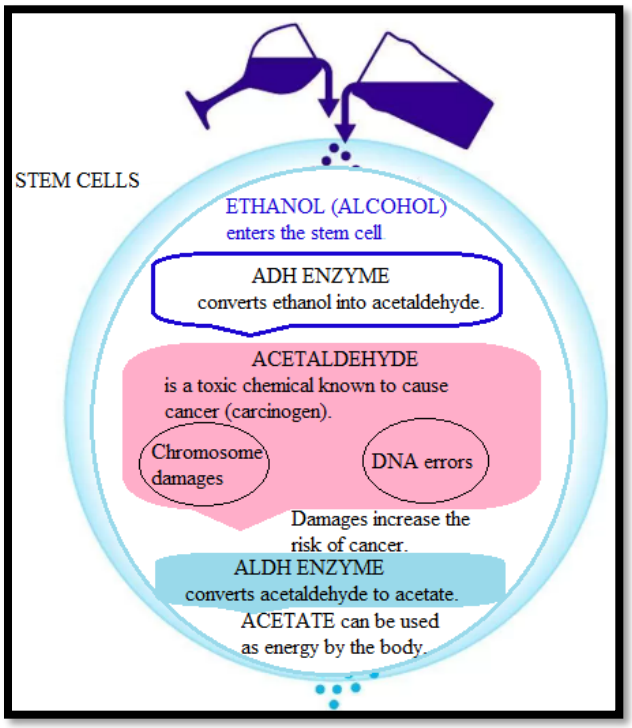

Figure 4: Mechanism by which alcohol causes cancer (Özdoğan, 2023)

1.6.Chemicals

Many chemicals have a carcinogenic effect when exposed to them for a long or short time. Carcinogenic chemicals can be structurally divided into organic chemicals and inorganic chemicals. These compounds, which have been proven to have carcinogenic effects, are shown in Table 2 (Coşkunses, 2008).

Carcinogenic organic compounds	Carcinogenic inorganic compounds
Halogenohydrocarbons	Beryllium and some of its compounds
Aromatic Amines	Cadmium and some of its compounds
Aromatic Hydrocarbons	Cobalt and some of its compounds
Nitro compounds	Chromium and some of its compounds
Azo compounds	Lead and some of its compounds
Nitroso compounds	Nickel and some of its compounds
Epoxides	Arsenic and some of its compounds
Acrylic acid derivatives	Some aluminum compounds
Chloroalkyl and bromoalkyl groups	Potassium bromate

Table 2: Carcinogenic organic and inorganic compounds (Coşkunses, 2008).

There are many chemicals used indoors and outdoors as biocides in agriculture. Biocides used in the market, such as carbamate, organic chlorine and carbinol groups, have been declared chemical carcinogens with the approval of EPA (US Environmental Protection Agency) and IARC (International Agency for Research on Cancer) (Vural, 2005).
In a study, the cancer risk of children whose parents were occupationally employed in a pesticide-contact sector and whose parents were not exposed to pesticides was evaluated. It has been revealed that the risks of cancer such as leukemia and central nervous system (CNS) tumors are higher in children whose parents are occupationally exposed to pesticides compared to the other group. Many cancer risks such as leukemia, brain tumors and lymphoma have been detected in children directly exposed to pesticides (Bhatia et al. 1999).
Water is the source of life and is absolutely necessary for the continuation of life. Water, which covers 2/3 of the world, causes the formation of colon and bladder tumors if it is not taken into the body in a clean and pure form. It has been proven by some in vivo and in vitro animal model experiments that if the phenol compound in water is above the limit determined by WHO (0.2 mg/L), it causes leukemia and lymphoma in older age groups. As a result of these experiments, cell changes that cause mutation and cancer were also detected (Saito, 1999).

2. Genetic Factors

Reproductive and Somatic cell mutation:

It occurs as a result of mutation of reproductive cells. Germline mutation, which is the cause of hereditary cancers, can be transmitted genetically (Klug et al. 2009). Somatic cell mutation occurs as a result of the mutation of body cells. Somatic mutation is not inherited (Knippers, 2006).

Proto- oncogenes:

Cell proliferation occurs in a controlled manner in line with physiological needs. Proto-oncogenes are known as genes that have a negative effect on cell proliferation. The functions of proto-oncogenes are as follows;

- Transcription factors
- Growth factor and growth factor receptors
- Suppression of apoptosis and modification of chromatins
- Intracellular signal transduction pathways
- The relationship of G proteins with the cell membrane

- Oncogenes occur when proto-oncogenes mutate and remain in constant activity (Croce, 2008).

Tumor suppressor genes:

These genes, also called "guardian genes" that suppress proliferation, have a negative effect on cell proliferation. Genes that lead the cell to apoptosis are included in this group. TP53 gene is a tumor suppressor gene that controls the cell cycle and directs the cell to apoptosis (McKusick, 2006). These genes; There are guard type genes that suppress the proliferation of cells, and caretaker type genes that prevent mutation by providing DNA repair that ensures genome integrity. If caretaker type genes, guard type genes and proto-oncogenes are mutated, mutations also occur in the genomes. Mutation of germline tumor suppressor genes is associated with genetic types of cancer (Çefle, 2009)

