AGRIBALKAN 2022

IV. BALKAN AGRICULTURAL CONGRESS

31 AUGUST – 02 SEPTEMBER 2022,

EDIRNE, TURKEY

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31 AUGUST – 02 SEPTEMBER 2022,

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In Trakya University Balkan Congress Center, Edirne, Turkey

Organized by Trakya University

with

Trakya Universities' Union, Balkan Universities' Union, Namık Kemal University, Onsekizmart University - Turkey, Uludag University, Turkey, Agriculture University of Plovdiv, Trakia University-Stara Zagora - Bulgaria, Democritus University of Thrace – Greece and with contribution of other Balkan Institutions…

Dear Colleagues,

You are welcome to our congress will be organized by Trakya University supporting with Trakya Universities Union, Balkan Universities Association and together with other Balkan Universities and Institutions.

The aim of our international congress is to present the newest research results and research goals, analyze current conditions and perspectives in agriculture.

Conference activities;

Plenary sessions with oral and poster presentations are on 29 August – 01 September 2021.

You are welcome to our congress and Edirne, TURKEY,

Yours sincerely,

Prof. Dr. Erhan TABAKOĞLU

Rector of Trakya University
Honorary Chair of Congress

Prof Dr Yalcin KAYA

Head of Organizing Committee

FOREWORD

Agriculture is so important sector feeding all humankind, but it needs new developments and technologies to supply enough food for increasing world population year by year. Turkey is one leading agricultural economy in the world. Balkan region is one the important agricultural areas of the world having rich soils producing different crops vastly and keeping enormous biodiversity for our future.

As there have been many different scientific meetings around the world, we intended to bring three communities together, namely science, research and private investment, in a friendly environment of Edirne / Turkey to share what they have and get benefit from each other. Trakya University intended to aim that agricultural community in Balkan areas should come together in that important event. Our congress goal is the agricultural subjects should be kept broad in order to provide opportunity to the science community to present their work that can be off value for agriculture.

First Balkan Congress was organized by Trakya University in 2014 as the biggest agricultural congress in Turkey and Balkan region. In the first congress, over 700 participants were presented total 830 papers (650 poster and180 oral presentations) and invited speakers presented country reports from all Balkan countries. 2nd Balkan Agriculture Congress was organized by Tekirdağ Namık Kemal University in 2017. The 3rd Balkan Agriculture Congress was hosted again in Trakya University in Edirne, Turkey in 2021 due to 40th anniversary of Trakya University. There was a worldwide participation from 41 countries with 406 papers contributed by 988 authors with 288 oral, 118 e-poster presentations.

As fourth one, Trakya University hosted again in Edirne, Turkey in 2022. We would like to thank all participants for great interest to our AGRIBALKAN 2022 congress. There is a worldwide participation from 41 countries with 388 papers contributed by 888 authors.

We hope that this congress will help to solve our problems with establishing good network collaborations, joint projects and better relationships among countries with sharing our knowledge and experiences together. We wish success for this meeting and hope a great scientific achievement with your contributions.

Edirne is very nice, lovely and historical city at just the edge of Europe, but just right at the heart of Balkan region and history endowed with monuments reminding imperial past. We are much pleased to host you all in Edirne and in Turkey. We would like to thank you to join this congress and we would like to give also special thanks our sponsors and collaborators for giving us big supports to organize this event.

We wish you nice stay in Edirne for truly rewarding days.

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CONTENTS

AN ECOTOXICOLOGICAL EVALUATION OF TRACE ELEMENTS IN THE WATER OF IRRIGATION PONDS LOCATED IN THRACE REGION OF TURKEY

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ABSTRACT

In this research, boron, selenium and manganese accumulations in water of irrigation ponds located in the Thrace Region of Türkiye were determined and probable non-carcinogenic health risks of these elements via daily human intake were evaluated. Total od 12 irrigation ponds were selected in the region and water samples were collected during the summer (dry) season of 2020. The element levels were measured by using an ICP-MS device and Estimated Daily Intake (EDI) and Hazard Quotient (HQ) of boron, selenium and manganese were calculated seperately. Boron concentrations were varied from 44.541 – 716.984 ppb; selenium concentrations were varied from 0.307 – 1.266 ppb; and manganese concentrations were varied from 1.621 – 1577.944 ppb. All the calculated HQ values in all the investigated locations in terms of all the investigated age groups were recorded as less than the critical limit of 1.

Keywords: Thrace Region, Irrigation ponds, Boron, Selenium, Manganese, Health risk assessment

INTRODUCTION

Thrace Region is located in the north – west part of Marmara Region and has a great agricultural potential due to its fertile clayey soil and there are many irrigation ponds in the region. Therefore, intensive agricultural activities in the region have a significant impact on freshwater and soil quality (Anonymous, 2018; Tokatlı, 2019; Onur and Tokatlı, 2020; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021a; Tokatlı, 2021).

Selenium and manganese, which are commonly found in rocks and soil, are naturally occurring elements. Boron is also a widely occurring element in minerals found in the earth's crust. They are all trace elements and essential nutrients for humans and necessary for good health. However, they can be harmful when regularly taken in amounts higher than those needed for good health. Significant amounts of boron can be released from agricultural fertilizer and pesticide usage. Also, manganese is known as being used in a wide variety of products including fertilizer. However, the principal release of selenium into the environment from anthropogenic sources is reported as coal combustion (ATSDR, 2003; 2010; 2012).

Probabilistic non-carcinogenic health risk assessment techniques are being widely used all over the world in especially recent years. They are very useful and effective tools in estimating non-carcinogenic hazards, which may occur when people are exposed to certain toxicants (Ustaoğlu and Aydın, 2020; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021a; 2021b; Varol and Tokatlı, 2022). The aim of this research was to determine the levels of boron, selenium and

manganese in water of 12 irrigation ponds located in the Thrace Region and to evaluate the probable non-carcinogenic health risks of these essential elements via daily human intake.

MATERIALS AND METHODS

Water Collection

In this research, total of 12 irrigation ponds located in the Meriç – Ergene River Basin in the Thrace Region were selected. Coordinate information and station codes are given in Table 1. The topoFigureic map of Meriç – Ergene River Basin and selected sampling stations are given in Figure 1. Water samples were collected in the summer of 2020 (dry season) with a telescopic water sampling device approximately 3 meters from the shore into the pre – cleaned polyethylene bottles.

Element Analysis

pH values of water samples were decreased to 2 by adding about 2 ml of nitric acid per 1 L into each. The samples were filtered by using a 0.45 µm – cellulose nitrate filter. The volumes of samples were made up to 50 ml with ultrapure water. In the acidic – filtered water samples, a total of 3 metal(loid)s (B, Se and Mn) were determined with an inductively coupled plasma – mass spectrometry (ICP – MS) in the laboratory of Technology Research Development Application and Research Centre of Trakya University – an international accreditation certificated institution. All the element analyses were determined as means of triplicate reads (TS EN / ISO IEC 17025) (EPA, 2001; APHA, 2005). The accuracy of analytical method was controlled by using a certified reference material (CRM) (CPAchem – Ref Num: 110580.L1).

Health Risk Assessment

In the current research, population was divided into four age groups based on their physiological and behavioural differences as follow: infants (< 2 years old), children (2 – 6 years old), teenagers (6 – 16 years old) and adults (> 16 years old). The daily exposure to boron, selenium and manganese were calculated in these four different groups by using the equation 1. Hazard Quotient (HQ) describes the non-carcinogenic risk of b, Se and Mn and it was calculated by using the equation 2 (Liu et al., 2018; Zango et al., 2019; Ghosh et al., 2020). A lower value of HQ than one reflects a negligible risk of non-carcinogenic effects and a higher value of HQ than one reflects an important health risk.

$$EDI = \frac{Csample\ x\ Cd}{Bw} \quad (1)$$

$$HQ = \frac{EDI}{RfD} \quad (2)$$

EDI: Estimation of fluoride consumption – daily (mg/kg/day)

C_{sample}: B, Se and Mn levels in the investigated water samples (mg/L)

C_d: Mean daily water intake (L/day) (the mean water consumption rates in infants, children, teenagers and adults were 0.1, 0.85, 2 and 2.5 L/day, respectively)

B_w: Body weight (kg) (body weights of investigated groups were considered 5, 10, 50 and 75 kg, respectively)

RfD: Reference dose (mg/kg/day) (it is 0.13, 0.005 and 0.14 mg/kg/day for B, Se and Mn respectively) (EPA, 1992)

Table 1. Coordinate information of selected stagnant water bodies

Name of Habitat	Station Code	GPS – North	GPS – East	Name of Habitat	Station Code	GPS – North	GPS – East
Sülecik	P1	41.823	26.848	**Muzalı**	P7	40.943	26.657
Taşlımüsellim	P2	41.823	26.780	**Boztepe**	P8	40.851	26.529
Değirmenci	P3	41.328	26.722	**Kocadere**	P9	40.872	26.642
Kurtbey	P4	41.187	26.581	**Bayramşah**	P10	41.131	27.202
Alıç	P5	41.080	26.664	**Küçükhıdır**	P11	40.909	27.056
Karasatı	P6	40.975	26.687	**Bıyıkali**	P12	41.012	27.389

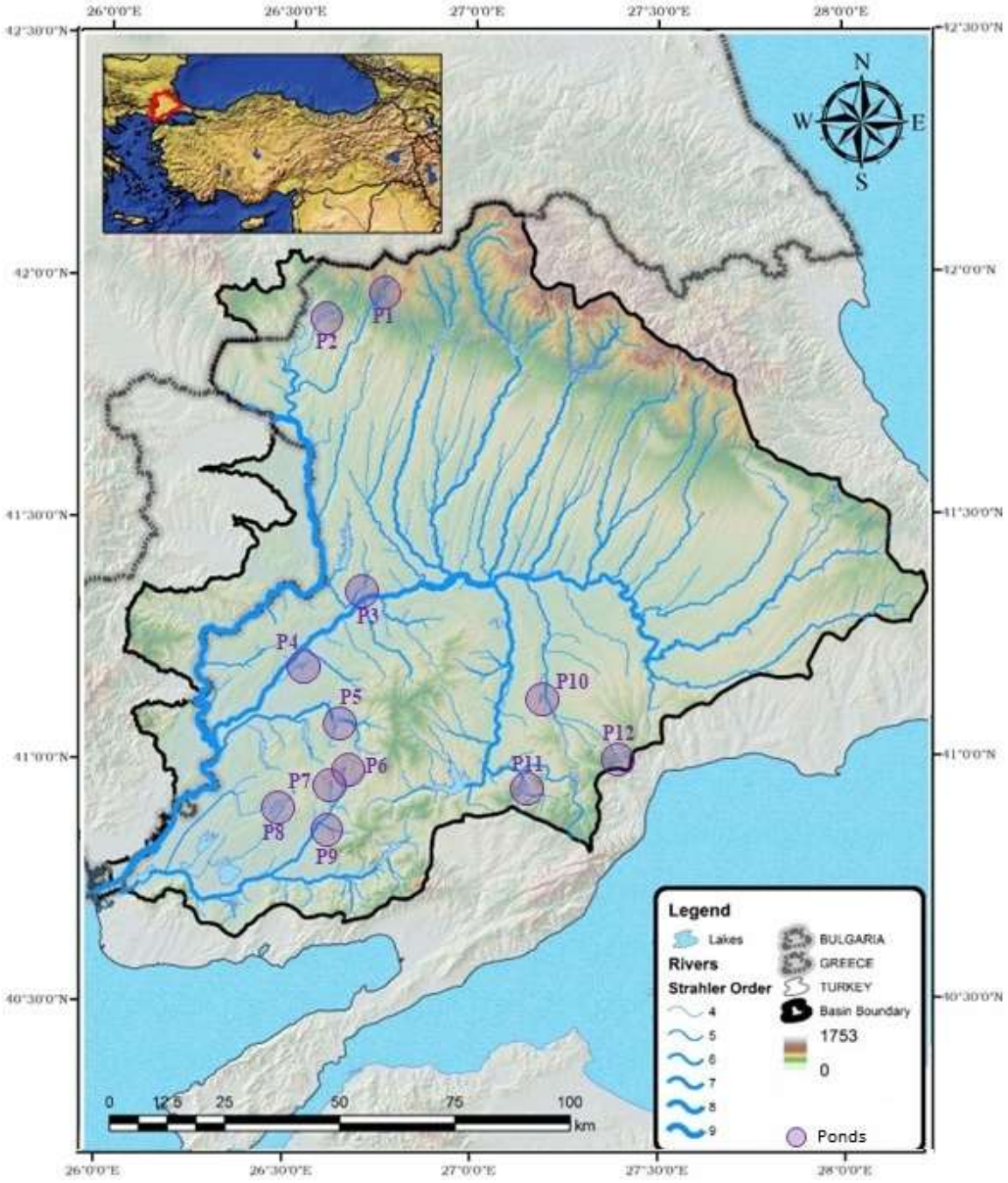

Figure 1. TopoFigureic map of Meriç – Ergene River Basin and selected lentic habitats

RESULTS AND DISCUSSION

Boron, selenium and manganese accumulations in water of investigated irrigation ponds located in the Thrace Region are given in Figure 2 and detected EDI and HQ coefficients in investigated stagnant water bodies are given in Table 2.

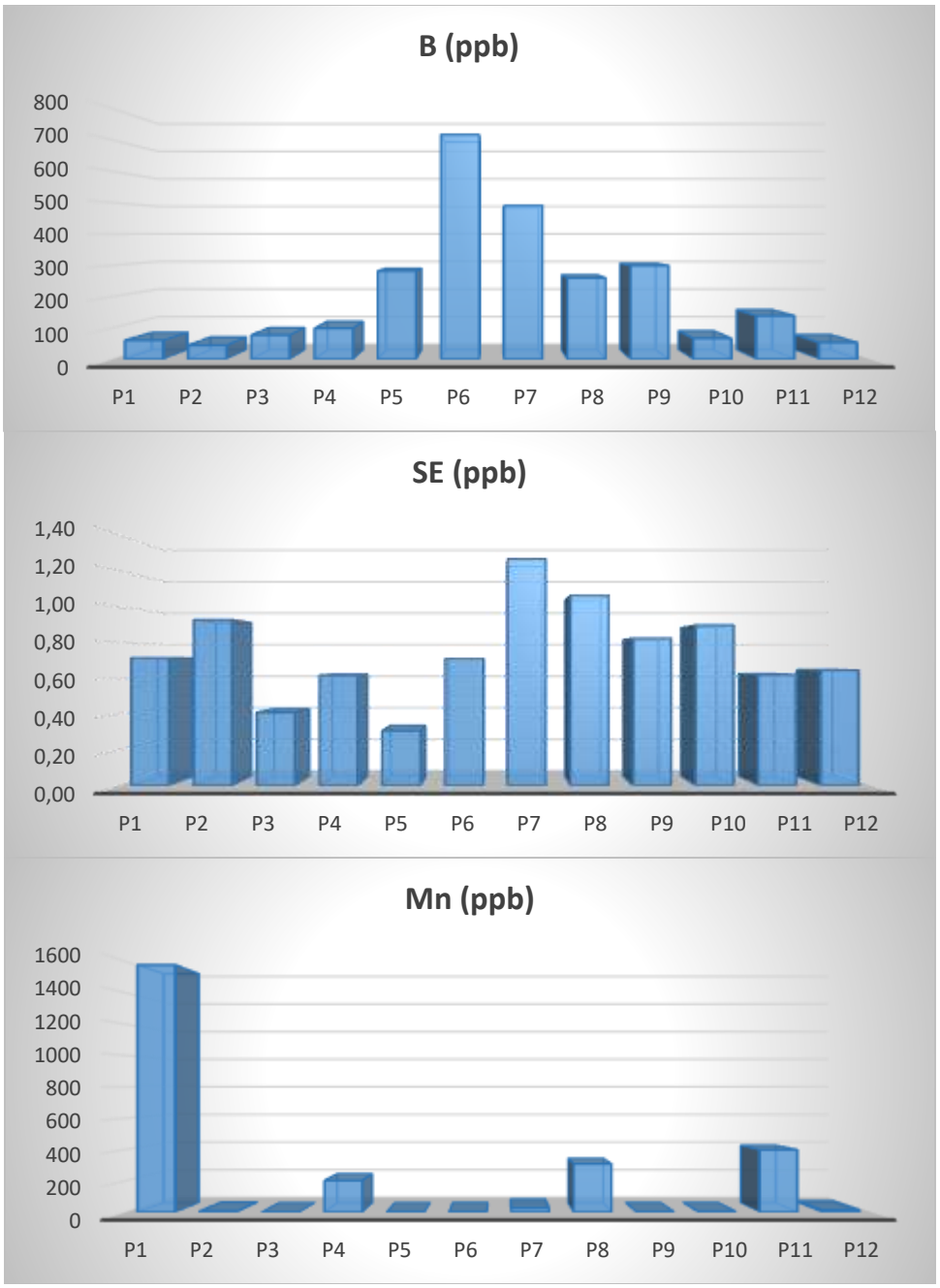

Figure 2. Boron (up), selenium (middle) and manganese (down) levels in water samples

Boron concentrations were varied from 44.541 (Taşlımüsellim Pond) – 716.984 (Karasatı Pond) ppb with an average of 215.619 ppb; selenium concentrations were varied from 0.307 (Alıç Pond) – 1.266 (Muzalı Pond) ppb with an average of 0.749 ppb; and manganese concentrations were varied from 1.621 (Değirmenci Pond) – 1577.944 (Sülecik Pond) ppb with an average of 212.649 ppb.

All the calculated HQ values in all the investigated locations in terms of all the investigated age groups were recorded as less than the critical limit of 1 with a spatial mean of 0.03317, 0.00300 and 0.03038 for infants; 0.14098, 0.01275 and 0.12911 for children; 0.06634, 0.00600 and

0.06076 for teenagers; and 0.05529, 0.00500 and 0.05063 for adults in terms of boron, selenium and manganese respectively.

The EDI and HQ risk rankings among the investigated different age groups were found as follows: children > teenagers > adults > infants in all the investigated stagnant water bodies including irrigation ponds, in general. In many of researches on the assessment of probable health risks associated with boron, selenium and manganese in water performed in many different countries confirmed that the probable chronic health risks in children due to the intake of these elements are more possible than the other age groups (Liu et al., 2018; Zango et al., 2019; Ghosh et al., 2020). In this research, as similar with the literature data, children were found as the riskiest age group for the probable health risk due to the intake of boron, selenium and manganese in water of irrigation ponds of Meriç – Egene River Basin.

Table 2. EDI and HQ values

		EDI				HQ			
		Infants	**Children**	**Teenagers**	**Adults**	**Infants**	**Children**	**Teenagers**	**Adults**
Boron	**L1**	0.00645	0.02742	0.01290	0.01075	0.04963	0.21093	0.09926	0.08272
	L2	0.00582	0.02474	0.01164	0.00970	0.04478	0.19032	0.08956	0.07464
	R1	0.00091	0.00388	0.00183	0.00152	0.00703	0.02987	0.01406	0.01171
	R2	0.00075	0.00320	0.00151	0.00126	0.00580	0.02465	0.01160	0.00967
	R3	0.00006	0.00026	0.00012	0.00010	0.00047	0.00200	0.00094	0.00079
	R4	0.00109	0.00463	0.00218	0.00182	0.00838	0.03564	0.01677	0.01397
	R5	0.00196	0.00832	0.00391	0.00326	0.01506	0.06399	0.03011	0.02509
	R6	0.00574	0.02442	0.01149	0.00957	0.04419	0.18781	0.08838	0.07365
	R7	0.00024	0.00103	0.00048	0.00040	0.00186	0.00791	0.00372	0.00310
	R8	0.00036	0.00154	0.00072	0.00060	0.00278	0.01182	0.00556	0.00464
	R9	0.00032	0.00137	0.00065	0.00054	0.00248	0.01056	0.00497	0.00414
	R10	0.00040	0.00170	0.00080	0.00067	0.00308	0.01310	0.00617	0.00514
	R11	0.00033	0.00139	0.00065	0.00054	0.00251	0.01065	0.00501	0.00418
Selenium	**L1**	0.00002	0.00008	0.00004	0.00003	0.00373	0.01585	0.00746	0.00622
	L2	0.00001	0.00006	0.00003	0.00002	0.00281	0.01196	0.00563	0.00469
	R1	0.00002	0.00009	0.00004	0.00004	0.00431	0.01832	0.00862	0.00718
	R2	0.00002	0.00010	0.00005	0.00004	0.00461	0.01958	0.00921	0.00768
	R3	0.00000	0.00001	0.00000	0.00000	0.00027	0.00114	0.00054	0.00045
	R4	0.00002	0.00009	0.00004	0.00003	0.00409	0.01740	0.00819	0.00682
	R5	0.00003	0.00011	0.00005	0.00004	0.00516	0.02195	0.01033	0.00861
	R6	0.00002	0.00007	0.00003	0.00003	0.00348	0.01481	0.00697	0.00581
	R7	0.00000	0.00001	0.00000	0.00000	0.00038	0.00160	0.00075	0.00063
	R8	0.00000	0.00001	0.00001	0.00000	0.00054	0.00228	0.00107	0.00089
	R9	0.00000	0.00001	0.00001	0.00000	0.00054	0.00228	0.00107	0.00089
	R10	0.00000	0.00001	0.00001	0.00001	0.00070	0.00296	0.00139	0.00116
	R11	0.00000	0.00001	0.00000	0.00000	0.00032	0.00137	0.00064	0.00054
Manganese	**L1**	0.05306	0.22550	0.10612	0.08843	0.37900	1.61073	0.75799	0.63166
	L2	0.00012	0.00051	0.00024	0.00020	0.00086	0.00364	0.00171	0.00143
	R1	0.00025	0.00104	0.00049	0.00041	0.00175	0.00745	0.00351	0.00292
	R2	0.00097	0.00412	0.00194	0.00161	0.00692	0.02941	0.01384	0.01153
	R3	0.00002	0.00009	0.00004	0.00004	0.00016	0.00068	0.00032	0.00027
	R4	0.00008	0.00033	0.00016	0.00013	0.00055	0.00236	0.00111	0.00092
	R5	0.00014	0.00058	0.00027	0.00023	0.00098	0.00414	0.00195	0.00163
	R6	0.00007	0.00029	0.00014	0.00011	0.00049	0.00208	0.00098	0.00082
	R7	0.00001	0.00005	0.00002	0.00002	0.00009	0.00037	0.00017	0.00014
	R8	0.00006	0.00024	0.00011	0.00009	0.00041	0.00172	0.00081	0.00068
	R9	0.00001	0.00004	0.00002	0.00001	0.00006	0.00025	0.00012	0.00010
	R10	0.00010	0.00040	0.00019	0.00016	0.00068	0.00289	0.00136	0.00113
	R11	0.00000	0.00002	0.00001	0.00001	0.00003	0.00013	0.00006	0.00005

CONCLUSIONS

In the current research, spatial variations of boron, selenium and manganese concentrations and the probable non-carcinogenic health risks of these elements via daily human intake in water of 12 irrigation ponds located in the Meriç – Ergene River Basin in the Thrace Region of Türkiye were investigated. As a result of this study, the detected HQ values of all age groups were found as less than the critical limit of 1 and the risk rankings among the investigated different age groups were found as follows: children > teenagers > adults > infants, in general.

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CHLORINE AND PHOSPHATE LEVELS IN DRINKING WATER OF İPSALA DISTRICT AN AGRICULTURAL STRESSED REGION IN TÜRKIYE: A GIS BASED EVALUATION

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ABSTRACT

Anthropogenic applications causing a transformation of the natural phosphorus cycle is considered as one of the most fundamental environmental issues. The aim of this research was to determine the chlorine and phosphate accumulations in drinking water of İpsla District located in the Meriç Plain in the west of Thrace Region of Türkiye. Tap – drinking water samples were taken during the winter season of 2021 from 23 selected locations including İpsala District and 22 connected villages and the water quality parameters were measured by spectrophotometric method. Chlorine concentrations in drining water samples were varied from 27 – 167 mg/L and phosphate concentrations in drinking water samples were varied from 0.001 – 0.639 mg/L.

Keywords: İpsala District, Drinking water, Chlorine, Phosphate

INTRODUCTION

About 3/4 of the earth's crust is covered by water and pollution of these water ecosystems are a threat to life in the biosphere today (Isiuku and Enyoh, 2019). One of the major contaminants of the freshwater habitats is phosphate, which emanate from fertilizers and wastes from animals and humans (Ali et al., 2018; De Girolamo et al., 2019). Chlorine and phosphate are essential ions and available to man through diets. However, when food and water sources are polluted by phosphate, this may cause several health problems.

Thrace Region is located in the north – west part of Marmara Region and has a significant agricultural potential and about 80% of the region consists of agricultural lands. İpsala District is located in the west part of the Thrace Region. The district is very suitable for wet agriculture applications due to its rich groundwater and surface water resources. Therefore, surface and groundwater resources of Thrace Region, Meriç Plain and İpsala District have been facing various pollution problems due to intensive agricultural applications and runoff from these lands (Anonymous, 2018; Tokatlı, 2019; Onur and Tokatlı, 2020; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021; Tokatlı et al., 2022; Varol and Tokatlı, 2022).

The aim of this research was to determine the levels of chlorine and phosphate levels in tap – drinking water of İpsala District and a total of 22 villages connected to the İpsala. Also the data detected were evaluated according to the Water Quality Control Regulations in Türkiye.

MATERIALS AND METHODS

Water Collection and Chemical Analysis

In this research, 23 sampling points were selected for the İpsala District. The topoFigureic map of study area and selected stations (İ1 – İ23) are given in Figure 1. The coordinates with the names of locations are given in Table 1. Drinking water samples were taken from the tap waters of the villages located in the İpsala District in winter season of 2021 by using polyethylene bottles. Chlorine and phosphate parameters were determined with a spectrophotometer device (Hach Lange, DR3900) during the laboratory studies.

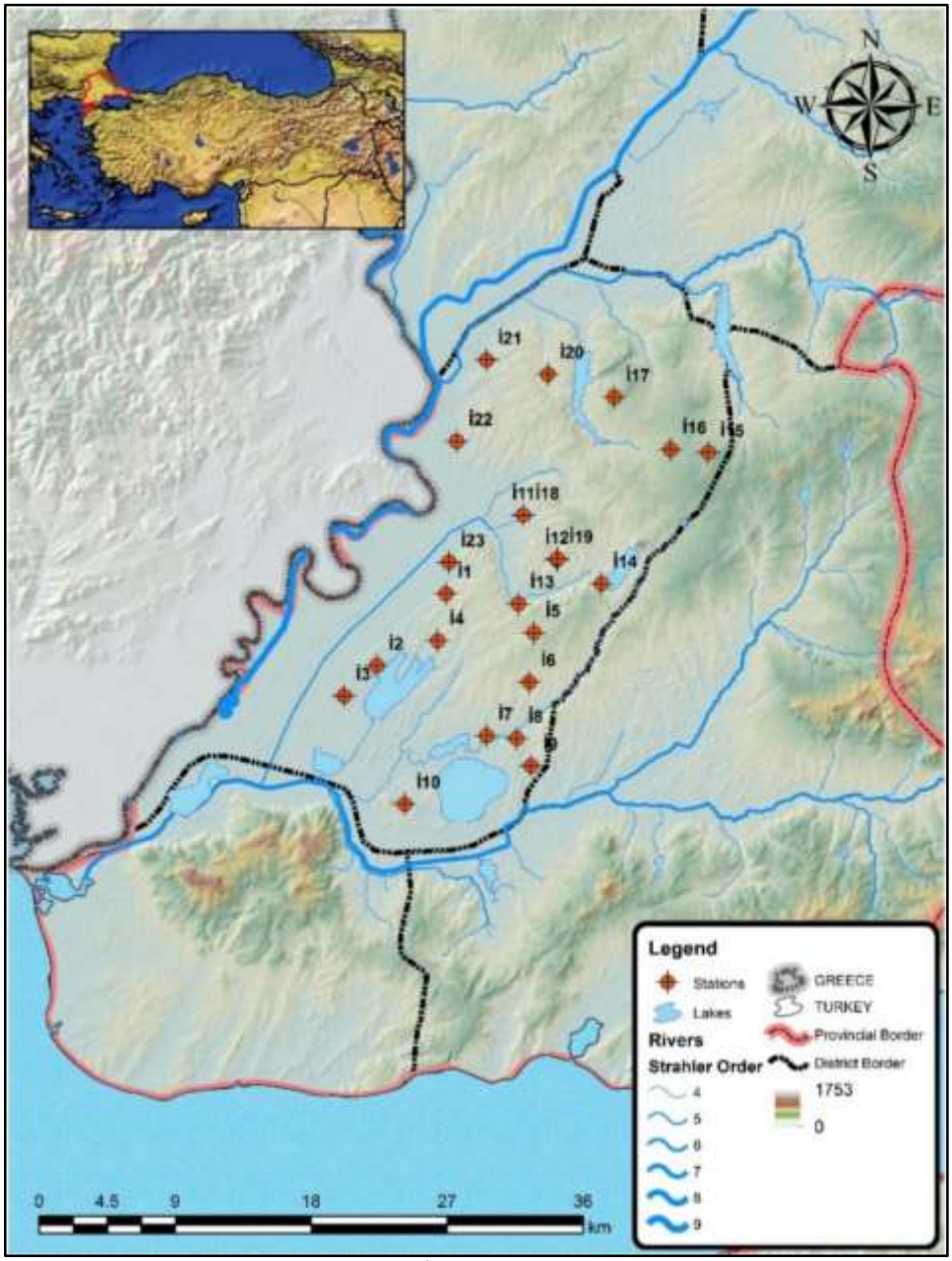

Figure 1. TopoFigureic map of İpsala District and selected lentic habitats

Table 1. Coordinate information and the locations of the investigated stations

Stations Code	Location	GPS – North	GPS – East
İ1	Ahırköy Village	40.894	26.374
İ2	Paşaköy Village	40.850	26.320
İ3	Yenikarpuzlu Town	40.832	26.295
İ4	Kumdere Village	40.866	26.368
İ5	Esetçe Town	40.871	26.443
İ6	Aliçopehlivan Village	40.841	26.440
İ7	Kocahıdır Village	40.809	26.407
İ8	Küçük Doğanca Village	40.808	26.430
İ9	Yapıldak Village	40.791	26.442
İ10	Koyuntepe Village	40.768	26.343
İ11	Turpçular Village	40.941	26.434
İ12	Hıdırköy Village	40.914	26.461
İ13	Sarpdere Village	40.888	26.431
İ14	Korucu Village	40.900	26.496
İ15	Pazardere Village	40.979	26.580
İ16	Hacıköy Town	40.981	26.550
İ17	İbriktepe Town	41.012	26.505
İ18	Karaağaç Village	40.941	26.434
İ19	Hıdırköy Village	40.915	26.461
İ20	Sultanköy Town	41.025	26.453
İ21	Balabancık Village	41.033	26.404
İ22	Sarıcaali Village	40.985	26.382
İ23	İpsala District	40.913	26.376

RESULTS AND DISCUSSION

GIS distriburiion maps of chlorine and phosphate concentrations in tap – drinking water of investigated villages are given in Figure 2. Chlorine concentrations were varied from 27 (Hacıköy Village) – 167 (Kumdere Village) mg/L with an average of 72 mg/L; and phosphate concentrations were varied from 0.001 (Yenikarpuzlu Town) – 0.639 (Pazardere Village) mg/L with an average of 0.174 mg/L.

Phosphate levels in the tap - drinking water samples were recorded as quite high levels and according to the Water Quality Control Regulations in Türkiye (WQCR, 2015), tap - driking water of the İpsala District has 2. – 3. Class water quality in terms of phosphate contents.

In a study conducted approximately 1 year before the current study by Tokatlı et al. (2022) in the same region, groundwater and tap water qualities were investigated in the Meriç Delta. As a result of this study, as similar with the present data, groundwater and tap water of the Meriç Plain was found as having 3. Class water quality in terms of phosphate accumulations.

It is known that phosphate is a signifcant organic pollutant, and it may reach quite high levels in drinking water in especially rural areas (Çiçek et al., 2013; Köse et al., 2014). Also, phosphate is known to be reached quite high levels in drinking waters especially in areas, where there is no sewage system and septic tanks, such as the present study area of İpsala District (Tokatlı, 2014; 2019; Tokatlı et al., 2022).

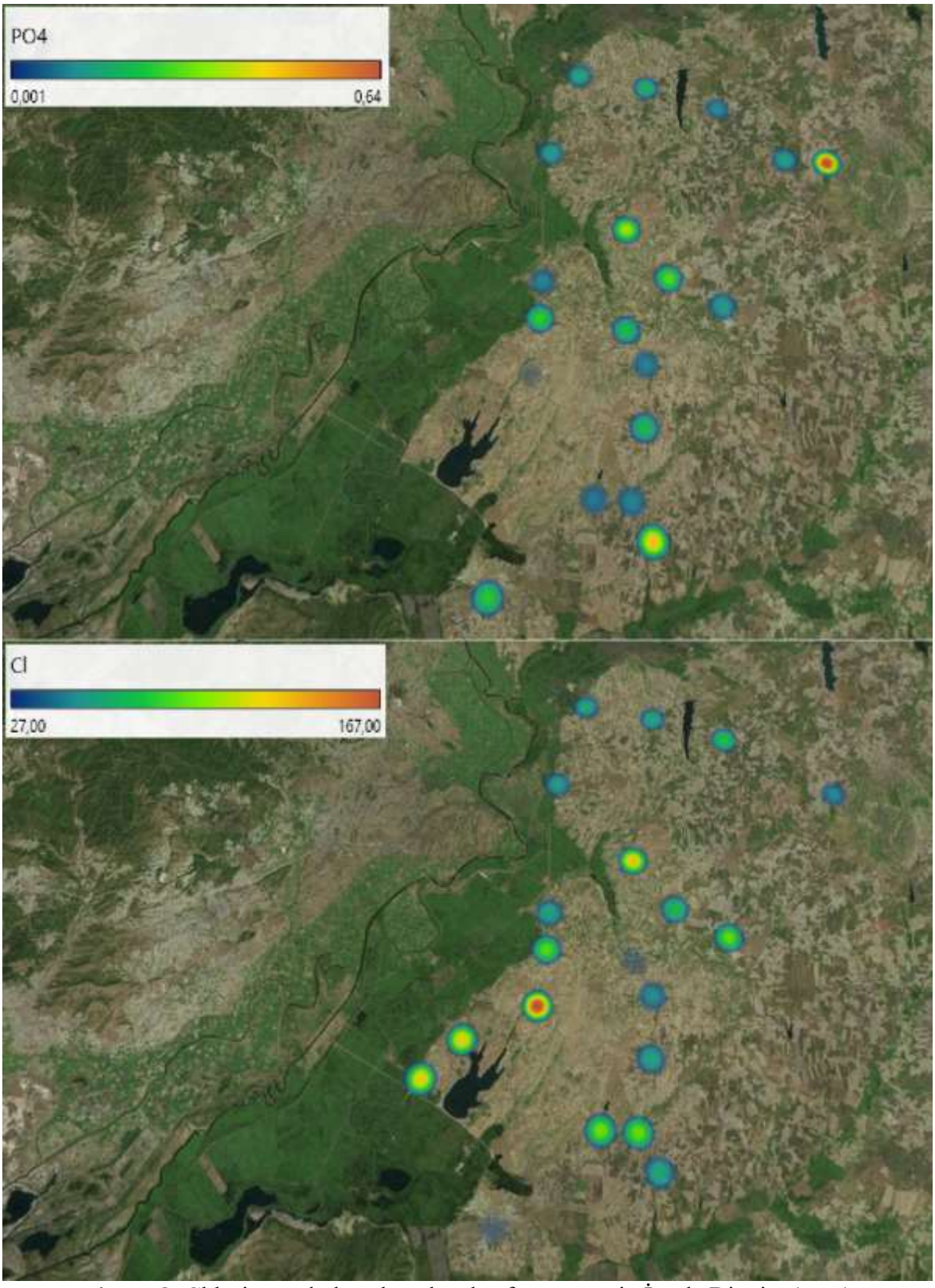

Figure 2. Chlorine and phosphate levels of tap water in İpsala District (ppm)

CONCLUSIONS

The agricultural production area of the Meriç Plain is about 500,000 decares and most of these agricultural lands in the plain consists of paddy fields requiring the use of quite high amounts of agricultural fertilizers and pesticides. Consequently, monoculture agricultural activities and the deficiencies of sewer system in the region are thought to be the main reason for the quite high phosphate levels in the drinking water. As a result of this research, it is recommended to expand the polycultural practices in the region.

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EFFECTS OF COMPOST APPLICATION RATE AND MULCH THICKNESS ON SOIL PHYSICOCHEMICAL PROPERTIES UNDER SALT AFFECTED SOIL OF DUGDA DISTRICT OF ORAMIA REGEON, ETHIOPIA

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ABSTRACT

Soil is increasingly recognized as an important non-renewable natural asset that should be properly managed to ensure sustainable development. The salt affected soils in Middle Awash Valley have been a challenge to agricultural production. Composts are used in agriculture and horticulture to improve soil fertility and quality because they can increase organic matter content, especially in sandy soils which have low water and nutrient holding capacity. However, the compost and mulch technology options were not demonstrated for irrigated agriculture in the study area. This objective was aimed to determine the effect of mulch and compost application rate on soil physicochemical property under salt affected soil for tomato production. The experiment was conducted in factorial experiment arranged in a Randomized Complete Block Design of three replications. The treatment combinations included four compost rate (0, 2, 4, and 6 ton/ha) and four mulch thickness (0, 5, 10, 15cm). Data on some soil physical and chemical properties and economic benefits were recorded and analyzed. Results showed that, Mg, Om%, CEC, Ec (ds)/m, Na, and Available (Av.P) ppm were significantly ($p<0.01$) affected by the interaction effects of compost application rates and mulch thicknesses. However, pH, TN%, OC%, and Ca are significantly ($p<0.01$) affected by the main effects of compost application rate. The net farm income and benefit cost ratio calculated confirm that, application of 6ton/ha compost rate and 10cm mulch thickness makes farmers' benefited from tomato yield. Further examinations are essential to make reliable technologies for compost rate and mulch thickness and draw recommendations on irrigated tomato production.

Key words: Soil salinity, compost, mulch, profitability

INTRODUCTION

Sustainability in agricultural production is guided by the principal philosophy of "feed the soil to feed the plant". This basic precept is implemented through a series of practices designed to increase soil productivity and fertility in order to improve soil physicochemical properties by adding organic matter, biological activity and nutrient availability. Adding organic materials such as green manure, crop residues, and composts to cultivated soils builds levels of soil organic matter and increasing soil fertility. Soil fertility is fundamental in determining the productivity of all

farming systems and is most commonly defined in terms of the ability of a soil to supply nutrients to crop (Wild, 1993). Farmers are suffering from declining soil fertility and are complaining about weak responses of their soil fertility management (Tonfack *et al*., 2013). Investigations have shown that farmers mostly rely on a single option of conventional nutrient replenishment, without considering the soil mineral balance (Tonfack *et al*., 2009

Salinity is the amount of salt in the soil or water and the causes are: cultivation of naturally-saline lands, rise in secondary salinity because of the inflow of mineralized groundwater with intensive irrigation, increase in the salt content of irrigation water because of the disposal of drainage water into irrigation canals (Shirokova *et al*., 2000).The dominant salt in most saline soil is common salt-sodium chloride (NaCl), varying amounts of calcium, magnesium and potassium chlorides and sodium sulfates (Thomas and Morini, 2005). The ions responsible for salinization are: Na^+, K^+, $Ca2^+$, $Mg2^+$ and Cl^-. When Na^+ (sodium) predominates, salt affected soil is called sodic soil (Krause and Whitfield, 2010). The types of salinity based on soil and groundwater processes are groundwater-associated salinity (dry land salinity), transient salinity (dry saline land) and irrigation salinity (water) (Rengasamy, 2010).

The Central Ethiopian Rift valley is characterized by a chain of lakes and wetlands with unique hydrological and ecological characteristics. Increasing population size and economic developments put an increasing claim on the precious fresh water resources. Large parts of the natural vegetation in the Rift valley have already been converted and degraded due to the pressing need for natural resources by the local population. Poor drainage and lack of appropriate irrigation water management were the main causes, which facilitated secondary salinization. Many reports indicate in Ethiopia, especially in the rift valley areas that, the low productivity of vegetables including tomato, is attributed to low or depleting soil fertility, poor agronomic practices such as imbalanced fertilization (Fekadu *et al*., 2006). Large sodium content exist in the lower part of the Central Rift Valley where soil pH present from 8.5 to 10.0 (OWRDB, 2009). Although salinity and sodicity are common phenomena for arid and semiarid regions of the world, salt-affected soils have been recorded in all climatic regions and in a wide range of altitudes in the country (Tena, 2002; and Paulos *et al*., 2002). Similar problems were observed in the Central Rift valley and occurred in the majority of small holder farmers. Soil health represents critical measures of the degree of sustainability of farming practices, which is a relative judgment that is made according to expectations of soil fitness for agriculture. Hence, farming practices that improve soil organic carbon are essential to enhance soil health and promote sustainable development (Wang et al. 2018). Farm management practices such fertilizers, mulching, manure, and pesticides can affect soil health and influence sustainable development.

Compost application increase water content at both field capacity and permanent wilting point, increase shoot and root growth under stressed condition and also can decreases the effects of salinity by increase soil water availability and nutrient uptake by plants (Nguye *et al.*, 2013). Mulching has demonstrated efficacy to enhance soil health by reducing evaporation, increasing moisture retention, regulating temperature, enhancing nutrient availability and root absorption, suppressing weeds, decreasing salinity, encouraging biological activity, and controlling crop pests and diseases.

Objectives

To determine the effects of Compost and Mulch Application on soil physicochemical Properties under Salt affected Soil of Dugda District in the Central Rift Valley of Ethiopia

MATERIALS AND METHODS

2.1 Description of the Experimental Site

The study area is 130 km to south of Addis Ababa and is located at 6^0 91' and 8^0 12' N and38^0 46'and 38^0 59' E and an elevation of 1641-1680 m.a.sl. Dugda is part of the CRVE (Central Rift Valley of Ethiopia) and the area has a semi-arid and arid climate (Mengistu, 2008). More broadly, the soil of the area is calcareous derived from mix of parent material including: basalt, ignimbrite, lava, genesis, volcanic ash, pumice, riverine and lacustrine alluvium parent materials (EGMOA, 1975). Generally, the texture of the soils range was from sandy loam to sandy clay loam. The average annual rainfall in the study area was about 677.84 mm with a maximum and minimum temperature of 27.8ºC and 14.4ºC, respectively National Meteorological Agency of Ethiopia, 2005.

2.2 Experimental Design and Procedures

Site selection and soil samples were randomly taken in September 2014 from five farmer of the selected kebele. Then, levels of soil salinity were identified from those analyzed soil and one that were shown high saline soil was used for experimental field. The compost was prepared at Genesis farm from the mixture of animal manure, poultry manure and crop residue under plastic shade. After Land preparation was done, the compost was applied on to the prepared plots and was incorporated in to the soil 20 days before transplanting. The well prepared rows in the plot were irrigated in the mid of the day of transplanting day and 100kg DAP/ha was applied before transplanting (Mengistu, 2008).Then wheat straw was applied to the transplanted field by hand per treatment on the same date of transplanting., 50kg Urea was side dressed at second hoeing and the left half was applied at last hoeing. The experiment was laid out in a factorial experiment arranged in Randomized Complete Block Design (RCBD).

2.3. Data Collection

2.2.1 Soil Sample

Soil samples were collected at random from Girisa kebele of five fields separately within the area at 0-30 cm depth before planting and after harvesting from each plot of the experiment field. These samples were then composited and two duplicate samples per collected soil samples were prepared for determination of soil chemicals and physical properties such as texture, organic matter, bulk density, organic carbon, (EC), pH, and amounts of phosphorus (P), nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and cation exchange capacity as showed below in Table 1.The soil samples were cleaned from root and other dusts, air dried thoroughly, mixed and ground to pass a 2 mm size sieve before laboratory analysis. Finally, the soil samples like PH, OM, %OC, %TN, AVP, EC (ds)/m were analyzed in Jimma University College of Agriculture and veterinary medicine soil laboratory. Cation CEC(c mol kg^{-1}), Mg, Na and Ca. were analyzed at Oromia water works design and supervision enterprise soil laboratory. For texture, hydrometer method (Gee & or, 2002); OM percentage by multiplying the organic

carbon (obtained by wet digestion method) with a factor of 1.724 as the procedures described by (Ryan *et al*., 2001). Organic carbon by Walkley and Black method (Nelson and Summers, 1996); EC by conductivity meter in 1: 5 soils: water ratio, pH by using pH meter; amounts of available phosphorus (P) by using Olsen procedure as described by (Olsen *et al*., 1954). Nitrogen (TNAV) by micro- Kjeldahl digestion procedure (Bremmer, 1996), Ca and Mg were analysed by AAS (Thomas, 1982), K and Na are determined by flame photometer and cation exchange capacity (CEC) was determined by using ammonium saturation method (Jackson, 1968).

Table1. Initial chemical and physical properties of soil and compost

Where Cmol = cent mole, Ph= power of hydrogen, %OC = percent of organic matter, %OM = percent of organic matter, %TN = percent of total nitrogen, Av.P. ppm = available phosphorus in parts per million, EC(ds) m = electrical conductivity in dessicemen, CEC = cation exchange capacity, Na = sodium, K = potassium, Mg = magnesium, Ca = calcium, % = percent, Trl class = textural class, Nd = not determined.

	Chemical properties							Cmol (+) Kg^{-1}				Physical properties of soil			
Tested item	Ph	% OC	% OM	% TN	Av. P ppm	EC (ds)/m	CEC	Na	K	Mg	Ca	% sand	% Clay	% Silt	Trl class
Soil	9.7	2.8	5.7	0.3	5.1	3.3	17.3	2.2	1.3	3.2	8.8	47	23	30	loam
Compost	7.8	25.6	44.3	2.2	18.2	1.8	43	2.1	6.3	9.6	16.6	Nd	Nd	Nd	Nd

2.2.2 Profitability calculation

Profitability analysis (Adeniyi, 2001) was used to access the net return of each of the treatment used. This was based on the formula:

Net Profit = TR – TC

Where; TR= Total Revenue

TC = Total Cost

Benefit-cost ratio was calculated as the ratio of crop total value to total cost of production.
Benefit-cost ratio= TR/TC
Whereas TR= Total Revenue (crop total value)
TC = Total Cost of production

2.2.3 Statistical Data Analysis

Data were subjected to analysis of variance (ANOVA) using the GLM procedure of data statistical software package (Genstat Version 13). Significance differences of treatments were delineated by LSD (Least Significance Difference) test of significance. Correlation analysis was determined using Pearson's simple correlation coefficients for some intended tomato parameters Laboratory seed germination was determined by SAS version 9.2 (1987)

RESULT AND DISCUSSION

3.1. Soil Analysis before sowing and after harvest

Pre-cropping soil analysis was indicated that, the soil was sodic saline, texturally loam, Low in organic carbon and phosphorus content. Medium in total nitrogen, calcium and cation exchange capacity. High in pH, organic matter, potassium and magnesium as showed in table1. The chemical properties of compost showed, high amounts of organic carbon, organic matter, total nitrogen, available phosphorus and other exchangeable cations like Mg, K and Ca those can boost agricultural productivity on the soil through gradual release of nutrients.
The soil result after experiment indicated significant ($P<0.01$) differences on Om%, CEC, Ec(ds)/m, Na, and Av.P ppm due the main effects of compost and mulch and also their interactions. On Mg, the main effect of compost and its interaction effect showed significant difference. But, Mg was not affected by the main effect of mulch (Table 2 and appendix Table 1). The maximum number for OM% and Av.P was observed from 10cm mulch thickness and 6 ton compost rate. The highest number was recorded for CEC and Mg, on 5cm mulch thickness and 6 ton compost rate and the lowest was from control plots. The lowest EC was recorded on 15 mulch thickness and 6 tone compost rates. And the lowest sodium concentration was found from a treatment combination of 15cm mulch and 4 tone compost and from 15 cm mulch and 6 ton compost but the highest concentration was observed from the control plots. This finding showed that, compost and mulch application reduces soil salinity effects and increase plant nutrient. Ouni *et al*. (2014) reported that reducing salinity reduces the monovalent Na^+ and this is particularly evident when the replace of the monovalent K^+ to the humate (salt) of the humic complex occurred.

The significant ($P<0.01$) differences were observed on OC%, Ph, TN% and Ca due to the main effects of compost. However, mulch as a main effect nor as the interaction with compost did not result to significant differences for any of the variables (Table 3 and appendix table1). Silt, sand, K and clay were not affected by any of the treatments nor their interactions. The highest percentage of total nitrogen, Organic carbon, and calcium but the lowest Ph values were recorded at 6 tone compost rate comparing with other treatments.

Generally, the chemical properties of the soil analysis that carried out after the harvest were increased in percent of organic matter, organic carbon, total nitrogen, available phosphorus, CEC (magnesium, calcium and potassium) contents and decreased in sodium concentration, Ph and electro conductivities (EC (ds)/m. The organic fertilizer application and as a consequence, the compost distribution decrease soil Na, EC and pH likely due to high supplies of Ca, Mg and K. This was in agreement with Lakhdar *et al*. (2009), who reported that Ca, Mg and K mineral elements keep the cation-exchange sites on soil particles; minimized adsorption of Na and enhanced Na leaching losses. Brady and Weil (2005) also found Cations such as Ca^{2+}, Mg^{2+} and K^+ are produced during decomposition. The effects of different levels of compost amendments combined with mulch showed positive effects on soil chemical properties. Similarly, Wanerley and Mitton, (2004) reported that, addition of organic manure to the soil enhanced microbial activity and increased their ability to conserve irrigation water and consequently increasing their fertility and productivity of the soil.

Table2. Effects of Compost and Mulch Interaction on the Soil Chemical Properties after Harvest

Treatment number	Compost rate and mulch thickness interaction	Parameters						
		% OM	Av.P.ppm	EC(ds)m	CEC	Na	mg	Textural class
1	0*0	4.13^{e}	8.07^{e}	2.20^{a}	17.83^{d}	2.64^{a}	2.75^{fg}	Loam
2	5 * 0	6.77^{d}	9.51^{de}	2.19^{a}	17.83^{d}	1.65^{b}	2.56^{g}	Loam
3	10 * 0	7.53$^{cd[}$	12.07^{bcde}	2.03^{a}	17.83^{d}	0.82^{cd}	3.01^{ef}	Loam
4	15 * 0	7.86^{bcd}	12.86^{bcd}	1.96^{ab}	19.94^{cd}	0.76^{cd}	3.11^{de}	Loam
5	0 * 2	7.86^{bcd}	11.68^{cde}	1.61^{c}	22.06^{ab}	1.11^{bc}	3.12^{de}	Loam
6	5 * 2	8.11^{bcd}	13.50^{bcd}	1.65^{bc}	19.94^{cd}	1.06^{c}	2.92^{ef}	Loam
7	10 * 2	8.19^{bc}	12.82^{bcd}	1.14^{d}	19.95^{cd}	0.91^{cd}	3.12^{de}	Loam
8	15 * 2	8.67^{abc}	10.09^{de}	1.10^{d}	22.07^{bc}	0.73^{cd}	3.40^{d}	Loam
9	0 * 4	8.18^{bc}	12.07^{bcde}	1.07^{de}	22.04^{bc}	0.12^{e}	4.31^{ab}	Loam
10	5 * 4	8.82^{abc}	13.57^{bcd}	1.10^{d}	24.1^{ab}	0.61^{cde}	4.32^{a}	Loam
11	10 * 4	8.18^{bc}	16.11^{b}	1.10^{d}	19.95^{cd}	0.71^{cd}	3.99^{bc}	Loam
12	15 * 4	8.87^{abc}	12.37^{bcd}	0.76^{ef}	24.23^{a}	0.37^{de}	3.77^{c}	Loam
13	0 * 6	8.95^{ab}	15.80^{bc}	1.62^{c}	22.07^{abc}	0.92^{c}	4.32^{a}	Loam
14	5 * 6	8.99^{ab}	22.17^{a}	1.28^{d}	24.06^{ab}	0.61^{cde}	4.23^{ab}	Loam
15	10* 6	9.62^{a}	24.88^{a}	0.71^{f}	24.23^{a}	0.57^{cde}	4.22^{ab}	Loam
16	15 * 6	8.47^{abc}	11.21^{de}	0.60^{f}	24.12^{ab}	0.37^{de}	3.99^{bc}	Loam
SEM(±)	0.47	1.44	0.11	0.76	0.10	0.11	0.11	
LSD(0.05)	1.36	4.16	0.32	2.19	0.28	0.32	0.32	
CV	10.00	18.20	14.10	6.10	19.10	5.40	5.4 0	

CV= Coefficient of variance, LSD = least significance difference, PH = Hydrogen power, % OC = percentage of organic carbon, % OM = percentage of Organic matter, % TN = percentage of total nitrogen, AV.P = Available phosphorus, EC = Electrical conductivity, CEC = cation exchange capacity, Na = sodium, K = Potassium, mg = magnesium.

Table 3. The Main Effect of Compost and Mulch on the Soil Chemical and Physical Properties after harvest

Compost (kg)	PH	TN%	Oc%	Ca	K	% sand	% Silt	% Clay
0	9.56[a]	0.29[d]	4.13[b]	8.86[c]	1.31	50.00	39.00	18.00
2000	9.41[ab]	0.35[c]	4.32 [b]	10.48[b]	1.37	49.50	39.75	17.00
4000	9.21[bc]	0.43 [b]	4.81[a]	11.72[a]	1.39	50.50	39.50	17.75
6000	8.99[c]	0.49[a]	5.15[a]	12.37[a]	1.49	49.25	40.25	18.75
SEM(±)	0.10	0.02	0.23	0.37	0.07	0.98	0.65	0.61
LSD(0.05)	0.29	0.06	0.46	1.08	NS	NS	NS	NS
Mulch (cm)								
0	9.50	0.36	4.31	10.49	1.36	49.50	39.00	18.50
5	9.28 ab	0.37	4.46	10.95	1.38	50.50	39.50	18.00
10	9.17	0.44	4.90	10.95	1.41	50.00	40.25	18.50
15	9.23	0.39	4.75	11.04	1.42	49.25	39.75	16.50
SEM(±)	0.10	0.02	0.16	0.37	0.07	0.98	0.65	0.61
LSD(0.05)	NS	Ns	NS	NS	NS	NS	NS	NS
CV	3.70	19.00	12.00	11.90	18.30	6.80	5.70	11.80

Ca = calcium, % sand = percentage of sand, % silt =percentage of silt, % clay = percentage of clay, CV= SEM = standard error of mean CV=Coefficient of variance, LSD = least significance difference

3.2 Profitability Analysis of Mulch and Compost

The profitability analysis of this study showed positive relationship between fruit yield enhancement and viability in tomato production. Using Compost and mulch was some expensive. However, it is the most profitable and viable with reference to net profit and benefit-cost ratio. As indicated in (Table 4), the highest total cost of compost and mulch 76810 ETH Birr was recorded for the application of 6t/ha compost rate and 15cm mulch thickness and the cheapest cost of production 61933 ETH Birr was recorded on the control plot. The maximum compost rate and mulch thickness incurs the maximum cost of production which was the most expensive. However the greater profit from the marketable yield was from 6t/ha compost rate with 10cm mulch thickness. The minimum marketable yield from the control plot was the result of the minimum

input on this plot. The total revenue obtained was also directly proportional to the marketable yield; in that, the maximum 260610 ETH Birr was found when 6t/ha compost was sown with 10cm mulch thickness and the minimum 151690 ETH Birr was on the control plot. The net income and benefit cost ratio was also positive relation to the marketable yield and total revenue; in that the maximum and minimum net income was 184832 and 89756.78 ETH Birr respectively and the maximum and minimum benefit cost ratio were 3.44 and 2.45 respectively. Ogbomo (2011) found the same result with the present finding.

Table 4. Effect of Compost and Mulch on the Economic benefit of tomato production.

Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	0* 0	5 * 0	10 *0	15*0	0 * 2	5 * 2	10 *2	15 * 2	0 * 4	5 * 4	10 *4	15 *4	0 * 6	5 * 6	10* 6	15* 6
Land preparation	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300
Seed	2331	2331	2331	2331	2331	2331	2331	2331	2331	2331	2331	2331	2331	2331	2331	2331
DAP and UREA	2230	2230	2230	2230	2230	2230	2230	2230	2230	2230	2230	2230	2230	2230	2230	2230
Mulch and compost	0	1232.1	2364.3	3396.4	7253.5	8485.6	9617.8	10649.9	8977	10209	11341	12373	11481	12713	13845	14877
Seedling and transplanting	2350	2350	2350	2350	2350	2350	2350	2350	2350	2350	2350	2350	2350	2350	2350	2350
Field management	12150	12150	12150	12150	12150	12150	12150	12150	12150	12150	12150	12150	12150	12150	12150	12150
Stalk	17488	17488.5	17488.5	17488.5	17488.5	17488.5	17488.5	17488.5	17488.5	17489	17488.5	17488.5	17488.5	17489	17489	17488.5
Stalking	1600	1600	1600	1600	1600	1600	1600	1600	1600	1600	1600	1600	1600	1600	1600	1600
Chemicals	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4	7080.4
Fuel	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8	7575.8
Harvesting	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5	5827.5
Total Cost	61933	63165	64297.5	65330	69187	70418.9	71551	72583.1	70910	72142	73275	74307	73414	74646	75778	76810
Marketable yield	21670	25160	26430	30290	29500	28870	27890	31620	27320	31400	33030	31650	30460	30980	37230	26970
Total Revenue	151690	176120	185010	212030	206500	202090	195230	221340	191240	219800	231210	221550	213220	216860	260610	188790
Netfarm income	89757	112955	120713	146700	137313	131671.2	123679	148757	120330	147658	157936	147243	139806	142214	184832	111980
Benefit: Cost Ratio	2.45	2.79	2.88	3.25	2.98	2.87	2.73	3.03	2.70	3.05	3.16	2.98	2.90	2.91	3.44	2.46

CONCLUSSION AND RECOMMONDATION

Organic fertilizer needs to be rationally used in order to avoid a negative ecological impact and undesirable effects on the sustainability of agricultural production system. Irrigation helps to diversify production and answers against risk for subsistence farmers and intensifies and diversifies production in time and space dimensions in this area. However continuous using of this irrigation affects the soil of the area for salinity. Mulch and compost applications are the best technology used to decrease the effects of salinity on vegetable crops and increase soil organic matter, organic carbon and essential nutrients in the soil for the area where the majority of the income of the farmers depends on irrigation. The experiment contains four level of compost rate (0, 2, 4, and 6t/ha) and four levels of mulch thickness (0, 5, 10, 15cm) in randomized complete block design with three replications. enerally practicing organic fertilizers are used to maximize fruit yield and is more appropriate in terms of soil fertility improvement mainly in the off-season where irrigation is used to secure family food and cash security and salinity effects hinders the production; because, the present result indicates the yield obtained from the fertilized tomato are greater than the yield obtained from the control plot as some authors reported. Further investigation on the selection of best compost method and types as well as its rate for decreasing soil salinity and vegetable production should be studied.

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AGRICULTURAL TRAINING CHALLENGES FACED BY THE SMALLHOLDER FARMERS IN THE ZULULAND DISTRICT, KWAZULU NATAL IN SOUTH AFRICA

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ABSTRACT

Agricultural training plays a strategic role in improving the competitiveness and the productivity of the agricultural sector. Hence, smallholder farmers need training to be able to obtain good agricultural production and produce good agricultural products in order to satisfy the market and household food security. In addition, an assessment was conducted in the Zululand District by the Agricultural Research Council (ARC) in collaboration with the Department of Agriculture, Land Reform and Rural Development (DALRRD) to address some of the smallholder farmers training needs. This study aimed at identifying the agricultural training challenges faced by smallholder farmers in the Zululand district, Kwazulu Natal in South Africa. The following objectives were followed: To identify the socio economic characteristics of the smallholder farmers and To identify and describe the training challenges faced by the smallholder farmers. A representative sample consisting of 50 smallholder farmers with 418 beneficiaries was taken and visited in the following seven local municipalities: Mahlabathini (14%), Abaqulusi (16%), Dumbe (20%), Pongola (32%) and Nongoma (18%). Quantitative and qualitative design were used for a detailed questionnaire written in English. Stakeholder's discussion and field observations were also part of the data collected. A purposive sampling technique was used to select fifty (50) smallholder farmers, in order to cover uniformity and homogenous characteristics such as infrastructure requirements, skills availability, production challenges, agricultural training needs, water source needs, educational level and others. Data was coded, captured and analyzed with the software Statistical Packaged for Social Sciences (SPSS version 24). Descriptive and Univariate analysis were conducted. The results indicated that 76% of the smallholder farmers received various training before ARC intervention. The smallholder farmers further indicated that the training received previously was not enough as it occurred for few days (1 – 5 days) with no after intervention/training monitoring and limited practical demonstrations, hence the following agricultural training challenges were identified: Soil Preparation, Seed Sowing, Pests and Diseases, Marketing, Harvesting, Transplanting and Post-Harvest Storage. Furthermore, the Univariate analysis showed a high level of positive association among Pest and Diseases (Dependent variable) and the following Independent variables: Water source, Crops planted, Land size, Education and Farming experience. The model fit was predicted by the r^2 at 0.937 (94%) and is always between 0 and 100%. In general, the higher the r^2, the better the model fits the data and the better interaction between dependent and independent variables. It is thus concluded and recommended that the transfer of agricultural knowledge to support smallholder farmers should be a priority for the government especially the seven training challenges identified by smallholder farmers. Smallholder farmers should therefore, be trained in line with the seven training challenges identified by the study.

Keywords: Smallholder Farmers, Agricultural Training, Zululand District, Kwazulu Natal Province and South Africa.

INTRODUCTION

According to FAO (2012), the well-being and sustainability of smallholder farmers features as priority from an international, continental, regional, as well as the national level. Sustainable agriculture can be described as "development that meets the needs of the present without compromising the ability of the future generations in meeting their needs. In addition, agricultural training plays a strategic role in improving the competitiveness and the productivity of the agricultural sector. Hence, smallholder farmers need training to be able to obtain good agricultural production and produce good agricultural products in order to satisfy the market and household food security. In sub-Saharan Africa and Asia, 80% of the food supply comes from small family farms, but most of these farms are producing only a fraction of their potential (FAO, 2012).

According to Opportunity International (2022) smallholder farmers trainings benefits includes:

- Increase their yields. Learning how to properly utilize inputs can drastically improve a farmer's productivity throughout the season.

- Build their skills in farm management. Through training, farmers learn how to better manage both the business and agricultural aspects of their farms.

- Earn more income. With significant yield increases, farmers can earn more at harvest and repay their loans in full without straining their household's cash flow.

- Improve the local economy. As their farms grow, they also create more demand for products such as fertilizer, pesticides, and irrigation systems, improving the entire agricultural economy.

According to Moloi (2021) a R57.2 million support programme is enabling smallholder farmers in KwaZulu-Natal (KZN) to buy the tractors, implements, fertiliser, seeds, provision of training and seedlings needed to make their farms commercially successful. "The introduction of the Farmer Support Programme today, is a clear statement that we are determined and stand ready to provide a dedicated and efficient support package to all the agricultural value chains that are key drivers to the province's sustainable economic growth and transformation," (Moloi, 2021).

In Zululand district there are small areas with extremely high agricultural potential and there have been opportunities initiated by different governmental departments and NGO's aimed at assisting smallholder farmers produce (Kruger, 2006). This has resulted in the increase in agricultural production but in order to sustain the development of the sector, smallholder farmers, beneficiaries of land reform programs, irrigation schemes and community gardens need to be provided with training and direct access to markets (Kruger, 2006). Hence, according to Maponya et al. (2016) improving the productivity and success of smallholder farmers is imperative to end extreme poverty and to improve food security.

Furthermore, an assessment was conducted in the Zululand District by the Agricultural Research Council (ARC) in collaboration with the Department of Agriculture, Land Reform and Rural Development (DALRRD) to address some of the smallholder farmers training needs. This study aimed at identifying the agricultural training challenges faced by smallholder farmers in the Zululand district, Kwazulu Natal in South Africa. The following objectives were

followed: To identify the socio economic characteristics of the smallholder farmers and To identify and describe the training challenges faced by the smallholder farmers.

METHODOLOGY

2.1Study Area

The research focused on the Zululand District as indicated in Figure 1. A total of (50) smallholder farmers with 418 beneficiaries participated in the study and were from the following local municipalities: Mahlabathini (14%), Abaqulusi (16%), Dumbe (20%), Pongola (32%) and Nongoma (18%).

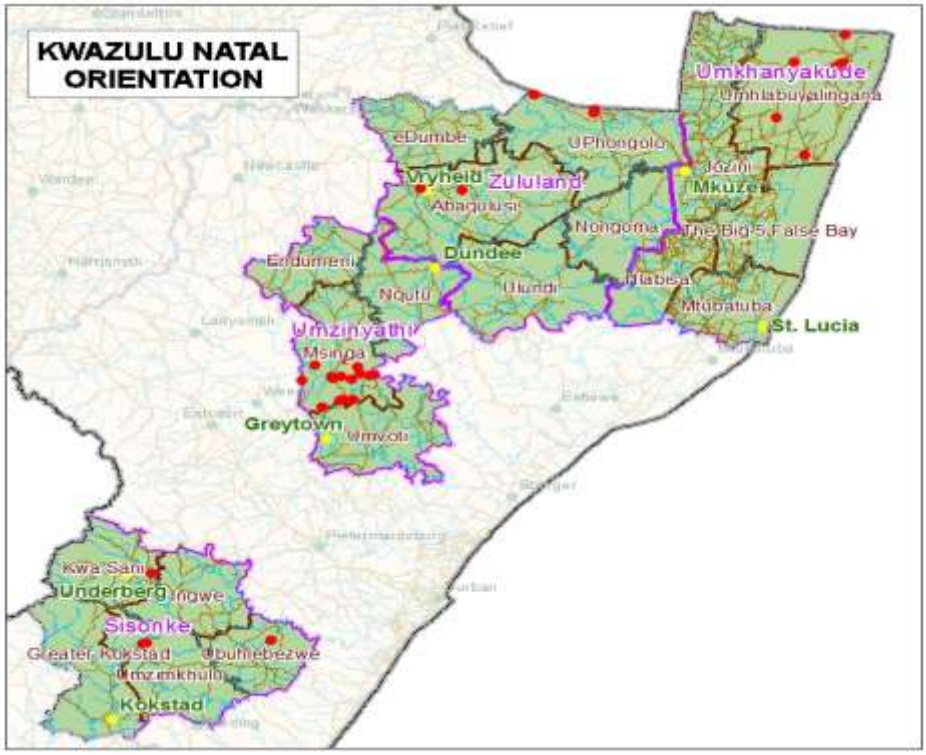

Figure 1: Study Area Map (Own Source)

2.2Study Design

The research employed both qualitative and quantitative methods concurrently and this was applied with the aim on establishing the limitations, balance and strength of the data. Furthermore, the methods included participatory action research as the smallholder farmers, community growers and stakeholders benefitted while the research was ongoing. Data collection methods were via interviews, site observations, focus groups, past researches, web and governmental reports. Pre and post-intervention questionnaires were developed and pilot tested with researchers working on community development within the Agricultural Research Council (ARC, 2014).

A desk-top audit on natural resources was also conducted for the development of crop suitability maps. The activity assisted in determining the biophysical viability of smallholder farmers and community growers in the Zululand district. The audit results gave provision of an overview of the districts in terms of orientation, water availability, selected climate and soil attributes as well as crop suitability.

2.3Sampling Procedure and Analytical Technique

A purposive sampling technique was used on selected 50 smallholder farmers in the Zululand district. The list of smallholder farmers were supplied by the Department of Agriculture, Land Reform and Rural Development (DALRRD) and the sample size was agreed with the stakeholder. A rule of thumb was applied, which is the minimum selection of 10% of the population and it is considered as a good sample size. Furthermore, data collected was analysed quantitatively using the Statistical Package for Social Sciences (SPSS) windows version 24. Descriptive and univariate analyses were applied.

2.4Econometrics Model

Descriptive Analysis was used to describe data and Univariate Regression Analysis was conducted to demonstrate the relationship and association of variables. Univariate regression analyses is used to test the association of one explanatory variable at a time with the outcome without worrying about other variables or confounders (unconditional association). This is essential in order to shortlist variables for multivariable analysis, especially if there are a large number of explanatory variables. It also excluded the variables from further analysis that do not show any significant association with the outcome. Results of univariate logistic regression analyses included Wald, likelihood ratio, chi-square test statistics and P-values, parameter estimates and standard errors, and odds ratios and their confidence limits. For logistic regression, values of parameter estimates are not very intuitive as they are calculated on a log scale. Therefore, odds ratios are examined, which are calculated after exponentiation parameter estimates. An odds ratio of <1 indicated negative association, whereas values >1 indicated positive association of the tested variable with the outcome. The following econometric model was used to determine association of variables (Greene, 1993):

Wi = _ + _Xi + _i (1)
Wi is the dependent variable value for person i (2)
Xi is the independent variable value for person i (3)
_ and _ are parameter values (4)
_i is the random error term (5)
The parameter _ is called the intercept or the value of W when X = 0 (6)
The parameter _ is called the slope or the change in W when X increases by one (7)

RESULTS AND DISCUSSION

3.1. Smallholder Farmers Socio-Economic Characteristics

The majority of smallholder farmers interviewed were female (60%) as compared to 40% male. In terms of educational attainment (Table 1), 26% had incomplete primary education, 18% had completed primary education, 2% had incomplete secondary education, 36% of

smallholder farmers had completed secondary education, 4% had incomplete tertiary education and 14% had completed tertiary education. According to (Maponya et al. 2016) training and education plays an important role in smallholder farmer development and failure to address some of the training needs has led to constrained agricultural growth in some districts in South Africa (Maponya et al. 2014 and Maponya et al. 2015). The results further indicated that 76% of the smallholder farmers received various training before Agricultural Research Council (ARC) intervention. The smallholder farmers further indicated that the training received previously was not enough as it occurred for few days (1 – 5 days) with no after intervention/training monitoring and limited practical demonstrations, hence the seven agricultural training challenges were identified. All smallholder farmers were farming fulltime. Results on land acquisition (Table 1) indicated that the majority of smallholder farmers got land through Permission to Occupy (PTO) (86%) while others received land through the following: Leased (4%), Inheritance (4%) , Own Finance (6%) and Land Redistribution for Agricultural Development (LRAD) (4%).

The age distribution of the smallholder farmers indicated that the majority were in the age group of 46 - 60 (34%) %). As indicated in Table 1, youth involvement is very low (12 %), 36 – 45 (22%) while 60>had 32%. A large percentage (56%) of smallholder farmers utilize 1-5 hectares (Table 1) while 18% utilize (6 – 10 hectares). A number of smallholder farmers (14%) utilize >50ha as indicated in table 1. Furthermore, majority of the smallholder farmers (26%) had 1- 5 & 21 – 49 years farming experience. Quite a number of smallholder farmers (24%) had 6 – 10 years farming experience. Only 8% of smallholder farmers had 50>farming experience while 16% had 11 – 20 years farming experience.

Table 1: Distribution of smallholder farmers according to their socio-economic characteristics.

Variables	Smallholder Farmers	% Smallholder Farmers Socio-Economic Characteristics
Gender		
Female	30	60
Male	20	40
Total	50	100
Age Categories		
18 – 35	6	12
36 – 45	11	22
46 – 60	17	34
>60	16	32
Total	50	100
Level of Education		
Primary Education Completed	9	18
Primary Education Incomplete	13	26
Secondary Education Incomplete	1	2
Secondary Education Completed	18	36
Tertiary Education Incomplete	2	4
Tertiary Education Completed	7	14
Total	50	100
Employment Status		
Full Time Farmer	50	100
Total	50	100
Land Size		

1 - 5	28	56
6 - 10	9	18
11 - 20	4	8
21 - 49	4	8
>50	7	14
Total	50	100
Land Acquisition		
Land Redistribution for Agricultural Development (LRAD)	2	4
Own Finance	3	6
Permission to Occupy (PTO)	41	86
Inheritance	2	4
Lease	2	4
Total	50	100
Farming Experience		
1 - 5	13	26
6 - 10	12	24
11 - 20	8	16
21 - 49	13	26
>50	4	8
Total	50	100
Training Provided		
Yes	38	76
No	12	24
Total	50	100

The results from Table 2 – 8 should be interpreted as follows: 1st – 3rd training challenges = Most challenging; 4th – 5th training challenges = Moderate challenging and 6th – 7th training challenges = Least challenges.

The results in Table 2 showed that 42 percent of smallholder farmers recognised soil preparation as the 1st training challenge and 20 percent of smallholder farmers recognised soil preparation as the 2nd training challenge. Table 2 also indicated that 10 percent, 12 percent and 4 percent of smallholder farmers indicated soil preparation as a 3^{rd} , 4^{th} and 5^{th} training challenges respectively. Only 10 percent and 2 percent of smallholder farmers recognised soil preparation as the 6th and 7th training challenges respectively. It is very clear that attention should be given to soil preparation as indicated by the 1^{st} , 2^{nd}, 3^{rd} and 4^{th} training challenges respectively.

Table 2. Soil Preparation as a Training Challenge

Training Challenges	Number of Smallholder Farmers	Percentages
1st Training Challenge	21	42
2nd Training Challenge	10	20
3rd Training Challenge	5	10
4th Training Challenge	6	12
5th Training Challenge	2	4
6th Training Challenge	5	10
7th Training Challenge	1	2
Total	**50**	**100**

The results in Table 3 showed that 4 percent of smallholder farmer's recognised seed sowing as the 1st training challenge and 2 percent of smallholder farmer's recognised seed sowing as the 2nd training challenge. Table 3 showed that 6 percent, 10 percent, 28 percent and 48 percent of smallholder farmers indicated seed sowing as the 3rd , 4th , 5th and 6th training challenges respectively. Only 2 percent of smallholder farmer's recognised seed sowing as the 7th training challenge. It is very clear that moderate attention should be given to seed sowing as indicated by the 4th , 5th and 6th training challenges.

Table 3. Seed Sowing as a Training Challenge

Training Challenges	Number of Smallholder Farmers	Percentages
1st Training Challenge	2	4
2nd Training Challenge	1	2
3rd Training Challenge	3	6
4th Training Challenge	5	10
5th Training Challenge	14	28
6th Training Challenge	24	48
7th Training Challenge	1	2
Total	**50**	**100**

The results showed that 6 percent of smallholder farmers recognised transplanting as the 1st training challenge, 2 percent of smallholder farmers recognised transplanting as the 2nd training challenge and 8 percent of smallholder farmers recognised transplanting as the 3rd training challenge. Table 4 showed that 12 percent, 18 percent and 48 percent of smallholder farmers indicated transplanting as a 4th, 5th and 6th training challenge respectively. Only 6 percent of smallholder farmers recognised transplanting as the 7th training challenge.

The results showed that 30 percent of smallholder farmers recognised pest and diseases as the 1st training challenge and 6 percent of smallholder farmer's recognised pest and diseases as the 2nd training challenge. Table 5 showed that 20 percent, 24 percent and 2 percent of smallholder farmers indicated pest and diseases as the 3rd, 4th , 5th and 6th training challenges respectively. Only 8 percent of smallholder farmers recognised pests and diseases as 7th training challenge. Almost 80 percent of smallholder farmers emphasised pests and diseases as the most training challenge as indicated by 1st , 2nd , 3rd and 4th training challenges respectively. This is

a worrying situation and a clear indication that the management of pests, diseases and weeds is still very challenging to many smallholder farmers in Zululand District. Hence, the study also run univariate analysis to determine factors contributing to pest and diseases as the training challenge among smallholder farmers (See Table 9).

Table 4. Transplanting as a Training Challenge

Training Challenges	Number of Smallholder Farmers	Percentages
1st Training Challenge	3	6
2nd Training Challenge	1	2
3rd Training Challenge	4	8
4th Training Challenge	6	12
5th Training Challenge	9	18
6th Training Challenge	24	48
7th Training Challenge	3	6
Total	**50**	**100**

Table 5. Pest and Diseases as a Training Challenge

Training Challenges	Number of Smallholder Farmers	Percentages
1st Training Challenge	15	30
2nd Training Challenge	3	6
3rd Training Challenge	10	20
4th Training Challenge	12	24
5th Training Challenge	5	10
6th Training Challenge	1	2
7th Training Challenge	4	8
Total	**50**	**100**

The results showed that 6 percent of smallholder farmers recognised harvesting as the 1st training challenge while 6 percent of smallholder farmers recognised harvesting as the 3rd training challenge. Table 6 showed that 14 percent, 26 percent and 40 percent of smallholder farmers indicated harvesting as the 4th, 5th and 6th training challenge respectively. Only 8 percent of smallholder farmers recognised harvesting as the 7th training challenge.

Table 6. Harvesting as a Training Challenge

Training Challenges	Number of Smallholder Farmers	Percentages
1st Training Challenge	3	6
3rd Training Challenge	3	6
4th Training Challenge	7	14
5th Training Challenge	13	26
6th Training Challenge	20	40
7th Training Challenge	4	8
Total	**50**	**100**

The results showed that 8 percent of smallholder farmers recognised post – harvest handling as the 1st training challenge, 30 percent of smallholder farmers recognised post – harvest handling as the 2nd training challenge and 22 percent of smallholder farmer's recognised post-harvest handling as the 3rd training challenge. Table 7 showed that 16 percent, 14 percent and 8 percent of smallholder farmers indicated post-harvest handling as the 4th, 5th and 6th training challenges respectively. Only 2 percent of smallholder farmers recognised transplanting as the 7th training challenge. Post-harvest handling and storage is one of the key constraints faced by majority of the smallholder farmers across South Africa (Maponya et al. 2014) and it is thus critical that smallholder farmers in the Zululand District be bettered with postharvest handling skills and knowledge as indicated in the 1st to 5th training challenges (90% of smallholder farmers).

Table 7. Post-Harvest Handling/Storage as a Training Challenge

Training Challenges	Number of Smallholder Farmers	Percentages
1st Training Challenge	4	8
2nd Training Challenge	15	30
3rd Training Challenge	11	22
4th Training Challenge	8	16
5th Training Challenge	7	14
6th Training Challenge	4	8
7th Training Challenge	1	2
Total	**50**	**100**

The results showed that 36 percent of smallholder farmers recognised marketing as the 1st training challenge, 26 percent of smallholder farmer's recognised marketing as the 2nd training challenge and 4percent of smallholder farmers recognised marketing as the 3rd training challenge. Table 8 showed that 14 percent, 8percent and 10 percent of farmers indicated harvesting as the 4th, 5th and 6th training challenges respectively. Only 2 percent of smallholder farmers recognised harvesting as a 7th training challenge. It is very clear that attention should be given to marketing as indicated by the 1st , 2nd, 3rd and 4th training challenges (78 percent of smallholder farmers).

Table 8. Marketing as a training challenge

Training Challenges	Number of Smallholder Farmers	Percentages
1st Training Challenge	18	36
2nd Training Challenge	13	26
3rd Training Challenge	2	4
4th Training Challenge	7	14
5th Training Challenge	4	8
6th Training Challenge	5	10
7th Training Challenge	1	2
Total	**50**	**100**

3.2 Smallholder Farmers Univariate Analysis

The odds of a farmer's land size, water source, crops planted, education, and farming experience were all more than one, as shown in Table 9. As a training task, this clearly suggested a favourable link with pests and diseases. The discovery of a positive relationship between water source, crops grown, and land area was not surprising, given pests and diseases are primarily transferred by water sources, particularly dirty water. Furthermore, this pest and disease problem can damage any crop grown on any area of land. This necessitated the use of a better educated and experienced smallholder farmer to combat pests and diseases on their crops. Many smallholder farmers, according to ARC (2014), are unaware of the life cycles of various pests, illnesses, and weeds, making it impossible to distinct qualities. As a result, they are unable to take appropriate preventative and control measures. Smallholder farmers must be effectively taught and monitored to use economical and effective pest and disease management strategies to avoid large crop losses (Maponya et al. 2016).

Table 9. Univariate Analysis among Factors Contributing to Pest and Diseases as a Training Challenge in the Zululand District

Variables	**OR and 95% CI**
Water Source	1.299[0.41–21.1]1
Crops Planted	1.29[9.3–70.9]1
Land Size	1.11[0.24-0.77]1
Education	1.55[0.23 – 4.99]1
Farming Experience	1.98[1.41 – 15.8]1

(N = 50); OR = Odds Ratio; 95%CI = 95% Confidence Intervals; 1< = No Association; 1> = Association.

4. Selected Photos of the smallholder farmers Trainings

Photo 1: Smallholder Farmers Training in the Zululand District, Kwazulu Natal. This After Intervention Training was conducted by the Agricultural Research Council – Vegetables, Industrial and Medicinal Plants Team to address the identified seven smallholder farmers training challenges.

Photo 2: Smallholder Farmers Practical Demonstrations Training in the Zululand District, Kwazulu Natal. This After Intervention Practical Demonstrations Training was conducted by the Agricultural Research Council– Vegetables, Industrial and Medicinal Plants (VIMP) Team to address the identified seven smallholder farmers training challenges as well as the smallholder farmers concern on limited practical demonstrations.

CONCLUSION

Smallholder farmers in the Zululand District confronted seven training problems, according to the study: Soil preparation, Seed sowing, Harvesting, Transplanting, Pests and Diseases, Post-harvest handling, and Marketing. As a result, it is concluded and advised that the government prioritize the transfer of agricultural knowledge to support smallholder farmers, particularly the seven training difficulties mentioned by smallholder farmers. Furthermore, smallholder farmers should be trained in accordance with the study's seven training difficulties and should have access to research and training organizations, such as the Agricultural Research Council (ARC, 2014), to increase their skills and knowledge in order to achieve these goals. Higher yields, increased revenue, increased knowledge and ability, food security, and resistance to a changing environment will all be achieved.

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POMEGRANATE (*Punica granatum* L) PEEL BIOACTIVE COMPOUNDS AS NATURAL POULTRY FEED ADDITIVES

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ABSTRACT

This review aimed to discuss the ability of pomegranate peel bioactive compounds as poultry feed additives. Plant bioactive compound extracts are commonly used as feed additives in the poultry industry due to the prohibition of antibiotics' application as growth promoters. Punica granatum, which belongs to the Punicaceae family, has long been regarded as a fruit with numerous health benefits. Turkey is the world's fourth-largest producer of pomegranates, with around 600,000 tons in 2020. The antimicrobial and antioxidant activity of pomegranate peel extract (PPE) is related to its phytochemical compounds including catechin, epicatechin, gallocatechin, gallic, ellagic, caffeic, ellagitannins, and gallotannins. PPE significantly increased broiler live weight, body weight gain, and egg production of laying hens and quail. Dietary PPE reduced coliform population in the quail caecum, total aerobic bacteria in the broiler intestine, and malondialdehyde (MDA) level in serum and stored broiler breast meat. It concluded that PPE can be applied as a feed additive, due to its bioactive compounds which are proven to act as antimicrobials and antioxidants that can improve poultry performance. Based on the literature, PPE can be used at levels of 0.01% to 0.05% in poultry feed. However, it is necessary to consider its use at high levels since the largest bioactive compound in PPE is polyphenol tannins which in high doses can bind to protein in the intestinal tract which causes a decrease in protein digestibility.

Keywords*:* Antimicrobial, antioxidant, extract, feed additives, pomegranate peel, poultry

INTRODUCTION

The application of antibiotics as a growth promoter (AGP) in poultry production has been banned for safety reasons. The prohibition of AGP is estimated to cause significant economic losses since poultry are more susceptible to disease, decrease productivity and increase mortality rates as well as increase the cost of disease treatment (Hamady et al., 2015). It is critical to find alternative antibiotics that are effective while not causing resistance or residue in the product. The use of plant extracts in the poultry diet has been reported to be successful and beneficial to poultry. The phytobiotic compounds derived from plant extracts are proven to be efficacious as a source of natural antimicrobials and antioxidants.

Pomegranate peel (*Punica granatum*), which is a common waste product in the juicing process, is one of the most valuable medicinal herbs. The pomegranate peel's biological activities have been evaluated including antioxidant and antibacterial properties (Akarca and Başpınar, 2019; Benchagra et al., 2021). Polyphenols of pomegranate peel are reported to be higher than that of arils and seeds including catechin, epicatechin, gallocatechin, gallic, ellagic, caffeic, ellagitannins, and gallotannins (Kandylis and Kokkinomagoulos, 2020). Pomegranate peel extract (PPE) supplementation improved the live weight, body weight gain, feed efficiency, total antioxidant blood capacity, and egg production (Sharifian et al., 2019; Kishawy et al., 2016; Eid et al., 2021). PPE administration improved villus length and villi length/village depth ratio and lactic acid bacteria (Abbas et al., 2017; Perricone et al., 2020). According to the mentioned points, this review aimed to discuss the ability of pomegranate peel bioactive compounds as poultry feed additives**.**

The production of pomegranate and its fruit parts

Punica granatum is a *Punicaceae* family that has long been regarded as a fruit with numerous health benefits (Sharma et al., 2014). Pomegranate is a subtropical fruit that can grow up to 1800 meters above sea level and adapt to a variety of climatic conditions. During the period of fruit development and ripening, pomegranate trees require a dry climate with an optimum temperature of 38 ^{0}C. Pomegranate can grow well under drought conditions with a sweet taste despite low fruit production, and it sheds its leaves during the winter months in areas with low temperatures (Kumar and Singh, 2021). The production of pomegranate is estimated to be about 8.1 million tonnes in the world of which Turkey is the fourth-largest producer after India, China, and Iran (Pienaar, 2021). Pomegranate production in Turkey has increased with production reaching 600 thousand tons in 2020 (TUIK, 2022). The most important varieties in Tuki are Hicaznar and Canernar with a characteristic red skin, and dark red arils whereas Canernar has a sweeter taste with a smaller size than Hicaznar. Turkey's pomegranate-producing regions include the provinces of Antalya and Mersin, followed by Mugla, Denizli Adana, Gaziantep, Sanhurfa, and Adiyaman (Ozkaya, 2021).

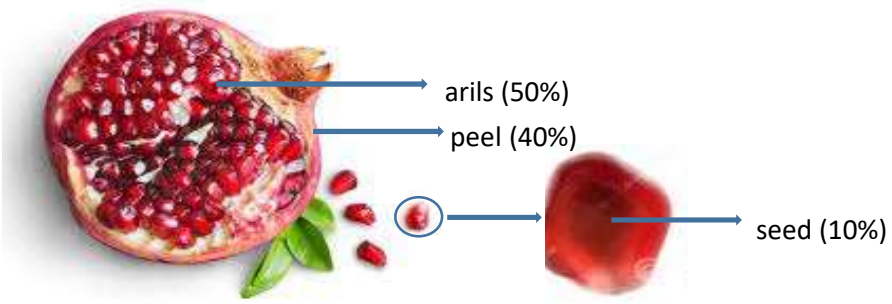

Figure 1. Pomegranates composition

Figure 1 shows the composition of the weight percent pomegranate. The details show that pomegranate consists of about 50% (arils) which is commonly used as a pomegranate juice. Pomegranate peel and seeds are waste consisting of 40% and 10%, respectively. Pomegranate seeds are used as a source of oil with a percentage of about 7.6 to 20% depending on the cultivar, climate, fruit ripeness, and storage (Sreekumar et al., 2015). This oil is high in polyunsaturated fatty acids (linoleic acids, linolenic acids), stearic acid, palmitic acid, and oleic acid (Viuda-Martos et al.2010). Pomegranate peel has the most promising polyphenols compounds compared to other parts. It has been reported that pomegranate peel contains high flavonoids, including flavonols, phenolic acids, and anthocyanins (Akhtar et al.2015).

Pomegranate Peel Bioactive Compounds as Poultry Feed Additives

Several studies reported that pomegranate peel extract has higher antioxidant activity than seed and aril extracts and butylated hydroxyanisole (Hamady et al., 2015; Benchagra et al., 2021). In addition, pomegranate extract also has antibacterial ability against gram-positive and gram-negative bacteria such as *Salmonella, E. coli,* and *Staphylococcus aureus* (Akarca and Başpınar 2019; Hamady et al., 2015). These abilities are due to the high polyphenol content in pomegranate peel which is reported to be higher than that of arils and seeds including phenolic acid, flavonoid, and hydrolyzable tannin (Figure 2) (Kandylis and Kokkinomagoulos, 2020). The phenolic content in pomegranate peel extracted by ultrasound-assisted extraction method is 177.54 mg GAE/g (More and Arya, 2021), and by enzyme supercritical fluid extraction method is 301.53 mg GAE/g (Musthaq et al. 2015). Recent results reported by Kuprik et al. (2022) by conventional methods (maceration and Soxhlet) yields 24.41 to 40.55 mg GAE/g of phenolics. This result showed that the yield of phenolic is affected by the extracted method which modern method improves extraction efficiency due to its ability in protecting the seconder metabolites against thermal degradation and oxidation (Latiff al,2021).

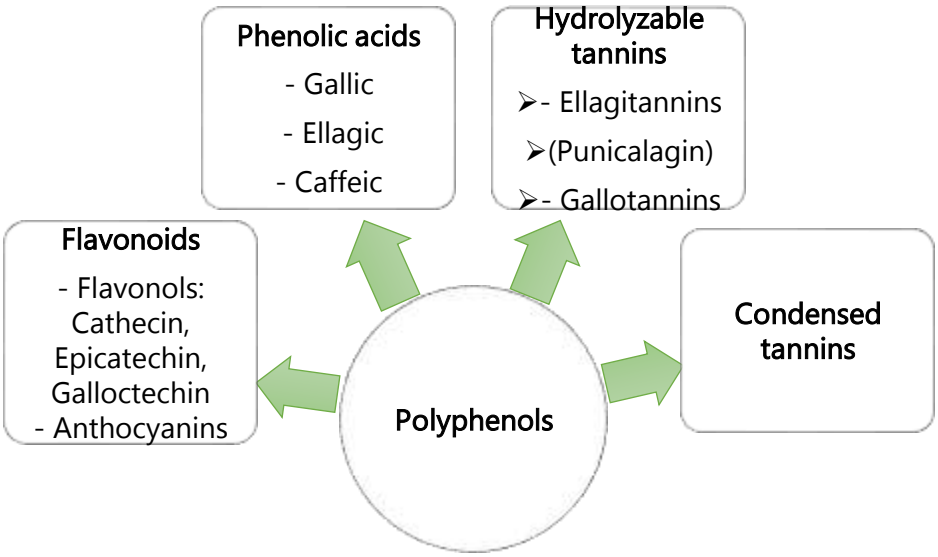

Figure 2. Main polyphenols of pomegranate peel

PPE and powder form have been used as poultry feed additives in numerous studies. For instance, dietary up to 650 mg/kg of PPE improved the body weight gain and plasma lipid profile of heat-stressed broiler chickens as well as preserved meat quality during refrigerated

storage (Sharifian et al., 2019). The addition of 0.05 % PPE to the broiler diet increased live weight, body weight gain, and feed efficiency (Kishawy et al., 2016). PPE administration at 2ml/l drinking water increased anti-radical activity in the blood and improved the ecology of intestinal microbial of broiler chickens by enhancing the lactic acid bacteria (Perricone et al., 2020). Lactobacilli have been shown to protect against pathogenic bacteria colonization through lumen acidification and bacteriocin production (Messaoudi et al., 2012) thus it supports poultry production performance and health.

In quail, pomegranate peel powder substitution at 7.5 % as a substitute for yellow corn resulted in the greatest villus length and villi length/village depth ratio, which might increase nutrient absorption, as expressed in improved egg production, egg weight, and egg mass (Abbas et al, 2017). Meanwhile, in laying hens, dietary pomegranate peel powder of up to 4% in the laying hen diet reduced malondialdehyde but improved the antioxidative enzymes, total antioxidant blood capacity, and egg production (Eid et al., 2021). Furthermore, PPE is reported to be able to inhibit the activity of pancreatic lipase which inhibits fat absorption in the intestine and is excreted through feces (Kishawy et al., 2016). This statement is supported by the findings of Kumar et al. (2018) that a diet of 100 mg/kg of PPE recorded the lowest serum cholesterol levels. According to Rao et al. (2019), the antioxidant effect of PPE was demonstrated by a reduction in lipid peroxidation and an increase in the activity of glutathione peroxidase in broiler chickens. The possible mechanism of action is that polyphenols are bound by blood cells after being absorbed in the intestines, especially erythrocytes, which leads to an increase in the total antioxidant capacity of the blood (Ginsburg et al., 2011). Despite the efficacy of pomegranate phenolic compounds being proven, it needs to be considered at high doses since polyphenols are also able to bind to feed proteins and endogenous proteins such as digestive enzymes in the luminal of the intestinal tract which leads to a decrease in the digestibility of protein in the diet. The interaction of polyphenolic hydroxyl groups with protein carbonyl groups results in a reduction of protein and amino acid digestibility (Pascariu et al., 2017). The positive impact of PPE application with optimal dosage on the poultry diet is presented in Table 1.