“Two strikes” hypothesis

The "two-hit" hypothesis put forward by Alfred Knudson regarding genetic and sporadic retinoblastoma is known as an eye tumor that occurs in childhood (Knudson, 1971). 40% of retinoblastoma factors occur through germline mutations. This constitutes genetic phenomena. Genetic phenomena cause mutation in RB1 genes located on the chromosome. 60% of

retinoblastoma factors are known to be sporadic. Due to genetic factors, tumors occur at earlier ages compared to sporadic ones (Newsham et al. 1998).
According to the hypothesis put forward by Alfred Knudson in 1971, there must be two types of mutations for the development of retinoblastoma. The first is the occurrence of somatic mutations in two copies of the RB1 gene. The other one is familial and one of the mutations is genetically transferred to the individual via germline mutation. Due to this situation, it shows the presence of congenitally heterozygous RB1 gene mutation in all cells of the body. Since this situation is not sufficient for tumor development, its effectiveness must be eliminated by somatic mutation of one copy. As can be understood from here, there should be a "two hit" pattern in the types of retinoblastoma (Knudson, 1971). In sporadic and hereditary cancers, both copies of the tumor suppressor gene are inactivated by a “double hit”. The difference with the genetic one is that the first of the strokes is present from birth. Loss of the other copy is sufficient for the tumor to develop. In sporadic cases, there must be two consecutive hits in the same cell after birth (Figure 5) (Çefle, 2009).

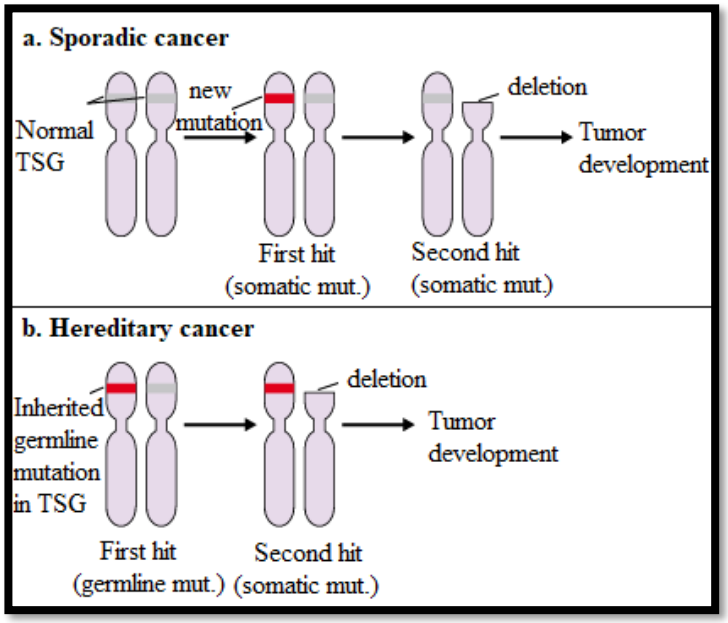

Figure 5: Two-hit hypothesis (Çefle, 2009).

Loss of heterozygosity (LOH):

In genetic eye tumors (retinoblastoma), heterozygous individuals have the RB1 mutant allele in the cancer area but the normal allele is absent. Because during oncogenesis, interstitial losses occur in the 13q14 area of RB1. Low heterozygosity in the RB1 gene region in the tumor is detected by genetic and molecular tests. Apart from interstitial deletions, chromosomal deletions are another striking mechanism. In this mechanism, in cases where LOH cannot be detected, a point mutation occurs during the second hit. LOH has been found in the RB1 gene sequence in sporadic eye tumors. These observations are not only related to retinoblastoma but are also valid for other types of cancer. In sporadic cancer cells, loss of heterozygosity is observed in chromosome areas carrying suppressor genes. The two-hit mechanism has been found to be effective in the emergence of many cancers, especially breast and colon tumors (Table 3) (McKusick, 2006).

LOH	TSG	**Associated cancer**
17p	*P53*	Colon, breast cancer
10q	*PTEN*	Prostate cancer
3p	*VHL*	Central nervous system hemangioblastoma
5q	*APC*	Colon cancer
13q	*RB1*	Osteosarcoma
18q	*DCC*	Colon cancer

Table 3: Chromosomal regions with LOH in various types of cancer (Çefle, 2009).

2. Oncogenesis:

It is known that during the process of a tissue becoming cancerous, gene parts within the cell mutate and initiate tumorization. These genes are generally known to be pro-oncogenes or suppressor genes. When scientific studies examine the onset and development of the tumor for oncogenesis in the colon, pro-oncogenes and tumor suppressor genes must also be mutated in addition to hereditary events. Oncogenesis is inherited multistage. In this multi-stage process, mutation formation occurs spontaneously or due to radiation (Fearon and Vogelstein, 1990).

2.1 Oncogenes

It has been determined that the cell culture medium obtained from cancerous tissues and the cell culture medium obtained from non-cancerous tissues have different properties. In this type of cell culture, the description transformed is used. Transformation is the development of a cell by feeding on fewer resources. Transformed cells can be round or elliptical and can adhere to soft surfaces. They can continue their lives with lower serum levels than normal. Instead of spreading as a layer on the ground surface, it can continue to develop as a mass structure. When these types of cells are added to the physiology of experimental animals, they provide the cells with the ability to become immortal, creating chain genetic changes and allowing tumor initiation. Many genetic factors must change for a cell or tissue to become cancerous. These genetic changes increase the transformation process of many carcinogenic substances in the cell. The cell often acts as an promoter and initiator of tumor formation through the transformations it undergoes after being exposed to carcinogenic substances for many years. The fact that this is the case tells us that there are different stages in cancer cases. There are two gene groups that cause transformation as a result of mutagens. Genes of viruses that cause transformation within the cell are called oncogenes. Uncontrolled development of genes within the cell called proto-oncogenes is associated with tumor growth. To date, 100 different oncogenes have been identified. The random transformations caused by these oncogenes combine with some chemical agents in the body and cause infection. When transformation occurs within the cell, oncogenes produce large amounts of oncogene proteins. By inhibiting the normal physiological functioning in the cell, it first copies itself as a single chain in RNA, and then turns itself into a double chain in DNA, taking over the functioning of the cell and using the cell to reproduce itself. As a result of the transformation event, some genes are integrated into the genetic structure of the cell and expressed differently. These oncogenes have no cellular similarities but produce transformed proteins called "oncoproteins" that inhibit cellular cancer cell suppressors (Pazarbaşı and Kasap, 2003).

2.2.Tumor Suppressor Genes

The balance that exists between life and death signals in a cell, which is known to be constantly monitored, determines the lifespan of that cell. Cell growth and cell proliferation are achieved when more than one genetic material functions together. Cells; maintaining its vitality, reproduction, division, etc. It synthesizes some special proteins in order to carry out its actions such as (growth factors in the cell). If a possible problem occurs in the repair of an acid or base error in a DNA sequence, deviations in life and death signals occur. Life signals stop functioning and death signals come into play. In other words, the cell's growth factors (proliferation, division) stop and the cell transitions to the death phase. Genes that produce proteins that can prevent the cell from growing and dividing are called 'tumor suppressor genes'. Proteins that trigger cell growth and division are called 'proto-oncogenes'. It is observed that an error in proto-oncogenes causes proteins to be released in larger amounts than normal in these genes. This excess protein triggers the uncontrolled proliferation of cells, leading the cell to cancer. Now, proto-oncogenes appear as an 'oncogene (tumor-triggering gene)' as a result of this cancer. With mutations in tumor suppressor genes, proteins in the gene may lose their function. With the loss of function in proteins, there is no obstacle to cell proliferation and the cell does not transition to the death phase. This maintains the cell's growth factors, but creates an environment for the cell to become cancerous (Köse, 2018).

2.3. DNA Repair Genes

Cancer Genes and Syndromes

TP53 (Tumor Protein 53) Gene:

TP53 gene, a tumor suppressor gene, is the guardian of the genome and is known as the molecule gene of the century. The TP53 gene takes part in DNA repair mechanisms, preventing the cell from reproducing and multiplying when there is damage to the genetic material in the cell and preventing this damage from reaching other cells. If this damage cannot be repaired, the apoptosis (cell death) mechanism comes into play and death occurs in the damaged cell. Tumor protein 53 is a gene active in cancer formation. This gene is found mutated in 50% of cancer patients. Mutated TP53 gene can cause Li-Fraumeni syndrome (LFS). Patients with LFS syndrome, which plays a dominant role during gene transfer, are particularly likely to suffer from diseases such as bone cancer, breast cancer, brain cancer and blood cancer (Özdoğan, 2021c).

- Von Hippel- Landau (VHL) Gene:

The Von Hippel-Landau gene, known as a growth-inactivating gene in cancer cells, provides dominant transition during gene transfer (Figure 6). Patients with VHL syndrome are especially likely to be diagnosed with CNS (Central Nervous System) cancers, kidney cancer and neuroendocrine tissue pancreatic cancers (Özdoğan, 2021c).

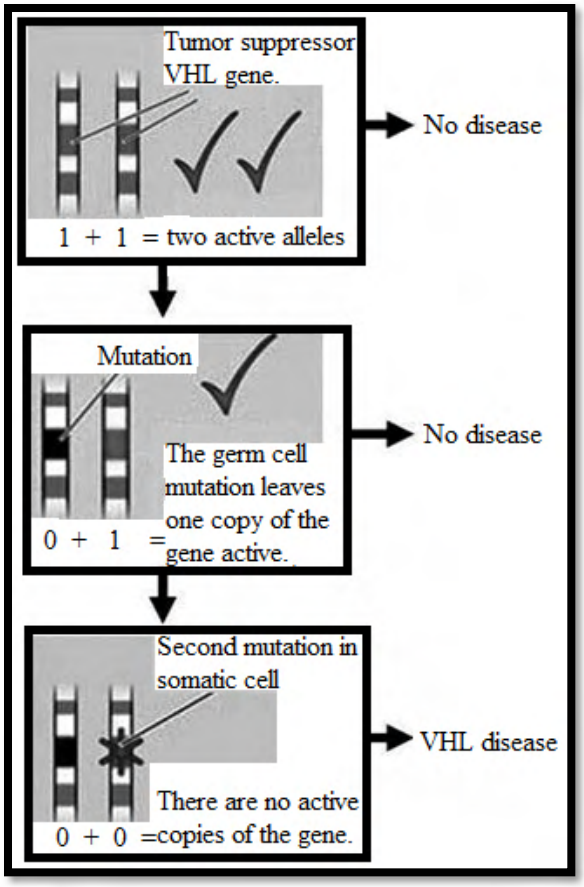

Figure 6: **Von Hippel-Landau gene. (Bayraklı vd. 2009).**

MutL homolog1 (MLH1) Gene:

The MLH 1 gene causes Lynch syndrome. MutL homolog1 gene plays a damage repair role in genetic material. Patients with Lynch syndrome, caused by the MHL1 gene, are particularly likely to suffer from diseases such as colon cancer, uterine cancer, ovarian cancer and stomach cancer (Figure 7 and figure 8) (Özdoğan, 2021d).