Table 1. The positive impact of PPE application on poultry diet

Optimal Dosage	Result	References
0.01%	Showing great antimicrobial activity against *B. cereus* and *S. aureus* in chicken meat product	Kanatt et al. (2010)
0.01%	Yielding in greater productive performance, body weight, and carcass weight percentage	Hamady et al. (2015)
0.02%	Increasing antioxidant activity and quality index of broiler breast meat	Saleh et al. (2016)
0.04%	Reducing oxidative stress, the total population of coliform bacteria, and improving egg quality in heat-stressed quail	Mutlu and Güler. (2021)
0.05%	Improving live weight, body weight gain, and feed efficiency	Kishawy et al. (2016)
0.05%	Decreasing total cholesterol and low-density lipoprotein of broiler serum and improving high-density lipoprotein	Yaseen et al. (2014)

In general, the antioxidant mechanism of polyphenols is by activating antioxidant enzymes, inhibiting pro-oxidant enzymes (xanthine oxidase), eliminating reactive oxygen species (ROS) through electron transfer, and increasing the activity of other antioxidant elements such as vitamin E and vitamin C (Procházková et al., 2011). Meanwhile, the antimicrobial mechanisms of polyphenols include: 1) precipitating cell membrane proteins causing a decrease in their fluidity and bacterial cell lysis, 2) inactivating cell proteins by reacting with protein sulfhydryl groups causing enzyme and growth inhibition, 3) reducing microbial oxygen consumption resulting in limited NADH oxidation, and 4) disruption of microbial metabolism through the interaction of hydroxyl groups (-OH) on the bacterial cell membrane and interacting with the active site of the enzyme ((Han et al., 2007; Pereira et al., 2006; Akhtar et al., 2015).

CONCLUSION

PPE can be applied as a feed additive, due to its bioactive compounds which are proven to act as antimicrobials and antioxidants that can improve poultry performance. Based on the literature, PPE can be used at levels of 0.01% to 0.05% in poultry feed. However, it is necessary to consider its use at high levels since the largest bioactive compound in PPE is polyphenol tannins which in high doses can bind to feed protein and endogenous proteins such as digestive enzymes in the intestinal tract which causes a decrease in protein digestibility

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OVERCOMING HEAT STRESS IN POULTRY THROUGH A NUTRIGENOMIC APPROACH

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ABSTRACT

The most problem of poultry farming in tropical countries is heat stress condition that has a negative impact on poultry health and productivity. High ambient temperatures not only result in inferior egg and meat quality but also cause heavy economic losses. This review aimed to discuss the mitigation of poultry heat stress through a nutrigenomic approach, especially on its effect on the heat shock protein 70 gene (HSP 70) expression. Nutrigenomic is the comprehensive study of how nutrition affects gene expression. Reducing the negative effect of heat stress can be modified by the nutrients approach such as proteins (amino acid), energy density, prebiotics, probiotics, vitamins, minerals, and phytogenics. The most studied gene as an indicator of the rapid response of poultry to heat stress is HSP 70. Under heat stress, chickens naturally attempt to maintain thermal homeostasis, which causes an increase in reactive oxygen species (ROS) in some organs including the heart, liver, and brain as well as induces HSP 70 to protect cells from oxidative damage. The proper nutrient application results in a decrease in HSP 70 gene expression indicating a decrease in the heat stress effect. It concluded that the nutritional approach can be used to overcome heat stress in poultry. Furthermore, the nutrigenomic approach can assist in the implementation of a more precise poultry feeding strategy.

Keywords: Heat stress, chicken, heat shock protein, nutrigenomic

INTRODUCTION

The most problem of poultry farming in tropical countries is heat stress conditions which have a negative impact on poultry health and productivity. Heat stress contributes to disease susceptibility and reduces nutrient absorption due to impaired intestinal mucosal integrity. It also harms skeletal muscle cells and lowers the system of immune, resulting in poor growth and low product quality, and huge economic losses (Roushdy et al., 2018). In heat stress conditions, chickens attempt to adapt by improving the flow of peripheral blood to reduce body heat, decreasing feed consumption to reduce metabolic heat, and increasing panting as an effort to cool evaporation. Heat stress triggers the production of excessive amounts of ROS, which damage the phospholipids in cell membranes to varying degrees depending on the intensity and length of heat exposure (Murugesan et al., 2017). Furthermore, long-term exposure to acute heat stress can cause serum corticosterone and heat shock protein (HSP) expression, particularly

in the liver, heart, and kidneys, to prevent cells against oxidative damage (Tamzil et al., 2013). HSP performs crucial functions in protein translocation as well as in the unfolding and refolding of damaged proteins. It plays a role in both extracellular and intracellular immune functions. The families of HSP, which are based on various molecular weight classes, include HSP47, HSP60, HSP70 HSP90, and HSP110. However, HSP 70 is the most widely studied as a heat stress biomarker (Al-Zhgoul et al., 2013). Overcoming heat stress can be conducted by a nutrigenomic approach by providing certain nutrients such as protein (amino acids), prebiotics, probiotics, vitamins, minerals, and phytogenics.

Nutrigenomics is the comprehensive study of how nutrition affects gene expression. Nutrients are not only needed for metabolic functions but also play a role in information or signaling systems in cells. This process includes changes in gene expression (transcription, translation), RNA and messenger protein stability, and activity of the protein. Specificity is a nutritional control principle of gene expression in which each receptor must be capable of binding to a nutrient signaling molecule and initiating adaptive change (Reen *et al.,* 2015). For instance, the administration of antioxidant vitamins and minerals can increase glutathione peroxidase and superoxide dismutase enzymes activity and reduce malondialdehyde (MDA) and the expression of the HSP70 gene in several broiler organs under stress conditions (Jang *et al.,* 2014), indicating a reduced negative effect of heat stress. Positive results were also obtained with the strategy of regulating dietary energy density and protein (amino acids), which can reduce serum corticosterone and HSP70 gene expression (Raghebian *et al.,* 2016). Likewise, the inclusion of phytogenics can increase intestinal mucin 2 gene expression further increasing the secretion of digestive enzymes to induce nutrient digestibility (Dong *et al.,* 2020). Based on the positive effect of nutrient modification in decreasing the adverse heat stress impact in poultry, this review aimed to discuss strategies for overcoming heat stress through a nutrigenomic approach, especially on its effect on HSP 70 gene expression.

Nutrigenomics

Nutrigenomics is the comprehensive study of how nutrition affects gene expression. Nutrigenomics applications connect multiple disciplines, including diet's effects on epigenomic changes, genome stability, RNA and protein expression, and metabolite changes. The nucleus can turn on and off the gene based on metabolic signals received from both internal (hormones) and external (nutrients) factors (Asmelash *et al.,* 2018). Nutrients are not only needed for metabolic functions but also play a role in information or signaling systems in cells. When a system transmits information, the signal must have a sensor or receiver to decode and convey the information. Furthermore, receptors, which are cellular proteins that receive and transmit information, convey this information to the cell nucleus via transduction mechanisms that are able to reprogram the cell to adapt to the changing environmental conditions. This can include changes in gene expression (transcription, translation), RNA and messenger protein stability, and protein activity. Specificity is a nutritional control principle of gene expression in which each receptor must be capable of binding to a nutrient signaling molecule and initiating adaptive change (Reen *et al*,2015). The gene expression is modified by numerous nutrients such as macronutrients (e.g. carbohydrates, proteins, fats), vitamins and minerals (e.g. Fe, Ca, Se, Zinc), and phytogenics compounds (Reddy *et al.,* 2018). For example, carbohydrate intake is closely related to glucose and insulin concentrations. Glucose is the main stimulus for insulin regulation and secretion in pancreatic cells which triggers the expression of genes encoding glucose transporters, lipogenic and glycolytic enzymes, and L-type pyruvate kinases such as acetyl-CoA carboxylase, L-type pyruvate kinase, and fatty acid synthetase, as well as the suppression of gluconeogenic pathway genes (Rui, 2014).

Nutrigenomics Applications in Poultry

Nutrigenomics in poultry helps in determining the relationship between nutrition and genetics. As a result, it is possible to match the feed formulation to the genotype of the animals to support improved productivity and health. Currently, DNA microarray and Polymerase Chain Reaction (PCR) techniques can be applied to evaluate the interaction between diet and genes by screening specific genes and providing a comprehensive picture of their expression. Nutrigenomics applications, particularly to combat the deleterious effects of heat stress, can be carried out through the provision or modification of specific nutrients such as energy density, protein (amino acids), prebiotics, probiotics, vitamins, minerals, and phytogenics. Heat stress in poultry has negative physiological and metabolic effects including reduced growth rate, feed intake, feed efficiency, immunity, and product quality (Figure 1). In addition, heat stress triggers excessive amounts of ROS production, which damage the phospholipids in cell membranes (Rajkumar *et al.,* 2018). Furthermore, acute heat stress conditions trigger serum corticosterone and HSP 70 gene expression to protect cells from oxidative injury (Tamzil *et al.*, 2013). The expression of HSP70 in chickens increased significantly after 2 hours of exposure to high temperatures, especially in the heart, liver, and kidneys (Murugesan *et al.*, 2017).

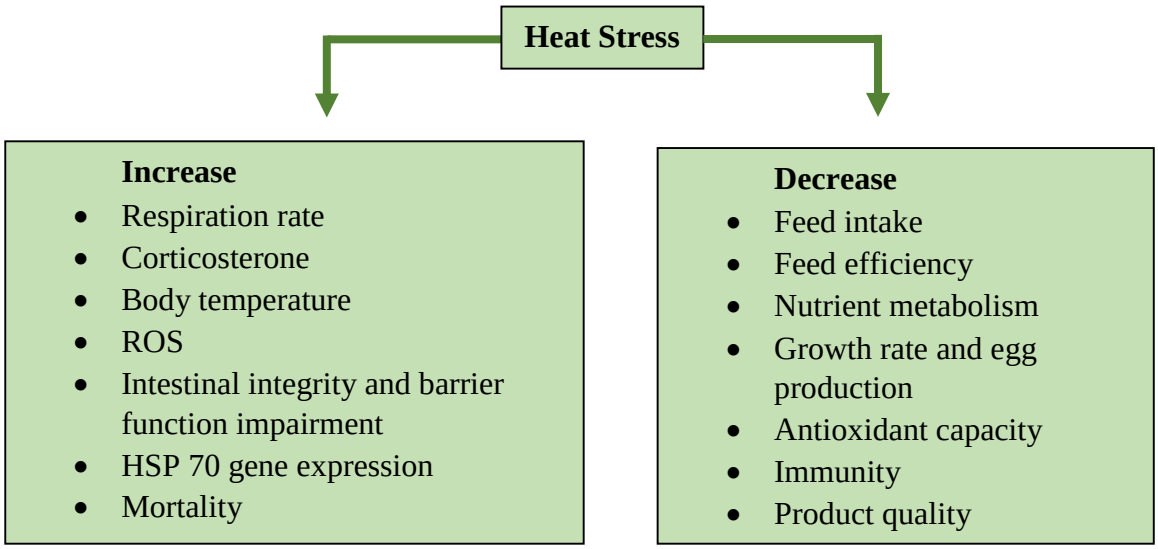

Figure 1. Effect of heat stress on the chicken

Energy and Protein

The use of energy sources from fat/oil improves chicken heat environment tolerance which sources and metabolizable energy density affect corticosterone hormone and hepatic HSP70 gene expression, and the broilers' performances during heat stress (Raghebian *et al.*, 2016). Another study by Gabriel *et al.* (2000) discovered that broilers exposed to heat stress had lower hepatic HSP70 gene expression due to lower energy density. The use of both lower energy density and high energy density with oil under high environment temperature lowered levels of corticosterone and HSP70 gene expression and thus improved broiler performance (Raghebian *et al.*, 2016). This demonstrates that increasing energy density by using oil can reduce the effects of heat stress through increasing energy efficiency due to its less heat increment than carbohydrates. Emadinia *et al*. (2020) discovered that an increase in threonine in the low crude protein diet resulted in increased jejunal mucin 2 (MUC2) gene expression, which was required for the maintenance of the intestinal mucosal layer. Elnesr *et al.* (2020) also

found that *in ovo* injection of sulfur amino acids improved the GSHPx gene and decreased expression of HSP 70 as an antioxidant activity indicator in serum and tissue (jejunum, muscle, liver heart) of broilers after hatching. Additionally, *in ovo* methionine injection increased antioxidant enzymes (SOD, GSH-px) and growth and metabolism-related genes (somatostatin, thyroid-stimulating hormone) of newly hatched broilers in the liver tissue (Elwan *et al.*, 2019).

Prebiotics and Probiotics

Prebiotics or probiotics have a positive effect on gut morphology, microbial profile, and broiler performance during heat stress. Mannan oligosaccharides (MOS) promote the beneficial organism's growth in the intestine and also serve as competitive attachment sites for pathogenic bacteria (Jahanian and Ashnagar, 2015). Supplementation of MOS into feed helps improve poultry performance, gut health, inhibition of pathogenic microflora, digestion and nutrition absorption, and immunity, through antioxidant activity under heat stress conditions. Rokade *et al.* (2018) found that the expression of HSP70 from jejunal tissue was decreased in MOS-treated birds compared to controls. Lowman *et al.* (2014) also found that the reduction in the expression level of HSP70 was more pronounced in the group of broilers exposed to heat stress given MOS supplementation. This is believed to be associated with an increase in the total beneficial gut microbes related to MOS, which have the potential to release bioactive substances that can prevent oxidative damage and ultimately decrease HSP70 expression (Sohail *et al.*, 2013). Additionally, probiotic (*Bacillus subtilis*) supplementation significantly increased glutathione peroxidase activity in broilers during heat stress, but it had no impact on the HSP70 gene (Cramer *et al.*, 2018). In contrast to that reported by Wang *et al.* (2018) that decreased HSP70 expression in the liver occurred in heat stress broilers fed *Bacillus subtilis* (1 × 106 CFU/g). The probiotic mechanism in reducing HSP70 protein levels is related to its ability to reduce stress reactions in poultry by decreasing the activity of the hypothalamic-pituitary-adrenal axis, one of which is indicated by decreased panting activity (Mohammed *et al.*, 2018)

1. Vitamins

Several studies have demonstrated that supplementing with antioxidant vitamins is proven to be effective in reducing the harmful heat stress effects. Chickens' bodies can produce ascorbic acid under normal circumstances. However, during heat stress, the need for ascorbic acid increases and requires the supplementation of vitamin C into feed or drinking water, meanwhile vitamin E is not synthesized by poultry, and therefore exogenous administration is necessary during heat stress (Ajakaiye *et al.*, 2010). In cell membranes, these vitamins act as antioxidants to stop the progression of lipid peroxidation. As a result, dietary vitamins C and E stabilize antioxidant status in chickens under heat stress, which inhibits the mRNA expression of proinflammatory cytokines and HSP70 (Jang et al., 2014). For instance, giving vitamin C to heat-stressed chickens resulted in a reduction in HSP70 expression in the heart (Piestun *et al.*, 2013). Also, dietary vitamin E increased total superoxide dismutase and glutathione peroxidase activity while the malondialdehyde and HSP70 expression decreased (Jang *et al.*, 2014). Sahin *et al.*, (2009) found that 500 mg of vitamins C and E each or combination supplementation increased egg production and feed consumption, and decreased serum corticosterone and Hsp70 in the brain and ovaries of heat stress quails. However, under thermoneutral conditions, supplementation of vitamin E and C had no impact on the expression of Hsp70 in the ovary or the brain.

2. Minerals

Trace mineral supplementation in poultry is beneficial in overcoming heat stress and improving poultry performance. Trace minerals play a crucial role in metabolic processes,

antioxidant and immune systems in poultry. A previous study reported that chromium (Cr) supplementation on the broiler diet affected the microRNA expression profile in muscle tissue and protein synthesis. Under heat stress conditions, broiler chickens fed a 1200 g *chromium picolinate* had lower levels of HSP70 expression than chickens fed a control diet (Ezzat *et al.*,2017). Cr is an essential mineral required for the metabolism of nucleic acids, lipids, and carbohydrates. Also, they confirmed that Cr works synergistically with antioxidant and total antioxidant capacity in the suppression of heat stress. The addition of 0.30 ppm organic selenium (Se) to broiler feed significantly reduced HSP70 gene expression in the pectoral muscle and brain as well as MDA value of chickens in the tropical environment compared to without selenium supplementation (Amizar *et al.,* 2017). The addition of zinc, Cr, and Se significantly reduced the expression of HSP70 gene in the body's organs (heart, spleen, muscle, liver) in broiler chickens under high temperatures (Rajkumar *et al.,* 2018). Se and zinc are able to facilitate the induction of endogenous antioxidant defense systems and improve antioxidant status. Especially Se is part of the glutathione peroxidase enzyme which functions to detoxify H_2O_2 and organic hydroperoxides into stable products (Surai, 2003), thereby decreasing HSP 70 expression in broiler chickens induced by heat stress.

3. Phytogenic Compounds

Phytogenic compounds are commonly used as natural feed additives as substitutes for antibiotics and can affect the expression of specific genes, which then enhances the performance of poultry. Its efficacy is related to its antioxidant activity in scavenging radicals, metal chelation, and synergism with other antioxidants (Hajati *et al.,* 2015). For instance, phytogenic substances can influence the intestinal mucosa's gene expression and encourage the release of digestive enzymes to increase nutrient digestibility (Liu *et al.,* 2014). They found that including resveratrol in broiler feed during heat stress reduced intestinal mucosal damage by altering gene expression, including HSP protein expression. Dietary 400 ppm of quercetin increased mucin 2 (MUC2) gene expression, encouraged the growth of *Lactobacillus* in the cecum, and stabilized the redox balance after oxidative conditions (Dong *et al.,* 2020). Grape seed extract at 300 mg/kg supplementation reduced the negative effects of heat stress on blood metabolites and suppressed HSP70 gene expression (Hajati *et al.,* 2015). Cinnamon, thyme, and turmeric supplementation enhanced MUC2 expression in the small intestine, which improved intestinal digestion and immunity in the broiler (Sangani *et al.,* 2014). Also, thymol is essential for improving the function of the intestinal barrier and decreasing the expression of cytokine genes under inflammation (Omonijo *et al.,* 2018).

CONCLUSION

The nutritional approach can be used to overcome heat stress in poultry. The inclusion of antioxidant vitamins and minerals, prebiotics, probiotics, phytogenics as well as modification of protein (amino acids), energy source, and energy density of feed can increase antioxidant enzyme activity and reduce the expression of the HSP70 gene in broiler exposed heat stress, indicating a reduced adverse effect of heat stress. Furthermore, the nutrigenomic approach can assist in the implementation of a more precise poultry feeding strategy.

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ANTIBIOTICS IN HONEY- ANTIMICROBIAL RESISTANCE RISK

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ABSTRACT

The use of antibiotics is among the most frequently cited factors in pollinator population decline, and their use poses a risk to bees and consumers. Many research shows that residues of antibiotics in honey originate from improper beekeeping practices than the environment. In this context, this paper aims to assess the beekeeping practices regarding antibiotics, specifically the knowledge of the risk posed by antibiotics. A structured online questionnaire was developed with beekeepers having bee-stabilized parks distributed in a wide geoFigureical area in Albania. The results showed that many beekeepers (56.6%) use antibiotics for bee treatment. The majority of beekeepers (69.7%) are not aware of drug-resistant infections that may come from the consumption of honey obtained from hives treated with antibiotics. The results also showed that 37.4% of beekeepers use antibiotics without following the labelled instructions. About 40% of beekeepers obtained information on the Internet or other uncontrolled sources regarding the source of information on antibiotics use. These findings suggest a lack of knowledge on antibiotics among the beekeepers in Albania. Also, the potential antimicrobial resistance risk among consumers. This is an important food safety issue that needs to be tackled by Albania's food safety and quality policy.

Keywords: Honey, antibiotics, antimicrobial resistance, Albania, food safety

INTRODUCTION

Honey is one of the beekeeping products that is produced in an environment polluted by different sources of contamination where antibiotic residues in it have recently become a major consumer concern. The aim of this study was to evaluate the level of knowledge and awareness that Albanian beekeepers have on antibiotics use inside the hives because consumption of honey without source and safety information can adversely impact the public health. Sources of honey contamination can be divided into environmental (indirect pollution) and apicultural (direct pollution) contaminations. Indirect way of contamination (environmental contamination) reflects the transport of toxic substances to the hive during the collection of nectar, water,

pollen, propolis or the theft of honey from other contaminated hives. Antibiotic residues originating from agricultural use should be carefully monitored as they can be allergenic and carcinogenic factors, and may contribute to bacterial resistance (Wassenaa TM. 2005; Baggio A et al. 2004 [1,2]). The direct way of contamination (apicultural contamination) which is the most important, is related to the contamination of bee products from the use of antibiotics and pesticides from beekeeping practice. The residues of antibiotics in honey originate mostly from improper beekeeping practices.

The term antibiotic initially refers to any agent with biological activity against living organisms. Antibiotics are medicines - therapeutically used to protect the health and welfare of humans and animals, it inhibits or abolishes the growth of microorganisms such as bacteria, fungi or protozoa (Johnson S., Jadon N. 2010[3]). Antibiotics are worldwide used in apicultural practices for the control of honeybee diseases, particularly American and European foulbrood (Hansen H., Brødsgaard C.J. 1999[4]) and nosemosis, a parasitic disease affecting adult bees. Obviously, beekeepers use antibiotics at relatively high doses to treat infections, or at low doses as "growth promoters". Maximum residue limits (MRLs) have been established for most foods produced by animals treated with sulfonamides and tetracyclines. However, there are no MRLs for bee products such as honey (Noori Al-Waili., Khelod SalomAntibiotic. 2012[5]). The use of antibiotics in beekeeping is illegal in some EU countries. However, there are no MRLs established for antibiotics in honey according to European Community regulations, which means that honey containing antibiotics residues are not permitted to be sold (Forsgren E. 2010., Mutinelli F. 2003[6,9]). The extensive use of antibiotics leads to an accumulation of antibiotic residues in honey, thereby leading to decreased quality and difficulty in marketing (Tillotson GS et al. 2006[7]).

The use of antibiotics are among the most frequently cited attributors to pollinator population decline. In Albania, many chemicals aimed at controlling colony parasites or pests have been shown to have negative effects on the health of honey bees (Kuliçi M et al. 2022[11]). But antibiotics used in animal foods poses a risk not only for bees but also for consumers because honey with antibiotic residues can produce resistance in the bacterial population which can evolve into more powerful organisms in the consumers (Noori Al-Waili., Khelod SalomAntibiotic. 2012[5]) and can affect public health. These bacteria might then cause difficult-to-treat human infections. Many cases of infant botulisms have been attributed to contaminated honey (Noori Al-Waili., Khelod SalomAntibiotic. 2012[5]). Some drugs have the potential to produce toxic reactions in consumers directly while some others are able to produce allergic or hypersensitivity reactions (Velicer C. M et al. 2004[10]). The latter requires an extremely long treatment. According to European Union regulations, honey as a natural product must be free of chemicals (Directive. 74/409/eec. 1974[8]). Currently in Albania, there are no exact data about the amount of antibiotic residue in produced honey, so we suggest further studies to monitor the presence of antibiotics in honey.

MATERIALS AND METHODS

The research was conducted from January to February 2022. Household surveys, field observations and quantitative structured survey (Christopher Robinette et. al. 2021[13]) collected through the beekeeping associations in Albania were used as a main tool for data collection.

One hundred fifty beekeepers with large bee-stabilized parks (most of which are registered) have participated in this study. The majority of the respondents were males and well distributed in all considered group ages. The questionnaire is composed of two sections: in the first one, demoFigureic characteristics of the beekeepers are collected. The second section collects data about the knowledge of the risk posed by antibiotics.

RESULTS AND DISCUSSION

The benefits of using an online survey were evident in the number of responses received (150 beekeeper responses). The results show (table 1) that the average beekeeper's age included in this study is 38.5 years of age and the majority of the respondents are males with experience 1-5 years.This result is evaluative and shows that despite the different professions and maybe not enough knowledge in the field of beekeeping, people are also dealing with beekeeping. These beekeepers (36.3%) keep honeybees as a hobby. Most honey produced by hobbyists is consumed at home, given to friends and relatives, or distributed through local outlets. On the other side 30.1% of beekeepers included in this study operate beekeeping primarily for profit and production efficiency and some of them are specialized in the production of queens and other bee productions. These full-time beekeepers can be divided into two groups: migratory and non-migratory beekeepers. Most full-time beekeepers relocate their bee colonies several times during the year to provide pollination services, to reach more sources of nectar, or to escape from adverse climatic conditions or pesticides from agricultural use. The non-migratory beekeepers move their colonies over significant distances but most of the time their colonies are normally left in the same location.

Related to the use of antibiotics the results showed that high deegre of beekeepers (56.6%) use antibiotics for bee treatment. This is a worrying result as beekeepers have not yet understood the consequences of using these antibiotics on bees and this may have been one of the main reasons for the large losses that beekeepers have had in Albania during the winter of last year (Kuliçi M et al. 2022[11]). These effects of antibiotics on bees have been studied by various researchers. According to Yarira Ortiz-Alvarado (2020) (Yarira Ortiz-Alvarado et al. 2020[12]) antibiotic treatments have an effect on the amount of lipids and rate of behavioral development of honey bees. Also, the timing of antibiotic treatment had distinct effects for the age of onset of behaviors, starting with cleaning, then nursing and lastly foraging. However this high rate of antibiotic use may come from a lack of information on the part of beekeepers or from the fact that antibiotics are relatively cheap and easy to take for use in various treatments. Most of these respondents (43.4%) indicated that they either did not use antibiotics in their beekeeping or that it was illegal to use them. After analyzing the obtained results, we identified that for question how much knowledge do you have about the use of antibiotics in bees? (using the *Likert scale = 1 No knowledge 5 =Very good knowledge)*, 14.1% of beekeepers indicated "no knowledge", 25.3% indicated "little knowledge", 27.8% indicated "enough knowledge", 12.6% indicated "moderately knowledgeable" and 20.2% indicated "very good knowledge".

Table 1. Socio-demoFigureic variables of the sample and population.

	Category	**Albania %**
Age	18-24 years old	6.2
Average= 38.5	25-34 years old	26.7
	35-44 years old	17.1
	45-54 years old	30.1
	More than 55 years old	19.9
Gender	Male	89
	Female	11
Experience	0-5 years	36.3
	6-10 years	16.4
	11-20year	17.1
	Over 20 years	30.1
Number of hives	1-10	16.4
	11-20	17.1
	21-50	30.1
	Over 50	36.3

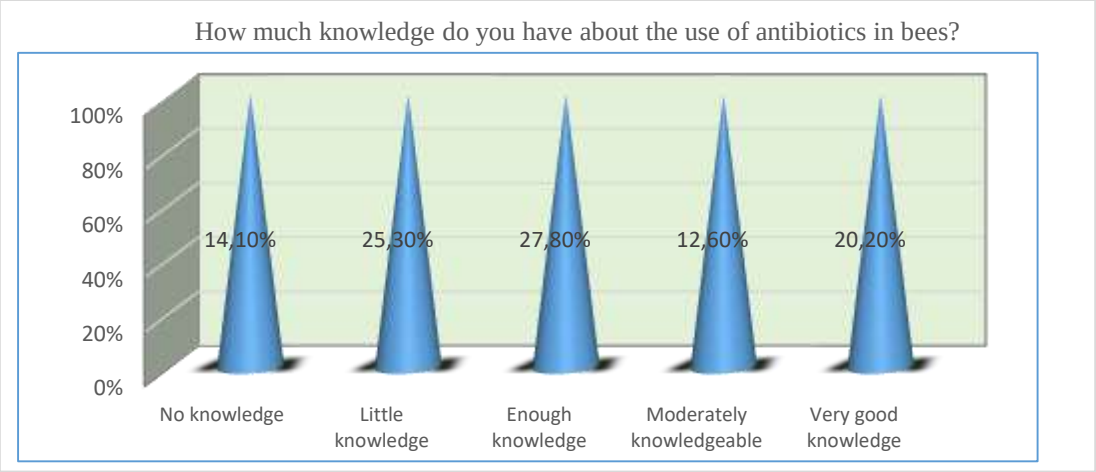

Figure 1: Likert scale: How much knowledge do you have about the use of antibiotics in bees?

As seems 39.4% of beekeepers have very poor knowledge about antibiotics and their use. Such results pose a threat to the hive because antibiotics may have been misused for the appropriate disease, or used altogether against the wrong disease, or may not have been used at the right time and in the right dosage and this may lead to the onset of new infections or may increase frequency of treatment failures. Such results also pose a threat to consumer food safety. Long-term effects of exposure to antibiotic residues consumed with honey include microbiological hazards, carcinogenicity, reproductive effects, and teratogenicity (Noori Al-Waili., Khelod SalomAntibiotic. 2012[5]). Microbiological effects are one of the major health problems in human beings. Certain drugs like nitrofurans and nitroimidazoles can cause cancer in human being, similarly, some drugs can produce reproductive and teratogenic effects at very low doses (Noori Al-Waili., Khelod SalomAntibiotic. 2012[5]). The use of antibiotics due to the treatment of bee diseases dominates (30.8%) in the results obtained for the question if they used antibiotics, for what reason did they use them? They use them mostly in order to control European foulbrood (EFB), American foulbrood (AFB) and Nosemosis. 20.7% of beekeepers who participated in this study said that they used antibiotics to prevent diseases and 4.5% said they used them to control bacterial infections.

About 40% of beekeepers obtained information on the Internet or other uncontrolled sources regarding the source of information on antibiotics use. 48.9% of beekeepers obtained information from veterinary pharmacy and supervising veterinarian and 11.1% from other beekeepers and beekeeping associations. Also by asking the beekeepers: How often do you think beekeepers use antibiotics without following the labeled instructions, from 1 to 5, Albanian beekeepers reported that 37.4% of them use antibiotics without following the labelled instructions. It is therefore a critical issue that beekeepers aren't properly informed about the risks incurred by antibiotic issues and they have lack of knowledge about antibiotics. That is not much to say that one source of information is less accurate than another, as many veterinarians lack knowledge and expertise with regards to the beekeeping industry (Christopher Robinette et. al. 2021[13]). However, we suggest getting information by trained professionals and also suggest training of beekeepers for antimicrobial interventions.

As we mention above, antibiotic residues consumed along with food and honey can produce resistance in bacterial populations.The majority of beekeepers included in this study (69.7%) are not aware of drug-resistant infections that may come from the consumption of honey obtained from hives treated with antibiotics and 30.3% appeared more aware of drug-resistant infections. Antibiotic resistance is a global public health concern. In this way, if policymakers do not interfere with training and information policies for beekeepers, the number of bacteria resistant to antibiotics consumed with honey may increase, and many bacterial infections may become resistant to the most common antibiotic treatments. To the question: Do you know that honey / honeycombs obtained from antibiotic-treated hives should not be consumed? 81.8% 3 beekeepers answered that they were aware and 18.2% of them were not aware. As we see most beekeepers reported that recently treated hive products should not be consumed, but are not aware that antibiotic waste in the hive takes time to eliminate, this was evident from the results of the above questions.

CONCLUSIONS

These findings suggest a lack of knowledge on antibiotics among the beekeepers in Albania and a potential antimicrobial resistance risk among bees and consumers. This is an important food safety issue that needs to be tackled by Albania's food safety and quality policy. We suggest extent of this study on identifying antibiotics residue among different honeys or the extent of this study on the health impact of honey contaminated with antibiotics. Also we suggest policymakers apply training politics to beekeepers in order to ensure in-depth awareness and understanding of antibiotic applications.

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COMPARATIVE STUDY OF HONEY PRODUCTION PRACTICES AND HONEY QUALITY IN DIFFERENT AREAS IN TIRANA DISTRICT

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ABSTRACT

Honey is the natural product made by one of our planet's most important species *Apis mellifera*. Nowadays, the quality of honey is the most determining issue and some of the reasons why honey samples differ in quality are processing (honey production practice) and storage conditions. This study was focused on comparison of processing conditions of honeys produced in beekeeping households and Honey Production Industry. In addition, a qualitative evaluation has been made for ten honey samples produced with these two technical practices to determine their quality. Closely observations in beekeeping households spread in 20 different locations during different periods of honey harvest are performed. For the household surveys, field observations and semi-structured interviews were used as the primary tool for data collection. At the same time, sensorial and physicochemical analyses for honey samples were performed in the University honey laboratory. Five honey samples were randomly collected, directly at five different bees stabled colonies in some areas of Tirana (Albania): (1-Dajt, 2-Tufin, 3-Babrru, 4-Sauk, 5-Vaqarr), and the other five industrial honey were randomly collected in the main markets of Tirana, selecting samples from producers and packers in the country as well as imported samples. Attributes analysed included different monofloral and polyfloral honey. All physicochemical parameters analysed were: soluble dry matter, invert sugar, humidity, free acidity, pH, electrical conductivity and HMF (5-hidroksimetilfurfural). Physiochemical properties were determined using the Harmonised Methods of the International Honey Commission. Results show that market honey samples, produced with advanced equipment and techniques, differed significantly for some of the analysed parameters (humidity, acidity, pH, HMF). Industrial honeys were less rich in minerals and may have been heat treated as in some of them the HMF values do not comply with the permitted European Standard. This study supports consumer preferences for honey produced under artisanal conditions, the parameters of which turned out to be very satisfactory. There is a need for practical oriented training on honey harvest for the beekeepers also, there is a need to enhance equipment and services in traditional beekeeping and credit provision needs to be facilitated to supply accessory equipment.

Keywords: honey, honey production practices, honey quality

INTRODUCTION

According to the definition of the Traditional Honey Standard named by the FAO/OMS Food Codex in 1989 "Honey is a food product produced by honey bees getting started from flower nectar or secretions derived from living parts of plants or insects found in to". Honey is a source of food on the table because of the energy values it has, the content of sugar, minerals, proteins, enzymes, flavonoids and active phenols. The composition and the flavor of honey depends on a huge number of variables, the most important of which is the type of plant that provided the source nectar, climatic condition during productions, processing and storage condition, beekeeping practices in removing and extracting honey (White, J.W . 1978; Nour, M. 1988; Bogdanov, S. 2004[1,2,3]). Knowing honey characteristics allows the packaging and storage of honey in appropriate conditions so as to preserve their qualities (Clement, H et al. 2002[4]). There are three types of honey production: wild honey collection, small honey production and industrial honey production. The latter is the most criticized beekeeping type and therefore, the aim for conducting this study was to evaluate the comparison of parameters of honey samples produced with different technical practices.

MATERIALS AND METHODS

2.1 Methodology

This study has two components: the first part was a survey which was conducted to assess the honey production practices in the study area. Household surveys, field observations and semi-structured interviews were used as a main tool for data collection. Honey extraction operation consists of several important stages such as: cleaning of frames from bees, uncapping, extraction, filtration, decantation, packaging and labeling. We have conducted comparisons between the processes in which the honey harvesting technology in the industry passes with the honey harvesting in the 20 different beekeeping households in Tirana district. The second part of this study consist of laboratory analysis which was aimed at determining the quality of different honeys. Ten samples taken in the analysis were subjected to chemical analysis which determined the physical, chemical characteristics and composition of honey of different types that were taken for study. These characteristics were then compared with those standards, which belong to a fresh and natural honey, defined according to the international honey commission.

2.2 Analyzed attributes

The attributes studied in this paper include: monofloral and polyfloral honeys extracted with different beekeeping practices. We have monofloral honey when bees have collected predominantly in one nectar source. Polyfloral honey is a honey collected from bees that have migrated to various pastures. It has several botanical sources, none of which are predominant.

2.3 Collection of Samples

Ten traditional and market honey samples were collected from different beekeeping households and markets in Tirana district. Five honey samples (three monofloral and two polifloral honeys) were collected directly at five different bee stabled colonies: (1-Dajt, 2-Tufin, 3-Babrru, 4-Sauk, 5-Vaqarr) and other five samples were collected in local markets (three monofloral and two polifloral honeys-same with traditional parks) spread in different areas of Tirana. The honey samples were kept in clean bottles and stored in a dark cupboard at room

temperature. The samples were taken to the laboratory for investigation. The analyses were performed in the honey laboratory of our faculty.

2.4 Physico-Chemical Analyses

The chemical analyzes performed for this study are as follows: Moisture content: Refractometer is the most common way to accurately determine the water content of honey. The instrument that can be thermostated at 20^0C, regularly calibrated with distilled water (International Honey Commission. 2009[5]). Directly after homogenisation, cover the surface of the prism evenly with the sample. After 2 minutes (Abbe refractometer) read the refractive index. Measure each honey twice and take the average value (International Honey Commission. 2009[5]). Determination of total solids: The percentage total solid of each sample was determined using the following formula: Total solids (%) =100 – Moisture content.

Determination of pH: The pH was measured using a digital pH meter. We calibrate the pH meter to pH 3.0, 7.0 and 9.0. Dissolve 10 g sample in 75 ml of carbon dioxide-free water in a 250 ml beaker. Stir with the magnetic stirrer, immerse the pH electrodes in the solution and record the pH (International Honey Commission. 2009., D.W. Lord. 1988[5,7]). Free acidity: Ten grams of honey were weighed in a glass beaker and then 75ml of deionized water were added. This solution was titrated with NaOH 0.1 mol. L-1 until reaching pH 8.5 using 1-2 drops of phenolphthalein until pink color appears at pH 8.3. Free acidity, express as milliequivalents acid/kg honey= ml of 0.1M NaOH x 10 (Weight of honey taken in the analysis) (International Honey Commission. 2009[5]).

Determination of electrical conductivity: Conductivity meter, is used to determine electrical conductivity. The electrical conductivity of a solution of 20 g dry matter of honey in 100 ml distilled water is measured using an electrical conductivity cell. The determination of the electrical conductivity is based on the measurement of the electrical resistance, of which the electrical conductivity is the reciprocal (International Honey Commission. 2009., D.W. Lord. 1988[5,7]).

HMF (Hydroxymethylfurfural): Determination of hydroxymethylfurfural is done according White (International Honey Commission. 2009., J.W. White. 1979; V. Figueiredo. 1991[5,8,9]). The determination of the hydroxymethylfurfural (HMF) content is based on the determination of UV absorbance of HMF at 284 nm. In order to avoid the interference of other components at this wavelength the difference between the absorbances of a clear aqueous honey solution and the same solution after addition of bisulphite is determined. The HMF content is calculated after subtraction of the background absorbance at 336 nm. Invert sugar-Determination of apparent reducing sugars: ‘Apparent reducing sugars’ are defined as those sugars which reduce a Fehling’s reagent under the conditions specified. ‘Apparent sucrose’ is defined as 0.95 of the difference in ‘apparent reducing sugars’ before and after the prescribed hydrolysis procedure (International Honey Commission. 2009[5])..

2.5. Data Management and Statistical Analysis

The survey data was entered to the computer using Microsoft Excel and (SPSS) program. Different categories of the samples were compared using t-test.

RESULTS AND THEIR DISCUSSION

3.1 Honey production practices

The comparison of honey extraction technology in amateur parks, with the extraction of honey in industrial ways, has been done for all the main stages of its extraction: The first process involves cleaning of frames (three-quarters of which must be sealed). The honey collection in amateur parks (beekeeping households) is done directly from the hives. It is done with the help of cleaning bee brushes for bee removals. Industries, on the other hand, provide a part of the honey from the bee parks they have and the other part from the purchase of honey from other beekeepers, the quality of which is always judged.

The second process has to do with uncapping. This involves removal of the wax caps from the honeycomb cells. Beekeeping households do this manually with uncapping knife or spoon. Large processors use uncapping machines with a fully automated process. Next the honey must be removed from the cells using an extractor. Beekeeping households do this with a manual extractor. Large processors use electric extractor. After that large processors (industries) often apply heat to 66°–77°C to decrease its viscosity prior to filtration. The heating process also increases the brown color, reduces the moisture content, delays crystallization, and destroys yeast cells, while beekeeping households don't apply this. Once the honey is extracted, it must be filtered. This process is done in order to remove wax, pollen, bee parts, some bacteria and any other foreign organic or inorganic substance. Beekeeping households perform filtration with sieves, as well as membrane filters are most commonly used. Industrial producers apply this form of macrofiltration with special double sieves but often they also perform microfiltration which also removes yeast cells, coal dust, and some bacteria. But this method is highly criticized because it eliminates all pollen in honey and thus its botanical origin. A very important process after filtration is decantation. Beekeepers who have a very small number of colonies, leave the honey overnight at the bottom of the extractor inside a warm room, then release it directly into containers for use. While beekeepers with a larger amount of honey, and especially those who want to sell some of it, pass it through a special reservoir that serves to ripen honey. Every day the foam that can ferment honey is removed from them.

Honey manufacturing industries have numerous honey processing equipment. They often use these equipment for various honey processing as it is creaming of honey: Creamed honey contains a large number of small crystals, which prevent the formation of large crystals. Small processors don't apply this. Honey after it has finished its processing must be packaged and labeled. In amateur producers, honey is packaged in various glass containers, plastic boxes or buckets, but none of these are labeled and above all in some cases are not completely hermetically sealed. Almost 90% of beekeepers sell honey in their homes, only a small number of beekeepers produce packaged honey with special names. Whereas, regarding the production of honey in an industrial way, the packaging of honey is done by means of machines suitable for these processes, in glass or plastic containers. At the end the labels are placed and the product is ready for the market.

The above results show that honey production industries have become high tech as new innovations in technology are made. This has facilitated, hygienized and standardized the honey extraction process compared to beekeeping households but the application of heat from them to reduce honey viscosity, to delays crystallization, or to destroys yeast cells and the treatment of honey (its creaming) with technological equipment does not improves the honey qualities.

Also, industrial ultrafiltration removes all pollen in honey, thus eliminating a very important component of honey and hiding its botanical origin, but it remains to see the results of chemical analysis to judge whether this advanced technology in industry, which was part of all honey extraction processes has a positive effect on the quality of honey produced and marketed by them.

3.2 Results of physico-chemical analyzes

Results of physico-chemical characteristics (table 1) show that the average values of chemical analysis for the parameters measured in the ten samples analyzed (5 traditional and 5 market) do not in all cases differ from each other. The observed differences are estimated in the range 0.146 to 37.36. The smallest differences in the analysis results were estimated for the analyses: electrical conductivity (0.146), pH (0.376) and invert sugar (0.43). The largest differences were observed for the analyses: Free acid (11.26) and HMF (37.36). The degree of acidity (free acid) depends not only on the total concentration of organic acids in honey but also on the mineral composition. As can be seen from the average values for this parameter, the honey harvested according to the traditional method has a higher value of acidity and as a result is richer in minerals and organic acids. Regarding the analysis of HMF, we can say that HMF is an organic ingredient which is formed from sugar in an acidic environment during the treatment of honey with heat (evaporation of fructose) or during long-term storage of honey. As can be seen from the average values for this parameter, market honey has the highest HMF value and this can be a guide index for the age of honey and / or the amount of heat that has occurred to that honey because the honeys just extracted have the HMF value close to zero.

Table1: Results of chemical analysis for samples taken in beekeeping households and markets in Tirana district

Variables	Traditional (Samples 1-5) Mean ± SD	Market (Samples 6-10) Mean ± SD	T-Test (SPSS output)	Difference
Soluble dry matter	83.04± 0.9	80.42± 2.951	0.095**	2.62
Moisture content	16.96± 0.923	19.58± 2.951	0.095**	2.62
pH	4.422± 0.496	4.046± 0.529	0.28*	0.376
Free acid	25.96± 6.179	14.7± 6.723	0.025**	11.26
Electrical conductivity	0.458± 0.246	0.312± 0.119	0.27*	0.146
HMF	5.19± 4.357	42.56± 25.34	0.012***	37.36
Invert sugar	73.148± 3.204	72.716± 3.223	0.83*	0.43

(* p>0.05, ** 0.01< p <0.05, ***p<0.01)

The results of different parameters of all the honey samples collected, were compared with the Codex Alimentarius (CODEX STAN 12-1981) and honey standard in EU (2001/110/EC) (Codex Alimentarius Commission Standard for Honey. 2001; Council of the European Union. 2014[6, 10]). It was observed that averages and ranges of values for analysis: soluble dry matter and invert sugar (for honey obtained by two methods) were within the required ranges set in the standard. As it is shown in Table 1, means of traditional and market honey samples for water content is below 20%, which is the maximum limit set in the standard. The values ranged between 16.037-17.883% for traditional honey samples, and 16.629-

22.531% for market samples. As we see the maximum value of moisture content in market honey samples is higher than the maximum limit of the standard. The moisture content contributes directly to honey quality, its viscosity, its fermentation and savor. We judge that this honey may have been harvested prematurely.

Related to pH, the values ranged between 3.926-4.918 for traditional honey samples, and 3.517-4.575 for market samples. All the honey samples investigated were acidic low enough to inhibit the growth of microbial organisms. These values were in conformity with the limits set in the standard 3.5-5. The free acidity as recorded in this study ranged between 19.781-32.139 meq/kg for traditional honey samples, and 7.977-21.423 meq/kg for market samples. The total acidity of all the honey samples analyzed were within the maximum value of 50meq/kg allowed by European standard. The values reported in this study which are lower than 50meq/kg suggest that all the honey samples will not be fermented. From this analysis we judge that traditional honey samples that had the highest values are richer in free acids and minerals.