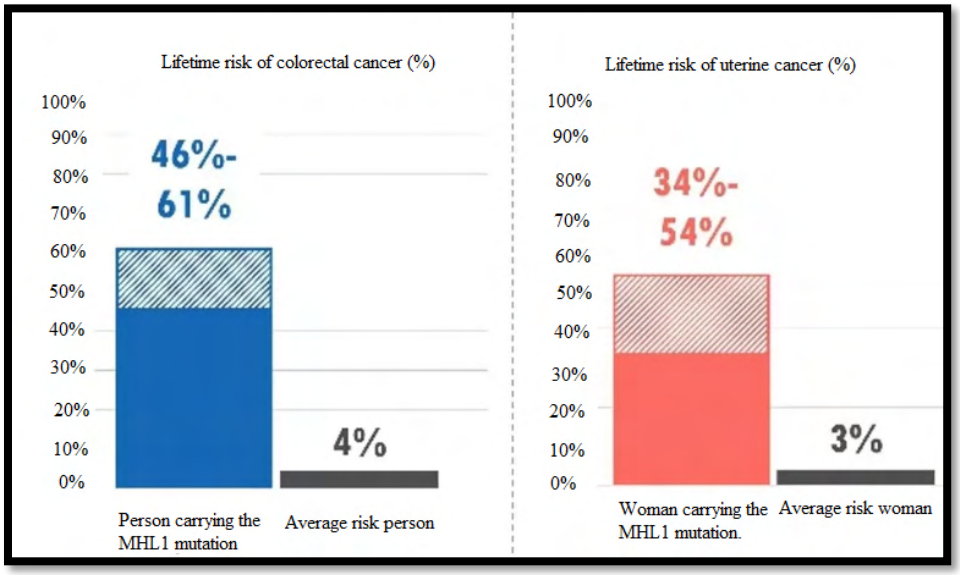

Figure 7: Risk of colorectal and uterine cancer in individuals with MHL1 mutation (Özdoğan, 2021d)

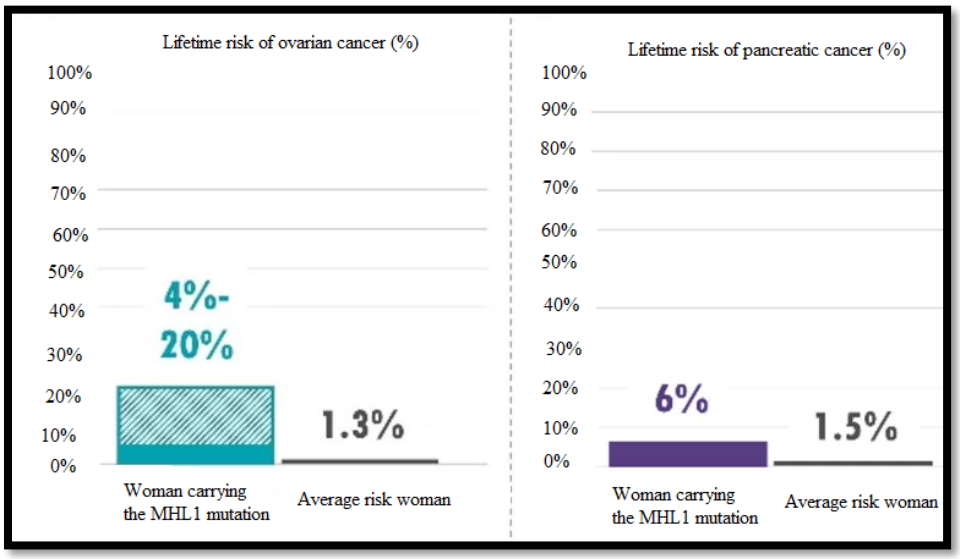

Figure 8: Risk of ovarian and pancreatic cancer in individuals with MHL1 mutation (Özdoğan, 2021d).

Excision Repair (ERCC) Gene:

ERCC gene, like other DNA cancer genes, is a DNA repair gene. It causes Xeroderma Pigmentosum syndrome, which causes recessive transmission during gene transfer. Individuals with this syndrome have a particularly high risk of skin cancer, and they need to protect themselves against sun and UV rays (Özdoğan, 2021c).

Breast Cancer (BRCA1 and BRCA2) Genes:

BRCA1 and BRCA2 genes are DNA repair genes that cause ovarian and breast cancer syndrome. The type of cancer in which genetic mutations are mostly seen in these genes is breast cancer. As a result of research conducted in recent years, it has been reported that BRCC genes can also cause prostate cancer (Özdoğan, 2021c).

MATERIAL AND METHOD

In this review, it is aimed to identify environmental carcinogens, pollution agents and genetic factors that cause cancer by scanning existing scientific resources. The evaluation of cancer cases caused by environmental carcinogens and pollution agents was handled in the Thrace Region. Carcinogenic factors that cause cancer in the Thrace Region have been identified by scanning scientific studies and research conducted in this region.

RESULTS AND DISCUSSION

Thrace Region is known as the region with the highest cancer death rates, according to the 2018 data of the Turkish Statistical Institute (TUIK). Looking at the data of TUIK, Thrace Region is one of the regions with the highest cancer cases in our country (Korkusuz, 2019).

When we consider the increase in cancer cases in the Thrace Region in recent years, 4 main factors stand out in terms of carcinogens. These are the old age rate of the people living in this region, the exposure of the Ergene River and agricultural areas to industrial pollution, the excessive use of pesticides and the fact that the fertile soils in the region are affected by acid rain after the Chernobyl disaster.