Electrical conductivity varied from 0.212-0.7 mS/cm for traditional honey samples, and 0.193-0.431 mS/cm for market samples. The value of electrical conductivity for all honey samples is below 0.8 mS/cm, as indicated in Codex STAN 12-1981 and EU Directive 2001/100/CE (EC) (Codex Alimentarius Commission Standard for Honey. 2001; Council of the European Union. 2014[6, 10]). Electrical conductivity is directly related to the content of Ash (ash), minerals and honey acids. The higher their content, the higher the resulting conductivity. As it seems from the results traditional honey samples had the highest value and as a result these honeys are richer in minerals and free acids, the same result as that of free acidity.

Related to HMF the maximum limit set by the European standard is 40 mg/kg. Processing of honey with heat treatment above 35°C, increases the amount of HMF. For traditional honey samples the values ranged between 0.833-9.547 mg/kg, this ensures that honey has not been overheated during processing and is safe for consumption. For market honey samples the values ranged between 17.22-67.9 mg/kg, this ensures that honey has been overheated during processing thus affecting its quality. As can be seen from the above results, industrial honey samples compared with traditional honey samples were natural, pure, less rich in minerals, acids and processed thermally. We suggest the addition of chemical analysis and the extension of sampling in different beekeeping households and markets of Tirana.

CONCLUSIONS AND RECOMMENDATION

This study shows that the use of advanced honey technologies in almost all honey extraction processes in industry, makes it possible to simplify the honey extraction procedure but does not improve the quality of honey. Comparison of the means of chemical analysis for traditionally harvested honeys with market honeys showed that the largest differences were observed for the analyses of free acid and HMF. This indicates the low presence of minerals and free acids in Industrial honeys and that may have been heat treated during processing. There is a need to enhance equipment and services in traditional beekeeping and credit provision needs to be facilitated to supply accessory equipment for beekeeping households.

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MELLIFEROUS PLANTS- THE CASE STUDY OF ALBANIA

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ABSTRACT

Bee is an inexhaustible source of value, is a biological indicator for nature, pollinate of plants and manufacturing of some products in the hive such as honey, royal milk, pollen, propolis, bee venom. Recognition of mellifer species has a great importance for the beekeeper, it helps them in identifying sources used for production of honey, in orientation of adequate pasture for bees, for their rational use. The variety of plant species that are grazed by bees is so wide that the overview presented in this paper aims to create a panorama on the possibility of grazing bees, to be at the service of beekeepers who apply migratory beekeeping. These data have indicative character because the real production depends on many factors: soil, climate, seasonal performance, humidity etc. The period when the research is conducted is April-June 2021 which coincides with the active period of beekeeping. The collection of information was done through online questionnaires, observation and discussions with beekeepers, about the melliferous plants in the places where their parks are located and based on the mellifer classification of plants according to melissopolenic analysis of honey (Pignatti, 1982 and E. Crane, 1975), which can fluctuate for areas with large geoFigureical differences, and in our case apply to the Mediterranean region. The analysis of the results show that the in interest for beekeeping is maintenance of main existing melliferous pastures as *Trifolium spp*, *Thymus*, *Prunus L*, *Robina pseudoacacia L*, *Carduus L.*, and various medicinal plants, which are the pride of our honeys, and the increase of them harmonized with general human interest. In central and southern Albania there is no pasture predominance, the pasture is more diverse. Additional melissopaynological studies are needed to complete the panorama of melliferous plants in Albania.

Keywords: bee, melliferous plants, climatic zone

INTRODUCTION

Our country is exposed by a geoFigureical position and very favorable climatic conditions for the development of melliferous plants, with a time span in all months of the year, with a variety of melliferous plants about 250 genera and in species they reach several thousands (Paparisto, K. and Balza, E. 2003[1]). The bees and some plant species have developed a close interdependence

with each other. Such reciprocal adaptation is the result of a long co-evolutionary relationship. Without bees plants would not blossom and biodiversity would not be so great. On the other hand bee food is provided entirely by the plants they visit. To attract bees (and consequently as a reward for their services) plants provide nectar, leaf dew and pollen. Nectar is a food source rich in energy, honeydew is a source of carbohydrates and pollen is a source of protein, used by young bees.

Humans benefit greatly from the natural relationships between bees and plants. When talking about the benefit of bees it is not only about the useful products it gives to man, their great contribution to the economy of the country is achieved indirectly through the pollination of agricultural crops and spontaneous flora. As bees and other insects visit flowers for food, they transport pollen from one plant to another, enabling them to pollinate and regenerate ecosystems.

Bees, according to geoFigureical differences, adapt to different regions, climate factors and vegetation, but also to the predominance of pests and pathogens. The purpose of this paper was to reflect the general considerations of the honey flora of the country as little interest is shown in the planting and preservation of melliferous plants. Knowledge of this flora is important in the orientation of nectar pastures most suitable for bees, in the best possible organization of bee migration by beekeepers a few days before blossom. Melliferous plants have been studied by many authors (Hasaj, S. 2004[2]), (Kukleci, F. 2012[3]) and their systematization in the bees interest has several rankings according to the importance of the study. Based on the bees interest, i.e. the honey potential (Thomo, K. 2010[4]) honey-producing plants are divided into 3 categories: nectar plants, which are plants that are visited by bees only for nectar, pollenic plants that are visited by bees only for pollen, and nectaropollenic plants that produce nectar, pollen and honeydrew. This includes the largest group of plants.

MATERIALS AND METHODS

2.1 Study area

The study area includes 20 districts of Albania. The districts represent four climatic zones (Berxholli, A., Kola, B. 2009[5]): *The area with a Mediterranean plain climate*. This area covers the entire coastal area of the Adriatic and Ionian Sea, occupying more or less 1/5 of their surface. *The area with Mediterranean hilly climate* includes the entire peripheral hilly system of the Western Lowlands, the pre-mountain system of the Ionian coast and some of the valley environments: Shushica, Vjosa, Drino, Shkumbini i Mesëm, partly Devolli and Osumi. *The area with Mediterranean pre-mountainous climate*. This area has an island-like extension along the Drini valley, Valbona; it includes the Domosdova field, the plain of Korça, the hollow of Kolonja and an extension to the NE in the plain of Dukagjin and Pollog. *The area with a Mediterranean mountain climate* includes mountain heights above 1000 m, such as the Albanian Alps, Bjeshkët e Namuna, the ranges between the Drini in the north and the Upper Osum in the south, Sharr and the southern mountains. The districts that have been subjected to our study are: Tropojë, Pukë, Kukës, Shkodër, Dibrër, Burrel, Lezhë, Krujë, Tiranë, Elbasan, Gramsh, Librazhd, Korçë, Ersekë, Fier, Lushnjë, Berat, Përmet, Vlorë and Sarandë.

2.2 The method used

Literature study, field observations, semi-structured interviews with beekeepers who have applied migratory and stationary beekeeping were used as the primary tool for data collection. The

period in which the melliferous flora research was conducted was April-May 2021, which coincides with the active period of beekeeping. In each district, surveys and distribution of questionnaires were carried out in the areas of three different parks. Based on the melliferous classification of plants according to melissopolenic analyses of honey (according to Pignatti, S. 1982[6] and Crane, E. 1975[7]), which can fluctuate for areas with large geoFigureical differences, and in our case are valid to the Mediterranean region. Information in total was collected from 20 districts of the country (from 60 bee parks).

RESULTS AND DISCUSSION

Surveys conducted in 20 Albanian districts showed that the cultivated and natural melliferous flora potential of the area makes it very favorable for beekeeping. As a result of the observations made, the species that are most visited by honeybees (Apis mellifera L) in 60 different parks, divided by climatic zones, are:

The area with a Mediterranean plain climate:

Shkodër

- Muriqan: 40% Clover (*Trifolium spp*) , 20% Fruit plants (*Prunus L*), 20% Lisra (*Thymus*).
- Rec: 50% Clover (*Trifolium spp*), 20% The spring heather (*Erica arborea L*), 20% Lisra (*Thymus*).

Berat

- Kuçovë: 30% Fruit plants (*Prunus L*), 30% Citrus (*Citrus*).
- Poshnje: 30% Citrus (*Citrus*), from 20% Fruit plants (*Prunus L*), Lisra (*Thymus*) and The autumn heather (*Erica manipuliflora Salisb.*).

Sarandë

- Nevicë: 40% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).
- Delvinë: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*). -Ksamil: 30% Citrus (*Citrus*), 20%, Fruit plants (*Prunus L*), 20% Lisra (*Thymus*).

Krujë

- Bubq: 30% Clover (*Trifolium spp*), from 20% Lisra (*Thymus*), The spring heather (*Erica arborea L*) and Gjëmbaçi (*Carduus L.*) .
- Thumanë: 50% Clover (*Trifolium spp*), 20% Lisra (*Thymus*).
- Qëndër: 40% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Fier

- Roskovec: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Lezhë

- Zejmen: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*), 30% Gjëmbaçi (*Carduus L.*).
- Manat: 40% Sage (*Salvia officinalis L*), 30% Clover (*Trifolium spp*).
- Balldren: 40% Sage (*Salvia officinalis L*), 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Tiranë

- Ib: 30% Clover (*Trifolium spp*), from 20% Fruit plants (*Prunus L*), Lisra (*Thymus*) and The spring heather (*Erica arborea L*).
- Prezë: From 20% Fruit plants (*Prunus L*), Marea (*Arbutus unedo*) and Gjëmbaçi (*Carduus L.*).
- Frakull: 30% Clover (*Trifolium spp*), 20% Lisra (*Thymus*).

Lushnjë

- Krutje: 40% Clover (*Trifolium spp*), 20% Lisra (*Thymus*).
- Dushk: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).
- Grabian: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Figure 1. The area with a Mediterranean plain climat

The area with Mediterranean hilly climate

Pukë

- Pukë Qëndër: 40% Clover (*Trifolium spp*), 20% Gjëmbaçi (*Carduus L.*).
- Iball: 30% Lisra (*Thymus*) and 40% Chestnut (*Castanea sativa Miller*).

- Gjegjan: 40% Gratheli (*Erica herbace*), 40% Clover (*Trifolium spp*).

Burrel

- Klos: 30% Fruit plants (*Prunus L*) and 40% Clover (*Trifolium spp*).

-Macukull: 30% Clover (*Trifolium spp*), and from 20% Lisra (*Thymus*), Thyme (*Satureja montana L.*) and Marea (*Arbutus unedo*).

Vlorë

- Dukat: 30% Clover (*Trifolium spp*), and from 20% Lisra (*Thymus*), Thyme (*Satureja montana L.*), Gjëmbaçi (*Carduus L.*) and The autumn heather (Erica manipuliflora Salisb.).

Përmet

- Petran: 30% Clover (*Trifolium spp*) and 30% Lisra (*Thymus*).

- Kutal: 30% Clover (*Trifolium spp*), 30% Marea (*Arbutus unedo*), 30% Acacia (*Robina pseudoacacia L*).

Tiranë

-Kamëz: From 20 % Fruit plants (*Prunus L*), Clover (*Trifolium spp*), Marea (*Arbutus unedo*) and The autumn heather (*Erica manipuliflora Salisb.*).

Gramsh

- Plakë: 30%Clover (*Trifolium spp*), 30% Lisra (*Thymus*), 20% Gjëmbaçi (*Carduus L.*).

Elbasan

- Shtërmen: From 20% Citrus (*Citrus*), Clover (*Trifolium spp*), Lisra (*Thymus*) and Marea (*Arbutus unedo*).
- Paper: 30% Clover (*Trifolium spp*), from 20% The spring heather (*Erica arborea L*), Marea (*Arbutus unedo*) and Lisra (*Thymus*).

Fier

- Ballsh: 30% Clover (*Trifolium spp*), 20% Lisra (*Thymus*), 20% Gjëmbaçi (*Carduus L.*).

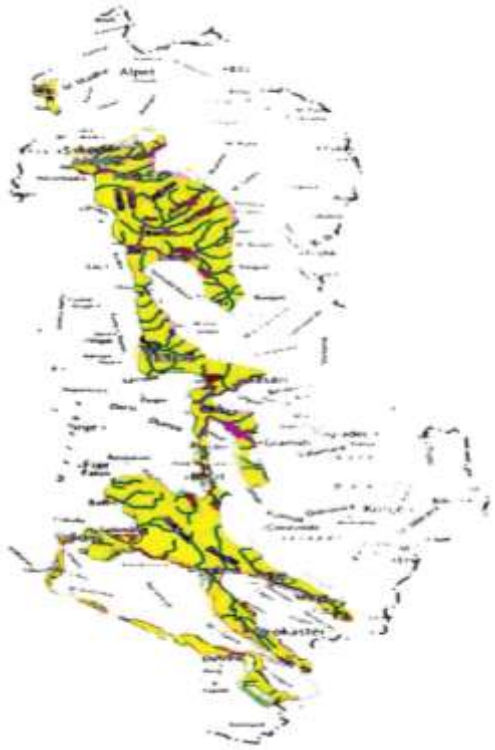

Figure 2. The area with Mediterranean hilly climate

The area with Mediterranean pre-mountainous climate

Kukës

- Kolesjan: 40% Clover (*Trifolium spp*), from 20% Thyme (*Satureja montana L.*), Lisra (*Thymus*) and Fruit plants (*Prunus L*).

Burrel

- Baz: 30% Clover (*Trifolium spp*), and from 20% Gratheli (*Erica herbace*), Fruit plants (*Prunus L*) and The spring heather (*Erica arborea L*).

Dibër

- Sohodoll: 60% Clover (*Trifolium spp*), 20% Fruit plants (*Prunus L*), 20% Thyme (*Satureja montana L.*).

Berat

- Poliçan: From 20% Fruit plants (*Prunus L*), Thyme (*Satureja montana L.*) and Gjëmbaçi (*Carduus L.*).

Elbasan

- Shushicë: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Gramsh

- Trashavicë: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Korçë

- Devoll: 30% Acacia (*Robina pseudoacacia L*), 30% Clover (*Trifolium spp*).
- Moravë: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*), 30% Fruit plants (*Prunus L*).

Librazhd

- Qukës: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).
-Qëndër: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Figure 3. The area with Mediterranean pre-mountainous climate

The area with a Mediterranean mountain climate

Tropojë

- Markaj: 70% Chestnut (*Castanea sativa Miller*), 20% Bliri (*Tilia spp*).
- Viçidol: 50% Thyme (*Satureja montana L.*), 30% Lisra (*Thymus*).
- Qafë Morinë: 30% Fruit plants (*Prunus L*) Lisra (*Thymus*) and 30% Clover (*Trifolium spp*).

Kukës

- Shtiqen: 40% Clover (*Trifolium spp*), 20% Fruit plants (*Prunus L*), 20% Lisra (*Thymus*), 20% Thyme (*Satureja montana L.*).
- Topojan: 40% Clover (*Trifolium spp*), from 20% are Lisra (*Thymus*), Chestnut (*Castanea sativa Miller*) and Thyme (*Satureja montana L.*).

Dibër

- Maqellar: 30% Fruit plants (*Prunus L*), 30% Clover (*Trifolium spp*).
- Melan: 40% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Vlorë

- Radhimë: 30% Lisra (*Thymus*), from 20% Fruit plants (*Prunus L*), Thyme (*Satureja montana L.*) and Citrus (Citrus).
- Llogara: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Ersekë

- Rehovë: 30% Lisra (*Thymus*), from 20% Clover (*Trifolium spp*), Thyme (*Satureja montana L.*) and Acacia *(Robina pseudoacacia L).*
- Borovë: 30% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

- Leskovik: 40% Clover (*Trifolium spp*), 30% Lisra (*Thymus*).

Librazhd

- Lunik: From 30% Lisra (*Thymus*), Bliri (*Tilia spp*) and Clover (*Trifolium spp*).

Shkodër

- Kir: 40% Gratheli (*Erica herbace*), 30% Bliri (*Tilia spp*).

Korçë

- Maliq: From 20% Lisra (*Thymus*), Fruit plants (*Prunus L*), Acacia (*Robina pseudoacacia L),* Tërfili (*Trifolium spp*) and Mimosa (*Acacia dealbata*).

Përmet

- Përmet qëndër : From 30% Clover (*Trifolium spp*) Marea (*Arbutus unedo*) and Lisra (*Thymus*).

Gramsh

- Ishëm: 30% Clover (*Trifolium spp*) and 30% Lisra (*Thymus*).

Figure 4. The area with a Mediterranean mountain climate

The major honey flow season of Albanian district is from May to June and the minor flow season is from October to December (Thomo, K et al. 2002[8]). The respondents indicated that even though there are different types of bee plants in honey flora seasons, there is a shortage of bee food during the dry seasons where ground and surface water resources are limited, for this reason beekeepers make migrations of their parks to avoid unfavorable conditions.

Figure 5. Some of the honey bee forage plants

List of melliferous plant species found in this study are presented below (in 20 districts) but additional melissopaynological studies are needed to complete the panorama of melliferous plants in Albania:

1.Tropojë: Lisra (*Thymus*), Chestnut (*Castanea sativa Miller*), Thyme (*Satureja montana L.*).
2. Kukës: Clover (*Trifolium spp*), Lisra (*Thymus*), Thyme (*Satureja montana L.*), Fruit plants (*Prunus L*).
3. Pukë: Clover (*Trifolium spp*), Thyme (*Satureja montana L.*), Chestnut (*Castanea sativa Miller*).
4.Dibër:Clover (*Trifolium spp*), Fruit plants (*Prunus L*), Lisra (*Thymus*).
5.Burrel:Clover (*Trifolium spp*), Fruit plants (*Prunus L*).
6.Shkodër:Clover (*Trifolium spp*), Lisra (*Thymus*), Gratheli (*Erica herbace*).
7. Lezhë: Clover (*Trifolium spp*), Lisra (*Thymus*), Sage (*Salvia officinalis L*), Fruit plants (*Prunus L*), Gjëmbaçi (*Carduus L.*), Thyme (*Satureja montana L.*), Acacia (*Robina pseudoacacia L*).
8. Krujë: Clover (*Trifolium spp*), Lisra (*Thymus*), Gjëmbaçi (*Carduus L.*), Fruit plants (*Prunus L*).
9. Tiranë: Fruit plants (*Prunus L*), Clover (*Trifolium spp*), Lisra (*Thymus*), Marea (*Arbutus unedo*), Gjëmbaçi (*Carduus L.*).
10. Elbasan: Clover (*Trifolium spp*), Lisra (*Thymus*), Marea (*Arbutus unedo*), Fruit plants (*Prunus L*), Gjëmbaçi (*Carduus L.*).
11. Gramsh: Clover (*Trifolium spp*), Lisra (*Thymus*), Gjëmbaçi (*Carduus L.*), Fruit plants (*Prunus L*), Acacia (*Robina pseudoacacia L*).
12.Librazhd: Clover (*Trifolium spp*), Lisra (*Thymus*), Bliri (*Tilia spp*).
13. Korçë: Clover (*Trifolium spp*), Lisra (*Thymus*), Fruit plants (*Prunus L*), Acacia (*Robina pseudoacacia L*).
14.Ersekë: Clover (*Trifolium spp*), Lisra (*Thymus*), Fruit plants (*Prunus L*).
15. Fier: Clover (*Trifolium spp*), Lisra (*Thymus*), Gjëmbaçi (*Carduus L.*), Fruit plants (*Prunus L*), Acacia (*Robina pseudoacacia L*).
16. Lushnjë: Clover (*Trifolium spp*), Lisra (*Thymus*), Citrus (*Citrus*), Fruit plants (*Prunus L*).

17.Berat: Fruit plants (*Prunus L*), Citrus (*Citrus*), Lisra (*Thymus*)
18.Përmet: Clover (*Trifolium spp*), Marea (*Arbutus unedo*), Lisra (*Thymus*), Gjëmbaçi (*CarduusL*),Acacia(*RobinapseudoacaciaL*).
19.Vlorë: Lisra (*Thymus*), Clover (*Trifolium spp*), Thyme (*Satureja montana L.*). Gjëmbaçi (*Carduus L.*), The autumn heather (*Erica manipuliflora Salisb*.).
20. Sarandë: Clover (*Trifolium spp*), Lisra (*Thymus*), Fruit plants (*Prunus L*), The autumn heather (*Erica manipuliflora Salisb*).
The areas with the highest diversity of melliferous plants in Albania are: Lezhë, Tiranë, Elbasan, Fier, Vlorë, Gramsh and Përmet.

CONCLUSIONS AND RECOMMENDATION

According to the climatic zones, the dominant melliferous plants are: The area with a Mediterranean plain climate: Fruit plants (*Prunus L*), Lisra (*Thymus*), Clover (*Trifolium spp*), Citrus (*Citrus*), Gjëmbaçi (*Carduus L.*). The area with Mediterranean hilly climate: Clover (*Trifolium spp*), Lisra (*Thymus*), Marea (*Arbutus unedo*), Gjëmbaçi (*Carduus L.*). The area with Mediterranean pre-mountainous climate: Clover (*Trifolium spp*), Lisra (*Thymus*), Fruit plants (*Prunus L*), Thyme (*Satureja montana L.*). The area with a Mediterranean mountain climate: Clover (*Trifolium spp*), Lisra (*Thymus*), Fruit plants (*Prunus L*), Thyme (*Satureja montana L.*), Bliri (*Tilia spp*). Melliferous plants found in all districts are: Clover (*Trifolium spp*), Lisra (*Thymus*), Fruit plants (*Prunus L*), also Acacia (*Robina pseudoacacia L*) and Gjëmbaçi (*Carduus L.*) although in slightly lower amounts. In central and southern Albania there is no pasture predominance, the pasture is more diverse, the places that contain a variety of melliferous plants are: Lezha, Tirana, Elbasani, Fieri, Vlora, Permeti, Gramsh. Based on the results above to support these pollinators we should stimulate farmers to plant melliferous plantations and manage crops with different flowering periods. Also, we should encourage municipalities to plant melliferous plants in cities and along roads.

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USE OF COMPUTER TECHNIQUES FOR PERFORMING MORPHOMETRIC MEASUREMENTS IN BIOLOGY

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ABSTRACT

In this study were performed morphometric measurements of the Hymenoptera *Apis mellifera carnica*, in the district of Tirana. Samples were collected at four different colonies: (1-Kamez, 2- Dajt, 3- Linze, 4-Lunder). From each sample 20 bees were prepared, scanned and measured using computer software. Measuring of 19 morphological characters of honey bees was carried out using advanced technique Scan Photo with Photoshop program (SPT) as well as using the binocular method. Averages estimated from the data of measurements made with the Binocular and SPT methods were: 6.411 and 6.373 for tongue length; 9.264 and 9.237 for fore wing length; 3.144 and 3.114 for forewing width; 2.63 and 2.601 for cubital index; 6.43 and 6.385 for hind wing length; 1.84 and 1.82 for hind wing width; 19.787 for 19.744 number of hooks; 2.557 and 2.552 for femur length; 3.235 and 3.204 for tibia length; 2.051 and 2.031 for metatarsus length; 1.22 and 1.19 for metatarsus width; 2.07 and 2.03 for longitudinal diameter of tergite IV; 0.812 and 0.8 for (TOM A); 0.519 and 0.52 for (TOM B); 0.325 and 0.277 for HLT 5; 2.776 and 2.75 for S3 longitudinal diameter; 1.338 and 1.308 for wax mirror longitudinal; 2.394 and 2.354 for wax mirror transversal; 0.371 and 0.359 for distance between wax mirrors. Comparison between SPT and using Binocular showed no significant difference between the two methods in measuring the chosen morphometric characters. These results reinforces the idea that SPT can be used extensively in Biology to make other morphometric measurements in the direction of Zoology or Botany.

Keywords: Biology, *Apis mellifera carnica*, Morphological traits, Scan Photo Technique

INTRODUCTION

Nowadays, many types of advanced instruments and techniques are used to perform morphological studies. In this study the morphological characters were measured by two different methods, the binocular method and the scan photo method and the results were compared in order to verify the accuracy of the SPT which is easily available for the identification of a large number of morphological characters of animals or plants. Specifically in this study the computer method Scan Photo Technique (El-Aw., et al. 2012[1]) was used to conduct the morphometric studies on a very useful insect such as the honey bee. This method in addition to the study of insects can also be used in botanics (Femat, A. 2011[2]). And for this it requires the collected plants, a scanner and the computer program as shown in the figure 1.

Figure 1: Scanning and measurement of plant parts (Patri Feher's photo)

MATERIALS AND METHODS

2.1 Study area and taking samples

The morphological characteristics of the Hymenopter *Apis mellifera carnica* were measured in four parks (1-Kamëz, 2- Dajt, 3- Linzë, 4- Lundër) in the district of Tirana. As a criterion it was chosen to take samples in the period April-June, which coincides with the active period of beekeeping and other indicators of breeding in general. For each park we took 3 samples representing three different families and a total of 12 samples were collected (Kuliçi M, Shehu L. 2021[3]). Since the bees are damaged during the taking samples and preparation, then in order to have a sufficient number of bees in which we would perform the measurements, we realized the taking of samples with 100 worker bees (Thomo, K., et al. 2002[4]) . Taking samples was done in solid hives. Solid families are those families that have a better queen and that have over 10 bee frames (Ravazzi, G. 2003[5]). The bees after drowning have been transferred to hermetically sealed containers and stored in ethanol 70 degrees (Ruttner, F. 1978[6]., Kuliçi, M 2021[7]).). They are transported by thermos to the laboratory and stored in cool places. Labeling was done on the samples taken. In them we have determined the date of receipt, the place and the characteristics of the family from where the sample was taken.

2.2 Morphological characters

Nineteen morphological traits (table 1) were measured for each worker bee (Ruttner, F. 1978[6]; Ruttner, F. 1988[8]). These include: head characters, articulated limbs in the thorax as well as abdominal characters. While measuring the length of these traits is shown in Figure 2.

Table 1: Morphological characters studied

Characters of the head and abdomen	***Characters of limbs articulated in the thorax***
1. Length of proboscis (**PL)**	10.Forewing Length (**FWL)**
2. Longitudinal diameter of tergitite 4 (**T4)**	11. Forewing Width (**FWW)**
3. Width of Tomentum, tergite 4 (**TOM A)**	12. Hind wing length (**HWL)**
4.Width of the dark stripe between tomentum and posterior rim of tergite 4 (**TOM B)**	13. Hind wing width (**HWW)**
5. Length of hairs on tergite 5 (**HLT 5)**	14. Femur Length **(FL)**
6. Sternite 3, Longitudinal (**LS_3)**	15. Tibia Length **(TL)**
7. Wax mirror of sternite 3 longitudinal (**WL)**	16. Basitarsus Length **(BL)**
8. Wax mirror of sternite 3 transversal (**WT)**	17. Basitarsus Width **(BW)**
9. Distance between wax mirrors St. 3 (**WD)**	18. Number of hooks

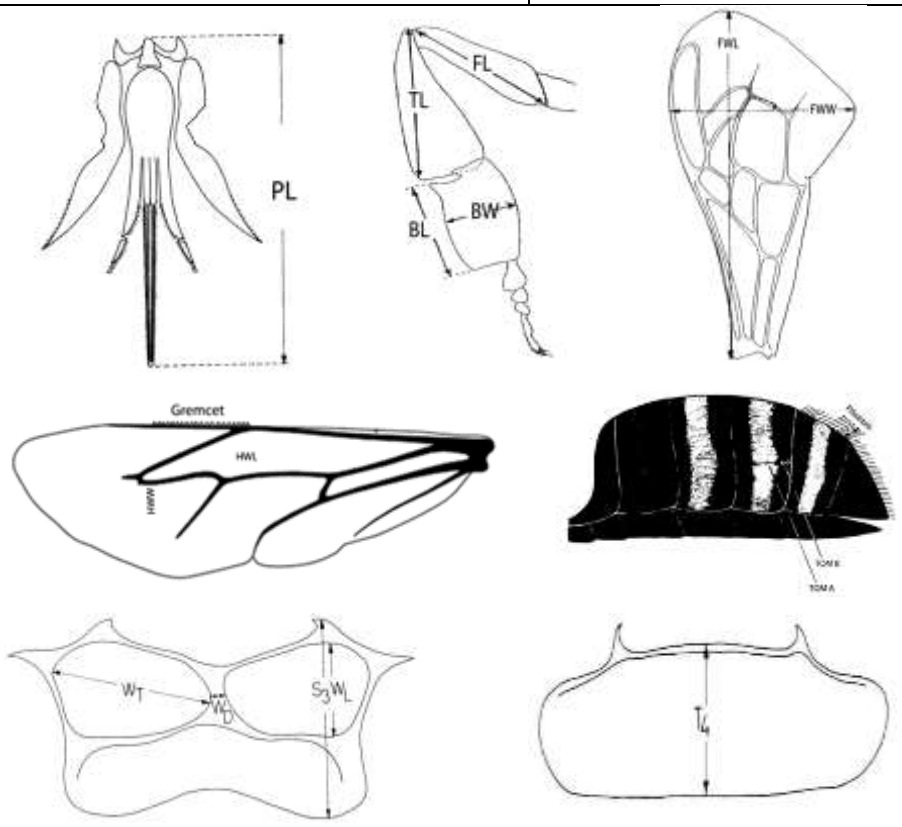

Figure 2: Morphological characters measured

2.3 Method used

In this study the measurement of morphometric characters was done using the Advanced Scan Photo Technique (El-Aw., M. A., Kh. A. Draz et al. 2012[1]) as well as the binocular method (traditional method with microscope). In the SPT technique all morphological characters were

measured using a high resolution scanner (Zoom 100%, color is red green blue (RGB), resolution 4800 ppi and units of mm), connected to a laptop, with screen resolution (1440 × 900 pixels). For this, first the dissection of the bee body parts and preparation of the scanning slides were done. After the slides have been scanned, their measurement has been done with the help of the program ruler (figure 3).

Figure 3: Images of morphological characters after scanning and opening with photoshop program

In each sample the measurements were made on 20 worker bees, so in total measurements were performed on 240 bees. As mentioned above, 19 morphological features were measured in each bee and a total of 4560 measurements were performed with the SPT method and 4560 measurements with the binocular method for the realization of this study. All measurements are in units of (mm) except the number of hooks (as a number).

2.4 Statistical analysis

The results obtained from the measurements made by the two different methods will be compared using the T-test. The statistical hypothesis to be studied is: The differences in the results of measurements with the binocular method and the Scan Photo method, do not bring attested statistical changes in the classification (grouping) of individuals.

RESULTS AND DISCUSSION

Table 2 gives the average values of morphological characters measured by the two methods for all parks. The results given in this table show that the average values of the various morphological characters measured by the Binocular method are, in all cases, higher than those measured by the Scan Photo method. This is a result similar to that communicated by Samborski et al. (2002)[9]; El-Aw, M. A et al. 2012[1]). As can be seen, on average, the results obtained from the use of the two methods for measuring morphological characters differ slightly from each other.

The observed differences are estimated in the range 0.001 to 0.048. The smallest differences in the measurement results with the two different methods were estimated for the characters: toumentum B (0.001), femur Length (0.005), longitudinal diameter of sternitis III (0.007), toumentum A (0.012), distance between wax mirrors (0.012) and width of hind wings (0.02). The largest differences were observed for the characters: hind wing length (0.045) and pilosity (0.048).

The statistical analysis shows that between the averages of the morphological characters considered, evaluated using the data of measurements performed with the binocular and Scan Photo method, there are no statistically attested differences. Consequently, it can be concluded that the Scan Photo method, compared to the binocular method, does not bring statistically attested changes in the quantitative evaluation of morphological characters, which

manage to influence the analysis and evaluation of differences between races (populations) of bees, when morphological characters will be used for this purpose. This is the same result as that reported by Al-Aw, M. A. et al. 2012[1]).

Table 2: Averages of morphological characters of worker bees measured by binocular and SPT

Nr.	Traits	Abbrev.	Mean±SD		Difference (mm)
			Binocular	Scan Photo	
1	Length of proboscis	PL	6.411±0.05	6.373±0.05	0.038
2	Forewing Length	FWL	9.264 ± 0.011	9.237 ± 0.011	0.027
3	Forewing Width	FWW	3.144 ± 0.04	3.114 ± 0.04	0.03
4	Cubital index	CI	2.63± 0.014	2.601±0.014	0.029
5	Hindwing Length	HWL	6.43± 0.04	6.385± 0.04	0.045
6	Hindwing Width	HWW	1.84±0.006	1.82 ±0.005	0.02
7	Number of hooks	HA	19.787±0.114	19.744±0.113	0.043
8	Femur Length	FL	2.557±0.008	2.552±0.008	0.005
9	Tibia Length	TL	3.235±0.023	3.204±0.023	0.031
10	Basitarsus Length	BL	2.051±0.009	2.031±0.008	0.02
11	Basitarsus Width	BW	1.22±0.003	1.19±0.003	0.03
12	Longitudinal diameter of tergitite 4	T4	2.07±0.008	2.03±0.008	0.04
13	Width of Tomentum, tergite 4	(TOM A)	0.812±0.01	0.8±0.01	0.012
14	Width of the dark stripe between tomentum and posterior rim of tergite 4	(TOM B)	0.519±0.002	0.52±0.002	0.001
15	Length of hairs on tergite 5	HLT5	0.325±0.004	0.277±0.004	0.048
16	Sternite 3, Longitudinal	S3	2.776±0.006	2.75±0.006	0.007
17	Wax mirror of sternite 3 longitudinal	WL	1.338±0.006	1.308±0.005	0.03
18	Wax mirror of sternite 3 transversal	WT	2.394±0.007	2.354±0.007	0.04
19	Distance between wax mirrors St. 3	WD	0.371±0.003	0.359±0.003	0.012

If we compare the two methods it is clear that the length of a trait can be measured in different units. Using the Scan photo method the length of the characters can be measured in several units (mm, cm, pixels and inches) while with the Binocular method usually in the unit of mm. Also the Scan photo method requires only a high resolution scanner and a computer with Photoshop program. The software and data required for identification are free to the general public and at a low cost while the other method requires a higher cost stereomicroscop.

The photoshop program ruler performs measurements automatically, is easy to use and saves measurements time, on the other hand in the Binocular method the calculation of measurements can result in errors, depending on the person performing the measurements and it requires a long time to carry out the measurements. This study reinforces the idea that the use of ordinary scanning technique and photoshop program makes it possible to simplify the procedure of measuring the morphological characters of bees and increase the number of characters that can be measured.

CONCLUSIONS AND RECOMMENDATION

The measurement of morphological features in worker bees by the Scan Photo method gives results statistically equal to the results of the Binocular method. Although for all traits the Scan Photo method gives higher values than the Binocular method, the effect of their multiplication on the classification of individuals into groups, races, populations statistically distinct is zero. We propose that such a technique can be successfully used in other representatives of the insect class to conduct morphological studies.

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QUALITY CHANGES IN DIFFERENT PARTS OF FRUITS OF SOME MORPHOLOGICALLY DIFFERENT WATERMELON HYBRIDS

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ABSTRACT

The study was carried out at Alata Horticultural Research Institute. In this study, the fruits of watermelon hybrids with different morphological characteristics were divided into six. The part where the fruits touch the ground was divided into 3 parts and the sun-exposed part was divided into 3 parts and examined in 6 parts in total. In terms of L* value, it can be said that part 5 has the highest values and part number 1 has the lowest values. In terms of ho value, it can be stated that the 2nd part is the highest value and the lowest value is the 3rd part in general. In terms of C* value, it can be said that part 6 has the highest values in general, and the lowest values are generally taken from part number 2. In terms of total soluble solid content, it can be stated that the highest value is taken from the 3rd part and the lowest value from the 2nd part. In terms of titratable acidity, the highest value is taken from part 6 and the lowest value from part 2. In terms of pH, the highest values are taken from the parts 5 and 6, and the lowest value is taken from the part number 2.

Keywords: Watermelon, Hybrid, Different Parts, Quality Changes,

INTRODUCTION

There are 101,620,420 tons of watermelons produced worldwide. After China (59.19%), Turkey (3.43%) is the second-largest producer. Turkey produces 3.49 million tons of watermelon on 78,179 acres of land (Faostat 2020).

Watermelon fruit is a vegetable with low protein, fat and starch content in terms of nutritional value. European consumers have started to be health conscious. This attitude has also started to be supported by governments with high investments to encourage fresh fruit and vegetable consumption. Watermelon provides a wide range of antioxidants such as carotenoids (lycopene and beta-carotene), phenols, vitamins (A, B, C and E) and certain amino acids (citrulline and arginine) in the diet (Perkins-Veazie, 2002; Perkins-Veazie et al., 2007), which is thought to play a protective role in reducing the risk of some types of cancer, cardiovascular diseases and age-related degenerative pathologies (Giovannucci, 1999; Rao, 2006). Watermelons are often prepared for the market in the field, and skin death, skin, pre-chilling, cold storage, and cold transportation are generally disregarded in watermelons that are being delivered to domestic and international markets. During marketing, watermelon fruit is subjected to high temperatures because its ripening period falls during the sweltering summer months. In particular when it comes to export, skin death, skin, cold storage, and transportation are crucial for extending the shelf life. In recent years, our nation has seen a sharp rise in the production of grafted watermelons. According to parameters like the auricles and leech on the

fruit stem drying, hair shedding on the stem, and the fruit growing to the specified size of the variety, skin death of watermelon fruits is monitored (Özdemir et al., 2014; Aras et al., 2015).

In this study, the fruits of watermelon hybrids with different morphological characteristics were divided into six and the changes in total soluble solid content, titratable acidity, pH, L and h° values in each part of the fruits were examined.

MATERIAL AND METHOD

The study was carried out on an open field at 36° 37' 08.03" N and 34° 21' 00.5" E that belonged to the Ministry of Agriculture and Forestry's Alata Horticultural Research Institute (Erdemli, Mersin, Türkiye). In the study, 36 hybrids from striped hybrids and 10 hybrids from varieties with dark green back ground color were used as material. The area where the study was conducted contains loamy soil with a pH value of 7,71, according to soil testing. Table 1 contains the results of the soil analysis.

Table 1. Soil analysis results of the parcel where the study was carried out

Analyzes	**Limit Values**	**Analysis Results (0-30 cm)**
Texture (100 g/ml)	30-50	48,00 (loamy)
Total Calcitic ($CaCO_3$ %)	5-15	40,00 (high calcareous)
Salinity E.C. ds/m (25 ^oC)	0-0.8	0,32 (slightly salty)
Organic matter (%)	3-4	2,20 (deficient)
pH 1: 2,5	6.0-7.0	7,71 (slightly high)
Available potassium (mg/kg)	244-300	70,60 (very low)
Receivable phosphorus (mg/kg)	20-40	21,30 (optimum)

Seed sowing was carried out on 4 March 2017, land preparation was carried out on 6 April 2017, and seedlings were planted on 11 April 2017. On the prepared banks with a width of 70 cm and a height of 40 cm, the seedlings were planted with 2,5 x 0,7 m spacings and distances, covered in black mulch. Using a drip irrigation system, irrigation was carried out. Gucdemir (2012) claims that fertilizations were carried out following soil analysis. Each irrigation included a drip irrigation system for fertilization. Spider mites and other pests were sprayed as soon as they were discovered. Mechanical and manual methods were used to control weeds. Harvests on 4July 2017, as soon as the tendril and auricles were dry, the fruits were harvested and brought to the cold storage. The climate values of the months in which the research was carried out are given in Table 2.

In this study, the fruits of watermelon hybrids with different morphological characteristics were divided into six. The part where the fruits touch the ground was divided into 3 parts and the sun-exposed part was divided into 3 parts and examined in 6 parts in total. The changes in total soluble solid content, titratable acidity, pH, C*, L and h° values in each part of the fruit were examined.

Table 2. Monthly climate data of the months in which the experiment was conducted.

March	**April**	**May**	**June**	**July**
Maximum Temperature Values (°C)				
23,5	28,2	30,0	34,2	40,5
Minimum Temperature Values (°C)				
2,6	6,1	2,0	14,7	19,3
Average Temperature Values (°C)				
13,4	16,8	20,0	24,6	28,8
Maximum Humidity Values (%)				
90,5	88,8	84,0	81,3	80,4
Minimum Humidity Values (%)				
39,6	41,4	59,0	61,5	43,5
Average Humidity Values (%)				
69,9	67,2	75,8	75,0	71,4
Average Precipitation Values (mm=kg÷m²)				
211,6	76,4	12,8	0,2	0,0

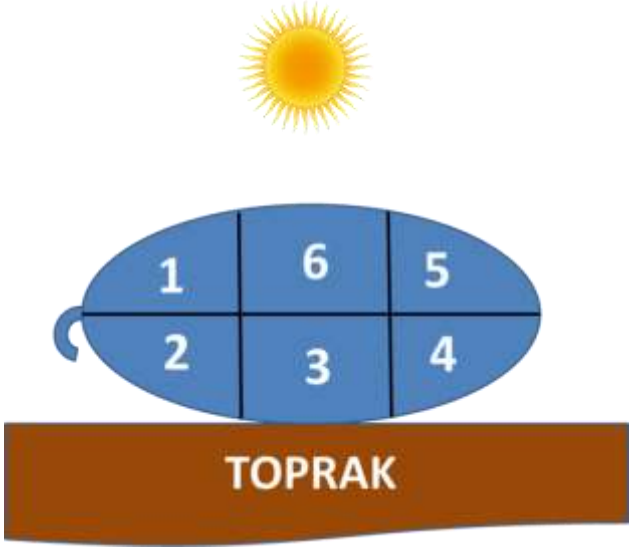


Figure 1. Locations of samples taken from different parts of the fruit.

In the study, 36 striped and 10 hybrids with dark green back ground color were used. For the purpose of analyzing watermelon hybrids, fruit samples from six different fruit-related areas were collected. Figure 1 shows the chronological order of the fruit samples. Total amount of titratable acid (TEA, potentiometric method), % determined by the Atago ATC-1E Model hand refractometer (Atago Co. Ltd., Tokyo, Japan) from fruit juice derived from fruits, and total amount of total soluble solid content (fruit obtained) With the help of the 0.1 N NaOH amount used as a result of the titration carried out until the pH reached 8.1, 5 ml of water was obtained and it was completed to 100 ml with pure water to determine the acidity value (Sadler, 2008).

Three replications of the experiment were set up using a randomized plot design. In each cycle, 3 fruits were employed. JMP statistical software (JMP®, Version 7, SAS Institute Inc.; Cary, NC, 1989-2007, NC 27513-2414, USA) was used to statistically analyze the data. T-Student was used to assess the differences between pairwise comparisons, Tukey test, and differences between multiple means ($P<0.05$). Statistical analyses were performed after applying angle transformation to percentage values.

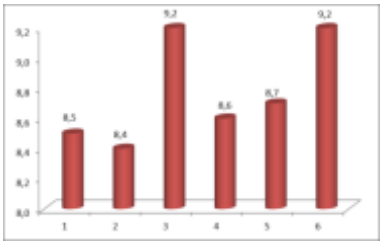
RESULTS AND DISCUSSION

Total Soluble Solid Content (%)

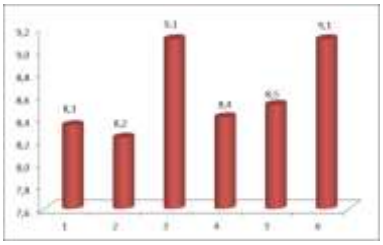
When all hybrids were examined in terms of SÇKM, the highest value was obtained from part 6 (9.2), and the lowest value was obtained from part number 2 (8.4). When the striped hybrids were evaluated within themselves, the highest values were obtained from the 6 (9,1) and 3 (9,1) numbers, and the lowest values were obtained from the 5 (8,5), 4 (8,4), 1 (8,3), 2 (8,2) parts. There was no statistical difference between the parts in hybrids with dark green ground color (Table 3, Figure 2). Aras et al. (2021) reported that soluble solid content ranged from 7,38 to 13,23 in their study.

Table 3. Changes of all hybrids, striped and background dark green in total soluble solid content according to parts.

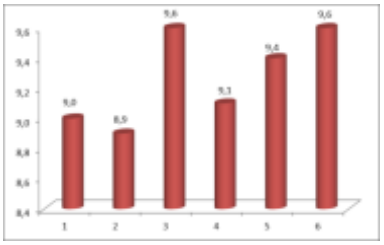
Parts	All Hybrids	Striped Hybrids	Background dark green hybrids
1	8,5 BC	8,3 B	9,0
2	8,4 C	8,2 B	8,9
3	9,2 A	9,1 A	9,6
4	8,6 BC	8,4 B	9,1
5	8,7 B	8,5 B	9,4
6	9,2 A	9,1 A	9,6
CV	5,6	5,7	5,5



All Hybrids



Striped Hybrids



Background dark green hybrids

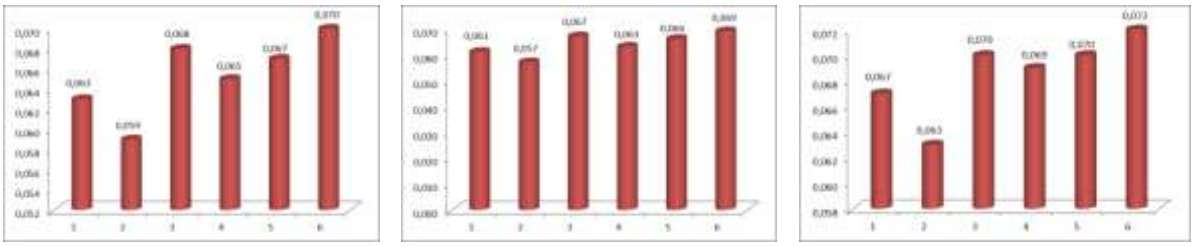
Figure 2. Total soluble solid content changes of all hybrids according, striped and dark green ground color to parts.

Titratable Acidity (%)

When all hybrids were examined in terms of titratable acid, the highest value was obtained from part 6 (0.070), and the lowest value was obtained from part number 2 (0.059). When the striped hybrids were evaluated within themselves, the highest value was obtained from the parts numbered 6 (0.069), and the lowest value was obtained from the part numbered 2 (0.057). In the hybrids with dark green ground color, the highest values were obtained from parts numbered 6 (0.072), 5 (0.070) and 3 (0.070), while the lowest value was obtained from parts numbered 2 (0.063) (Table 4, Figure 3). Aras et al. (2021) reported that pH values ranged from 0,20 to 0,49 in their study.

Table 4. Changes of all hybrids, striped and background dark green in titratable acidity according to parts.

Parts	All Hybrids	Striped Hybrids	Background dark green hybrids
1	0,063 D	0,061 D	0,067 B
2	0,059 E	0,057 E	0,063 C
3	0,068 B	0,067 B	0,070 A
4	0,065 C	0,063 C	0,069 AB
5	0,067 B	0,066 B	0,070 A
6	0,070 A	0,069 A	0,072 A
CV	5,70	5,74	5,58



All Hybrids Striped Hybrids Background dark green hybrids

Figure 3. Titratable acidity changes of all hybrids according, striped and dark green ground color to parts.

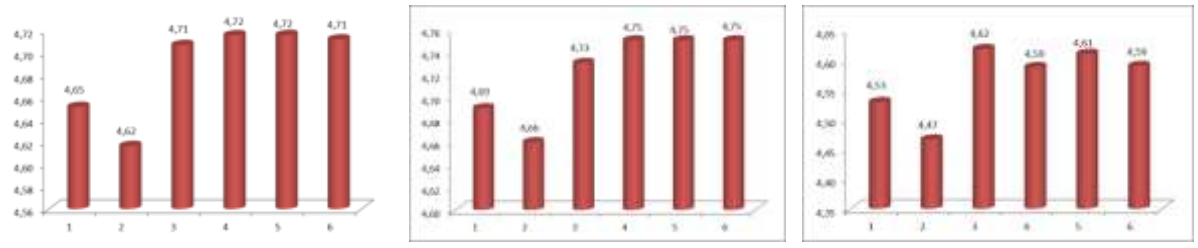
Fruit Juice pH

When all hybrids were examined in terms of pH, the highest values were obtained from parts 4 (4.72), 5 (4.72) and 6 (4.71), and the lowest value was obtained from part number 2 (4.62). When the striped hybrids were evaluated within themselves, there was no statistical difference between the sections. There was no statistical difference between hybrids with dark green ground color (Table 5 , Figure 4).

Aras et al. (2021) reported that pH values ranged from 4.20 to 6.44 in their study.

Table 5. Changes of all hybrids, striped and background dark green in fruit juice pH according to parts.

Parts	All Hybrids	Striped Hybrids	Background dark green hybrids
1	4,65 AB	4,69	4,53
2	4,62 B	4,66	4,47
3	4,71 AB	4,73	4,62
4	4,72 A	4,75	4,59
5	4,72 A	4,75	4,61
6	4,71 A	4,75	4,59
CV	5,53	5,53	5,53



All Hybrids | Striped Hybrids | Background dark green hybrids

Figure 4. Fruit juice pH changes of all hybrids according, striped and dark green ground color to parts.

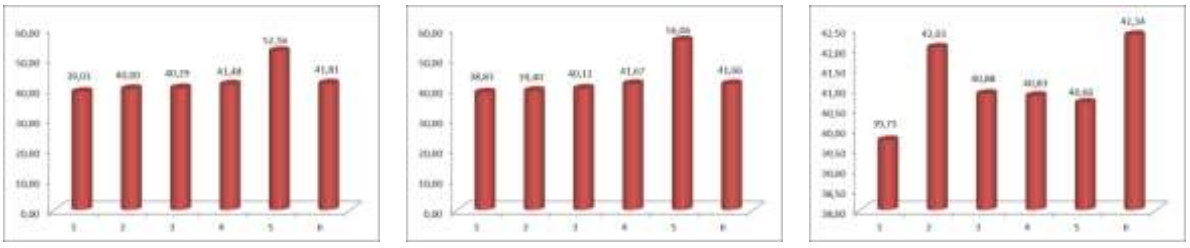
Fruit flesh color L value*

When all hybrids were examined in terms of fruit flesh L* value, the highest value was obtained from part 5 (52,56), and the lowest value was obtained from part number 1 (39,03). When the striped hybrids were evaluated within themselves, the highest value was obtained from part 5 (56,06), and the lowest value was obtained from part number 1 (38,83). In hybrids with dark green ground color, the highest values were obtained from parts 6 (42,34) and 2 (42,03), while the lowest value was obtained from part 1 (39,73) (Table 6, Figure 5).

Tokgöz et al. (2015) in his study, L* color value varied between 35,26 and 37,17. Aras et al. (2021) reported that L* color value ranged from 15,21 to 41,23 in their study.

Table 6. Changes of all hybrids, striped and background dark green in fruit flesh color L* value according to parts.

Parts	All Hybrids	Striped Hybrids	Background dark green hybrids
1	39,03 D	38,83 D	39,73 B
2	40,00 C	39,40 CD	42,03 A
3	40,29 C	40,12 C	40,88 AB
4	41,48 B	41,67 B	40,83 AB
5	52,56 A	56,06 A	40,66 AB
6	41,81 B	41,66 B	42,34 A
CV	6,32	6,51	5,58



All Hybrids | Striped Hybrids | Background dark green hybrids

Figure 5. Fruit flesh color L* value changes of all hybrids according, striped and dark green ground color to parts.

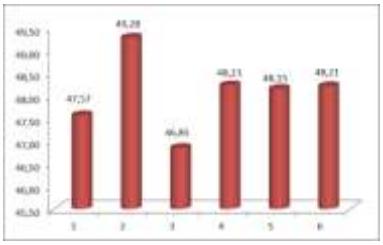
Fruit flesh color h° value

When all hybrids were examined in terms of fruit pulp h° value, the highest value was obtained from 2 (49.28) and the lowest value was obtained from 3 (46.85). When the striped hybrids are evaluated within themselves, the highest values are 2 (49.05), 6 (48.69), 4 (48.23) and (48.20), the lowest value is 1 (46.98) and 3 It was obtained from sections (46.47). In hybrids with dark green ground color, the highest value was obtained from part 2 (50.09), while the lowest value was obtained from part 6 (46.60) (Table 7, Figure 6).

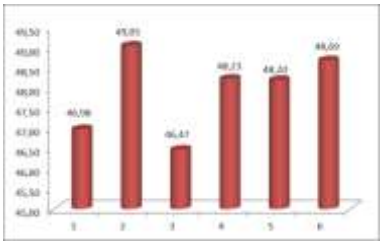
Tokgöz et al. (2015) in his study, the h value ranged between 26.67 and 33.28 on average. Karaca et al. (2012), on the other hand, the h^o values ranged between 35.2-42.3, respectively. Aras et al. (2021) reported that h^o value ranged from 36,21 to 69,12 in their study.

Table 7. Changes of all hybrids, striped and background dark green in fruit flesh color h° value according to parts.

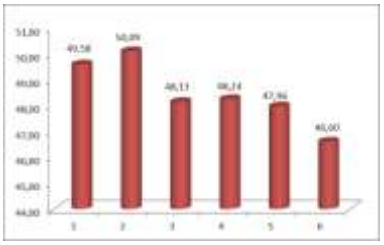
Parts	All Hybrids	Striped Hybrids	Background dark green hybrids
1	47,57 BC	46,98 B	49,58 AB
2	49,28 A	49,05 A	50,09 A
3	46,85 C	46,47 B	48,13 ABC
4	48,23 B	48,23 A	48,24 ABC
5	48,15 B	48,20 A	47,96 BC
6	48,21 B	48,69 A	46,60 C
CV	5,55	5,55	5,56



All Hybrids



Striped Hybrids



Background dark green hybrids

Figure 6. Fruit flesh color h° value changes of all hybrids according, striped and dark green ground color to parts.

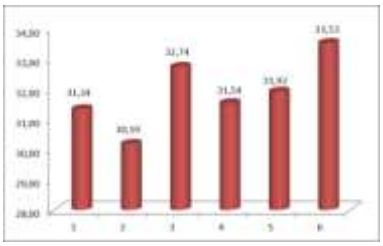
Fruit flesh color C value*

When all hybrids were examined in terms of fruit pulp C* value, the highest value was obtained from part 6 (33.53) and the lowest values were obtained from part 1 (31.34), 4 (31.54) and 5 (31.92). When the striped hybrids were evaluated within themselves, the highest value was obtained from the 6 (34.09) parts, and the lowest values were obtained from the 4 (31.54) and 1 (31.76) parts. In hybrids with dark green ground color, the highest values are obtained from 3 (31.91), 6 (31.62) and 4 (31.55) numbers, while the lowest values are 1 (29.92) and 5 (30.01). It was obtained from part numbered (Table 8, Figure 7).

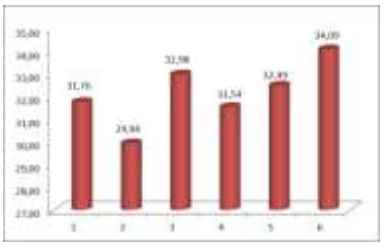
Tokgöz et al. (2015) determined the C* value between 26.72 and 33.25. Karaca et al. (2012), on the other hand, C* values ranged between 28.1-35.9. Aras et al. (2021) reported that C* value ranged from 14,88 to 47,09 in their study.

Table 8. Changes of all hybrids, striped and background dark green in fruit flesh color C* value according to parts.

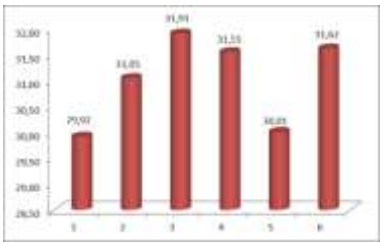
Parts	All Hybrids	Striped Hybrids	Background dark green hybrids
1	31,34 C	31,76 C	29,92 B
2	30,19 D	29,94 D	31,05 AB
3	32,74 B	32,98 B	31,91 A
4	31,54 C	31,54 C	31,55 A
5	31,92 C	32,49 B	30,01 B
6	33,53 A	34,09 A	31,62 A
CV	5,62	5,61	5,66



All Hybrids



Striped Hybrids



Background dark green hybrids

Figure 7. Fruit flesh color C* value changes of all hybrids according, striped and dark green ground color to parts.

CONCLUSIONS

In determining reported health levels, bioactive compounds and antioxidant levels of genotypes of fruits and vegetables are strongly affected by agrotechnical processes, environmental conditions, ripening stage, harvest and postharvest changes as well as external factors (Waterman and Mole, 1994; Abushita et al., 2000; Dumas et al. et al., 2003; Lenucci et al., 2009). In terms of L* value, it can be said that part 5 has the highest values and part number 1 has the lowest values. In terms of ho value, it can be stated that the 2nd part is the highest value and the lowest value is the 3rd part in general. In terms of C* value, it can be said that part 6 has the highest values in general, and the lowest values are generally taken from part number 2. In terms of total soluble solid content, it can be stated that the highest value is taken from the 3rd part and the lowest value from the 2nd part. In terms of titratable acidity, the highest value is taken from part 6 and the lowest value from part 2. In terms of pH, the highest values are taken from the parts 5 and 6, and the lowest value is taken from the part number 2.
In practice, researchers divide the fruit into four and use the middle of any ¼ slice. It is necessary to take a sample from the same place, from the whole watermelon fruit or by dividing it into 6 parts.

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NUTRITIONAL COMPOSITION OF BABY CORN (*ZEA MAYS* L.)

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ABSTRACT

Baby corn (*Zea mays L.*) or maize is dehusked immature maize ear, harvested when the silks have either not emerged or just emerged but prior to fertilization. Baby corn is very adaptable to different weather conditions and high-value crop, gaining popularity fast in domestic and international markets. It is consumed as food at various developmental stages from baby corn to mature grain. Besides fresh consumption, baby corn is used in the preparation of various foods such as canned foods, soups, pickles, snacks and sweet products. Baby corn has a rich profiles of nutrients and phytochemicals when compared with other whole grains. The desirable size of baby corn is 6-11 cm length and 1-1.5 cm diameter with regular row arrangement. The most preferred colour by the consumer is generally creamish to very light yellow. Baby corn is highly nutritive as containing high content of fat, protein, carbohydrate, ash, calcium, phosphorus, ascorbic acid. It has a rich profiles of nutrients and phytochemicals which include vitamins (A, B, E, and K), minerals (Mg, P, and K), dietary fiber, phenolic acids (ferulic acid, coumaric acid, and syringic acid), carotenoids and flavonoids. It is low in calories, high in fiber, and low in cholesterol. Baby corn is an emerging potential crop among the progressive farmers and there is limited information about the nutritional composition. In this presentation, it was aimed to review the chemical composition and nutritional value of baby corn.

Key words: Baby corn, chemical composition, nutrition, phytochemicals

INTRODUCTION

Baby corn (*Zea mays* L.) is immature dehusked, unfertilized maize ear, harvested 1 to 2 days after silking. The desirable size of baby corn is 6-11 cm length and 1-1.5 cm diameter with regular row arrangement. The most preferred colour by the consumer is generally creamish to very light yellow and consumed as vegetable due to its sweet flavor. Being a short duration crop (50-60 days) it can be sown and harvested 3 to 4 times in a year. Baby corn is a suitable crop for cultivation under diverse agroclimatic conditions. High nutritional value, eco-friendly and crispy nature of baby corn has made it special choice for various traditional and continental dishes apart from canning in the elight society (Singh and et al., 2010; Nguyen, 2020).

Chemical composition

Chemical composition composition of baby corn shows variations due to the climate, soil condition and agricultural applications. Fresh cobs of 100 g had moisture 88 g, protein 1.90 g,

carbohydrate 4.0 g, fat 0,2, calories 30 kcal, fiber 2.41 g, ash 46 g, total sugars 415 mg, reducing sugar 300 mg, thiamine 0.06 mg, riboflavin 0, 15 mg, calcium 23 mg and iron 1.97 mg. Organoleptic evaluation of the products showed that all the baby corn products were highly acceptable as compared to the control (Anitha and Rajyalakshmi, 2005). According to Hooda and Kawatra (2013) baby corn contained 5.43 mg/100 g of ascorbic acid and 670 mg/100 g of b-carotene. Calcium, magnesium and phosphorus content of baby corn was 95.00, 345.00 and 898.62 mg/100 g, respectively, baby corn contained 0.05, 2.85 and 0.675mg/g of methionine, isoleucine and leucine, respectively. Baby corn starch has oval shape granules whereas polyhedral shape granules were observed in starches from other corn types. Baby corn starch showed lowest swelling power, solubility, amylose content and mean granule diameter (Sandhu et al., 2004).

Nutritional Value

Baby corn may be consumed as raw or used as an ingredient in various preparations such as soups, deep fried with meat or rice, sauted with other vegetables, pickles, soups, sweets and snack foods. The nutritive value of baby corn is comparable to several high-priced vegetables like cauliflowers, cabbage, french beans, spinach, lady finger, brinjal, tomato, radish, etc. Baby corn, a new vegetable not so popular yet, is an emerging potential crop among the progressive farmers around big cities (Hooda and Kawatra, 2013). Baby corn has unique profiles of nutrients and phytochemicals when compared with other whole grains. Corn nutrients and phytochemicals include vitamins (A, B, E, and K), minerals (Mg, P, and K), phenolic acids (ferulic acid, coumaric acid, and syringic acid), carotenoids and flavonoids (anthocyanins), and dietary fiber. Baby corn is highly nutritive as containing high content of fat, protein, carbohydrate, ash, calcium, phosphorus, ascorbic acid (Das et al., 2009). Its phosphorus level was higher than other vegetables. It is low in calories, high in fiber, and low in cholesterol. It is also free from residual effects of pesticides because it is harvested within a week of silk emergence and pesticide need not be applied during this time frame. Therefore it is also considered as a green food. Regular consumption of baby corn lowers the risk of developing chronic diseases such as cardiovascular disease, type 2 diabetes, and obesity and improves digestive health (Nguyen 2020).

Baby corn is a highly perishable produce because of its high respiration rate. An inexpensive way to extend shelf life is the use of modified atmospheric packaging (MAP) in combination with low temperature. A research was conducted to enhance the shelf life by storing baby corn at 3 degrees C in low density polythene bags of 150, 200 and 300 gauges with different ventilations (0, 0.2, 0.4 and 0.6%) by Vani et al. (2013). Spoilage was delayed and shelf life was extended by 38 days over control and 8 days over the second best treatment when stored in unventilated 150 gauge polybags which had a maximum shelf life of 63 days. Reducing sugars, total sugars, proteins and ascorbic acid were retained with a superior organoleptic score. The longer shelf life and a better quality of cobs in polybags were due to the reduced permeability of the polybags for oxygen and accumulation of carbon dioxide coupled with low temperature and the resultant retarded respiration rate. Hooda and Kawatra (2012) studied the effect of frozen storage of 90 days on nutritional composition of baby corn. Moisture, crude protein, crude fat and crude fibre content of baby corn showed no significant

change during 90 days of frozen storage. A significant reduction was observed in calcium, magnesium, zinc and iron content of frozen baby corn. *In vitro* starch and protein digestibility showed a non-significant change during frozen storage. Ascorbic acid and β-carotene content of frozen baby corn decreased significantly by 11.60% and 10.75 %, respectively, by the end of 90 days of storage. The authors revealed that freezing is an effective processing technology to keep the nutritional value and to enhance the storage life of baby corn.

CONCLUSIONS

Baby corn is highly nutritive and its nutritional quality is at par or even superior to some of the vegetables and whole grains. Besides protein, vitamins and iron, it is one of the richest source of phosphorus and phytochemicals.

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ASSESSMENT OF SALT AND WATER STRESS TOLERANCE OF TUNISIAN SQUASH (*CUCURBITA MAXIMA* DUCHESNE) GERMPLASM DURING THE GERMINATION AND EARLY SEEDLING GROWTH

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ABSTRACT

High salinity in soil or irrigation water and drought are considered as the most common abiotic stresses affecting seed germination and seedling growth especially in arid and semi-arid regions. Seed germination is the most sensitive stage during the life-cycle of many species. Local squash landraces are the important vegetable crops in Tunisia cultivated in open field and under greenhouse. The present study was carried out to assess the salt and water tolerance of four local squash (*Cucurbita maxima* Duchesne) landraces (Batati, Galaoui, Karkoubi and Bejaoui). Different salt (NaCl) and D-Mannitol concentrations of 0, 100, 200 and 300 mM were selected in order to evaluate the response of the study germplasm based on germination potential and agro-morphological traits of seedlings. A varied effect of the salt and water stress level was observed among the studied landraces. Results showed that all landraces were drastically affected at high stress NaCl and D-Mannitol level with a significant variation in their stress response, indicating the existence of considerable genetic variability. Under salt stress, landraces NGB746 (Batai) and 747 (Galaoui) were the best performing cultivars across stress levels, while under drought stress NGB751 (Bejaoui) proved as the most capable to germinate under the high water deficit. The seedling traits (shoot and root length, shoot and root fresh weight...) were more sensitive under drought stress than NaCl stress, nevertheless, root elongation was more affected under both stress than shoot growth. These findings can be extrapolated into efforts to develop more salt and water tolerant squash landraces and exhaust the possibilities of using saline water or soils under changing climate conditions.

Keywords: *Cucurbita sp;* landraces; salinity and water stress; seed germination; seedling growth

INTRODUCTION

Soil salinity and drought are two major abiotic stresses affecting food crops and food security worldwide. More than 20% of all cultivated land are affected by salinity and approximately half of irrigated lands are affected by drought stress (Abdelraheem *et al*., 2019). The later authors reported that more than 50% of the arable land lost worldwide was caused by the combination of drought and salinity. It was pointed out that plant responses to salinity and drought are often similar; the first phase of salinity stress, the osmotic effect, is quite similar to that of drought stress (El Harfi et al., 2016; Peng-cheng et al., 2021). The sensitivity of many species to these two abiotic stresses can be expressed throughout their life cycle; however, the germination phase is the most sensitive. In fact, beside water scarcity, saline water used for irrigation or high soil salinity may exert an adverse effect upon seed germination in field, exposing the seeds to stress (Almodares et al., 2007). Increased salinity leads to a reduction and/or delay in germination of both halophyte and glycophyte seeds (Albregts and Howard, 1973). Plant roots have effective mechanisms to sense low water potential that result either from an actual lack of water in the environment, due to low precipitation, or to the presence of excess salt ions in the water present. In both cases, plants cannot uptake sufficient water for normal growth and development and common stress related signal transduction pathways are activated, for example increased levels of ABA. Immediately following the induction of these stress related signal transduction pathways, under drought or salinity, shoot growth, especially leaf growth, is greatly reduced. A significant contributor to this growth reduction is associated with a change in cellular oxygen and carbon dioxide levels due to the partial closure of stomata. Plant cells also produce various reactive oxygen species (ROS) in response to both salt and drought stress. Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Mohammadizad et al., 2013; El Harfi et al., 2016). For many species earlier growth stages are more sensitive to salinity and drought than subsequent ones (Abd El-Mageed and Semida, 2015). Although most plant species are salt-sensitive at all stages of their lifecycle, their sensitivity differs among growth stages (Acosta-Motos et al., 2017), with seed germination being viewed as the most critical stage when salt stress impairs water absorption during seed imbibition and turgescence (Golbashy et al.,2012). Flowers et al. (2015) and Orlovsky et al (2016) pointed out that at this stage, salt stress is expressed through the reduction in germination percentage, delayed germination rate and inhibited tissue elongation. The increased Na^+ and Cl^- ion concentrations induces ionic toxicity, oxidative stress and nutritional imbalance as well as water stress, by lowering the osmotic potential of soil solution, ultimately leading to inhibition of germination in many species (Shahid et al., 2020). OKÇU et al. (2005) reported that salinity has also been identified as the major seedbed factor influencing establishment in arid and semi-arid regions and the germination and seedling growth are reduced in saline soils with varying responses for species and cultivars.

Squash (*Cucurbita maxima* Duch.) belonging to *Cucurbitacae* family, is one of the most important cucurbits species cultivated in Tunisia from the north to the south regions (Hamdi et al., 2017). Although, soil salinization is gradually increasing due to the scarcity of rains and the increase in evapotranspiration, affecting plant germination, growth, development and fruit setting in salt-affected soils, as is the case of semiarid regions. Considering the climate change inducing both drought and salt stresses, the research for salt and water-tolerant germplasm is

one of the most effective means for enhancing squash production in Tunisia. This study aimed at investigating the response of the four main type of squash cultivated to salt and drought stress at germination stage and examining the potential of early selecting salt-water- tolerant genotypes at laboratory level. Different salt (NaCl) and D-Mannitol concentrations of 0, 100, 200 and 300 mM were selected in order to evaluate the response of the study germplasm, and their response was assessed on the basis of traits related to seed germination and seedling growth potential.

MATERIALS AND METHODS

Plant material and growth conditions

Based on the agro-morphological and physiological characterization of the collection of local landraces (Hamdi et al., 2019 a et b), we selected four landraces representing the main types of cultivated squash; Batati orange "746", Galaoui "747", Karkoubi orange "748" and Bejaoui green "751" to evaluate their responses to salt and drought stress using different NaCl and D-Mannitol concentrations (0, 100, 200 and 300 mM). Each landrace was assigned passport data and an inventory number, according to the National Gene Bank of Tunisia, while full details are available at the Germplasm Resources Information Network—GRIN (http://www.tn-grin.nat.tn/gringlobal/search. aspx, accessed on 15 February 2022). The description of fruit morphology (table 1) was performed based on the European Cooperative Program for Plant Genetic Resources (ECPGR) list of descriptors for Cucurbita spp. (ECPGR, 2008).

Table 1. Description of the Tunisian squash landraces employed in this study

Landrace Inventory #	Local Name	Origin	Short Description
NGBTUN746 ("746")	Batati Orange	Siliana (Sidi Hamada)	Globular fruit, orange skin, light orange flesh
NGBTUN747 ("747")	Galaoui	Ariana (Kalaa Andalous)	Raised fruit with basal tip, green skin, green flesh
NGBTUN748 ("748")	Karkoubi Orange	Sousse (SidiBouali)	Flattened fruit, dark yellow skin, yellow flesh
NGBTUN751 ("751")	Bejaoui Green	Siliana (Sidi Hamada)	Flattened fruit, dark green skin, light green flesh

The experiment was carried out at the Department of Horticulture, High Agronomic Institute of Chott Mariem-Sousse-Tunisia. Following the selection of seeds for size homogeneity, 50 seeds per landrace were surface-sterilized for 5 min in 10% H_2O_2 (v/v) and rinsed twice in sterile H2O. Sterilized seeds were primed via exposure to an eliciting solution of 1.5 mM gibberellic acid (GA_3) for 24 hours, to stimulate germination, and subsequently rinsed in sterile H_2O. Five to ten seeds, according to size, were placed on sterile petri dishes containing two

layers of filter paper moistened daily with 5 mL of appropriate solutions: H_2O (control), 100, 200 and 300 mM NaCl or D-Mannitol concentrations. Seedlings were grown under controlled conditions for 15 days (25 ± 2 °C, 50 ± 5% relative humidity, 18-h light/6-h dark photoperiod under white fluorescent light (40 µmol m^{-2} s^{-1}).

Determination of germination and seedling growth potential under salt and drought stress

Various parameters related to seed germination and seedling growth potential under salt and drought stress conditions were measured; seeds were considered germinated when the protruding radicle was at least 2 mm long.

- Germination percentage $GP = \frac{\text{number germinated seeds}}{\text{number of total seeds}} x\ 100$
- Shoot and Root length (cm)
- Shoot and Root fresh weight (g)
- Shoot length /Root length Ratio
- Germination Reduction (GP of control-GP of stress plant)
- Shoot and Root reduction length (Shoot or Root length of control – Shoot or Root length of stress plant)

Water seed absorbance $(WU) = \frac{\text{initial seed weight}}{\text{seed weight following water absorbance}} * 100$ (Partheeban et al., 2017)

- Seed vigor index (SVI) = (Root length + Shoot length) * GP % (Hannachi et al., 2018)
- Root length stress index (RLSI) :
  $RLSI = \frac{\text{RL under stress condtions}}{\text{RL under normal conditions}} x100$ (Partheeban et al.,2017)

- Shoot length stress index (SLSI): $SLSI = \frac{\text{SL under stress condtions}}{\text{SL under normal conditions}} x100$ (Partheeban et al.,2017)

Statistical analysis

The experimental layout was completely randomized with three replications. Data were analyzed using ANOVA tests ($P \leq 0.05$), according to the experimental design, combining salt and water concentrations and landraces. Differences between means were compared using the Duncan Multiple Range test (DMRT). Statistical analyses were performed using SAS software V9 (SAS Institute, Cary, North Carolina, U.S.) and Figures were drawn using Excel software.

RESULTS

For a better understanding of the effects of salt and water stress, the results will be presented separately while the discussion will be common.

Effect of the landrace and the salt stress level on traits related to seed germination and seedling growth potential

Among landraces NGB747 (Galaoui) and NGB746 (Batati orange) presented the highest germination percentage (GP) under normal conditions (98.7% and 97.3%, respectively), while NGB751 (Bejaoui green) showed the lowest GP (80%) indicating that seed longevity is a genotype dependant variable (Fig.1.1). Nevertheless, under salt stress the germination of all landraces was severely affected with the high NaCl concentrations. At 100 mM the GP was arranged between 30% and 45%; NGB747 landrace proved as the most capable with the high germination rate. The late landrace associated to NGB746 landrace showed a moderate GP at 200mM NaCl (35%) as well as at 300mM (25% and 20%, respectively) and proved as the best performing landraces under salt conditions, while NGB751 presented the lowest value (Fig.1.1). Furthermore, Figures 1.2 and 1.3 showed a drastically reduction of shoot and root length compared to the control with the root length being more affected by different salt stress concentrations. At 100 and 200 mM NaCl, NGB751 has the highest root length followed by NGB746 indicating, despite the low GP of NGB751, their abilities to withstand high levels of salt stress and would be recommended in salt soil or salt water irrigation. The shoot fresh weight and root fresh weight decrease as NaCl stress increases in all landraces (Table 2) with the distinction of NGB751 landrace at both 100 and 200mM NaCl, while NGB746 presented the lowest values. Although at all stress levels, the shoot/root reduction (SRR)was more affected especially at 300mM NaCl with landraces showing varying ratio ranging from 1.32 (NGB751) and 7 (NGB747). These results reflected the more drastical effect of NaCl stress on the root than on the shoot plant parts.

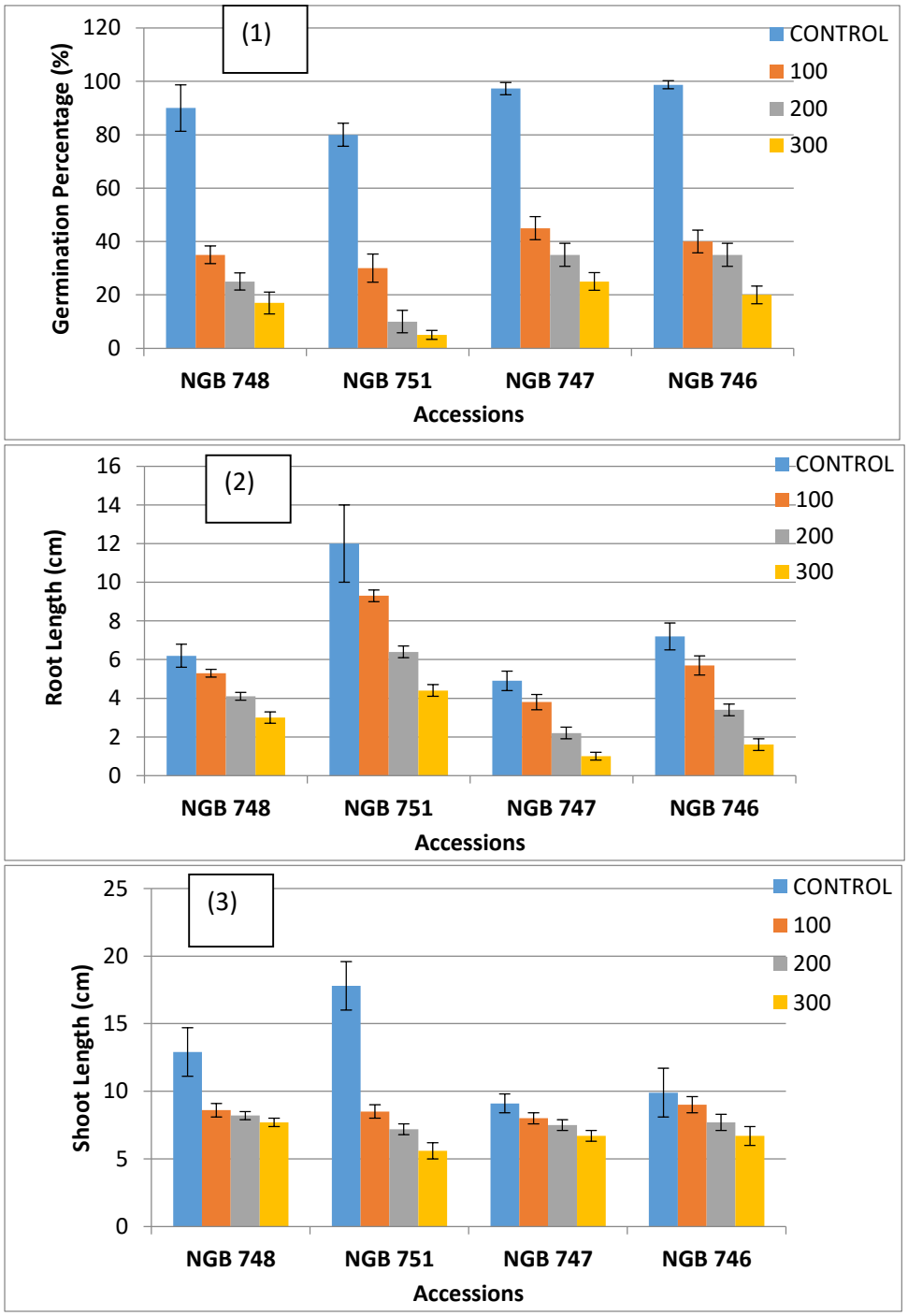

Fig. 1: Response (means ± SD, n=50) of the four squash landraces to salt stress treatments at different concentrations, depicted for the Germination percentage (1), Root length (2) and Shoot length (3)

Table 2. Response of squash germplasm to varying salt stress levels (0, 100, 200 and 300 mM NaCl) in relation to traits (means ± SD) related to Shoot fresh (SFW), Root fresh weight (RFW) and Shoot and Root length reduction (SRR)

NaCl concentration (mM)	Accession	SFW (g)	RFW (g)	SRR
Control	NGB746	0.82* ± 0.1	0.04 ± 0.01	1.41 ± 0.2
	NGB747	0.71 ± 0.4	0.07 ± 0.03	1.92 ± 0.2
	NGB748	1.10 ± 0.3	0.15 ± 0.02	2.11 ± 0.4
	NGB751	1.33 ± 0.2	0.14 ± 0.02	1.51± 0.1
100	NGB746	0.62 ± 0.2	0.03 ± 0.05	1.63± 0.2
	NGB747	0.68 ± 0.04	0.04 ± 0.01	2.12 ± 0.2
	NGB748	0.92 ± 0.2	0.10 ± 0.03	1.61 ± 0.1
	NGB751	1.00 ± 0.04	0.11 ± 0.01	0.93 ± 0.1
200	NGB746	0.50 ± 0.04	0.01 ± 0.00	2.32 ± 0.3
	NGB747	0.55 ± 0.03	0.01 ± 0.00	3.51 ± 0.5
	NGB748	0.74 ± 0.03	0.08 ± 0.03	2.00 ± 0.1
	NGB751	0.85 ± 0.04	0.08 ± 0.01	1.12 ± 0.1
300	NGB746	0.50 ± 0.01	0.01 ± 0.00	4.00 ± 0.9
	NGB747	0.47 ± 0.03	0.01 ± 0.00	7.00 ± 1.4
	NGB748	0.52 ± 0.1	0.07 ± 0.01	2.63 ± 0.2
	NGB751	0.35 ± 0.03	0.04 ± 0.01	1.32 ± 0.1

* Mean ±SD

Effect of water stress and landraces on traits related to seed germination and seedling growth potential

Based on the analysis of variance applied on individual data, all traits related to germination and seedling growth potential under water stress conditions were differentially affected by the landrace and D-Mannitol stress level applied. In the absence of stress, germination was considerably affected by the landrace, thus substantiating a variable germination potential, which could be mainly attributed to the median longevity of seeds. Among landraces, "751" and "748" presented the highest and lowest GP under normal conditions (97.77 % and 85.77 %, respectively) which also justifies that seed longevity is a genotype-dependent variable (Table 3). Upon water stress, the germination of all landraces was severely affected. At 100 mM D-Mannitol, "751" proved to be the most capable landrace of retaining a high germination rate (96.55 %). In contrast, "747" landrace showed difficulties in germination and had low GP (68.33 %) compared to other landraces which justifies that "747" landrace was incapable to germinate at D-Mannitol stress levels. Also, at 200 mM D-Mannitol, "751" presented the highest GP (83.33 %), whereas "747" ranked as the landrace with the lowest GP value (55.55 %). Finally, at 300 mM D-Mannitol, "746" followed by "751" proved to the best performing landraces (66.77 % and 63.33 %, respectively). While, "747" suffered great losses at 300 mM D-Mannitol level (33.33 %). Furthermore, the response of all landraces to water stress involved a drastic reduction in both SVI and WU. At 100 mM D-Mannitol level, "748" presented the highest value for SVI (856.27 %) and "751" ranked as the landrace with the highest WU value (96.55 %). Moreover, at 200 mM D-mannitol level, "751" presented the highest values for SVI and WU (676.69 % and 83.33 %, respectively). At 300 mM D-Mannitol, for SVI, the highest value was recorded to"751" landrace (512.61 %) and for WU, the highest value was recorded to "746" landrace and it was equal to 66.77 % (Table 3).

Generally, watering with increasing concentrations of mannitol in germination phase showed inhibitory effect on the length of squash shoots for all landraces. Additionally, statistical analysis showed their inhibitory effects on the growth of squash roots. The highest concentrations of mannitol (300 mM) were completely inhibited the development of shoots and roots of Galaoui landrace (NGBTUN747). Plants watered with mannitol during germination phase, had the shortest remainder of shoot and root in comparison with control (Table 5).

Table 3. Response of squash germplasm to varying D-Mannitol levels (0, 100, 200 and 300 mM) in relation to germination and seedling characteristics.

D-Mannitol concentration (mM)	Landrace	GP (%)	SVI (%)	WU
Control	" 748 "	85.77*±2.64	1776.88±38.53	88.77±2.64
	" 751 "	97.77±1.44	1287.58±40.90	96.66±1.44
	" 747 "	96.66± 2.22	1581.41±28.90	97.77±2.22
	" 746 "	92.22±2.64	1572.39±50.82	92.22±2.64
100	" 748 "	74.11±3.86	856.27±49.23	74.11±3.86
	" 751 "	96.55±1.05	755.28±20.76	96.55±1.05
	" 747 "	68.33±3.90	778.76±9.76	68.33±3.90
	" 746 "	82.88±1.73	789.74±43.48	82.88±1.73
200	" 748 "	68.88±2.68	548.13±13.52	68.88±2.68
	" 751 "	83.33±4.63	676.69±13.85	83.33±4.65
	" 747 "	55.55±1.30	636.055±29.70	55.55±1.30
	" 746 "	75.77±1.35	426.03±12.77	75.77±1.35
300	" 748 "	50.00±4.08	183.54±29.90	50.00±4.08
	" 751 "	63.33±1.44	512.61±13.53	63.33±1.44
	" 747 "	33.33±5.27	330.08±9.11	33.33±5.27
	" 746 "	66.77±1.47	215.34±18.59	66.77±1.47

* Mean ±SD

GP: germination potential, SVI: seed vigor index; WU: water seed absorbance.

Biometric analysis of four local accessions squash, growing from grains germinated on Petri's dishes with different concentrations of mannitol (0, 100, 200, 300 mM) during growth, showed statistically significant differences in the length of roots and shoots) (**Table 4**). Plants of NGBTUN748 landrace watered with mannitol solution at a concentration of 300 mM during growth had the shortest roots (2.55 cm), a decrease of 32.69 % in comparison with control group (7.80 cm). The roots of the NGBTUN747 landrace treated with 300 mM D-Mannitol wilted after treatment. Statistically significant differences in shoot length were observed in different concentrations of mannitol (100, 200 and 300 mM) in relation to the shoots of seedlings growing on the Petri's dishes with distilled water (control). In the case of high concentration of

mannitol (300 mM), shoots of Karkoubi pink landrace (NGBTUN748) reached about 5.14 cm, a decrease of 35.58 % in comparison with length shoots of control seedlings. In low concentration (100 mM), the length of shoots of the seedlings was about 32.83 % less than the control length shoots of the seedlings. For Bejaoui green landrace (NGBTUN751), in the case of high concentration, a decrease of 73.74 % was observed. In low concentration, the length of shoots was about 19.74% less than the length of shoots of the control seedlings. For Galaoui landrace (NGBTUN747), Plants watered with mannitol solution at a concentration of 200 mM during germination phase had the shortest shoots (4.40cm) in comparison with control group (10.60 cm), a decrease of 58.49 %. However, shoots of Galaoui landrace (NGBTUN747) treated with 300 mM D-Mannitol wilted after treatment. For Batati orange landrace (NGBTUN746), Significant differences in the length of shoots was found between squash plants which had grown from grains germinating on dishes with 100, 200 and 300 mM of D-Mannitol and plants watered with distilled water (**Table 4**).

Table 4. Effect of Mannitol levels and landraces on traits related to seedling growth potential of the four landraces.

Treatment								
Accessions	Control		100 mM		200 mM		300 mM	
	RL (Cm)	SL (Cm)	RL (Cm)	SL (Cm)	RL (Cm)	SL (Cm)	RL (Cm)	SL (Cm)
NGBTUN748	7.80± 1.78	7.98 ± 1.12	5.56± 1.01	5.36± 0.97	3.00 ±0.96	5.96 ±1.19	2.55± 1.10	5.14± 0.90
NGBTUN751	6.76± 1.83	10.13± 1.15	4.65± 1.19	8.13± 1.09	4.60± 1.31	6.23± 1.82	3.00± 0.87	2.26± 1.01
NGBTUN747	7.85± 0.85	10.60± 1.22	6.93± 0.96	5.86± 1.16	5.81± 1.05	4.40± 0.95	0.00± 0.00	0.00± 0.00
NGBTUN746	4.56± 1.07	10.02± 0.52	3.94± 1.07	5.63± 1.16	4.25 ± 0.89	5.55 ± 1.19	3.38 ± 1.17	5.53± 1.60

According to the results of fresh matter of roots and shoots of four landraces of squash seedlings, it was indicated that mannitol-induced reduction of fresh matter of shoots and roots for all landraces. The shoot/root ratio (SSR) ranged from 1.629 (control) to 1.852 (100 mM D-mannitol) for Bejaoui green landrace (NGBTUN751) and ranged from 1.087 (control) to 2.149 (200 mM D-Mannitol) and 2.365 (300 mM D-Mannitol) for Karkoubi pink landrace (NGBTUN748), suggesting that water stress is more pronounced on root elongation than on shoot (Table 6).

The reduction of germination percent (RG %), was more important for seeds treated with 100 mM of D-Mannitol for the landrace Galaoui (NGBTUN747) to reach a value of 16.66 %. For seeds treated with 200 mM of D-Mannitol, the reduction of germination percent was more important for the landrace Galaoui (NGBTUN747) and less important for the landrace Batati orange (NGBTUN746) and ranged from 3.33 % (Batati orange) to 30 % (Galaoui). The reduction of germination percent was important with increasing D-Mannitol levels (300 mM) and the landrace Bejaoui green (NGBTUN751) has the highest value (50 %) and the local accession Batati orange (NGBTUN746) has the lowest value (6.66 %).

Table 5. Effect of Mannitol levels and landraces on fresh weight of shoot and root of the four squash landraces.

Treatment	Accessions	RFW (g)	SFW (g)	SL / RL
Control	NGBTUN748	0.67 ± 0.08	0.59 ± 0.19	1.08 ± 0.31
	NGBTUN751	0.03 ± 0.02	0.32 ± 0.09	1.62 ± 0.57
	NGBTUN747	0.02 ± 0.02	0.32 ± 0.10	1.35 ± 0.14
	NGBTUN746	0.07 ± 0.02	0.57 ± 0.11	2.30 ± 0.58
100 mM	NGBTUN748	0.59 ± 0.19	0.32 ± 0.08	0.97 ± 0.17
	NGBTUN751	0.02 ± 0.01	0.26 ± 0.09	1.85 ± 0.52
	NGBTUN747	0.014 ± 0.01	0.19 ± 0.08	0.83 ± 0.12
	NGBTUN746	0.05 ± 0.02	0.45 ± 0.13	1.46 ± 0.22
200 mM	NGBTUN748	0.18 ± 0.09	0.27 ± 0.14	2.14 ± 0.77
	NGBTUN751	0.02 ± 0.03	0.18 ± 0.10	1.46 ± 0.58
	NGBTUN747	0.013 ± 0.01	0.17 ± 0.08	0.74 ± 0.07
	NGBTUN746	0.04 ± 0.01	0.40 ± 0.13	1.31 ± 0.14
300 mM	NGBTUN748	0.06 ± 0.02	0.22 ± 0.08	2.36 ± 1.18
	NGBTUN751	0.004 ± 0.001	0.17 ± 0.09	0.73 ± 0.20
	NGBTUN747	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	NGBTUN746	0.02 ± 0.01	0.28 ± 0.09	1.95 ± 1.28

RFW: root fresh weight; SFW: shoot fresh weight; SL/RL: shoot length/root length ratio

Table 6. Effect of Mannitol levels and landraces on root length reduction (RLR), shoot length reduction (SLR) and germination reduction (GR).

Treatment	Accessions	RLR (%)	SLR (%)	GR (%)
100 mM	NGBTUN748	2.233* ± 1.423	2.038 ± 0.850	11.666 ± 2.134
	NGBTUN751	2.211 ± 1.332	2.000 ± 1.574	0.000 ± 0.000
	NGBTUN747	0.916 ± 0.327	4.783 ± 1.041	16.667 ± 2.285
	NGBTUN746	0.622 ± 0.389	4.388 ± 1.041	3.337 ± 1.855
200 mM	NGBTUN748	4.800 ± 1.814	2.622 ± 0.720	16.888 ± 4.164
	NGBTUN751	2.166 ± 0.868	3.900 ± 0.851	14.444 ± 5.299
	NGBTUN747	1.983 ± 0.392	6.200 ± 1.257	30.000 ± 3.611
	NGBTUN746	0.316 ±0.146	4.472 ± 1.038	3.337 ± 1.732
300 mM	NGBTUN748	5.244 ± 0.724	2.842 ± 0.978	35.777 ± 4.155
	NGBTUN751	3.766 ± 0.513	7.866 ± 1.695	50.000 ± 1.689
	NGBTUN747	7.850 ± 0.892	10.600 ± 1.224	30.000 ± 5.031
	NGBTUN746	1.183 ± 0.420	4.488 ± 1.609	6.666 ± 2.279

* Mean ±SD

The physiological indices such as reduction root length (RRL), reduction shoot length (RSL), root length stress index (RLSI) and shoot length stress index (SLSI) were calculated, and the significant differences were recorded. The most important reduction of the root length (RRL) was recorded for landraces Karkoubi pink (NGBTUN748) and Bejaoui green (NGBTUN751) treated with 100 mM of D-Mannitol (2.23 and 2.21 % respectively). The lowest value of reduction root length was recorded to landraces Galaoui (NGBTUN747) and Batati orange (NGBTUN746) treated with 100 mM of D-Mannitol (0.91 and 0.62 % respectively). For 200 mM of D-Mannitol, the most important reduction of the root length (RRL) was recorded to the landrace Karkoubi pink (NGBTUN748) (4.80 %), whereas the lowest value was recorded to Batati orange landrace (NGBTUN746) (0.31 %). Galaoui landrace (NGBTUN747) treated with 300 mM of D-Mannitol recorded the highest value for the reduction root length (RRL) (7.85 %) and Batati orange landrace recorded the lowest one, 1.18 % (**Table 7**). The reduction of shoot length (RSL) was more important for Galaoui landrace (NGBTUN747) with increasing D-Mannitol levels and ranged from 4.78 % (100 mM D-Mannitol) to 10.60 % (300 mM D-Mannitol) (**Table 7**). The lowest values of RLSI were noted on stressed seedlings of Bejaoui green landrace (NGBTUN751) with 100 mM D-Mannitol (70.52 %), on stressed

seedlings of Karkoubi pink landrace (NGBTUN748) with 200 mM D-Mannitol (39.60 %) and on stressed seedlings of Galaoui landrace (NGBTUN 747) with 300 mM D-Mannitol (0.00 %).