Old age is defined by the World Health Organization as the life span of 65 years and above. The incidence of cancer is high in people aged 65 and above, which we call the last quarter of

the human life cycle. Since cancer formation requires a long process, it is normal that this disease is more common in older individuals (Çınar and Taş, 2015).

According to TÜİK data, while the average rate of the elderly population in Turkey is 9.9%, the elderly population rate of Edirne is 16.2% compared to the total population. Edirne is among the provinces with the highest proportion of elderly population (TUIK, 2022).

As risk factors increase and cell repair mechanisms weaken as we age, a history of cancer occurs in various tissues and organs, and the incidence of cancer also increases. There is an increase in trachea, bronchus, lung, prostate, bladder, stomach, kidney and colorectal cancers in male individuals at older ages (Figure 9). In older female individuals, there is an increase in thyroid, trachea, bronchus, lung, uterine corpus, stomach, ovary, cervix, breast and colorectal cancers, depending on age (Figure 10) (Kaygusuz, 2018).

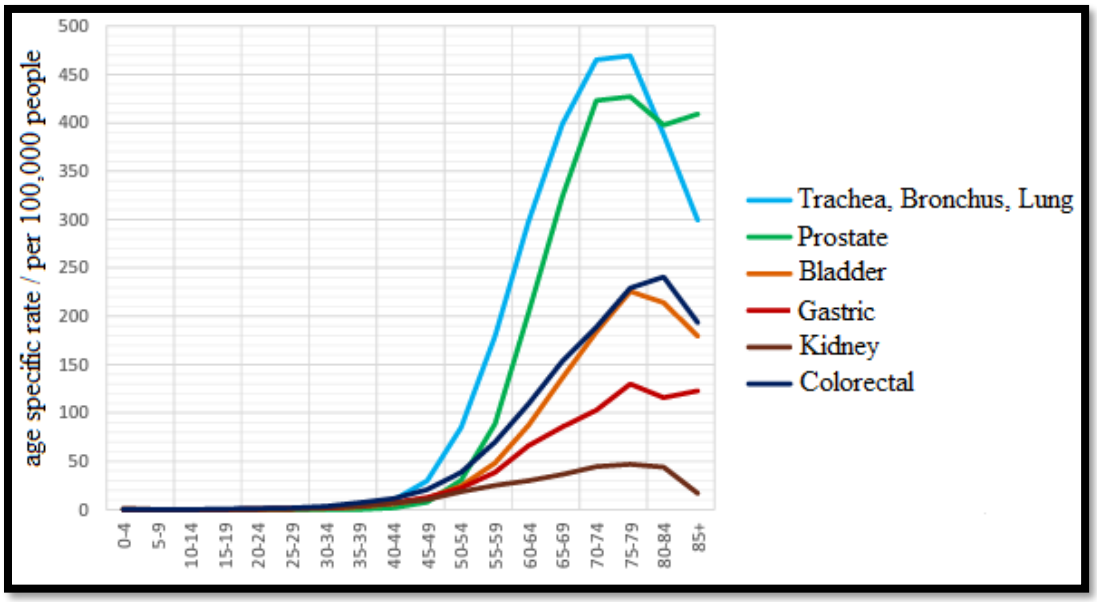

Figure 9: Age-specific rates of some cancer types seen in men (Kaygusuz et al. 2018).

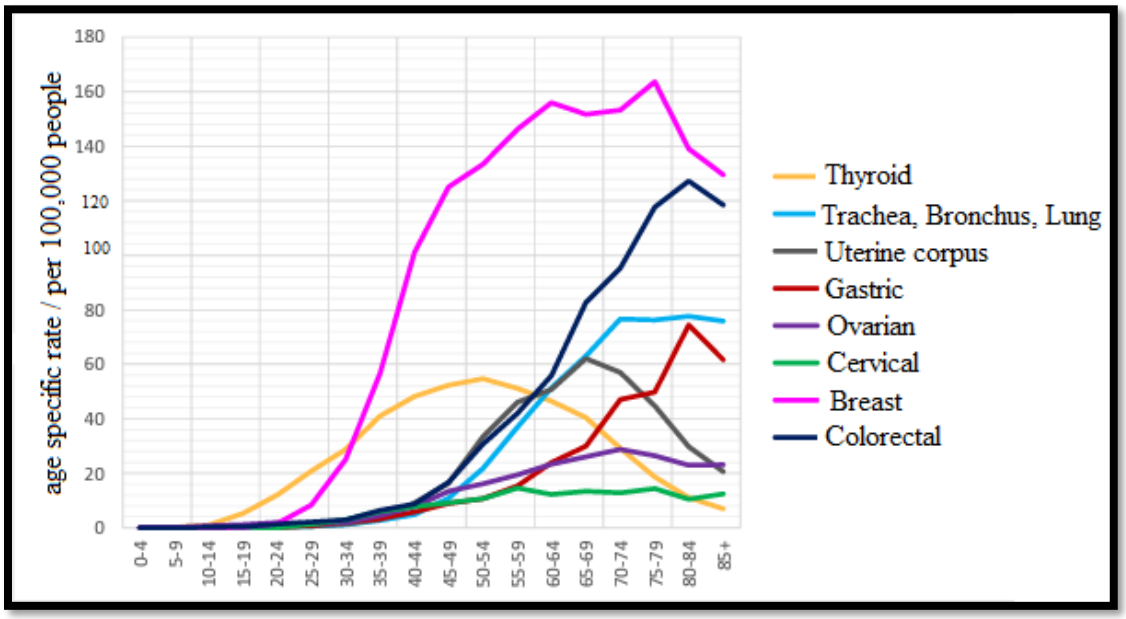

Figure 10: Age-specific rates of some cancer types seen in women (Kaygusuz et al. 2018).