Table 7. Effect of Mannitol levels and landraces on root length stress index, shoot length stress index and drought stress index.

Treatment	Accessions	RLSI	SLSI	DSI
100 mM	NGBTUN748	73.084 ± 4.183	67.120 ± 2.484	86.122 ± 2.644
	NGBTUN751	70.524 ± 5.792	81.092 ± 4.332	100.000 ± 0.000
	NGBTUN747	88.173 ± 4.471	54.442 ± 1.741	70.138 ± 4.127
	NGBTUN746	86.089 ± 2.781	56.106 ± 3.465	90.167 ± 1.786
200 mM	NGBTUN748	39.602 ± 4.254	75.480 ± 5.488	81.053 ± 4.297
	NGBTUN751	71.955 ± 8.249	62.901 ± 6.903	85.555 ± 5.298
	NGBTUN747	74.312 ± 2.157	41.677 ± 2.848	80.972 ± 3.546
	NGBTUN746	93.910 ± 2.800	55.283 ± 3.586	83.604 ± 1.520
300 mM	NGBTUN748	34.897 ± 6.116	64.827 ± 3.259	58.388 ±4.579
	NGBTUN751	45.310 ± 3.698	22.849 ± 3.618	17.777 ± 1.689
	NGBTUN747	0.000 ± 0.000	0.000 ± 0.00	33.888 ± 5.127
	NGBTUN746	76.812 ± 9.122	55.246 ± 5.180	72.719 ± 1.864

RLSI: root length stress index; SLSI: shoot length stress index DSI: drought stress index.

DISCUSSION

In view of the constraints imposed by climate change, which generate abiotic stresses (salinity and drought), the selection of germplasm tolerant to these stresses has become an international priority. Kavi Kishor et al (2018) reported that screening of some species genotypes for abiotic stress tolerance and subsequent attempts to breed them for drought and salt stresses has been slow due to the rarity of alleles for these stresses. Further, gene introgression from the wild-type into the cultivated varieties will be difficult due to incompatibility barriers and laborious backcrossing methods. Based on the sensitivity of the first phase of the life cycle of sexually reproducing species such as squash, the evaluation of stress tolerance during the germination phase is an approach for early selection of plant material (Matkovic Stojšin et al., 2022). Evaluating the tolerance of 4 local squash landraces towards salt and drought stress during germination process and seedling growth, our findings revealed that these abiotic stresses affect all the traits associated to the germination potential and seedling characterization compared to untreated seeds (Fig.1 and Table 3). These results are consistent

with Sayar et al. (2010) who evaluated the tolerance of 2 wheat genotypes towards salt and drought stress and affirm that the germination and emergence rates were delayed by both stress in both varieties, with differences between genotypes among growth stages. Salinity (NaCl) may also affect germination by facilitating the intake of toxic ions, which may change certain enzymatic or hormonal activities such as ABA of the seed (Smith and Comb, 1991). These physicochemical effects upon the seed seem to result in a slower and/or lower rate of germination or seedling growth. All landraces were drastically affected by these stresses, especially at high stress level (200 and 300mM of NaCl or D- Mannitol), their tolerance varied significantly indicating the existence of an important genetic variability in the studies 4 landraces. In fact, NGB747 (Galaoui) and NGB746 (Batati orange) were the best landraces under salt stress but didn't show the same tendency under water stress (Table 3) indicating that the effect of these stress is different at the germination stage . The seedling traits (shoot and root length, shoot and root fresh weight...) were more sensitive under drought stress than NaCl stress, nevertheless, root elongation was more affected under both stress than shoot growth. Such result support previous evidence that root and shoot lengths are the most suitable traits for evaluating salt and drought stress (Janghel et al., 2020; Azeem et al., 2020) since roots are responsible for water uptake and shoot for supplying aerial parts with water. These findings are in accordance with those of Shahi-Gharahlar et al. (2010b) who reported that increasing salinity concentration decreased seedling fresh weight of cumin and with Jamaati-e-Somarin et al. (2010) who indicated that increasing osmotic potential decreased seedling fresh weight in canola and amount of effect was different depend on cultivar and levels of stress. It is evident that the germination and seedling emergence from laboratory results does not necessarily represent germination and seedling emergence from field soils. Still the most important agronomic question is whether the observed differences in salt and water tolerance during early stages are representative of these stresses of the four landraces during the whole growth cycle.

CONCLUSIONS

Soil salinization and water deficit are gradually increasing over the world due to the scarcity of rains especially in arid and semi-arid regions such as Tunisia. These two abiotic stresses limit the germination potential and seedling growth as well as the flowering and fruiting in many vegetable species. Germination phase is the most sensitive stage to these abiotic stresses. The evaluation of stress tolerance during this life cycle phase is an approach for early selection of plant material and can be useful for breeding programs. Despite the scientific research in relation to salt and drought stress, for squash, representing the most important cucurbits in Tunisia, there is a lack of data and breeding programs. The development of salt and water stress tolerant germplasm, based on germination potential and seedling growth, is (i) the most effective means for improving squash productivity and farmers incomes and (ii) and constitutes a source of plant material for varietal improvement programs of this species, remaining until now, dependent on standard imported varieties or self-produced local accessions.

Authors' Contributions

Conceptualization: N.T. and W.S.; Data curation: W.S. H.F. and R.K.; Formal analysis: K.H.; Funding acquisition: L.B. and R.K; Methodology: N.T. and W.S.; Visualization: L.B. and H.F.; Writing - original draft: N.T. and W.S; review and editing/ L.B.

All authors read and approved the final manuscript.

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CONTRIBUTIONS OF LIVESTOCK MARKETS TO THE MUNICIPAL BUDGETS IN THE REPUBLIC OF BENIN

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ABSTRACT

Like many African countries, Benin has adopted the decentralization system as its mode of state management. It is in this perspective that after the decentralization, the municipalities have acquired the full power to mobilize resources to be independent from the central administration. In view of this, resources are mobilized at the local level to contribute to the revenues of the municipal administration. The financial resources mobilized for this purpose by municipalities include the Local Development Tax (TDL). Market infrastructure, including livestock markets, also contributes to the TDL. Livestock markets, through TDL, contribute to the financial mobilization of municipalities of Benin. In this study, primary data were collected from Self-Managed Livestock Markets (MBA) and Traditional Livestock Markets (MT) of Gogounou, Nikki, Bassila, Matéri, Savè and Kétou municipalities in the Republic of Benin. The MTs are the oldest livestock trading system in the study area. The MBAs are the modern livestock markets where transactions are under strict control. In both livestock markets, cattle, sheep and goat are traded. The results showed that the MBAs contributed 12.74%, 5.22%, 8.25%, 3.12%, 2.86%, and 8.46% respectively to the annual municipal budgets in Gogounou, Nikki, Bassila, Matéri, Savè, and Kétou. However, MTs in the same municipalities contributed to the municipal budgets 1.43%, 0.52%, 0.54%, 0.56%, 0.36%, and 0.28% respectively. Given the economic importance of livestock markets in the municipalities of Benin, it is imperative to make substantial investments to increase their potential. This would increase the financial autonomy of these municipalities.

Keywords: Self-managed livestock markets, Traditional livestock markets, Local Development Tax, Municipal budget, the Republic of Benin

INTRODUCTION

Livestock markets, especially in rural areas, represent an important part of annual household income and capital and are important financial resources for municipalities. Some municipalities even rely on animal markets to finance local projects (Onibon, 2004; Moussa, 2014).

In most democratic African countries, the central state, through decentralisation laws, gives municipalities full power to create and manage market infrastructure, including livestock markets (Onibon, 2004; Abdoulaye, 2014). Therefore, the creation or rehabilitation of markets will make it easier for municipalities to obtain resources and thus make them more independent of external income variability (Samba et al., 2013).

Self-managed livestock markets (MBA) and traditional livestock markets (MT) are the two types of market studied in this paper. MBAs are the new model of animal marketing. It was created by farmers and traders. Whereas MTs are the old system of animal marketing. MBA are not a government creation but they are subject to the law. That is why they pay taxes to the municipalities. As for the MTs, they are public and therefore governmental.

This study aims to evaluate the contribution of livestock markets, here MBA and MT, to the budget of six municipalities in the Republic of Benin. For this purpose, the budgets of the six communes, the taxes paid by the MBAs and the MTs to the municipalities were evaluated.

MATERIAL AND METHOD

In this study, primary data were collected from both Self-Managed Livestock Markets (MBA) and Traditional Livestock Markets (MT) of Gogounou, Nikki, Bassila, Matéri, Savè and Kétou municipalities in the Republic of Benin. For comparison, data and information for both types of animal markets were obtained from the municipal authorities in the six districts selected in the study, by means of questionnaires filled in accordance with the purpose. In order to determine the contribution of animal markets to the annual budget of the municipalities, the annual budgets of the municipalities were calculated first. The survey of the research was carried out between June and August in 2016. Data obtained in local currency have been converted into the Turkish Lira using World Bank data as the study will be presented in Turkey.

Formation of municipal budgets and contribution of livestock markets to the budgets of municipalities

The different sources of revenue that make up the annual budget of municipalities are shown in the formula below (Hartono and Rohaeni, 2014; MEF, 2019).

$Y = Gs + Ci + Oi$

In the formula

Y = annual budget of municipalities (TL/year)
Gs = government grants and subsidies (TL/year)
Ci = own income (TL/year)
Oi = other income (TL/year)

The contribution of animal markets to the annual budget of municipalities is as shown in the formula below:

$Z = (X/Y) \times 100$ (Marwati et al., 2020)

In the formula

Z = Contribution of animal markets (%)
X = Income of animal markets (TL/year)
Y = annual budget of the municipality (TL/year)

RESULTS AND DISCUSSION

Municipality sources of income

Figure1 shows the sources of income of the six municipalities included in the scope of the research in 2016. The municipality's income sources are mainly composed of its own income, government grants and subsidies and other revenues. However, a large share of the budget still comes mainly from grants from the central government (MEF, 2019). According to the principle of decentralisation, each municipality should self-finance its development and have its own fiscal policy. However, none of Benin's 77 municipalities is financially self-sufficient (Azon, 2018). Government subsidies account for more than 50 per cent of municipal budgets. Therefore, the development of income-generating activities such as animal markets should be among the priorities of local development projects.

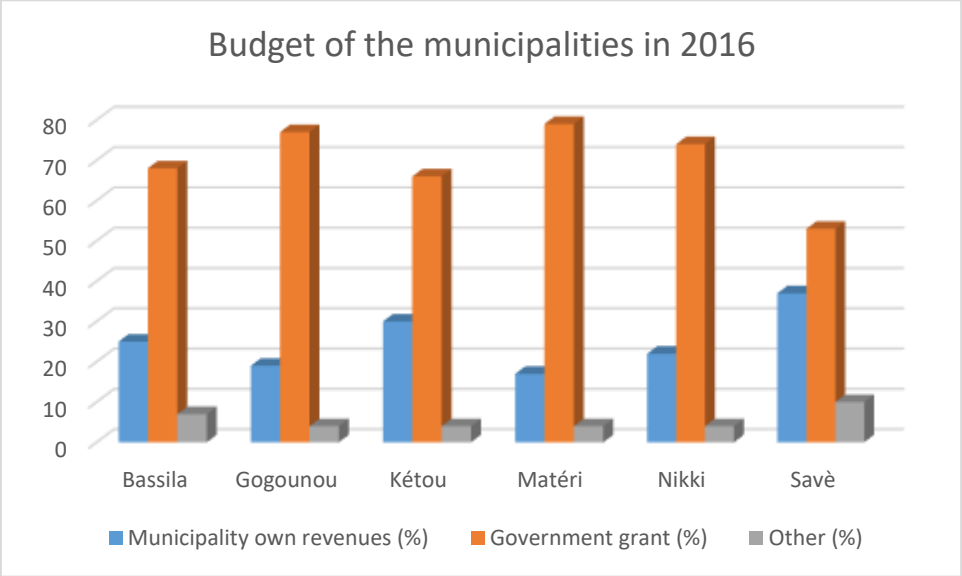

Figure 1: Budget of the Municipalities in 2016 (Source: MEF (2016).

Contribution of MBA livestock markets to municipal budgets

The contribution of MBAs to the municipal budget varies from one market to another (Figure 2). MBA markets contribute between 7.72 % and 65.50% to the municipalities' own income and between 2.86% and 12.74% to their annual budget. Gogounou's MBA is the largest contributor to the municipality's annual budget. Gogounou is the first MBA created in Benin. The commercial flow in Gogounou is very important, even the management system is very good because the government, private and international organisations provide the most management training and support there.

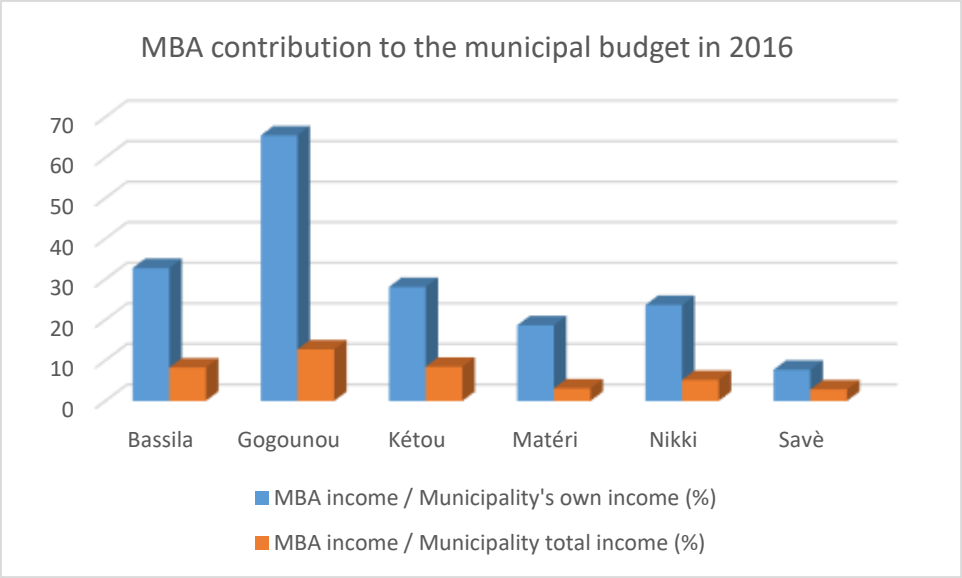

Figure 2: MBA Contribution to the Municipal Budget in 2016

Contribution of MT livestock markets to municipal budgets

The contribution of MTs to the municipal budget differs from one district to another (Figure3). MT markets contribute between 0.92% and 7.33% to the municipalities' own income and between 0.28% and 1.43% to their annual budget. Gogounou's MT is the largest contributor to the municipality's annual budget. The development of MBAs has a positive impact on MTs. However, the contribution of MTs to municipal budgets remains very low due to inadequate collection of revenues and lack of control over the inflow and outflow of funds in the markets.

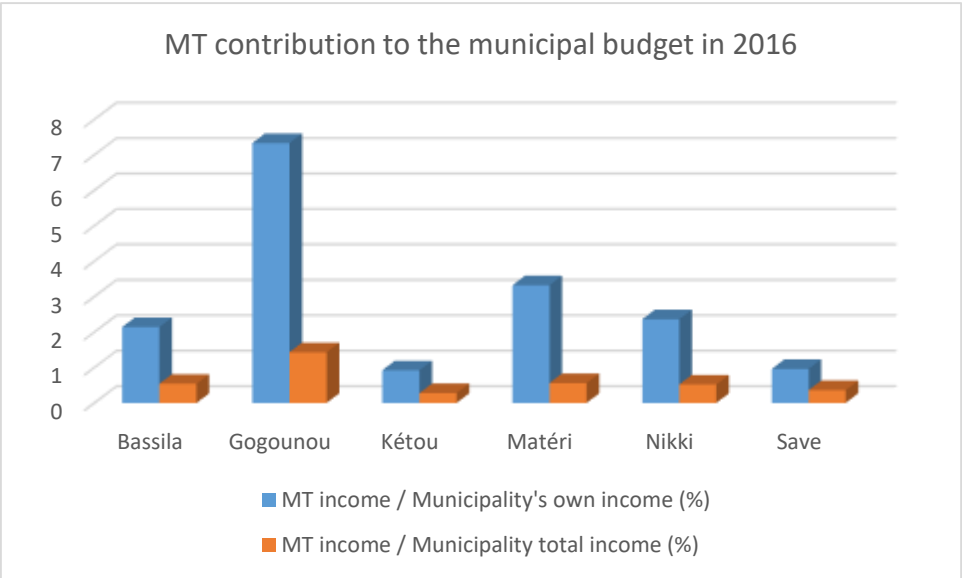

Figure 3: MT Contribution to the Municipal Budget in 2016

The contribution of MBAs and MTs to municipal budgets is mainly made up of taxes collected from the transactions of animals in these markets. These taxes collected by municipalities are known as Local Development Taxes (TDL).

TDLs are authorised by the central government and allow municipalities to raise local resources to finance local development plans. The taxes transferred to municipalities from animal markets depend mainly on the number of animals sold because tax is charged per animal sold. The rate of participation of animal markets in municipal annual budgets can also be interpreted as the rate of municipal financial independence. When this ratio increases, municipalities become financially independent. There are also many factors that directly and indirectly affect the contribution of markets to the municipal budget. Some of these are listed below.

Taxes

Taxes collected per animal vary according to the type of animal (small and large ruminants), the region where the market is located and the contract between the municipality and the market. Taxes are paid equally by the buyer and the seller. Taxes collected per large ruminants are higher than that collected per small ruminants. Such taxes are collected during the marketing process. These taxes are determined jointly by the management committee of the MBA and the municipalities. But in MT markets, taxes are determined only by the municipalities. Because MT livestock markets are fully owned by the government.

The municipality has the right to manage local commercial infrastructures. But the management of MBAs isvery sensitive. There was disagreement between the municipality and the MBAs. The management of the MBAs is carried out by the farmers themselves. Hence the name "Marché a Bétail Autogéré" (MBA), a self-managed livestock market. Following this agreement, several contracts were signed between the municipality and the MBA management committee. These contracts include a method of paying taxes to the municipality. Some MBA pay their taxes through a monthly contract based on a fixed amount payable monthly to the municipality. Other MBAs pay taxes based on daily sales on market days. Regarding MTs, municipal representatives collect the taxes every market day. The disadvantages of the contract payment method for MBA markets are declines in sales. The drop in sales does not affect the payment of taxes to the municipality. Payment based on daily sales is subject to variations in the quantities sold on each market day.

Accounting

Accounting is an important determining factor in the revenue generated by MBAs. Indeed, in the absence of accounting, MBA revenues were mismanaged, revenues were low even though sales were high. Accounting allows MBAs to record revenues and therefore to participate fully in the formation of municipal budgets. Accounting is only available in modern markets, i.e. in the MBA. Since MBAs have a management committee, accounting is necessary for good management and transparency in financial matters. As traditional livestock markets (MT) are not organised, there is no accounting service. It should be noted that there are resource losses in MT markets. Not all transactions are reported, tax collection is not transparent.

Seasonality

Changes in animal supply in the markets throughout the year follow a seasonal trend, especially during the dry and rainy seasons. During the rainy season, breeders sell fewer animals. In the dry season, breeders sell some of their animals to feed the rest of the herd. More transhumance is practised. Transhumance allows animals to cope with seasonality by providing

access to fodder (Stenning, 1957; Boone et al., 2008). The size of the livestock herd in the market area and the size of the Peul ethnic group (Fulani). In Benin, the Peul ethnic group is most concentrated in the North. The Peul ethnic group live in villages and camps where they grow food crops and raise livestock (Jones, 1998). Their farms have a high degree of flexibility and autonomy through a characteristic union of livestock, farming and harvesting activities. A family-based agro-pastoral economy characterises the lifestyle of most Fulani farmers in Benin (Bierschenk, 1998). In this production system, cattle breeding plays an important role, providing meat, animal traction, milk for household consumption, marketing and manure used to fertilise farms.

Feast periods

Sales volumes increase during the New Year and Eid al-Adha periods, especially for sheep. Animal sales volumes are higher in MBAs than in MTs.

Animal prices

The prices of the animals vary according to the time of year on the one hand and the breed, quality or geoFigureical region on the other. Animal prices are higher in MBAs than in MTs (Onibon, 2004).

CONCLUSION

Animal markets, above all, play an important and strategic role in the development of rural areas. Few studies and poverty alleviation programmes have considered and examined animal markets, especially self-managed animal markets, as an important source of development, wealth creation and municipal financing in rural areas.

In the Sahel region, livestock markets are very important socio-economic units, when these units operate at full capacity, the economy of the region develops better and all crises (climate, security and politics) are affected at this level. Generally, an effective and efficient marketing system increases consumption, output and economic development (Achike and Anzaku, 2010). Since most of the population in developing countries live in rural areas and most of them are poor, for the effective development of rural areas, national or international poverty alleviation programmes should focus on socio-economic activities such as livestock markets.

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EVALUATION OF PHYSICO-CHEMICAL PARAMETERS OF SOME WHEAT VARIETIES AND RELATED MICROFLORA

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ABSTRACT

Wheat is one of the raw materials, most important for the production of many products such as flour, bread, pasta, flour, cookies, etc. The high physico-chemical and microbiological quality of wheat will be reflected in its best technological properties and of course in products quality closer to the standards. The aim of the study was focused on the evaluation of some physico-chemical parameters of the wheat that give us a database for the evaluation of the wheat quality and to ensure a better mix of wheat samples for technological processing and at the same time evaluating the suitability of them with the permitted values. Another important factor to evaluate is the total mesophilic microflora, the presence of which is related to the physical-chemical characteristics of the wheat samples. The analyzed samples represent wheat of different imported and nation varieties. The physico-chemical parameters analyzed are: hectoliter weight (kg/Hl), moisture content (%), absolute weight (gr). Total mesophilic microflora (bacteria, mould, yeast) was determined by the standard method of cultivation, in Petri dishes, medium PCA, PDA, Capek, Mc Concey incubation temperature 30^{o}C for 72 hours. In general, the wheat samples analyzed are within the permitted values referred to the physico-chemical parameters and the microbial load.

Key word: wheat, moisture, physico-chemical parameters, hectoliter weight, etc.

INTRODUCTION

Wheat is one of the most important of the cereal crops and it is used to make flour, bread, pasta, biscuits, etc. (FAO / UN, 2012) (Zeqiri V.2020). Due the worldwide importance, wide use of wheat and their products, the wheat's physico-chemical and microbiological characteristics are the two most important key points of the quality and safety evaluation of wheat. Based on this parameters the wheat samples will be selected for processing (Zeqiri V. 2020). The quality of the wheat will depend on the soil composition, environmental factors and agronomic practices (Trethowan, R. M., Reynolds, M., Sayre, K.,(2005), Britannica.com). Wheat varieties can be classified and used based on quality and safety parameters such as physico-chemical, microbiological and specific requirements of millers, bakers and consumers (Cardoso V.C. R, Fernandes A, Heleno A. S, 2018). The physico-chemical evaluation of wheat varieties is based on the evaluation of the moisture content of the wheat grain, hectoliter weight, absolute weight, glassy appearance (Iqbal Z, Pasha I, Abrar M, Masih Sh. 2015, Etong I. D., Mustapha O, Lawrence I.G. 2014). Grain moisture is one of the most important indicators for assessing its quality. High moisture content reduces the grain's ability to be stored. It can cause its rapid spoilage and reduces the yield of flour (Cardoso V.C. R, Fernandes A, Heleno A. S,.. 2018, Conroy, J. P., Seneweera, S., Basra, A. S., Rogers, G.,1994). The hectoliter weight is a

key indicator for rapid evaluation of the grains during acceptance. Hectoliter weight of grains means the mass of hectoliters of grains expressed in one kilogram. The hectoliter weight of grains and other seed crops depends on a number of factors, such as: chemical composition, moisture, the amount of wrapper and impurities etc. The absolute weight of the grain is also considered the weight of 1000 grains of grain expressed in grams and calculated on the dry substance. The absolute weight and the hectoliter weight, both gives information on the quality of the grain. The absolute weight depends on the condition of the grains, their size and density. Cereal grains, according to the degree of glassy appearance, can be whole glassy, not whole mealy or mixed. Based on this indicator, it is judged on the hardness of the grain, on the chemical composition and on its use (Binbashi H., Frasheri M.) (Troja R.). Referring to the microbial content is a key safety factor assessment, for wheat acceptance and its processing. So, it is important to determine the total aerobic mesophilic microflora, total coliforms bacteria *B.cereus, Salmonella spp E coli* etc. (Cardoso V.C. R, Fernandes A, Heleno A. S,. 2018). The selection of the wheat sample with characteristics as close as possible to the standard ones will ensure the production of the highest quality products (Shewry, P. R., Mitchell, R. A., Tosi, P., 2012). Thus, because of its important role, the wide range of use of different varieties of wheat and the change of its physicochemical characteristics depending on several factors, our study focused on the evaluation of its physico-chemical and microbiological quality parameters of some varieties of imported and nation wheat samples, used in a factory for its processing.

MATERIALS AND METHODS

A total of six wheat samples were taken. The analyzed samples represent import and nation samples used in a flour processing factory, Albania. Different varieties of wheat have different physico-chemical characteristics. They vary from soil, season, processing techniques, etc.(britannica.com). The physico-chemical characteristics evaluated (basic reference methods) are the moisture content (%), hectoliter weight (kg/Hl), absolute weight (gr), glassy appearance (%) based on the standard methods used in our country. Total aerobic mesophilic microflora and total coliforms were determined by the standard cultivation method, in Petri dishes, respectively, in PCA, PDA, Capek Mc Concey medium, incubation temperature 30 and 37 ^{o}C for 72 hours. (Frasheri M. dhe Prifti D). The determination of the absolute weight is the weight of 1000 grains obtained from the weights of 500 grains of two opposite sectors. It is calculated according to the formula: A=(100-l)*p /100. Where A: absolute weight in grams, l= moisture in %, p= weight of 1000 grains with moisture in grams. Determination of the hectoliter weight is done with Shoper's scale: First, the scale is settled, the stand is screwed, the arm is placed and the stones are fixed at the zero scale. On the left side hangs the empty measuring cylinder together with the cylindrical stone. The scale is placed in balance with the side of the regulator located on the left side. The measuring cylinder is taken and fixed on, in its place. A knife is placed in the slot of the cylinder and the cylindrical stone is placed on it. Then the intermediate cylinder is placed. The grain is thrown into the filling cylinder, which has an opening mechanism at the end. After being filled with grain, the filling cylinder is placed on top of the intermediate cylinder. The mechanism is opened, which allows the uniform flow of grain into the intermediate cylinder. Then the knife is removed from the measuring cylinder and the cylindrical stone is allowed to fall after this grain. The filling cylinder is removed, the excess grain is removed and the knife also. The grain measuring cylinder hangs on the left side of the scale and is weighed accurately to 1 g. Determining the shape of the cereal grain with the Farinotome. The knife is removed from the discs and they match each other. The 50 grains are placed in the 50 holes and the knife is pulled forcefully through the two discs. In order not to remove those of the upper disc during the cutting of the grains. The knife and the upper disc are

removed and the glassy degree is observed in the cross-sections of the grains remaining on the lower disc of the Farinotome. This analysis is performed twice and the difference between two parallel determinations should not be greater than 5%. The degree of glassy appearance of grain is expressed in percentage. To determine the moisture content, 5 g of wheat sample were weighed in a dish and then set to dry in a thermostat at a temperature of 105 degrees C, until a constant weight (this usually takes 4-6 hours). According to our standards, grain (wheat) must contain no more than 14% moisture. % of moisture content is calculated according to the formula: l%= (W.blank + W.wet sample) - W.after drying/W.sample*100 (Binbashi H., Frasheri M)

RESULTS

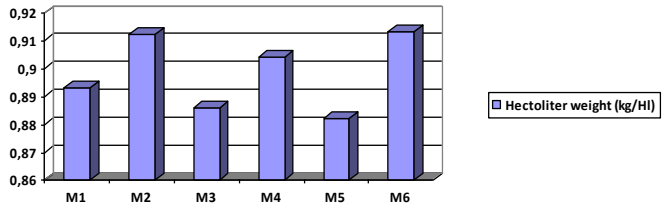
Table 1 Summary table of the physical and chemical characteristics of the analyzed wheat samples

		Hectoliter weight kg/Hl	***Absolute weight (g)***	***Determination of grain shape (%)***	***Moisture content (%)***
M1	Analysis1	0.891	34.257	83%	11.88
	Analysis 2	0.895		79%	11.82
M2	Analysis 1	0.914	34.966	61%	12.17
	Analysis 2	0.910		59%	11.73
M3	Analysis 1	0.884	32.253	42%	12.47
	Analysis 2	0.888		38%	12.63
M4	Analysis 1	0.906	32.745	50%	12.13
	Analysis 2	0.901		51%	12.01
M5	Analysis 1	0.883	30.018	48%	12.03
	Analysis 2	0.881		47%	11.87
M6	Analysis 1	0.915		80%	12.66
	Analysis 2	0.911	34.546	85%	12.35

Table 2 Summary table of the analyzed wheat samples microbial load

	Total mesophilic aerobic bacteria (cfu/gr)	**Total coliforms (cfu/gr)**
M1	$33*10^1$	71
M2	$37*10^1$	49
M3	$5*10^3$	41
M4	$3*10^2$	66
M5	$2*10^2$	118
M6	$19*10^2$	68

Figure 1 The hectoliter weight values (kg/Hl) for each analyzed wheat sample



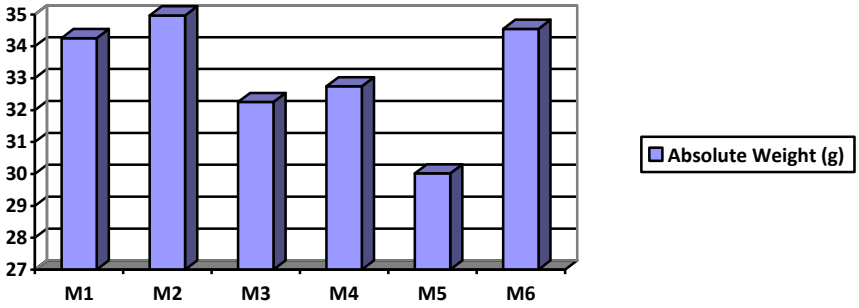


Figure 2 The absolute weight values (%) for each analyzed wheat sample

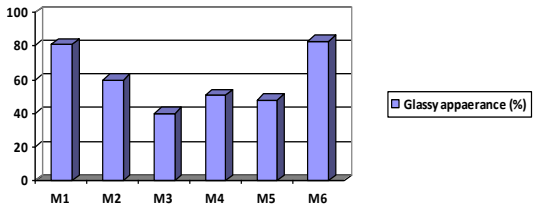


Figure 3. The glassy appearance values (%) for each analyzed wheat sample

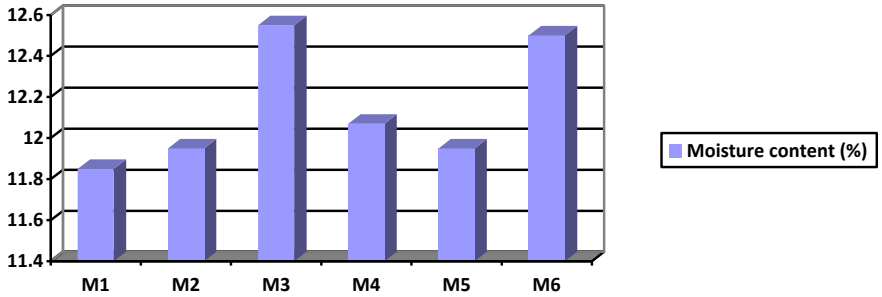


Figure 4. The moisture content values (%) for each analyzed wheat sample

The physico-chemical characteristics of the analyzed wheat samples are important to determine, to ensure the best mix of wheat samples and to obtain flour and other products of the highest quality within the required standards. From the moisture content table, it can be seen that for all the samples their value is within the permissible values, below 14%. Its values vary from 11.73% to 12.66%, where the highest value has M3, M6 wheat sample respectively and the lowest value has M1 wheat sample. The higher the moisture content, the more opportunities for microorganism development and this promotes protolytic and lipolytic activity, which leads to a loss of nutritional values and a reduction in storage time. (Cardoso V.C. R, Fernandes A, Heleno A. 2018). The absolute weight for all samples is within the permissible limits. This expresses the size of wheat grain and the density. The absolute weight of the analyzed wheat samples resulted from 30,018 to 34,966 g. Where the highest value of the absolute weight has the M2 sample of wheat (34.966 g) and the lowest has the M5 sample of wheat (30.018 g). The hectoliter weight is an indicator of quality and it results that it is within the permissible values. Its values vary from 0.881 to 0.915%. The highest value of the hectoliter weight results to have the M6 wheat sample (0.915 %) and the lowest M5 wheat sample (0.881%). The glassy appearance of wheat grain is an endosperm construction indicator and flour milling consistency. In general, for wheat samples, this parameter varies from 38 to 85%.

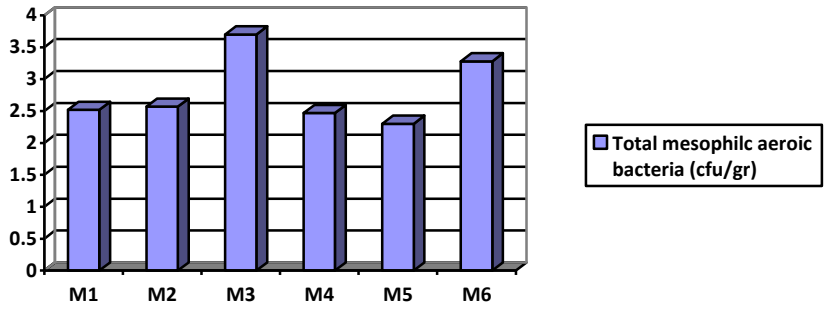


Figure 5 The logarithmic values of total mesophilic aerobic bacteria for each wheat sample analyzed

Referring to the Figure above, sample M3 has the highest total microbial load, then sample M6 is of the order of 10^3 cfu/gr (standard $5*10^3$ cfu/gr) (Microbial standard of grain safety) The lowest is sample M1, M2 of the order of 10^1 cfu/gr and then M4 of the order of 10^2 cfu/gr. For samples M3 and M6, care must be taken in controlling the factors (moisture content, processing techniques, etc.) that affect their microbial load.

CONCLUSIONS

Most important parameters that affect the assessment of the quality and technological properties of wheat for flour and other products are the physico-chemical parameters such as moisture content, hectoliter weight, absolute weight, shape of the grain of wheat. We resulted that for all the wheat samples these parameters were within the permissible values.

In general, the hectoliter weight for all analyzed wheat samples varies from 0.881 to 0.914%. The highest value of the hectoliter weight results in this sample of M6 wheat and the lowest in the sample of M5 wheat. Absolute weight for the wheat samples analyzed resulted from 30,018 to 34,966 gr. The highest value of the absolute weight has the M2 wheat sample and the lowest has the M5 wheat sample. Referring to the moisture content, its values vary from 11.73 to 12.66%, where the highest value has the M3 wheat sample and the lowest has M1 wheat sample. For all the wheat samples analyzed, it resulted to have a content of total aerobic mesophilic microflora and total coliforms. The main factors could be from the field to storage (variety, different processing operations such as harvesting, transport, cleaning, air insects, dust, season (temperature relative humidity) etc. M3 wheat sample has the highest total microbial load, then M6 wheat sample has 10^3cfu/gr microbial load. The lowest microbial load has M1, M2 wheat sample (10^1 cfu/gr) and then M4 wheat sample has 10^2 cfu/gr microbial load.

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CURRENT SITUATION OF THE RED MEAT SECTOR IN THE REPUBLIC OF BENIN: PROBLEMS AND SOLUTION RECOMMENDATIONS

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ABSTRACT

The red meat sector is one of the Benin government's leading projects, as it contributes significantly to food and nutritional security and to the creation of jobs and income. Red meat is produced by cattle, sheep and goat mainly from the pastoral farming system. The cost of the production system is one of the lowest in the world. However, meat on the market remains expensive and inaccessible on a regular basis for most households. In view of this problem, a thorough and complete analysis is essential for a necessary and rapid reaction of the local sector to remain viable and competitive. This study aims to establish the current state of the red meat sector in Benin in order to be able to propose solutions to optimize it and ensure its viability and sustainability.

Keywords: Red meat, Livestock, Pastoral farming system, Food security, the Republic of Benin

INTRODUCTION

Animal products are an important source of food, wealth, exchange and dynamism in West Africa (Duverge, 2006). The rearing of large and small ruminants is the main source of red meat in Benin (Dognon et al., 2018). It also plays a very important socio-economic role in the daily life of farmers. The production systems of the Sahel are characterised as pastoral and agropastoral, and have the lowest production costs in the world (Inter-Réseaux, 2015). Yet meat on the market remains expensive and inaccessible on a regular basis for the majority of households.

The objective of this study is to assess the current state of the red meat sector in Benin, identify the problems and propose solutions for its improvement. This study is a literature research prepared using data and information published online. In the evaluation of the secondary data obtained in accordance with the purpose of the study, simple statistical methods such as index and percentage calculation were used and the results were shown in tables in the study. The time scope of the research covers the last 17 years for which data can be obtained regularly.

GENERAL STRUCTURE OF LIVESTOCK SYSTEMS IN AFRICA AND IN THE REPUBLIC OF BENIN

Agropastoral systems

Also known as farmer systems, they are the most widespread and dominate the landscape of Sub-Saharan countries. Agropastoral systems use a combination of grazing on uncultivated land, forage crops, agricultural and agro-industrial waste products and purchased feed, in a mixed crop and livestock farming system. According to Seré (1994), pastoral systems hold one third of the cattle and half of the small ruminants in sub-Saharan Africa. They provide 60% of the beef meat, 40% of the small ruminant meat and 70% of the milk of this group of countries. Combining agriculture and livestock for self-consumption and for sale, these systems have grown significantly as a result of population growth. Nowadays there is an increase in agropastoral breeding systems to the detriment of purely pastoral systems. It is increasingly rare to find pastoralists who do not practice some form of agriculture. Livestock combined with agriculture provides manure, traction, and a capital reserve, which is essential wherever the banking system is weak and money is scarce. Individually or collectively owned cattle herds are run by paid herdsmen.

Livestock productivity in agropastoral systems is declining due to several factors including:

1) The intensity or frequency of transhumance
2) Recurrent trypanosomiasis disease
3) The reduction of pastoral space due to the expansion of crops and other land uses
4) The racial composition and management style of the herds. This is often characterised by the presence of trypanotolerant cattle (Tano et al., 2001)

In conclusion, pastoral and agropastoral systems across sub-Saharan Africa contribute to the majority of the supply of lives animal and animal products.

TRENDS IN ANIMAL NUMBERS

In Benin, cattle, sheep and goat assets have gradually increased in the last 17 years. In 2016, cattle herd increased by 51.82% with 2339 thousand heads, sheep herd increased by 37.11% with 915 thousand heads and goat herd increased by 48.04% with 1836 thousand heads. It appears that cattle experience more growth than sheep and goats. This can be explained by the spread of MBAs in the country, the sedentarisation of some pastoralists and the new livestock entrepreneurs (Table 1). Despite the large numbers of animals there is unsatisfied demand for meat in the major.

In 2016, the African cattle, sheep and goat herd represented 23.30%, 31.16% and 40.21% of the world cattle, sheep and goat herd, respectively. The West African cattle, sheep and goat herd represents 21.41%, 29.76% and 38.87% of the African cattle, sheep and goat herd, respectively. The Benin cattle, sheep and goat herd represents 0.68%, 0.25% and 0.44% of the African cattle, sheep and goat herd, respectively. The Benin cattle, sheep and goat herd represents 3.19%, 0.82% and 1.14% of the West African cattle, sheep and goat herd, respectively (Table 2). Compared to sub-Saharan Africa as a whole, the Sahel and West Africa contain 25% of cattle, 33% of sheep and 40% of goats (CSAO-OCDE/CEDEAO, 2008). In 2016, cattle meat production increased by 53.11% with a total production of 40 thousand tonnes, sheep meat production increased by 36.21% with a total production of 9 thousand tonnes and goat meat production increased by 48.04% with a total production of 9151 tonnes. Cattle and goat meat cover a large portion of red meat production (Table 3).

Table 1. The herd of large and small ruminants in Benin (1000 Heads)

Years	Cattle	Index (2000=100)	Sheep	Index (2000=100)	Goat	Index (2000=100)
2000	1541	100.00	667	100.00	1240	100.00
2001	1599	103.76	679	101.76	1266	102.10
2002	1639	106.40	683	102.39	1320	106.40
2003	1676	108.79	690	103.40	1306	105.30
2004	1718	111.51	708	106.09	1346	108.53
2005	1718	111.51	724	108.49	1386	111.75
2006	1810	117.48	742	111.19	1427	115.06
2007	1857	120.53	762	114.23	1454	117.21
2008	1905	123.65	781	116.99	1483	119.60
2009	1954	126.83	791	118.53	1570	126.59
2010	2005	130.14	808	121.08	1605	129.41
2011	2058	133.58	825	123.63	1640	132.23
2012	2111	137.02	842	126.17	1678	135.30
2013	2166	140.59	860	128.87	1716	138.36
2014	2222	144.23	878	131.57	1755	141.51
2015	2280	147.99	896	134.27	1795	144.73
2016	2339	151.82	915	137.11	1836	148.04

Source: FAOSTAT, 2022, FAOSTAT Database, www.fao.org/faostat (Accessed in February 2022).

Table 2. Herd in Benin, West Africa, Africa and the World in 2016 (1000 Head)

Animal type	Benin	West Africa	Africa	World	Benin / West Africa (%)	Benin /Africa (%)	West Africa /Africa (%)	Africa / World (%)
Cattle	2339	73344	342642	1470386	3.19%	0.68	21.41	23.30
Sheep	915	111062	373248	1197724	0.82%	0.25	29.76	31.16
Goat	1836	160847	413796	1029024	1.14%	0.44	38.87	40.21

Source: FAOSTAT, 2022, FAOSTAT Database, www.fao.org/faostat (Accessed in 02.2022).

Table 3. The red meat production in Benin (Metric Ton)

Year	Cattle (MT)	Index (2000=100)	Sheep (MT)	Index (2000=100)	Goat (MT)	Index (2000=100)
2000	26126	100.00	6607	100.00	6182	100.00
2001	27108	103.76	6724	101.76	6311	102.10
2002	27797	106.40	6765	102.39	6578	106.40
2003	28421	108.79	6832	103.40	6510	105.30
2004	29131	111.51	7010	106.09	6709	108.53
2005	29131	111.51	7168	108.49	6908	111.75
2006	30693	117.48	7347	111.19	7113	115.06
2007	31490	120.53	7548	114.23	7246	117.21
2008	32304	123.65	7730	116.99	7393	119.60
2009	33135	126.83	7832	118.53	7826	126.59
2010	34000	130.14	8000	121.08	8000	129.41
2011	35000	133.97	8000	121.08	8174	132.23
2012	36000	137.80	9000	136.21	8364	135.30
2013	37000	141.62	9000	136.21	8553	138.36
2014	38000	145.45	9000	136.21	8748	141.51
2015	39000	149.28	9000	136.21	8947	144.73
2016	40000	153.11	9000	136.21	9151	148.04

Source: FAOSTAT, 2022, FAOSTAT Database, www.fao.org/faostat (Accessed in February 2022).

CONSTRAINTS AND SOME PROPOSALS FOR ACTION

There are several constraints to the development of the livestock and red meat sector in Benin. These are as follows:

- ✓ Insufficient water points for livestock,
- ✓ The destruction of natural pastures by bush fires,
- ✓ The importance of cattle theft,
- ✓ The large distances between villages and veterinary posts
- ✓ Lack of market infrastructure, slaughter, processing, cold chain
- ✓ Very poor hygiene mesures

All these constraints result in higher meat prices for the consumer. Table 4 presents some proposals for improving the red meat sector in Benin.

Table 4. Proposals

Livestock farming	Livestock trade	Meat trade	Economic structure	Policies, organization
-Intensification, fattening, -Availability and accessibility of inputs.	-Improve sub-regional integration, - Professionalization, - Marketing chain reduction.	-Market segmentation, -Market information system, -Protect the internal market, -Raise consumer awareness.	-Access to credits, -Develop banking system, -Secure transactions.	-Structuring of the sector, -Creation of organs of professionals, -Market information system, -Installation and renewal of basic infrastructure

CONCLUSION

The consumption of animal products and meat in particular are important geoFigureical and social markers. The red meat sector in Benin is a source of important national and sub-regional trade. Red meat is mainly produced by cattle, sheep and goats. The pastoral and agro-pastoral system does not produce enough red meat to meet national needs. These needs are met by red meat imports. The red meat sector in Benin is hampered by several constraints that need effective action to be solved. Improving the sector through effective policies would lead to food self-sufficiency in red meat production in Benin.

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PRODUCTIVITY OF DIFFERENT CAPE GOOSEBERRY (*PHYSALIS PERUVIANA* L.) GENOTYPES INFLUENCED BY METEOROLOGICAL CONDITIONS IN SOUTH BULGARIA

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ABSTRACT

The purpose of the study is to determine which interphases periods are crucial for the productivity of the investigated varieties of cape gooseberry. The experiments were carried in 2015-2019 years with three genotypes of cape gooseberry in Experimental fields of Agricultural University – Plovdiv. In the stage of botanical maturity, the ripe fruits were harvest, total yield was established. The air temperature and the sum of precipitations were recorded. Based on them, the main agro-climatic indicators were established during the most important periods of the development of cape gooseberry and they were compared with the obtained yields by years and genotypes. Crucial periods depending on the climatic conditions of the cape gooseberry productivity are flower buds - flowering and fruit sets – fruiting.

Keywords: climate, phenology, precipitations, temperature, yield.

INTRODUCTION

Cape gooseberry is native from South America Andes, mainly Peru, Bolivia and Ecuador (Medina, 1991). The best development of this crop is under the conditions of Mediterranean climates (Obrecht, 1994; Fischer, 2000). It is characterized by great adaptability and tolerance to various environmental conditions (Muniz et al, 2014). The main factor for the successful development of the cape gooseberry is temperature conditions. Salazar et al. (2008) estimated that the base temperature for the calculation of the thermal time for node appearance is 6.29 ºC. He emphasizes that on the base of temperature and photosynthetically active radiation it is possible to build a potential production model for the growth of *Physalis peruviana* L.