Ergene River is an approximately 285 km long river that starts from the Yıldız Mountains that feed the agricultural lands of the Thrace Region, merges with the Meriç River and flows

into the Aegean Sea (Figure 11). Since the 1970s, industrial areas in the Thrace region, especially the industrial facilities in Çorlu and Çerkezköy, have been dumping all their waste and poison into the Ergene River, polluting this river, which is the water source of the region. Ergene River, where wastewater is dumped by industrial facilities, feeds the agricultural lands of the region. Thrace Region is a region with fertile agricultural lands in terms of food production such as rice, wheat and sunflower. However, these agricultural lands are exposed to Ergene's polluted waters containing heavy metals. The foods grown here negatively affect human health. Heavy metals, which are strong carcinogens, have been detected in foods grown in the region (Kocaman et al. 2011). 12% of Turkey's wheat production, 61% of sunflower production and 54% of rice production are produced in the Ergene basin (Bağdatlı, 2014, figure 11).

Ergene River is polluted by industrial facilities and contains heavy metals (cadmium, mercury, etc.) and toxic chemicals (paint, arsenic, etc.). Because of this problem that has not been solved for many years, people living around the Ergene River are exposed to diseases such as cancer and other health problems. In a study conducted on cancer patients and non-cancer individuals living around the Ergene River (Babaeski, Çerkezköy, Enez, Muratlı, İpsala, Meriç, Çorlu and Uzunköprü), it was found that individuals with cancer had higher levels of cadmium, mercury, dye and arsenic in their bodies than others. Heavy metals and chemicals were found in the river (Yolal, 2014). This shows that the Ergene River contains large amounts of carcinogenic substances.

The poisoning of the Ergene River by industrial waste not only pollutes the fertile agricultural lands in this region, but also causes pollution in many areas. It pollutes all food products fed by river water, all groundwater it passes through, and causes air pollution as a result of water evaporation. Individuals who breathe the air, consume agricultural foods whose soil is fed by river water, and directly consume groundwater mixed with the river also take many carcinogenic substances into their bodies. This causes other health problems, especially cancer (Yolal, 2014).

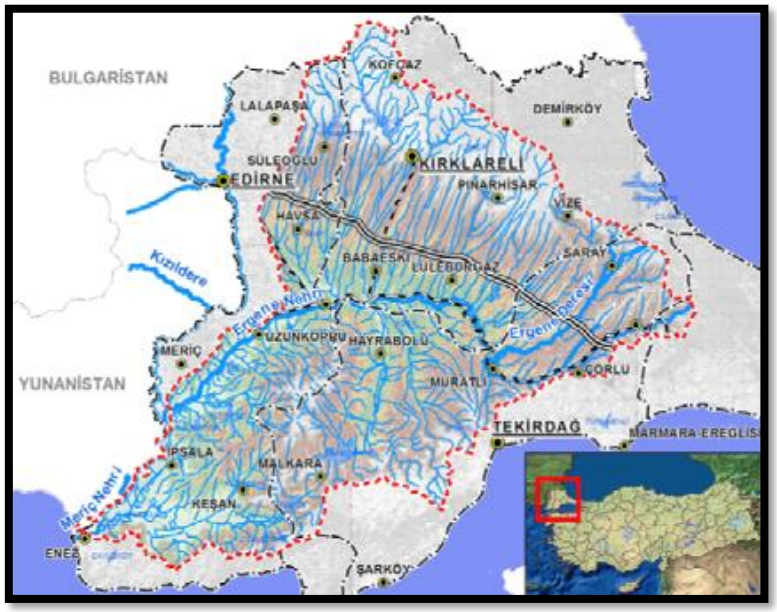

Figure 11: Geographical location of Ergene River (Bağdatlı, 2014)

In another study conducted in the region, high levels of heavy metals such as cadmium, zinc and lead were found in the nail pathologies of individuals with bladder, prostate and kidney

tumors living around the Ergene River (İnci et al. 2013a). In this study, patients with kidney tumors were specifically considered. Two groups were examined in the study. The groups are as follows; These are individuals with kidney cancer living around the Ergene River and individuals with the same diagnosis living in an area far from the river. As a result of the kidney histology and blood analysis examinations of the patients, it was seen that lead and cadmium elements were found in higher amounts in patients living near the river compared to others. Particularly as a result of blood analysis, it was seen that the amount of cadmium was 4 times higher than normal (İnci et al. 2013b).

The Chernobyl disaster is the nuclear power plant accident that took place on April 26, 1986 in the city of Pripyat, Ukraine, known as the largest nuclear accident according to the International Nuclear Event Scale (Figure 12 and Figure 13) (WHO, 2002).

Figure 12: Chernobyl disaster nuclear explosion (Demircan, 2019).

Figure 13: Chernobyl disaster after the nuclear explosion (Demircan, 2019).

It is known that approximately 8 tons of radioactive material was released into the air in this accident, which occurred during the electrical safety test at the power plant. The poisonous

clouds loaded with radioactive material that emerged after the explosion reached from Ukraine, Belarus, to Russia, parts of Europe, Turkey, America, Canada and Japan day by day. Approximately 7-8 million people were exposed to radiation after the accident, and the effects of the accident still continue today. The Chernobyl accident caused many deaths and diseases by exposing the air, soil and environment to intense radiation, affecting the food and plants grown in the region (Figure 14). Approximately 50 thousand square kilometers of agricultural land has become unproductive for 30-40 years. After the disaster, vision disorders and pathological diseases in newborns, especially thyroid cancer cases, increased (Figure 15) (Saraçoğlu 2006; TTB 2006).

Figure 14: Radioactive effects seen in foods after the Chernobyl accident (https://www.tarim.com.tr)

Figure 15: Anomaly in newborns and thyroid cancer in children after the Chernobyl accident (https://www.radyasyon.gen.tr).

In a study conducted on milk in the region 25 years after the Chernobyl disaster, cesium-137, known as a long-lived isotope, was found in 93% of the milk. Scientists say that currently there is still 1 ton of plutonium and 190-200 tons of uranium under the power plant, and all this residue can only be cleaned in 48-50 thousand years. As a result of the research, it was reported

that approximately 30-60 thousand people were diagnosed with a deadly type of cancer after the Chernobyl accident (Saraçoğlu 2006; TTB 2006).

While the normal radiation level measured in the Thrace Region before the Chernobyl accident was measured as 8-10 microroentgens/hour, after the disaster, this level was recorded as 30-40 microroentgens/hour. With the rain that fell in the Thrace region about a week after the Chernobyl accident, the air, soil and water in the region were completely under the influence of radiation (Saraçoğlu, 2006).

Although 37 years have passed since the disaster, the Thrace Region and the Black Sea Region are still experiencing the effects of this nuclear accident (Kara and Günay, 2013; TTB, 2006).

Agricultural drugs used to destroy organisms that harm the food produced are called pesticides. There are many types of these pesticides. Those used for insects are called insecticides, those used for fungi are called fungicides, and those used to destroy weeds are called herbicides. On the other hand, there are many commercial pesticides used against rodents, mollusks, nematodes and mites (Kaymak et al. 2015).

In a study conducted on rice samples collected from 25 different points around the Meriç River in the Thrace Region, many pesticide residues were determined in the rice. In this study, the pesticides azoxystrobin, cyproconazole, epoxyconazole, prochloraz, profoxidim, propoconazole, tebuconazole and trifloxystrobin were determined in the rice collected. Although the pesticide residue amounts are in accordance with the Turkish Food Codex and EU legislation, the fact that pesticides were obtained in the paddy fields in this region shows that higher doses of pesticides were used than necessary during pesticide application (Kulaksız and Akgün, 2020).

When pesticides were first discovered, it was predicted that they would break new ground in the field of agriculture. Because it is known that if thrown into the soil, it increases the yield by preventing the products from spoiling. Later, extensive studies on pesticides revealed that after using pesticides in agricultural lands, the products to be harvested mix with soil, groundwater and the atmosphere, causing great harm to the environment. Farmers who apply pesticides experience health problems by being exposed to both hormonal disorders and carcinogenic substances by inhaling the pesticide directly during application (Akdoğan et al. 2012, Tudi et al. 2022) (Figure 16).

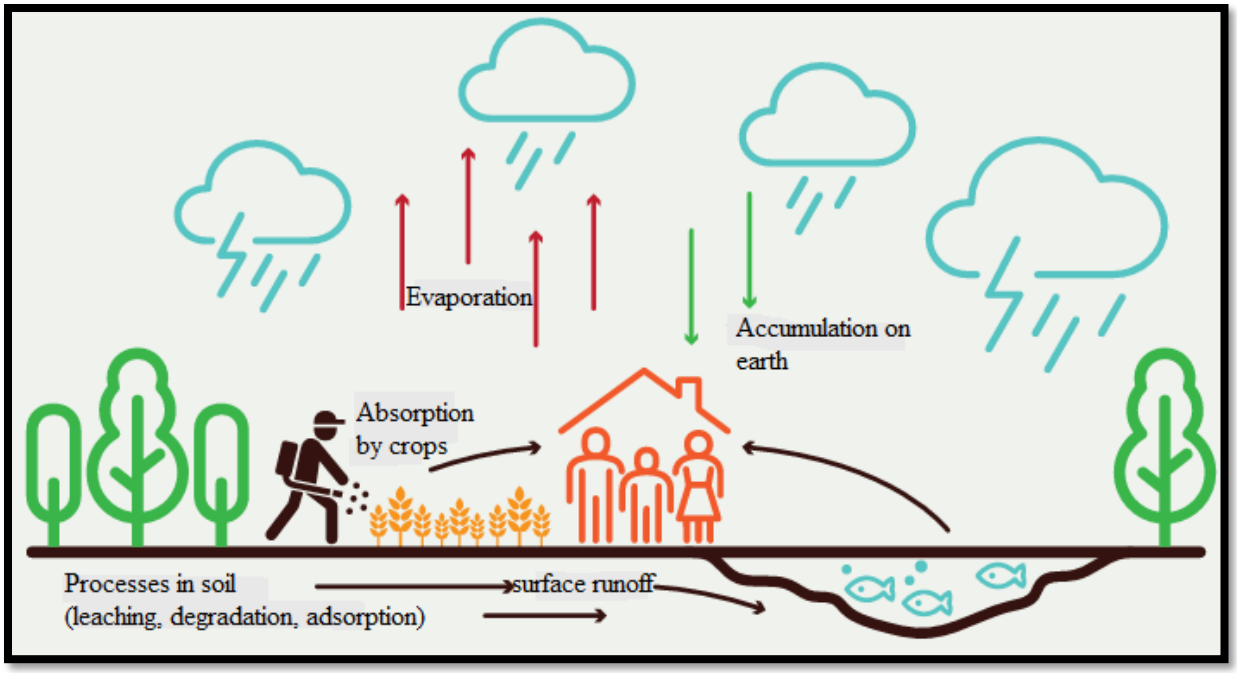

Figure 16: Environmental cycle of pesticides (Çağlayan et al. 2023).