The temperature mainly influences the course of physiological processes and the kinetic energy of enzyme systems (Fischer and Orduz-Rodriguez, 2012). The normal development of cape gooseberry fruits according to these authors is in the temperature range of 13-18^{0}C. The increase in the temperature can cause fruit deformation, which affects overall productivity. In

this species, the temperature above 30°C suppresses normal flowering, decreases the vitality of the pollen and the fruit set formation (Westwood, 1993). Cherenok (1997) also formulated a similar conclusion that, in cape gooseberry, the temperature is a limiting factor and it has specific temperature requirements. Fischer and Lüdders (1992) emphasize that these requirements depend on the ecotype. For Columbian ecotype, which is most widely used, normal root system development and dry mass accumulation is optimal at 22^0C daily and 14^0C overnight, while vegetative development and dry matter content, as well as fruit formation, are the best at 29^0C, while for South African ecotype this temperature is 22^0C. In addition to temperature, humidity has also great importance for the development of cape gooseberry. In waterlogged conditions for six to eight days plant biomass, especially those of the roots, flowering and fruit production are reduced and after eight days the plants may die under these conditions (Aldana et al., 2014). Villareal (2014) also points out that the presence of higher than optimal humidity suppresses the development of the root system and the leaves weight, decreases the intensity of leaf gas exchange processes, which negatively affects the yield.

The purpose of the study is to determine which interphases periods are crucial for the productivity of the investigated varieties of cape gooseberry.

MATERIALS AND METHODS

The experiments were carried out with three cape gooseberry (*Physalis peruviana* L.) genotypes: variety Plovdiv – Bulgarian selection, genotype 08-2010 – origin from Turkey and genotype 11-2012 – origin from Germany, during the period 2015-2019 years in the Experimental field and scientific laboratories of the Department of Horticulture and of the Department of Botany and Agrometeorology at the Agricultural University-Plovdiv, Bulgaria. The plants were grown from seedlings in a plastic unheated greenhouse The sowing date were: 26.03.2015, 24.03.2016, 22.03.2017, 27.03.2018 and 27.03.2019. The sowing rate was 80-100 g/ha, while per square meter it was 1.5 g. The plants were planted on the following dates: 20.05.2015, 21.05. 2016, 22.05.2017, 23.05.2018 and 21.05.2019. The scheme 70 x 50 cm (Panayotov and Tcorlianis, 2000) on furrows was applied. Each agro-technological and cultural practice, that are necessary were applied during vegetation. The experiments were carried out in four replicates with 25 plants in each and the experimental plot was 9 m^2. In the time of full maturity, each ripped fruits were harvest and productivity was determined. Three harvests were made.

Daily meteorological information from Plovdiv weather station for the last 5 years has been used. The main factors of the climate are the mean air temperature and the sum of precipitations. Based on them, the main agro-climatic indicators were established during the most important periods of the development of cape gooseberry and they were compared with the obtained yields by years and genotypes. Results were statically processed by a two-way

analysis of variance (ANOVA) and the influence of the variation factors (η %) and correlations were also established (Fowel and Cohen, 1992).

RESULTS AND DISCUSSION

According to several authors, in order to determine whether the specific conditions of a given region are suitable for the normal development of the cape gooseberry, it is necessary to evaluate the suitability and adaptability of different genotypes by preliminary tests (Obrecht, 1994; Muniz et al, 2014). Differences in the productivity of the cape gooseberry were observed, both between the tested varieties and by the years of the experiments (Table 1). The highest yield was recorded in 2015, while the lowest one was in 2018. The average productivity values for the three genotypes ranged from 1676.63 kg.ha^{-1} (2018) to 1851.2 kg.ha^{-1} (2015), i. e. the decrease between these the highest and the lowest yields is approximately 10%. The next two years are characterized also by relatively high productivity. The strongest difference between Plovdiv variety and the other two genotypes is in 2015, with 642.5 kg.ha^{-1} than 08-2010 and with 662.0 kg.ha^{-1} to 11-2012, while between the last two breeding lines this is observed in 2018 (132.9 kg.ha^{-1}). Among the three studied genotypes, Plovdiv is the most productive, with average yield for five years - 2163.4 kg.ha^{-1}. This is probably due to the fact that this variety was selected under the conditions of Southern Bulgaria, where the experiments were carried out and, accordingly, it has better adaptability and suitability for cultivation in this region compared to the other two genotypes. Similar results have been found for this variety in our previous study (Popova et al., 2010). Next place is the genotype 11-2012, originating in Germany - 1602.56 kg.ha^{-1}. The differences in the obtained results are determined primarily by the influence of the factor genotype, followed by the influence of the year and on the last place of their interaction. Todorova and Pevicharova (2018) also reported similar conclusions about the greater influence of variety and year on the productivity. Varietal differences in the productivity of the investigated genotypes of cape gooseberry, regarding the influence of agrometeorological conditions were found.

In the Plovdiv variety, the sum of precipitation during the period from flower buds to flowering and from fruit sets to fruiting are crucial and high correlation and regression dependencies (Figure 1 and 2) were found, with correlation coefficient r=0.8261 and r=0.8712, respectively for these both periods. A precipitation above 60 mm during the period from flower buds to flowering reduces the yield by more than 200 kg.ha^{-1}. Aldana et al. (2014) report a significant dependence of cape gooseberry plants on humidity. They emphasize that the presence of stronger moisture, even for not a very long period, causes the decrease in the accumulation of biomass, flowering and fruiting are impaired and in some cases can lead to the death of the plants. Precipitations below 80 mm during the period from fruit set to fruiting reduce yields below 2100 kg.ha^{-1}.

Table 1. Productivity of cape gooseberry (kg ha^{-1})

Varieties	2015	2016	2017	2018	2019	The average
Plovdiv	2386.1	2191.1	2159.1	1990.8	2089.9	2163.4
08-2010	1743.5	1632.5	1550.5	1453.1	1502.8	1576.48
11-2012	1724.1	1553.1	1520.1	1586.0	1629.5	1602.56
Average	1851.2	1792.23	1743.23	1676.63	1740.7	

Genotype (A)		Year (B)		Genotype x Year (AxB)	
MS	η%	MS	η%	MS	η%
16328.69**	37.28	10243.28**	31.19	1145.23*	24.12

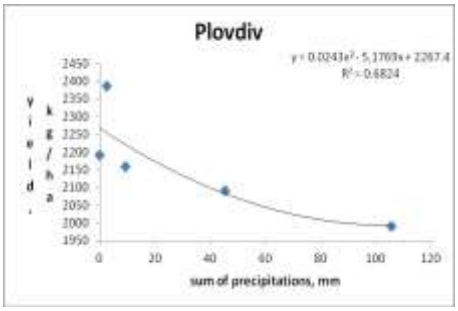

Figure 1. Yield dependence on the sum of precipitation during the period of flower buds to flowering in Plovdiv variety

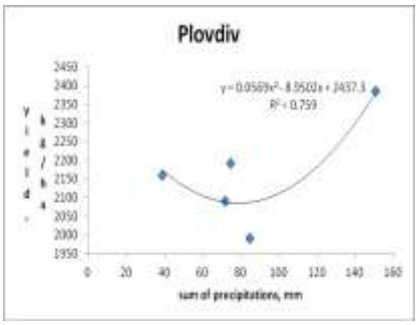

Figure 2. Yield dependence on the sum of precipitation from fruit sets to fruiting in Plovdiv variety

In variety 08-2010 during the period from flowering to the fruit sets formation the decisive factor for productive potential is the sum of precipitations (r = 0.8515), while from the period - fruit sets to fruiting these factors are air temperature (r = 0.8411) and the sum of precipitations (r = 0.7852) (Figures 3, 4 and 5). The increasing of the precipitations more than 30 mm, during the period from flowering to the fruit sets formation, causes the decreases of the yield by more than 200 kg.ha^{-1}. The highest yields were obtained at average temperatures of 24-25°C and precipitations above 120 mm during the period from fruit sets to fruiting. Temperature is a very essential element of the climatic conditions for the good development and high productivity of the cape gooseberry (Angulo, 2005; Rufato and De,

2010). These authors emphasize that both high (above 30^0C) and low especially nighttime (below 10^0C) temperatures adversely affect flowering and fruiting and inhibit plant development. In our experiments for a variety 11-2012, the most significant influence on the yield formation are the air temperature (r = 0.8716) during the period from flower buds to flowering (Figure 6) and the sum of precipitations (r=0.8532) during the period from fruit sets to fruiting and yield formation (Figure 7). The highest yields above 1600 kg.ha^{-1} were obtained at average temperatures of 22-23°C during the flower buds-flowering period and the sum of precipitations above 120 mm from fruit sets formation to fruiting Temperatures higher than 25°C reduce yield by more than 250 kg.ha^{-1}.

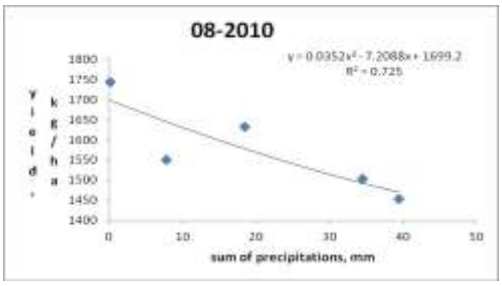

Figure 3. Yield dependence on the sum of precipitation during the period of flowering to fruit sets in 08-2010

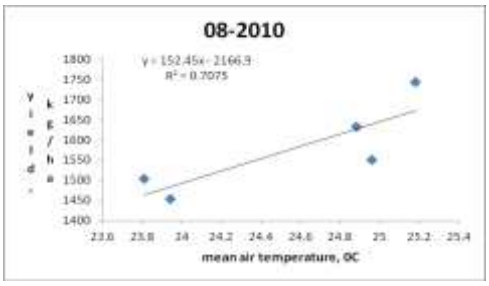

Figure 4. Yield dependence on the mean air temperature from the fruit sets to fruiting in 08-2010

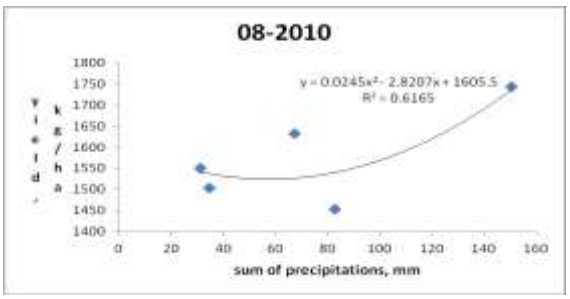

Figure 5. Yield dependence on the sum of precipitation during the period of fruit setting to fruiting in 08-2010

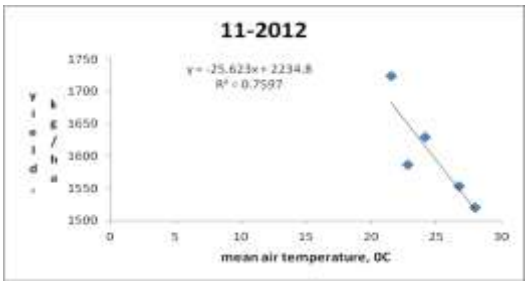

Figure 6. Yield dependence on the mean air temperature from flower buds to flowering in 11-2012

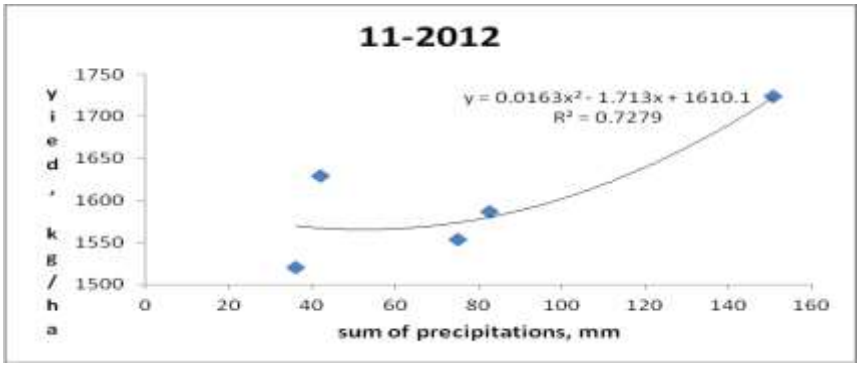

Figure 7. Yield dependence on the sum of precipitation during the period of fruit setting to fruiting in 11-2012

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CONCLUSIONS

A significant genotypic response was found regarding the influence of agrometeorological conditions on the productivity of the studied cape gooseberry genotypes and also significant differences during the separate growing seasons. The effect of agrometeorological conditions on the values of the yield is strongest during the period from fruit setting to fruiting, regardless of the variety. The most productive of the studied genotypes is Plovdiv, the Bulgarian selection of cape gooseberry, with an average yield for five years of 2163.4 $kg.ha^{-1}$, and the least productive variety is 08-2010, origin from Turkey, with an average yield of 1576.5 $kg.ha^{-1}$.

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EFFECT OF THE APPLICATION OF ULTRASOUND ON THE SEED QUALITY OF *CAESALPINIA PULCHERRIMA* (L.) SW. AND THE DEVELOPMENT OF THE SEEDLINGS

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ABSTRACT

The aim of the present study was to determine the effect of the pre-sowing application of ultrasound on the sowing qualities of seeds of *Caesalpinia pulchirima* (L.) Sw. The seeds were treated with ultrasound for 2, 4 and 6 minutes, and the control was untreated seeds. Seeds were placed in the germination chamber The germination energy and germination were recorded. On the day of the last germination, count mean germination time and uniformity of germination were calculated as well as the length of embryo root and length of hypocotyls of the seedlings were measured. The highest germination energy and germination were reported after application of 4 minutes. Also, this duration improved mostly the morphological features of the seedlings. Linear and polynomial regression between sonication and germination energy and germination with a high coefficient of determination R^2=0.56 and R^2=0.76, respectively were established. There is a strong negative correlation between the time for germination of 50% of seeds with germination energy and germination.

Keywords: seedlings, mean germination time, germination, regression, sowing quality,

INTRODUCTION

Caesalpinia is a flowering shrubby plant in the legume family, Fabaceae. Many species belong to this genus, and according to different authors, their number varies considerably. It is usually pointed out that it is between 70 to 165 species (Gledhill, David (2008). It is emphasized that some species can be used for medical needs as well as for food (Ferro et al., 2019; Jeya et al ., 2021). The species *Caesalpinia pulchirima* (L.) Sw. originates from Central America, northeastern Brazil (Ferro et al., 2019; Gilman and Watson, 2003). It is defined as a low tree or shrubby habit. It is characterized by very beautiful flowers, that retain their high decorative effect for a long time. This is due to its wide application in decorative gardening, both in groups and solitary. In addition, due to its relatively small size and great diversity in flowering, it is very often used in afforestation, as well as for building

living fences (hedges). An important feature is its biological properties, it is characterized by tolerance to high temperatures and drought, which makes it suitable for growing in a wide area with different conditions. (Ferro et al., 2019).

The seeds of this species are characterized by the presence of dormancy, which, according to some authors, is of varying intensity, depending to a large extent on the soil and climatic conditions of the area, therefore it is necessary to know them very well (Oliveira et al.,(2010; Marcos Filho, 2015; Ferro et al., 2019; Jeya et al., 2021). Bentsink and Koornneef (2008) emphasize that information on the biology and dormancy of seeds is extremely limited. Santos et al, 2019, reported that the seed viability of *Caesalpinia pulcherrima* (L.) Sw. depends to a great extent on their location on the plant and from there on their supply of vitally important proteins. The authors point out that the seeds with the median, proximal/median and proximal position have the best qualities, which also have the highest reserve protein content. The importance of the reserve proteins as a major factor responsible for seed germination was also reported by Taiz and Zeiger (2017).

Jeya et al. (2021) found that *Caesalpinia* seed dormancy was primarily associated with the presence of poor seed coat permeability. They are experimenting with different methods to overcome this dormancy, applying physical and chemical treatment of the seeds, as well as scarification. According to them, physical methods and especially scarification show the best results. Panayotov (2015) points out that physical methods and especially the application of ultrasound are very promising ways to remove dormancy in seeds. Mason et al., (2005) expressed an opinion that the application of ultrasound is a widely used method in agriculture. Through ultrasound, Rifna et al, 2019 achieved an effective increase in the rate of germination and in general the growth of seedlings.

The main objective of the present study was to investigate the influence of different durations of sonication with ultrasound on on the sowing qualities of the *Caesalpinia pulcherrima* (L.) Sw. seeds.

MATERIAL AND METHODS

The experiments were carried out in the scientific laboratory of the Department of Horticulture at the Agricultural University-Plovdiv, Bulgaria with seeds of *Caesalpinia pulcherrima* (L.) Sw. The seeds were collected at full botanical maturity, during the October-November period, from ten plants from the region of the city of Plovdiv, which is characterized by a typical moderate continental climate. Seeds from all plants were pooled together and an average sample was formed from which seeds were used for analyses.

An ultrasonic bath Nahita, model 620-1 of the company Auxilab, S.L. from Spain with parameters: frequency 40 kHz, bath volume 0.6 l, working with electricity supply of 220 V and

frequency 50-60 Hz.was used in the experiment. The following sonication durations with ultrasound of *Caesalpinia pulcherrima* (L.) Sw. seeds were tested:

1. Control;

2. Sonication with ultrasound for 2 minutes;

3. Sonication with ultrasound for 4 minutes;

4. Sonication with ultrasound for 6 minutes.

Daily germinated seeds were counted and germinating energy and germination were determined as described by Ferro et al. (2019) method and according to the rules of ISTA (2013) as 100 seeds in four replicates in 10 cm diameter Petri dishes with moistened filter paper and placed in a germination chamber. Seedling morphological parameters such as embryo root length and hypocotyls length were analyzed on 15 seedlings from each of the four replicates when germination was reported. The time for germination of 50% of the seeds was determined. The Mean germination time was calculated according to the method of Battle and Whittington (1966) by the equation

$$M.G.T. = \frac{\Sigma(G \times T)}{F}$$, where

T – day on which germination took place;

G – number of germinated seeds on this day (T);

F – final number of germinated seeds.

Uniformity of germination was determined according to Strona (1966).

The obtained data were subjected to analysis of variance (ANOVA) and correlation and regression relationships were determined (Fowel and Cohen, 1992)

RESULTS AND DISCUSSION

The effect of pre-sowing stimulation of the seeds is established most precisely by monitoring their sowing qualities. Table 1 presents data on the seed viability of *Caesalpinia pulcherrima* (L.) Sw. The application of ultrasound causes stimulation of germination processes in this species (Table 1). At the higher germinating energy, a larger part of the seeds germinated in a shorter period, from which it can be assumed that they are correspondingly more viable (Panayotov, 2015). The strongest increase in this sign was observed after treatment for 4 minutes, the difference with the control being 15.2%. In this variant, weak inhibition is found relative to the average duration of action.

The germination is the most important indicator for assessing seed status. This indicator significantly improves after 4 minutes of ultrasound and reaches 95.33%, which is 5.33% above the control. Next is the longer sonication of 6 minutes. The differences in these variants are statistically significant. The stimulating effect of ultrasound on seeds according to Miano et al. (2019) and Patero and Augusto (2014) is due to an increase in seed hydration processes associated with a stronger reduction in internal resistance, as well as changes in the microstructure through the formation of microchannels, i.e. the so-called effect of the sponge, causing an inertial flow.

Although weaker, an effect was also observed on the time for 50% germination of the seeds. The shortest is for sonication at 4 minutes, followed by 2 minutes. There is a weak suppression compared to the control in the 6-minute variant. A high negative correlation was found between the germination time of 50% of the seeds with the germination energy and with the germination, with correlation coefficients of r=-0.63 and r=-0.88, respectively. This indicates that as the germination time of 50% of the seeds decreases, the values of these two indicators increase.

The regression lines reflecting the changes in the vitality of the seeds depending on the steadily increasing duration of sonification with ultrasound are presented in figures 1 and 2. For the germination energy, it is linear, while of the germination it is a polynomial type. The coefficients of determination, which indicate what percentage of the dispersion of the resulting variable is explained by the action of the factor variable, are high. For germination energy, R^2=0.56 and germination, R^2=0.74. This means that in 56% and 74% of the cases, depending on whether it is for germination energy or germination, respectively, sonication will cause the observed changes in seed viability.

Table 1. Seed viability of *Caesalpinia pulcherrima* (L.) Sw., after ultrasound treatment

№	Variants	Germination energy (%)	Germination (%)	Time for 50% of seed germination (days)
1	Control	33,0	90.0	9,33
2	2 min.	39,1	90.33	8,66
3	4 min.	48,2	95.33	7,00
4	6 min.	42,3	80.33	10,33
p=5.0%		16,4	4,29	1,70
GD p=1.0%		23,75	6,25	2,46
p=0.1%		35,63	9,31	3,69
		*r=-0.63	*r=-0.88	

*correlation with Time for 50% of seed germination

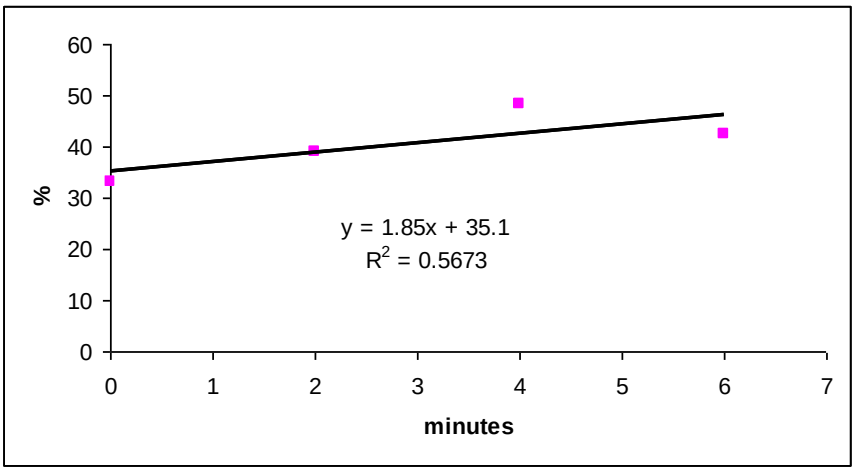

Figure 1. Regression dependence of germination energy of seeds of *Caesalpinia pulcherrima* (L.) Sw. after treatment with ultrasound

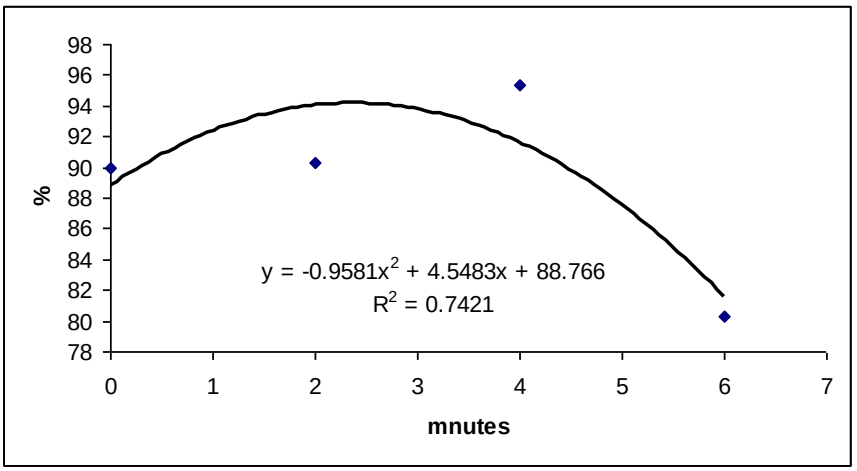

Figure 2. Regression dependence of seed germination of *Caesalpinia pulcherrima* (L.) Sw. after treatment with ultrasound

The mean seed germination time, indicating the time required for one seed to germinate, is a very good indicator for assessing the seed's vital status (Black et al. 2006). Its values vary within narrow limits (Table 2). The best results were obtained after sonication for 2 minutes, after which a longer period was required for the germination of one seed in the other variants. The effect of the highest tested duration was inhibitory. The uniformity of the seed's germination is improved more significantly. For all sonication, the values are higher compared to the control. The largest increase was at 4 minutes, with 12.37%, followed by 2 minutes. A decrease relative to these two variants, but higher than untreated seeds was observed for a duration of 8 min.

Seedling morphological characters are very closely related to seed vigor (Copeland and McDonald, 2001). Application of the ultrasound provoked enhancement of growth of *Caesalpinia pulcherrima* (L.) Sw. seedlings (Table 3). A stimulating effect is reported for all tested periods of sonification. The greatest increase in the embryo root was at 4 minutes, reaching a maximum length of 2.06 cm., followed by that of variant at 2 minutes. A decrease, but still with higher values, compared to the control was observed for the 6-minute variant. A similar trend was found for hypocotyl length. Again, at 4 minutes of sonication, the results were best, with an increase over control of approximately threefold. The stimulatory effect was weaker at 6 min, with twice as long hypocotyls than in untreated seeds, followed by 2 min treatment. The data obtained for both indicators are with mathematical significance at the highest level of evidence of p=0.1%

Table 2. Sowing characteristics of *Caesalpinia pulcherrima* (L.) Sw. seeds, after ultrasound treatment

№	Variants	Mean germination time (days)	Uniformity of germination (%)
1	Control	9,70	13,05
2	2 min.	8,33	22,31
3	4 min.	9,29	25,42
4	6 min.	9,86	20,89
p=5.0%		1,66	13,05
GD p=1.0%		2,40	18,9
p=0.1%		3,60	28,35

The daily germination temp of the seeds is presented in Figure 3. The earliest and relatively uniform course of germination was shown by the seeds from the control and sonication for 2 minutes. Higher durations of 4 and 6 minutes delayed germination, but it increased very sharply on days 9 and 10 and this continued until day 11. In all variants, the peak is at day 11, especially well expressed in 4 minutes. After that day, no germinated seeds were counted.

Table 3. Morphological features of *Caesalpinia pulcherrima* (L.) Sw. seeds, after ultrasound treatment

№	Variants	Length of embryo root (cm)	Length of hypocotyls (cm)
1	Control	0,93	0,64
2	2 min.	1,82	1,17
3	4 min.	2,06	1,74
4	6 min.	1,35	1,28
p=5.0%		0,18	0,02
GD p=1.0%		0,27	0,04
p=0.1%		0,41	0,06

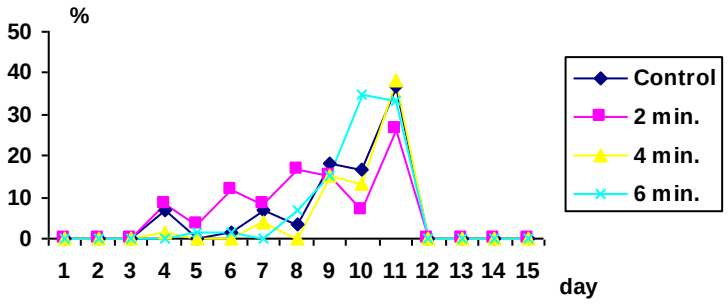

Figure 3. Daily temp of germination of *Caesalpinia pulcherrima* (L.) Sw. seeds after sounding with ultrasound

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CONCLUSIONS

The seed treatment of *Caesalpinia pulcherrima* (L.) Sw. with ultrasound causes a significant improvement in their vitality status. The highest germination energy and germination were observed when sonicated for 4 minutes. The established regression dependences on the duration of ultrasound have a high coefficient of determination, as for the germination energy it is of the linear type, while for the germination it is of the polynomial type. The uniformity of germination is significantly improved, and the effect of sonification on the mean germination time is weaker. The embryo root and hypocotyl develop more strongly when exposed to ultrasound for 4 minutes. To treat the seeds of *Caesalpinia pulcherrima* (L.) Sw. with ultrasound, a duration of 4 minutes is recommended.

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FACTORS AFFECTING MARKET PARTICIPATION: A CASE STUDY OF SMALLHOLDER FARMERS IN THE EHLANZENI DISTRICT, MPUMALANGA PROVINCE IN SOUTH AFRICA

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ABSTRACT

This paper highlighted the factors affecting market participation by small holder farmers in Ehlanzeni District. Project assessment was conducted in Ehlanzeni District by Agricultural Research Council (ARC) in collaboration with the Department of Agriculture, Land Reform and Rural Development (DALRRD) with an attempt to identify market needs and access in the district. A total of (110) smallholder farmers with 884 beneficiaries participated in the study and were from the following local municipalities: Thabachweu (20), Bushbuckridge (36), Mbombela (19), Umjindi (6) and Nkomazi (29). Quantitative and qualitative designs were used as a detailed questionnaire written in English, with a focus group discussion, a stakeholder's discussion and field observations as part of the data collection. A purposive sampling technique was used to select one hundred and ten (110) smallholder farmers in order to cover uniformity and homogenous characteristics such as infrastructure requirements, skills availability, production challenges, agricultural training needs, water source needs, educational level, market availability and other factors. Data was coded, captured, and analysed using Software Programme for Social Science (SPSS) version 21. The following analysis were conducted: Descriptive and Univariate Regressions. The results showed positive association among the following variables: age, educational level, farming experience, land size, land acquisition, farming fulltime, agricultural training and market participation. It is evident that interventions should be implemented focusing on identified factors in order to improve market participation by smallholder farmers and community growers in the Ehlanzeni district. The study recommends that for holistic market participation among smallholder farmers in Ehlanzeni District, the government and other policy makers should increase the knowledge, experience and marketing skills of smallholder farmers and community growers to enhance food security and improve sustainable livelihoods.

Key Words: Market Participation, Smallholder Farmers, Community Growers, Ehlanzeni District, Mpumalanga Province and South Africa.

INTRODUCTION

Smallholder farmers and community growers, irrespective of their selected farming niche are faced with many difficulties before being able to enter the market. It was lately emphasised by Maponya (2021a) that smallholder farmers and community growers who are engaged in agroforestry practice lack access to formal markets. A lack of formal contractual agreements,

market information, quality of the product and sanitary measures to enable them to meet market requirements further discourage these farmers from investing in such an enterprise (Maponya, 2021b).

South Africa is known as a semi – arid country vulnerable to water stress, particularly drought. According to ARC (2020), Mpumalanga Province annual rainfall varies from 400 mm in the lowveld to more than 1000 in most of the escarpment area. Substantial areas receive 600-700 mm. Likewise summer temperatures vary from cool in high-lying plateau and escarpment areas to hot in the lowveld (T_{max} in January 21-22 and 33-34°C respectively, with substantial areas between 31 and 32°C). Winter temperatures vary from cold in high-lying escarpment areas to mild in the lowveld (T_{min} in July 1-2°C and 9-10 respectively). Evaporation is moderately high in the lowveld at 900-1100 mm for the summer months of October to March. Frost does not occur regularly in the lowveld. The threshold for rainfall agriculture is averaged at 250 mm annually. In terms of forestry, the plantation forests of South Africa use just 3% of the country's total water resources and rainfall needs to be higher than 750 mm per annum to sustain commercial forestry.

Agriculture in Ehlanzeni District has annual turnover of R1, 5 billion consisting of subtropical fruit farming and processing, avocados, bananas, mangoes, litchis, macadamia nut, sugar cane cultivation by large and smallholder farmers and sugar production at two mills producing nearly 20% of South Africa's sugar (EDM, 2014). Ehlanzeni produces 16% of South Africa's citrus crop and export 70% (EDM, 2014). The Agricultural research Council (ARC) is currently involved in an agricultural and agroforestry projects in the Ehlanzeni district in Mpumalanga Province where beneficiaries were trained and mentored, allocated production inputs so as to implement sustainable production and develop markets (Maponya et al. 2015 and Maponya, 2021a). In addition, there is a great interest in agroforestry practice among the smallholder farmers and community growers in the Ehlanzeni district but the challenge is market access (Maponya et al. 2021c).

In the present study, research was conducted with the overall aim of establishing if sustainable agricultural markets are viable in the Ehlanzeni district. The major objectives were: (1) To identify and describe the socio economic characteristics of the selected smallholder farmers in the Ehlanzeni district. (2) To determine factors that affect decision making to participate in agricultural markets in Ehlanzeni district.

Methodology

Study Area

The research focused on the Ehlanzeni District as indicated in Figure 1. A total of (110) smallholder farmers with 884 beneficiaries participated in the study and were from the following local municipalities: Thabachweu (20), Bushbuckridge (36), Mbombela (19), Umjindi (6) and Nkomazi (29).

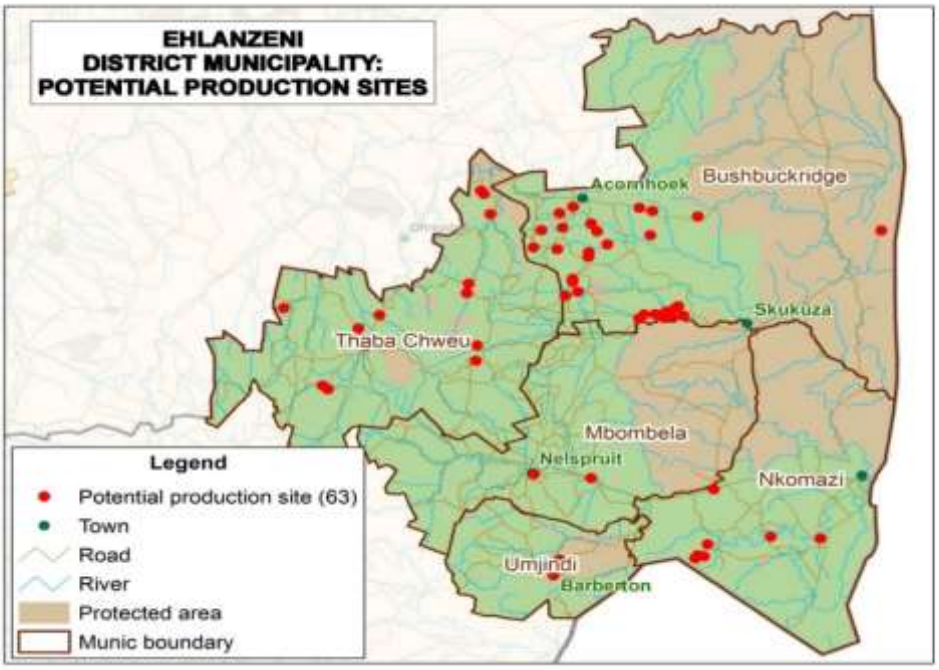

Figure 1: Ehlanzeni District Map

Study Design

The research employed both qualitative and quantitative methods concurrently and this was applied with the aim on establishing the limitations, balance and strength of the data. Furthermore, the methods included participatory action research as the smallholder farmers, community growers and stakeholders benefitted while the research was ongoing. Data collection methods were via interviews, site observations, focus groups, past researches, web and governmental reports. Pre and post-intervention questionnaires were developed and pilot tested with researchers working on community development within the Agricultural Research Council (ARC).

A desk-top audit on natural resources was also conducted for the development of crop suitability maps. The activity assisted in determining the biophysical viability of smallholder farmers and community growers in the Ehlanzeni district. The audit results gave provision of an overview of the districts in terms of orientation, water availability, selected climate and soil attributes as well as crop suitability.

Sampling Procedure and Analytical Technique

A purposive sampling technique was used on selected 110 smallholder farmers in the Ehlanzeni district. The list of smallholder farmers were supplied by the Department of Agriculture, Land Reform and Rural Development (DALRRD) and the sample size was agreed with the stakeholder. A rule of thumb was applied, which is the minimum selection of 10% of the population and it is considered as a good sample size. Furthermore, data collected was

analysed quantitatively using the Statistical Package for Social Sciences (SPSS) windows version 21. Descriptive and univariate analyses were applied.

Econometrics Model

Descriptive Analysis was used to describe data and Univariate Regression Analysis was conducted to demonstrate the relationship and association of variables. Univariate regression analyses is used to test the association of one explanatory variable at a time with the outcome without worrying about other variables or confounders (unconditional association). This is essential in order to shortlist variables for multivariable analysis, especially if there are a large number of explanatory variables. It also excluded the variables from further analysis that do not show any significant association with the outcome. Results of univariate logistic regression analyses included Wald, likelihood ratio, chi-square test statistics and P-values, parameter estimates and standard errors, and odds ratios and their confidence limits. For logistic regression, values of parameter estimates are not very intuitive as they are calculated on a log scale. Therefore, odds ratios are examined, which are calculated after exponentiation parameter estimates. An odds ratio of <1 indicated negative association, whereas values >1 indicated positive association of the tested variable with the outcome. The following econometric model was used to determine association of variables (Greene, 1993):

Wi = _ + _Xi + _i (1)
Wi is the dependent variable value for person i (2)
Xi is the independent variable value for person i (3)
_ and _ are parameter values
(4)
_i is the random error term
(5)
The parameter _ is called the intercept or the value of W when X = 0 (6)
The parameter _ is called the slope or the change in W when X increases by one
(7)

Table 1: Definition of Variables included in the Model

Variables	Description of variables	Unit	Expected Sign
PAR	1 if a farmer participate in the market, 0 otherwise	Dummy	+
AGE	Age of the farmer	Years	+
GEN	1 if male, 0 otherwise	Dummy	+
EDU	The highest educational level that a farmer possess	Number	+
WAT	1 if irrigation, 0 otherwise	Dummy	+
IMP	1 if tractor, 0 otherwise	Dummy	+
FSI	The size of the farm which is under crop production	Ha	+
EXP	Farming experience of the respondent(s)	Years	+
SOI	1 if soil is tested, 0 otherwise	Dummy	+/-

Table 2: Distribution of smallholder farmers according to their socio-economic characteristics.

Variables	**Smallholder Farmers**	**% Smallholder Farmers Soci Economic Characteristics**
Gender		
Female	49	45
Male	61	55
Total	110	100
Age Categories		
18 – 35	19	17
36 – 45	22	20
46 – 60	44	40
>60	25	23
Total	110	100
Level of Education		
Primary Education Completed	17	16
Primary Education Incomplete	24	22
Secondary Education Incomplete	5	4
Secondary Education Completed	52	47
Tertiary Education Completed	12	11
Total	110	100
Employment Status		
Full Time Farmer	110	100
Total	110	100
Land Size		
1 - 5	63	57
6 - 10	23	21
11 - 20	15	14
21 - 49	2	2
>50	7	6
Total	110	100
Land Acquisition		
Land Redistribution for Agricultural Development (LRAD	7	6
Own Finance	11	10
Permission to Occupy (PTO)	82	75
Inheritance	8	7
Lease	2	2
Total	110	100
Farming Experience		
1 - 5	33	30
6 - 10	21	19
11 - 20	34	31
21 - 49	13	12
>50	9	8
Total	110	100
Training Provided		
Yes	76	69
No	34	31
Total	110	100

RESULTS AND DISCUSSION

Smallholder Farmers Socio-Economic Characteristics

The results in Table 2 indicate gender composition with females at 45 % and males at 55 % and all smallholder farmers were farming fulltime. This results are in line with the sampling criteria, which targeted fulltime smallholder farmers. The age distribution of the smallholder farmers indicated that the majority were in the age group of 46 – 60 (40%). As indicated in Table 2, youth involvement is 17%, 36 – 45 (20%) while > 60 had 23%. Furthermore, 22% had incomplete primary education, 16% had completed primary education, 4% had incomplete secondary education, 47% had completed secondary education and 11% had completed tertiary education. The smallholder farming experience varies from farmer to farmer. As indicated in Table 2, majority of the smallholder farmers (31%) had 11 – 20 farming experience. Quite a number of farmers (30%) had 1 - 5 farming experience. Only 8% of farmers had 50>farming experience while other farmers had different farming experience as indicated in Table 2. Results on land acquisition (Table 2) indicated that the majority of smallholder farmers received land through permission to occupy (75%), own finance (10%), land for redistribution for agricultural development (LRAD) (6%), inheritance (7%) and lease (2%). The role of traditional leaders must be applauded in Ehlanzeni district as majority of smallholder farmers were using permission to occupy (PTO).

A large percentage (57%) of smallholder farmers utilize 1-5 hectares (Table 2) while 21% utilize (6 – 10 hectares). A few number of smallholder farmers (6%) utilize >50ha as indicated in table 1. The results indicated that Ehlanzeni district is dominated by smallholder farmers and more support is needed to enable them to be commercial. According to Maponya (2021a) and Maponya (2021b), most of these smallholder farmers own less than 1 hectare of land and those that own more than 20 hectares of land could be farmers who are migrating from a stage of smallholder to emerging or commercial farming. These farmers producing on less than one hectare mainly produce for household consumption and as the sizes of the farms increase the farmers produce for commercial purposes (Maponya, 2021a and Maponya, 2021b). As indicated in Table 2, 69% of smallholder farmers received some level of agricultural training, while only 31% received no agricultural training.

Smallholder Farmers Market Access Status

Table 3 indicated that most smallholder farmers were trading in informal markets (37%) with 25 % trading in both formal and informal markets. Very few (6%) had access to formal markets. Quite a number of smallholder farmers (32%) had no access to markets. In addition, the Agricultural Research Council (ARC) recently requested by the Mountain to ocean (MTO) plantation in the Ehlanzeni district to assist in market access. MTO has allocated 40ha to the nearby communities and smallholder farmers in 2021 to plant various crops in between their trees and the only challenge now is market information and access especially formal market. Hence, the rational to undertake this study was to determine factors that encourage market participation for smallholder farmers and community growers.

Table 3: Smallholder Farmers Market Access

Markets access	Smallholder Farmers	Percentage
Formal market	7	6
Informal market	41	3
Formal and Informal Markets	27	25
No Market Access	35	32
Total	110	100

The results from Table 4 should be interpreted as follows: 1st – 3rd training challenges = Most challenging; 4th – 5th training challenges = Moderate challenging and 6th – 7th training challenges = Least challenges.

The results showed that 66 percent of smallholder farmers recognised marketing as the 1st training challenge, 20 percent of smallholder farmers recognised marketing as the 2nd training challenge and 5 percent of smallholder farmers recognised marketing as the 3rd training challenge. Furthermore, table 4 showed that 3 percent, 2 percent and 2 percent of smallholder farmers indicated marketing as the 4th, 5th and 6th training challenge respectively. Only 2% percent of smallholder farmers recognised marketing as the 7th training challenge. It is very clear that attention should be given to marketing as indicated by the 1st, 2nd and 3rd training challenges.

Table 4. Marketing as a Training Challenge

Training Challenges	**Smallholder Farmers**	**Percentages**
1st Training Challenge	73	66
2nd Training Challenge	22	20
3rd Training Challenge	5	5
4th Training Challenge	4	3
5th Training Challenge	2	2
6th Training Challenge	2	2
7th Training Challenge	2	2
Total	**110**	**100**

Table 5 indicates that all smallholder farmers (100%) were receiving extension services. Of the group receiving extension services, majority of smallholder farmers (65%) received extension service through formal extension, formal extension, farmer to farmer, neighbours and family support while other smallholder farmers were receiving different types of extension services as indicated in Table 5.

Table 5: Smallholder Farmers Access to Extension Services

Extension Services Percentages	**Smallholder Farmers**	
Access to Extension Service		
Yes	109	100
Form of Extension Service		
Formal Extension	9	8
Formal Extension, Farmer to Farmers		115
Formal Extension, Farmer to Farmer, Neighbours and Family Support	71	65
Formal Extension, Family Support/ Neighbours	6	6
Formal Extension, Family Support, Neighbours	8	7
Total	**110**	**100**

Univariate Analysis among variables

Results in Table 6 showed that there is positive association among the following variables; age, education, farming experience, land acquisition, land size, agricultural training, farming fulltime and market participation. This was supported by more than 1 estimate values at the 95% confidence interval. The results in Table 6 further predicted 84 % (R - squared = 0.84) variation in the dependent variable was explained by the independent variables. Prediction accuracy were assessed based on the coefficient of determination (R - squared). The coefficient of determination R- squared was used to explain the total proportion of variance in the dependent variable explained by the independent variable. The R- squared removes the influence of the independent variable not accounted for in the constructs. R-squared is always between 0 and 100%. In general, the higher the R-squared, the better the model fits the data.

It must be emphasised that social-demoFigureic characteristics of the smallholder farmers play a very important role in either promoting or impeding their participation in agricultural markets. As indicated in Table 6, smallholder farmers age had a positive association with market participation (>1). Studies by Barret (2007); Makhura (2001); Maponya et al. 2014, Maponya et al. 2015 and Maponya et al. 2021a also concur with the positive relationship between smallholder farmers age and market participation.

There is a positive association among farming experience, farming fulltime, education and market participation as indicated in Table 6. A well-educated, fulltime farmer with experience will always understand market conditions better and will enhance smallholder farmer's market orientation. Advancement in education increases the education level and the ability to obtain better market information Makhura (2001), Maponya et al. (2015) and

Maponya et al. 2021a and Maponya et al 2021b. Agricultural training is also positively associated with market participation as smallholder farmers and community growers need to be equipped with technical skills to improve their productivity and to negotiate in the agricultural markets. As indicated in Table 6, there is a positive association among land acquisition, land size and market participation (>1). Land acquisition and size provides a necessary condition for agricultural growth and development and ultimately successful agricultural commercialisation of smallholder farmers and community growers. Smallholder farmers and community growers with land and bigger land size were found to participate more because of their ability to produce bigger volumes to the market. According to Maponya et al. 2021a and Maponya et al. 2021b land acquisition and land size enables smallholder farmers and community growers to produce surplus to the market.

Table 6: Univariate Regression Analysis of factors affecting market participation in Ehlanzeni District

Variable	**Total**	**(%)**	**OR [95%CI]**
Farming Experience	110	100	1.11[0.45-303]1
Age	110	100	1.34[0.058-2.9]1
Land Acquisition	110	100	1.09[0.272- 3.9]1
Education	110	100	1.29[0.226- 13.6]1
Farming fulltime	110	100	1.33[0.23–4.3]1
Land Size	110	100	1.05[0.24–5.57]1
Agricultural Training	110	100	1.12[0.28 – 4.88]1

OR= Odds ratio; 95% CI = 95% confidence intervals; 1< = no association; 1> = association

Selected Photos of the smallholder farmers and community growers

Fig 2: Ehlanzeni District Smallholder Farmers harvesting sweet potatoes for the informal market.

Fig 3: Ehlanzeni District community growers involved in agroforestry practice harvesting groundnuts for the informal market.