Some specific types of cancer such as blood cancer, Hodkin's, non-Hodkin's lymphoma, stomach cancer, prostate cancer, breast cancer, and brain cancer are observed in agricultural workers who apply pesticides. A scientific study was conducted on rats to prove that pesticides are carcinogenic and to detect their negative effects (Ito et al. 1995). In this study conducted by Ito et al., rats were given a mixture of 19 phosphorus and 1 chlorine pesticide for 2 months. At the end of the 8th week, it was determined that there was an increase in the number and area of prenenoplastic lesions in the subject's liver. DNA insertion is the state of cancer-causing compounds covalently bonded to genetic material. As another result of pesticide application, when some leukocytes were examined for genetic material, a significant increase in DNA inclusion was detected. This research conducted on rats proved that pesticides are carcinogenic chemicals by causing DNA damage, oxidative damage, liver enzyme system disorders and increased DNA admixture in living organisms (Kurutaş and Kılınç 2003).

CONCLUSION

By scanning the scientific studies and existing literature, pollution agents, environmental carcinogens and genetic factors in the Thrace Region were identified. The evaluation of cancer cases caused by these carcinogens and agents was handled in the Thrace Region. Environmental carcinogens have been identified as smoking, obesity, viruses and bacteria, ultraviolet rays, alcohol consumption and chemicals. According to scientific studies, it has been observed that cancer patients smoke more than patients who are not diagnosed with cancer (Doll et al. 1994). As a result of the irregular increase in body weight and fat tissues of obesity patients, hormonal diseases, chronic diseases and various types of cancer occur in these individuals (Urhan and Akbulut, 2017). As a result of oncogenes being attacked by microorganisms, the cell does not multiply and grow in a healthy way, resulting in tumor formation (Özdoğan, 2021b). As a result of UV rays reaching the individual, the immune system in the skin is disrupted and DNA is damaged and cancer cells are formed (Herring, 2010, Özdoğan, 2017). According to the results of scientific studies, it has been observed that individuals with low system repair capacity in the repair and restoration of genetic material due to alcohol consumption have a high risk of developing cancer (Garaycoechea et al. 2012). As a

result of a scientific study, it was revealed that the risk of leukemia and CNS tumors is higher in the children of parents who have occupational contact with pesticides. This shows us that pesticides are chemical carcinogens (Bhatia, 1999).

When we consider environmental carcinogens as the Thrace Region; The high rate of old age, the dumping of industrial and other wastes into the Ergene River, the negative impact of the region after the Chernobyl disaster, and the excessive use of pesticides in agriculture have been identified as the main reasons for the high number of cancer cases in this region.

As we get older, the risk of getting cancer increases as our genetic structure begins to deteriorate (Kaygusuz, 2018). It has been determined that cancer cases and the risk of developing cancer are higher in Edirne, especially as a result of the high old age rate and lower birth rates in Edirne (TUIK, 2022).

As a result of industrial facilities in the Thrace Region throwing their waste and poison into the Ergene River, known as the water source of the region; Foods grown in these soils also contain other carcinogenic substances such as heavy metals. It has been determined that people who consume these foods have an increased risk of many diseases and cancer (Kocaman et al. 2011).

As a result of the nuclear explosion that occurred during a safety test at a nuclear power plant in Ukraine, the Thrace Region, along with many countries and cities, was greatly affected by this nuclear accident. As the radioactive poison clouds that emerged after the explosion reached the region and acid rain fell on Thrace, the air and the soil and the environment were exposed to intense radiation. Therefore, in the Thrace Region; Living people have a high risk of contracting cancer as well as many diseases as a result of consuming grown plants and foods. As a result of the Chernobyl disaster; Along with many human deaths, 7-8 million people were exposed to radiation. Approximately 50 thousand kilometers of agricultural land has become unproductive for many years. Thyroid cancer cases have increased, especially in children, and visual impairments and pathological diseases have been observed in newborns. According to the results of the research, it was reported that 30-60 thousand people were diagnosed with a deadly type of cancer after the disaster (Saraçoğlu 2006; TTB 2006).

As a result of the excessive use of pesticides in agricultural areas in the Thrace Region, it has been determined that pesticides cause great harm to the environment by mixing with the harvested products, soil, groundwater and the atmosphere. It has been observed that the risks of hormonal disorders and cancer are high in farmers who apply pesticides, which are known to be carcinogenic substances, and in people who consume food and breathe the atmosphere (Akdoğan et al. 2012, Tudi et al. 2022).

In this study, genetic carcinogens; Oncogenes, tumor suppressor genes, DNA repair genes and other carcinogenic gene concepts have been identified. It has been observed that hereditary cancers occur as a result of mutations in somatic and reproductive cells (Knippers, 2006), proto-oncogenes (Croce, 2008) and tumor suppressor genes (McKusick, 2006). Retinoblastoma, explained by the two-hit hypothesis, occurs as a result of 40% germline mutations and 60% sporadic gene mutations (Newsham et al. 1998).

It has been determined that tumor protein 53 gene, Von Hippel-Landau (VHL) gene, MLH1 gene, Excision repair gene, BRCA1 and BRCA2 genes, also known as DNA repair genes, cause some syndromes in the body and that individuals have a higher risk of developing various types of cancer. (Özdoğan, 2021c).

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