CONCLUSIONS AND RECOMMENDATIONS

Various socio – economic characteristics of smallholder farmers were described and determined. The research indicated that the factors enhancing market participation in the Ehlanzeni district were: age, educational level, farming experience, land size, land acquisition, farming fulltime, agricultural training. The established factors will influence the manner in

which smallholder farmer's benefit from the opportunities availed in the agricultural markets especially formal in respect of the quality and quantity of the agricultural produce. It is expected that addressing such factors may create enabling conditions that would encourage smallholder farmers and community growers to access and participate more effectively in the markets especially formal. Based on the findings, the research recommends that for holistic market participation among smallholder farmers and community growers in Ehlanzeni District, the government and other policy makers should increase the knowledge, experience and marketing skills of smallholder farmers and community growers. This will go a long way in assisting smallholder farmers and community growers to supply quality products to the market especially formal and improve their livelihoods.

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INVESTIGATION OF THE EFFECT OF THE COVID-19 PROCESS ON THE AGRICULTURAL SECTOR: AYDIN PROVINCE EXAMPLE

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ABSTRACT

The covid-19 epidemic, which started in Wuhan city of China's Hubei province in December 2019 and spread all over the world, affected all sectors, especially health. The epidemic triggered the contraction of production, domestic trade and foreign trade in many different sectors. Especially in the agricultural sector in Turkey, problems have arisen in terms of supply, sharing and accessibility. This study was conducted to examine the effects of the pandemic on farmers working in the agricultural sector. For this, a face-to-face survey study was conducted with 100 farmers carrying out their activities in Aydın. According to the findings, it was determined that the farmers had difficulties in finding workers, supplying inputs and selling products, and the costs increased in this period. A significant portion of the farmers stated that they could easily obtain a permit to go out. 37.0% of the farmers stated that they delayed the harvest. The study can guide policy makers to solve the problems encountered in the agricultural sector in times of crisis.

Keywords: Agriculture, Farmer, Pandemic, Production

INTRODUCTION

The agricultural sector is of great importance in terms of healthy and balanced nutrition of human life. With the onset of the covid-19 epidemic in the world, it has become clear how important the agriculture and health sector is. After the full closure, people more than expensive items; They competed with each other for basic food products such as legumes, bread, fruit and vegetables. As the consumer began to have problems in these matters, the importance of domestic production and small family farmers emerged (Doğan and Doğan, 2020). The covid-19 epidemic, which started in Wuhan city of China's Hubei province in December 2019 and spread all over the world, affected all sectors, especially health. The Covid-19 process triggered the contraction of tourism, industry, services, production in sectors, domestic trade and foreign trade in the global economy. Common measures include closing schools, restricting travel, banning public gatherings, investing in emergency health facilities, contact tracing, social assistance, other practices in the spread of the epidemic, improving health systems, and executing the economic consequences of these actions. The Covid-19 outbreak has increased the strategic importance of the agricultural sector and created a social awareness. The pandemic may cause production declines and agricultural shortages (Uçak, 2020). The effects of the

epidemic are more related to supply, sharing and accessibility. The absence of problems in production and accessibility depends on ensuring the continuity of production, labor mobility and food supply. The rise in agricultural input costs can be listed as possible problems in accessing the market and labor force (Demir, 2020). This study was conducted to examine the effects of the covid-19 pandemic on the agricultural sector. This study can guide the measures to be taken for the agricultural sector during epidemic periods.

MATERIAL AND METHOD

The main material of this study was the survey data obtained from the producers registered in the farmer registration system and continuing their agricultural activities in Aydın Province. Due to time and cost constraints, the sample scope was limited to Koçarlı, Söke and Nazilli districts, which are important agricultural production areas of Aydın. According to the information received from the District Directorates of Agriculture, a total of 13,018 producers are registered in the Farmer Registration System in the covered settlements and these producers constitute the main population. The sampling formula used to determine the number of surveys is as follows: This formula (Newbold, 1995):

$$n = \frac{Np(1-p)}{(N-1)\sigma^2_{\hat{p}_x} + p(1-p)}$$

where n is the sample volume, N is the population volume (13018), p is the prediction ratio (0.5 for the maximum sample volume), the probability level confidence interval (95% confidence interval, 1.96 σp: 0.10 for 0.10 margin of error, σp: 0.05102) (Newbold, 1995). Population volume (N) As a result of the calculation, the sample volume was determined as 96 for this study, and face-to-face surveys were conducted with a total of 100 producers due to proportional losses.

FINDINGS

SociodemoFigureic Characteristics of Farmers

Table 1 shows that the average age of the farmers is minimum 24 and maximum 72. It has been observed that they have a minimum of 3 years, a maximum of 54 years and an average of 19 years of experience. If we look at the number of animals, 39% of them have animals, 61% of them do not. It has been observed that the land size is minimum 2 decares and maximum 1250 decares. Of the farmers participating in the survey, 38% have additional income and 62% do not have any additional income. In terms of land assets, there are 16% vineyards, 37% gardens and 79% fields. Our farmers are 21% low, 68% medium and 11% high in terms of income.

Table 1: SociodemoFigureic characteristics

	N	Minimum	Maximum	Average	Std. Error
Age	100	24,00	72,00	41,2100	10,70664
Experience	100	3,00	54,00	19,0800	11,73244
Number of animals	100	1,00	25,00	1,8600	2,39958
Land size	100	2,00	1250,00	183,1500	234,02402

Input Usage

In Table 2, statements regarding the difficulties in finding seasonal workers during the Covid-19 period are presented. According to this statement, 50.0% agree, 44.0% disagree and 6.0% are undecided. According to Table 3, it is observed that the pandemic has changed the planting decisions of the farmers.

Table 2: Finding a seasonal worker

I had a hard time finding seasonal workers	Frequency	%	Cumulative %
I strongly disagree	15	15,0	15,0
I do not agree	29	29,0	44,0
I'm undecided	6	6,0	50,0
I agree	28	28,0	78,0
Absolutely I agree	22	22,0	100,0
Total	100	100,0	

Table 3: October decision change

My October decision has changed	Frequency	%	Cumulative %
I strongly disagree	19	19,0	19,0
I do not agree	34	34,0	53,0
I'm undecided	12	12,0	65,0
I agree	25	25,0	90,0
Absolutely I agree	10	10,0	100,0
Total	100	100,0	

In Table 4, statements regarding the difficulties in obtaining fertilizers during the Covid-19 period are presented. According to this statement, 74.0% agree, 20.0% disagree and 6.0% are undecided. Accordingly, it was stated that it was difficult to supply fertilizer during the covid-19 period. Excess inventory was cited as the reason for difficulty in obtaining increased prices.

Table 4: Fertilizer supply situation

I had difficulty in obtaining fertilizer	Frequency	%	Cumulative %
I strongly disagree	9	9,0	9,0
I do not agree	11	11,0	20,0
I'm undecided	6	6,0	26,0
I agree	41	41,0	67,0
Absolutely I agree	33	33,0	100,0
Total	100	100,0	

In Table 5, statements regarding the difficulties in obtaining medicines during the Covid-19 period are presented. According to this statement, 66.0% agree, 31.0% disagree and 3.0% are undecided. Accordingly, it has been stated that it is difficult to obtain medicine during the covid-19 period.

Table 5: Drug supply status

I had a hard time getting medicine	Frequency	%	Cumulative %
I strongly disagree	12	12,0	12,0
I do not agree	19	19,0	31,0
I'm undecided	3	3,0	34,0
I agree	35	35,0	69,0
Absolutely I agree	31	31,0	100,0
Total	100	100,0	

According to Table 6, it was stated that farmers had difficulty in obtaining seeds during the covid-19 period.

Table 6: Seed supply status

I had a hard time getting seeds.	Frequency	%	Cumulative %
I strongly disagree	12	12,0	12,0
I do not agree	25	25,0	37,0
I'm undecided	6	6,0	43,0
I agree	30	30,0	73,0
Absolutely I agree	27	27,0	100,0
Total	100	100,0	

Price

53.0% of the farmers stated that they had difficulty in finding buyers for the sale of the products in this period (Table 7).

Table 7: Status of finding buyers

I had a hard time finding buyers for the sale of products	Frequency	%	Cumulative %
I strongly disagree	18	18,0	18,0
I do not agree	25	25,0	43,0
I'm undecided	4	4,0	47,0
I agree	35	35,0	82,0
Absolutely I agree	18	18,0	100,0
Total	100	100,0	

According to Table 8, it was stated by the farmers that fertilizer prices increased during the covid-19 period. According to Table 9, farmers stated that drug prices increased during the pandemic period. According to Table 10, farmers stated that seed prices increased during the pandemic period.
A significant part of the farmers did not find the support given to agriculture sufficient during the epidemic period (Table 11).

Table 8: Fertilizer price situation

I think fertilizer prices have increased	Frequency	%	Cumulative %
I strongly disagree	2	2,0	2,0
I do not agree	4	4,0	6,0
I'm undecided	4	4,0	10,0
I agree	17	17,0	27,0
Absolutely I agree	73	73,0	100,0
Total	100	100,0	

Table 9: Pharmaceutical price status

I think drug prices are going up	Frequency	%	Cumulative %
I strongly disagree	3	3,0	3,0
I do not agree	3	3,0	6,0
I'm undecided	2	2,0	8,0
I agree	18	18,0	26,0
Absolutely I agree	74	74,0	100,0
Total	100	100,0	

Table 10: Seed price situation

I think seed prices have increased	Frequency	%	Cumulative %
I strongly disagree	3	3,0	3,0
I do not agree	2	2,0	4,0
I'm undecided	1	1,0	5,0
I agree	18	18,0	23,0
Absolutely I agree	76	76,0	99,0
Total	100	100,0	

Table 11: Agricultural sector support status

The support provided to the agricultural sector was sufficient	Frequency	%	Cumulative %
I strongly disagree	54	54,0	54,0
I do not agree	19	19,0	73,0
I'm undecided	8	8,0	81,0
I agree	8	8,0	89,0
Absolutely I agree	11	11,0	100,0
Total	100	100,0	

CONCLUSION AND RECOMMENDATIONS

Covid-19, which emerged at the end of 2019, has posed a great threat to public health. Due to the high rate of transmission of the disease, strict restrictions such as travel-transport restrictions, interruptions in social life, pauses in working life have occurred with the curfew. This situation has affected the agricultural sector. In this study, the effects of the Covid-19 process on the agricultural sector production in Aydın province are discussed. In general, the results indicate that farmers have problems in input supply and supply chain rather than production. The reason for this may be reasons such as raw material price increases, procurement costs and shipping problems, export bans. In order to prevent these problems in the future, it may be suggested to improve internal control and to support domestic production instead of imported agricultural inputs.

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EFFECTS OF MEDETOMIDINE/KETAMINE ANAESTHESIA AND ATIPAMEZOLE ON OCULAR PARAMETERS IN DOGS

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ABSTRACT

This study was aimed at investigating the effects of the combined use of medetomidine with ketamine hydrochloride, and their reversal by atipamezole, on anesthesia monitoring (AM) and ocular parameters including intraocular pressure (IOP), tear production (TP), and horizontal pupil diameter (HPD) in dogs. Ten sexually intact female dogs (weighing 7.0-29 kg and aged 0.75-3.0 years), which were referred for ovariohysterectomy (OVH) from a local dog shelter, were included in this study. General anaesthesia was induced, 15 min after the intramuscular (IM) administration of 100 µg/kg of medetomidine premedication, with 10 mg/kg of IM ketamine. The animals were monitored for electrocardioFigurey, heart rate, respiratory rate, non-invasive blood pressure, pulse oximetry and rectal temperature throughout anaesthesia. At the end of the OVH procedure, atipamezole was administered at a dose of 500 µg/kg by IM route. Ocular measurements were performed in laterally recumbent dogs, such that the head was raised and care was taken to avoid the occlusion of the jugular veins and the placing of pressure on the eyeball while retracting the eyelids. While group showed a significant decrease in heart rate (HR) after the induction of anaesthesia (T0 vs. T2, $p<0.05$). While TP was significantly lower at T5 ($p=0.01$). In conclusion, while MED-KET was found to be effective in reducing TP and the HPD, and altering the anesthesia monitoring response to general anaesthesia, atipamezole could be used in dogs for a quick and safe recovery to baseline values after abdominal surgery, such as OVH.

Keywords: Anesthesia monitoring, Atipamezole, Dog, Ocular parameters

INTRODUCTION

Increase in intraocular pressure (IOP) is a major problem in ophthalmic surgery such that avoiding and controlling the rise of IOP before, during and after surgical interventions is a basic requirement (Banga et al., 2015; Miller, 2019). Anaesthesizing a patient with an ocular penetrating injury on a full stomach is challenging for the anaesthesiologist. In such cases, anaesthesia should be induced rapidly without increasing the IOP. It is imperative for the anaesthesiologist to weigh the risk of aspiration for the injured eye against the risk of blindness that could be caused by an increased IOP and ocular extrusion (Banga et al., 2019). Anaesthesia induction techniques impact IOP in humans (Badrinath et al., 1986; Lev and Rosen, 1994). Sudden increases in the IOP in cases of glaucoma or near-perforating corneal trauma may trigger dramatic effects. Ocular content prolapse not only complicates the surgical intervention, but also results in poor prognosis (Chmielevski et al., 1997; Smith et al., 2019). In cases of glaucoma, a slight increase in IOP would suffice to reduce the axoplasmic flow in the optic nerve, and thereby, cause predisposition to further injury (Hofmesiter et al., 2009; Smith et al., 2019; Muench et al., 2021). Thus, procedures to prevent the increase of IOP during the induction of anaesthesia would be of benefit in cases of ocular trauma and glaucoma and in

patients anaesthesized for intraocular surgery.

Anaesthesia monitoring (AM) is used for ensuring sedation, comfort, amnesia, and anxiety relief during diagnostic and therapeutic procedures involving sedation and analgesia (Gelatt et al., 2007; Karabağlı et al., 2014; Senthil et al., 2021). Given that respiratory depression causes grave injuries (Karabağlı et al., 2014; Park et al., 2019), optimal AM refers to maintaining anaesthesia and normal cardiovascular functions without airway obstruction and severe respiratory depression. Another basic requirement of AM is the ability to rapidly regulate the depth of general anaesthesia if needed. Various analgesics, sedativesand narcotics are used for these purposes with minimum adverse effects (Karabağlı et al., 2014).

Anaesthesia induced with medetomidine/ketamine (MED-KET) combinations, which can be antagonized by atipamezole, has been proven to be useful in animals (Badrinath et al., 1986; Lev and Rosen, 1994; Keating et al., 2020). The primary benefit of these combination is the competitive reversibility of medetomidine by atipamezole, which enables an improved control of anaesthetic depth, a shorter recovery period and reduced risk of hypothermia (Badrinath et al., 1986; Lev and Rosen, 1994; Keating et al., 2020).

While the pharmacokinetics and pharmacodynamics of various premedicants and general anaesthetics have been studied in detail, only scarce and controversial information is available on their effects on AM and functional ocular parameters, including IOP and pupil diameter in animals and particularly dogs (Karabağlı et al., 2014; Bruniges et al., 2016). To our knowledge, there is no previous study on the effects of MED-KET anaesthesia on AM and ocular parameters in dogs.

This study was aimed at investigating the effects of the combined use of medetomidine with ketamine hydrochloride, and their reversal by atipamezole, on AM and ocular parameters including IOP, tear production (TP), and horizontal pupil diameter (HPD) in dogs.

MATERIAL AND METHOD

This study was randomized and single-blinded. Ten sexually intact female dogs (weighing 7.0-29 kg and aged 0.75-3.0 years), which were referred for ovariohysterectomy (OVH) from a local dog shelter, were included in this study. The study design was approved by the Local Ethics Committee (Approval Number: 2016-03/2).

The animals were randomly included to group (T0, baseline). Carprofen was administered intravenously at a dose of 4 mg/kg (Rimadyl, Pfizer, USA) to all dogs for analgesia. General anaesthesia was induced, 15 min after the intramuscular (IM) administration of 100 µg/kg of medetomidine premedication (Tomidine, Provet, Turkey; T1: 10 min after premedication), with 10 mg/kg of IM ketamine (Ketamin, Egevet, Turkey; T2: 10 min after induction). Physiological saline solution (Eczacıbaşı, Istanbul, Turkey) was administered intravenously at a dose rate of 10 ml/kg/h during the operation. The animals were monitored for electrocardioFigurey, heart rate, respiratory rate, non-invasive blood pressure, pulse oximetry and rectal temperature (G9000, Guoteng Co Ltd, China) throughout anaesthesia (T3: 15 min after the start of the operation; T4: after the last suture). No other anaesthetic or analgesic drug was administered during the operation. At the end of the OVH procedure, atipamezole (Reversal 5 mg/kg, Provet, Turkey; T5: 10 min after reversal) was administered at a dose of 500 µg/kg by IM route.

The animals breathed spontaneously during anaesthesia. The dogs were monitored for respiratory rate (RR), heart rate (HR), mean arterial pressure (MAP), hemoglobin oxygen saturation (SpO_2), and body temperature (BT) with a multiparameter monitor (G9000, Guoteng Co Ltd, China). Clinical data, including the HR, RR, SpO_2, MAP, and BT were recorded at six

time points (T0, T1, T2, T3, T4, T5). Arterial blood pressure was measured by oscillometry (Rap100, Riester, Germany).

Ocular parameters were evaluated at the time points T0, T1, T2, T3, T4, and T5. Ocular measurements were performed in laterally recumbent dogs (except T3), such that the head was raised and care was taken to avoid the occlusion of the jugular veins and the placing of pressure on the eyeball while retracting the eyelids. One ophthalmologist, blinded to the anaesthetic technique, performed all of the IOP measurements and used a rebound tonometer (TonoVet, Tiolat, Finland). IOP was measured at the center of the cornea, in accordance with the instruction manual of the tonometer. The factory-calibrated tonometer was recalibrated before each measurement. Three readings were recorded for the right eye at each time point. The mean value of the three readings was calculated. No local anaesthetic was used prior to the IOP measurements. HPD measurements were performed with a caliper at the same time points. The Schirmer tear test I was conducted by placing commercial Schirmer strips (Schirmer Tear Test, Eickmeyer, Germany) in the lower fornix for 1 min. The dogs underwent OVH after the T2 time point. All surgical operations were performed by a single veterinarian with assistance from veterinary students. All dogs were discharged 24 h after surgery.

Mean±SD values were used for data estimation. Given the small sample size and abnormal distribution of data, statistical analyses were performed with nonparametric tests. The study group was compared with the Mann-Whitney U test, and differences between the measurements over time were evaluated with the Wilcoxon test. Based on an assumption of equal variance and a two-sided confidence interval, statistical significance was set at $P<0.05$. Statistical analyses were performed using the SPSS software (22.0, IBM Company, USA).

RESULTS AND DISCUSSION

The treatment group did not show any statistically significant differences from normal values for the body condition score (BCS), body weight (BW), age and baseline HR, RR, MAP, SpO_2, and RT values. The monitored baseline values of the anaesthetized dogs fell within the normal reference ranges (Table 1).

Table 1. Animal data and baseline (T0) physiological variables for both groups, Mean (±SE).

Patient variable	**MED-KET**
Body weight (kg)	10.58±1.06
Body condition score	3.00±0.00
Age (years)	1.2±0.17
Baseline heart rate (beats/min)	130.56±7.82
Baseline respiratory rate (breaths/min)	13.00±0.72
Baseline blood pressure (mmHg)	113.00±6.22
Baseline SpO_2 (mmHg)	91.11±0.42
Baseline rectal temperature (°C)	38.64±0.29

SPO_2: Hemoglobin oxygen saturation, MED-KET: medetomidine/ketamine.

A comparison of the serial data changes in group MED-KET are presented in Table 2. In Group MED-KET, HR displayed a significant decrease after medetomidine and ketamine administration. Statistically significant differences were determined between the time points ($p<0.001$). While significantly lower values were measured at T1, T2 and T4 compared to T0

(T1, T2, T4 vs. T0, p=0.005, p=0.008 and p=0.037, respectively), the values measured at T3 and T5 did not significantly differ from the baseline values (T3, T5 vs. T0, p=0.114, and p=0.074). Furthermore, significantly higher values were measured at T2, T3, T4 and T5 compared to T1 (T2, T3, T4, and T5 vs. T1, p=0.005, p=0.005, p=0.005, and p=0.005, respectively). While the HR values displayed a temporary decrease, the quartile values fell within the normal range except for the value measured at T1 [T1:41.90 (28.88-53.12), T2:79.80 (58.05-105.09), T3:109.40 (61.37-156.91), T4:106.60 (59.78-154.80), T5:107.40 (77.61-150.96)].

A comparison of the serial changes in the intraocular parameters of group MED-KET is presented in Table 3. In Group MED-KET, the HPD significantly decreased after the administration of medetomidine and ketamine. Statistically significant differences were observed between the time points (p=0.004). Significantly lower values were measured at T1, T2, T3 and T4 compared to T0 (T1, T2, T3, T4, vs. T0, p=0.004, p=0.005, p=0.040, p=0.034, respectively). Significantly lower values were measured at T1 and T2 compared to T5 (T1, T2 vs. T5, p=0.023, p=0.043). Moreover, TP significantly decreased after medetomidine and ketamine administration. Statistically significant differences were detected between the time points (p<0.001). Significantly lower values were measured at T1, T2, T3, T4 and T5 compared to T0 (T1, T2, T3, T4, T5 vs. T0, p=0.005, p=0.005, p=0.008 and p=0.005, p=0.015, respectively). The values measured at T1 and T2 significantly differed from those measured at T5 (T1, T2 vs. T5, p=0.032, p=0.008).

CONCLUSIONS

In conclusion, while MED-KET was found to be effective in reducing TP and the HPD, and altering the AM response to general anaesthesia, atipamezole could be used in dogs for a quick and safe recovery to baseline values after abdominal surgery, such as OVH. It is considered that a dilated HPD could aid in avoiding anterior sinechia during corneal surgery. Thus, the anaesthetic protocol tested may not be suitable for corneal surgery. Future research on the effects of other premedicants and induction agents on the HPD, IOP, TP, and AM would either confirm or disprove our results for metetomidine and ketamine, and contribute to the achievement of a better understanding.

Table 2. The serial monitorized data changes in MED-KET group.

	Group	T0	T1	T2	T3	T4	T5	P within group
HR	MED-KET	133.50±7.59	41.90±3.65*	79.80±7.76$^{*\pm}$	109.40±13.58$^{\pm}$	106.60±13.47$^{\pm *}$	107.40±11.03$^{\pm}$	**0.041**
O_2	MED-KET	91.20±0.39	88.50±2.18	88.90±1.00	91.44±1.68	92.50±1.67	93.70±0.52	0.45
RR	MED-KET	12.20±0.65	12.20±0.66	13.10±0.48	12.22±0.92	11.90±0.80	14.70±1.37	0.36
BP	MED-KET	113.00±6.12	110.70±7.63	116.400±5.13	126.78±4.49	120.90±5.53	106.67±6.47	0.55

*p<0.05: vs. T0 within the group, $^{\pm}$ p<0.05: vs. T1 within the group, $^{¥}$ p<0.05: vs. T2 within the group, $^{\perp}$ p<0.05: vs. T4 within the group, $^{+}$ p<0.05: vs. T5 within the group. HR: heart rate, O_2: oxygen saturation, RR: respiration rate, BP: blood pressure.

Table 3. The serial intraocular parameter changes in MED-KET group.

	Group	T0	T1	T2	T3	T4	T5	P within group
IOP	MED-KET	19.90±1.02	19.70±1.63	23.20±1.36	21.00±0.85	21.30±1.82	21.30±1,62	0.79
HPD	MED-KET	6.80±0.44	4.50±0.45^{*+}	4.00±0,30^{*+}	5.00±0.33*	4.80±0.55*	5.70±0.67	0.94
ETP	MED-KET	17.30±1.83	5.70±0.91^{*+}	4.40±0.48$^{*\pm+}$	5.00±0.50^{*+}	4.80±0.70^{*+}	11.20±2.06^{*+}	0.62

*p<0.05: vs. T0 within the group, ± p<0.05: vs. T1 within the group, ¥ p<0.05: vs. T2 within the group, ⊥ p<0.05: vs. T4 within the group, $^{+}$ p<0.05: vs. T5 within the group. IOP: intraocular pressure, HPD: horizontal pupil diameter, ETP: ear tear production.

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DETERMINATION OF CHANGES IN SOME NUTRIENT CONTENT OF *Agaricus bisporus* STEM PART FERMENTED WITH RUMEN LIQUID IN DIFFERENT ENVIRONMENTAL CONDITIONS

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ABSTRACT

This study aimed to determine the nutrient change in the stem part of Agaricus bisporus by using rumen liquid in the solid-state fermentation method. In this study, a total of 36 samples with three different fermentation times (3, 5, and 7 days), two different initial pHs (6 and 7), and two different inoculations (Yes or no) in a 3x2x2 factorial experiment design and three replications in each group. The stem part of the Agaricus bisporus was sliced into small pieces for fermentation in the laboratory. Rumen content was obtained from Bafra sheep on a farm in Samsun/TURKEY region. The rumen liquid was brought to the laboratory centrifuged at 1000 rpm for 10 minutes in pre-sterilized falcon tubes. Then it was made ready for inoculation. The fermentation medium was decontaminated by the autoclave method before adding rumen liquid, and then 1000 µl of rumen liquid was added to each medium. Fermentation was carried out in incubators set at 37 °C. The samples, which completed the fermentation period, were dried and analyzed for crude protein, cellulose, ash, acid detergent fiber, and neutral detergent fiber. At the end of the research, it was determined that the *A. Bisporus* stem part fermented with rumen liquid positively affects the nutrient content. According to the results obtained, the most effective fermentation time was seven days, and also best fermentation pH was seven, respectively.

Key Words: Fermentation, rumen liquid, nutrient composition, *Agaricus bisporus*.

INTRODUCTION

Agaricus bisporus has been used in human and animal nutrition for many years. The Asian region is the most critical producer worldwide (Sonnenberg et al., 2011). This production is followed by Italy and the USA (Atila et al., 2017). According to FAO (2019) data, it is reported that annual production of 10 million tons is made in the world. However, Turkey has an annual production of 55 thousand tons (TUIK 2021). As a valuable plant, *A. bisporus* has many biochemical substances and nutrients. It contains incredibly high levels of polysaccharides, fiber compounds, antioxidants, and vitamins C, B12, and D. In addition, folate, ergothioneine, and polyphenol compounds in its structure show that mushrooms have potential anti-inflammatory, hypoglycemic and hypocholesterolemic effects (Fukushima et al. 2000, Mattila et al. 2001, Koyyalamudi et al. 2009 (a), Koyyalamudi et al. 2009 (b)). The fermentation method using bacteria, yeast, or fungal inoculants improves the nutrient composition of the feedstuffs. In this way, it increases the use of feedstuffs in poultry diets (Altop et al., 2018). There are studies in which rumen liquid is used as an inoculant in fermentation. For example, it has been reported that when cottonseed meal is fermented with rumen liquid when suitable conditions are provided, the crude cellulose content decreases, the crude protein level increases,

and as a result, the nutrient composition improves (Koç et al., 2021). However, studies in which feedstuffs are fermented with rumen liquid are limited. The present study it is aimed to reduce the cellulose content and improve the nutritional composition of the stem of *A. bisporus* fermented using sheep rumen liquid. In addition, at the end of the study, it is aimed to determine optimal the fermentation time and pH conditions of fermented *A. bisporus*.

MATERIAL AND METHOD

The cap and stem part of the mushrooms brought to the laboratory were separated, and the stem part was sliced into small pieces for fermentation. The rumen content used in fermentation was obtained from Bafra sheep on a farm in Samsun. The rumen liquid brought to the laboratory was centrifuged at 1000 rpm for 10 minutes in pre-sterilized falcon tubes. Then it was made ready for inoculation. The study was carried out on a total of 36 samples, with three different fermentation times (3, 5, and 7 days), two different initial pH (6 and 7), and two different inoculation (Yes or no) in a 3x2x2 factorial experiment design and three replications in each group. Before the study, 100 g of stems, 400 ml of distilled water, and 8.4 g of urea (46%) as nitrogen sources were combined into each fermentation medium. The appropriate pH value of the fermentation medium was provided by using 1N HCl. The samples were then sterilized in an autoclave. Sterilized samples were inoculated around the burner flame using microorganisms obtained from rumen liquid. The study was carried out in an anaerobic environment at 37 ºC. The pH parameters of the samples and fermentation medium that completed the fermentation process were determined. Then after samples were dried in the air, dry matter, crude ash, and crude protein analyses were determined according to AOAC (2000). The data were analyzed using the Windows version of SPSS 21.0 (SPSS Inc., NY, and the USA) statistical package program. The Duncan test compared the differences between groups after the ANOVA test for the data variance. Results were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

The pH parameters of the fermented product and fermentation medium were affected by inoculation ($P<0.05$). When all parameters were examined, the pH values of the inoculated samples were found to be lower than the control group. It has been determined that the microorganisms in the rumen fluid affect the fermentation positively by keeping the environment between the healthy rumen pH values of 6-7 under suitable conditions. It was determined that the pH value was above 7 in the not inoculated samples. This result shows that fermentation is affected positively. Because the cellulolytic bacteria in the rumen liquid reduce the pH value of the fermentation medium. (See article on working pH of cellulosic bacteria)
Additionally, inoculation has increased crude protein levels ($P<0.05$). This result is similar to Koç et al. (2021). Microorganisms in the rumen liquid positively contribute to the protein level by using urea nitrogen. It was determined that the raw ash parameters decreased in the inoculated samples, and the difference between the control group was significant ($P<0.05$). It is thought that the reason for this is the increase of organic substances by fermentation of microorganisms. The results obtained determined that the highest dry matter value was reached on the fifth day in dry matter parameters. These results are followed by the third and seventh days, respectively.

CONCLUSION

It has been determined that the *A. bisporus* stem part fermented with rumen liquid positively affects the nutrient content. According to the results obtained, the seventh day and seven pH were the most effective fermentation time.

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ANTIOXIDANT ACTIVITY AND COMPOSITION OF THE ESSENTIAL OILS OF TWO ENDEMIC *SIDERITIS* SP. FROM TURKEY

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ABSTRACT

The genus *Sideritis* includes approximately 150 species distributed mainly in the Mediterranean region and represented in the Flora of Turkey by 46 species and altogether 55 taxa, 42 of them being endemic. The plants of *Sideritis* genus contain various types of plant secondary metabolites including predominantly terpenes, flavonoids, iridoids, coumarins, lignans and sterols which are supposed to be responsible for the various biological activities such as antioxidant, anti-inflammatory, antimicrobial, antiulcer, antispasmodic, anticonvulsant and anti-osteoporotic. Plants belonging to *Sideritis* species have been used since ancient times in folk medicine due to their anti-inflammatory, antirheu matic and antimicrobial properties.
This study was carried out to determine the antioxidant activities, essential oil content and composition of endemic *Sideritis hololeuca* Boiss. Et Heldr. Apud Bentham and *Sideritis argyrea* P.H. Davis from Turkey. The essential oil of the plant was obtained by hydro distillation method for 3 hours using Clevenger apparatus and essential oil components were determined by GC-MS. It was determined that the essential oil content of *S. hololeuca* was 0.64% and *S. argyrea* was 0.38%. The results of the analysis revealed that the main components of essential oils of *Sideritis* species are β-pinene, α-pinene and trans-caryophyllene. The radical scavenging activity values were obtained in *S. hololeuca* (74.27%) and *S. argyrea* (30.49%).

Keywords: *Antioxidant, Endemic, Essential oil, GC-MS, Sideritis sp.*

INTRODUCTION

Turkey is a very rich country in terms of plant diversity. This richness of Turkey is due to factors such as being at the intersection of 3 different phytogeoFigureic regions, the existence of the Anatolian Diagonal, and being under the influence of many different climate types, having height differences ranging from 0-4500 meters, hosting different ecosystems such as sea, river, lake, river and swamp (Yılmaz Sancar, 2021; Maral et al., 2022).

The *Lamiaceae* family includes about 236 genera and 6,900 to 7,200 species. The largest genera of the *Lamiaceae* family are *Salvia* (900), *Scutellaria* (360), *Stachys* (300), *Plectranthus* (300), *Hyptis* (280), *Teucrium* (250), *Vitex* (250), *Thymus* (220), *Nepeta* (200), while *Sideritis* has 100 species. Plants of the genus *Sideritis*, whose aerial parts are widely used as folk medicine in Turkey, Spain and Greece, are generally known as "mountain tea". *Sideritis* species are generally used in the preparation of herbal medicines and traditional teas and as a spice in Anatolia. Species belonging to the genus *Sideritis* are widely used in folk medicine as anti-inflammatory, anti-ulcer, cytostatic, antimicrobial, vulnerary, astringent, flu vaccine and

stimulating circulatory agents. In addition, essential oils obtained from *Sideritis* species are used for many therapeutic purposes such as lung disinfectants, diuretics, stomach remedies and nerve relaxants (Palomino et al., 1996; Senatore, 2000; Aligiannis et al., 2001; Özcan et al., 2001; Basile et al., 2006).

The genus *Sideritis* is represented by approximately 150 annual and perennial species in the world, and 46 species, 12 subspecies and 2 varieties in two sections in Turkey. Of these, 34 species, 4 subspecies and two varieties are endemic, and with this endemism rate of 78%, the genus *Sideritis* is one of the genus with the highest endemism among the plants grown in Turkey (Obon de Castro et al., 1994; Huber-Morath, 1982; Davis et al., 1988; Duman, 2000; Çarıkçı, 2010).

Extracts of *S. argyrea* species, which is endemic of the genus *Sideritis*, have been reported to have antibacterial and anti-inflammatory activity against carrageenan-induced edema in mice in Turkey. It has been revealed in previous studies that the main components of *S. argyrea* essential oil are α-pinene and β-pinene and exhibit antimicrobial activity (Kirimer et al., 2003; Gökbulut et al., 2017).

S. hololeuca, which is also an endemic species, is a perennial, 25-60 cm tall, white densely hairy plant. Middle and lower stem leaves are opposite, rounded or semi-rounded. Lamina 1–2.5 cm, leaf margins crenate (very small roundish) and dense petioles up to 1 cm short. Inflorescence corymbose, Middle leaves 0.5–0.8 x 0.5–1 cm, mucronate, appear connate from birth due to indumentum attachment. Calyx 6–8 mm, teeth 2–3 mm obtuse, Corolla yellow, 8–10 mm, hairy and brown stained inside. In Turkey; It is located at 1300 m from Ermenek-Sarıvadi and at 1000 m at the 20th km between Karaman and Mut (Çarıkçı, 2010).

Plant chemical compounds are divided into two groups as primary and secondary metabolites according to their metabolic pathways and functions. Free radicals play an important role in the pathogenesis of various diseases, therefore antioxidants have an important role in preventing diseases. Phenolic substances found in plants are bioactive compounds that are important sources of antioxidants (Topçu and Çölgeçen, 2015; Küçükboyacı et al., 2014; Zhang et al., 20165; Sevindik et al., 2021). In this study, it was aimed to determine the essential oil content and components, total phenolic content and antioxidant activities of endemic *S. argyrea* and *S. hololeuca* species.

MATERIAL AND METHOD

Plant Material

Sideritis argyrea P.H. Davis was collected on 13 August 2021 in the Anamur district of Mersin at an altitude of 1100 m. *Sideritis hololeuca* Boiss. Et Heldr. Apud Bentham was collected on 7 July 2021, on the Karaman-Mut road, around the Alaoda Church at an altitude of 1160 m. Herbarium specimens (*Sideritis argyrea* P.H. Davis ÖÇ 5371; *Sideritis hololeuca* Boiss. Et Heldr. Apud Bentham ÖÇ 4969) was deposited in Karamanoglu Mehmetbey University, Biodiversity Research and Application Center. The plants were identified by Dr. Ömer ÇEÇEN.

Isolation of the Essential Oils

The air dried aerial parts of the plants were hydro distilled for 3h using a Clevenger type apparatus. The obtained oils were stored at +4°C until analyzed.

GC-MS Analyses

The analysis of the main components of the essential oils of each samples were performed using Thermo Scientific ISQ Single Quadrupole Gas ChromatoFigurey- Mass Spectrometry device (Milan, Italy) fitted with TR- FAME capillary column (5% Phenyl Polysilphenylene-siloxane, 60 m × 0.25 mm i.d. × 0.25 µm film thickness). Helium (99.9%) was used as the carrier gas at a flow rate of 1 ml/min. Mass spectra were recorded at 70 eV, the mass range was from 1.2 to 1,200 m/z. Scan Mode was used for data collection. MS transfer line, ionization source and the injection port temperatures were 250, 230, and 220 ℃, respectively [Maral et al. 2022].

DPPH Radical Scavenging Assay

The DPPH method was used with some modifications for the antioxidant determination of the samples [Brand-Williams et al.1995; Blois, 1958]. Accordingly, after the 20µl sample was made up to 10 ml with methyl alcohol, 200µl of this solution was taken and 100µl of 0.004/100ml DPPH's methanol solution was added. After the solution obtained was made up to 1 ml with methyl alcohol and kept in the dark for half an hour, the absorptions at 517 nm were read. 0.9 ml of methyl alcohol and 0.1 ml of DPPH solution were mixed and read at 517 nm at 0 minutes and recorded as control. Methyl alcohol solution was evaluated as a blank sample. Radical scavenging activity (%) was calculated from the equation = $[(A_{Control}-A_{Sample}) / A_{Control}] \times 100$.

Assay for Total Phenolics

The total phenolic content of fresh and dry plant parts was determined by using Folin-Ciocalteu assay [Slinkard and Singleton, 1977; Singleton and Rossi, 1965]. In five different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg / ml) gallic acid solutions were prepared with 99.9% methanol. Twenty µl of the prepared solution was taken, 680 µl of distilled water, 400 µl of 0.5 N folin reagent (in water) and 400 µl of 10% Na_2CO_3 (in water) were added to absorption at 760 nm wavelength. In addition, a blank sample was prepared by mixing 700µl distilled water, 400µl 0.5 N folin reagent (in water), 400µl 10% Na_2CO_3 (in water) and the absorption values from the blank sample were zeroed for samples and standard samples. From the data, a plot of absorption versus concentration with R^2 value of 0.968 was obtained. Twenty µl of the extracted plant solution was diluted with methyl alcohol to 10 ml. Twenty µl of the solution was taken and 680 µl of distilled water, 400 µl of 0.5 N folin reagents (in water) and 400µl of 10% Na_2CO_3 (in water) were added to the absorption values at 760 nm at the end of 30 minutes. The read absorption values were replaced by y in the obtained equation, and x values, in other words, phenolic equivalents in gallic acid, were calculated.

RESULTS AND DISCUSSION

Essential Oil Characteristics

The essential oil content of *S. argyrea* hydro distilled by Clevenger apparatus was determined as 0.38%. 32 components were identified in the oil, representing 99.82% of the total oil. β-pinene (33.27%), α-pinene (30.44%), torreyol (5.20%), humulene (4.61%), cubenol (4.31%), α-cadinol (3.69%) and limonene (3.25%) were found to be the major constituents (Table 1).

The essential oil content of *S. hololeuca* hydro distilled with Clevenger apparatus was determined as 0.64%. 32 compounds were characterized, representing 99.41% of the oil. The major components were β-pinene (23.96%), trans-caryophyllene (22.94%), α-pinene (16.69%), bicycloelemene (7.50%), spathulenol (6.73%), caryophyllene oxide (4.32%), germacrene D (2.83%) and α-curcumene (2.25%) (Table 1).

In many studies on the chemical composition of *Sideritis* essential oils, it has been reported that there are some qualitative and quantitative differences between the components of the essential oil, and these differences vary depending on different reasons such as climatic, genetic factors, applied agricultural practices or plant chemotype.

Kirimer et al., (2003), in their study with five *Sideritis* species, reported the main components characterized in *S. argyrea* and *S. hololeuca* essential oils as β-pinene (20% and 30%) and α-pinene (14% and 13%). Ezer et al., (1996), reported in their study that *S. argyrea* essential oil is characterized by the presence of high monoterpene hydrocarbons, and α- and β-pinene are the main components. Iscan et al., (2005), reported β-pinene (48.40%) and α-pinene (31.90%) as the main components of *S. bilgerana*. Kan et al., (2018), The main components of *S. bilgerana* essential oil were determined as 19.82% β-pinene, 14.60% α-pinene, 9.86% β-bisabolene and 7.93% δ- cadinene. Özel et al., (2008), reported that the major components of *S. congesta* essential oil are β-pinene (17.15-25.34%), α-pinene (12.53-14.55%), and δ-cadinene (10.97-14.52%).

In previous studies with Turkish *Sideritis* species, it was reported that monoterpene hydrocarbons (α-pinene, β-pinene, α-phellandrene, sabinene and myrcene) were the main components in 57%. It was determined that the main components of the essential oils of *S. argyrea* and *S. hololeuca* species examined in this study were β-pinene, α-pinene and trans-caryophyllene.

Antioxidant Activity

Natural products derived from plants offer many opportunities to obtain new drugs. Phenolic compounds, also known as secondary metabolites, are among the most important and functional components produced by plants. These components take part in activities such as color, taste, aroma formation and plant defense systems in plants. The amount of phenolic compounds in plants varies depending on the variety, soil structure, habitat, climatic and seasonal characteristics (Aydin, 2020; Sasidharan et al., 2011; Çağlar and Demirci, 2017; Arion et al., 2014; Sevindik et al., 2021). The results of total phenolic content and antioxidant activities of *Sideritis* extracts are given in Table 1. The DPPH antiradical activity of *S. argyrea* was 74.27% and that of *S. hololeuca* was 30.49%. While the phenolic content of *S. argyrea* in terms of gallic acid was determined as 9.80 mg/100 ml, it was determined that *S. hololeuca* was 10.45 mg/100 ml.

Sagdic et al., (2008), reported that DPPH radical scavenging activities of *Sideritis ozturkii* and *S. caesarea* plant extracts were 41.68% and 72.47%, respectively. According to Koleva et al., (2003), reported that the radical scavenging activity of *Sideritis scardica, S. syriaca* and *S. montana* extracts obtained using different solvents was over 90%. Tunalier et al., (2004), reported that total phenol content values ranged between 191.6 and 402.5 mg/g among 27 *Sideritis* species Sagdic et al., (2008) reported TPC values of 39.35 and 93.79 mg/g in two endemic *Sideritis* species. The differences between the results obtained in this study and those reported in the literature may be due to differences in growing conditions, especially in species, method, or solvent.

Table 1. Chemical composition, DPPH˙ radical scavenging activities and phenolic substance content of *Sideritis argyrea* and *Sideritis hololeuca* (%)

RT	Compounds	*S. argyrea*	*S. hololeuca*
6.54	α-Pinene	30.44	16.69
8.35	β-Pinene	33.27	23.96
8.68	Sabinene	0.18	0.45
8.98	Myrcene	0.16	0.23
9.99	Limonene	3.25	0.80
11.98	Eucalyptol	0.19	0.34
12.69	o-Cymene	0.18	0.29
18.97	α-Copaene	1.68	1.09
20.27	β-Bourbonene	0.25	1.02
20.71	Linalool	---	0.79
21.26	Germacrene D	0.10	2.83
23.98	trans-Caryophyllene	2.84	22.94
24.65	Alloaromadendrene	---	0.19
25.16	Trans-pinocarveol	0.16	0.38
26.11	Pinocarvone	0.16	0.33
26.40	Humulene	4.61	1.29
27.10	isoledene	0.91	---
27.44	Borneol	0.25	---
28.37	Bicycloelemene	---	7.50
28.62	Myrtenal	0.18	0.48
29.50	Myrtenol	0.16	0.22
30.55	cis-Calamenene	0.74	---
31.19	junipene	0.11	---
33.70	Torreyol	5.20	0.29
35.43	Palustrol	0.11	0.32
36.71	Bicyclogermacrene	0.32	---
38.38	Cubenol	4.31	---
38.73	Veridiflorol	0.39	0.22
38.89	Cedrene	1.42	1.39
39.32	Globulol	---	0.40
40.10	Caryophyllene oxide	1.85	4.32
40.31	Spathulenol	0.17	6.73
40.89	α-cadinol	3.69	0.25
41.28	α-curcumene	1.22	2.25
40.90	Junipene	---	0.23
42.71	α-copaen-11-ol	0.55	---
43.61	β-Eudesmol	---	0.43
45.19	Sclareol	0.77	0.30
53.67	Levantenolide	---	0.24
56.29	Sclareol	---	0.41
Number of identified compounds		32	32
Total (%)		99.82	99.41
Essential oil content (%)		0.38	0.64
% radical scavenging activity		74.27±0.21	9.80±2.91
Phenolic substance content (mg /100ml)		30.49±1.12	10.45±2.63

RT: Retention time

CONCLUSIONS

This study was carried out to determine the phenolic compounds and essential oil components of *S. argyrea* and *S. hololeuca* plant extracts and to investigate their usability as a

natural antioxidant source. In previous studies, it has been reported that monoterpene hydrocarbons (α-pinene, β-pinene, α-phellandrene, sabinene and myrcene) are the main components in 57% of Turkish *Sideritis* species. It was determined that the main components of the essential oils of *S. argyrea* and *S. hololeuca* species examined in this study were β-pinene, α-pinene and trans-caryophyllene. In this study, the maximum total phenol content was determined in *Sideritis hololeuca*. The highest antioxidant activity value was obtained in *Sideritis argyrea*. As a result of our study, it was concluded that these two endemic *Sideritis* species can also be used in the pharmaceutical industry due to their antioxidant activities.

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EFFECT OF GRAPEFRUIT ESSENTIAL OIL ADDITION TO THE DIET ON PERFORMANCE, SLAUGHTERING CHARACTERISTICS AND MEAT QUALITY OF MALE QUAILS

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ABSTRACT

The current research was conducted to determine the effect of addition of 0, 100, 200, or 300 mg/kg grapefruit essential oil to the diet on the performance, slaughtering and meat traits of male quails. In the study, a total of 96 male Japanese quails aged 91 days were randomly distributed to 4 treatment groups with 6 subgroups and the quails were fed with experimental diets for 30 days. At the final of the study, body weight and feed intake did not affected by the addition of grapefruit essential oil to the diet ($P>0.05$). In addition, the supplementation of grapefruit essential oil to the diet did not affect carcass and visceral organ weights ($P>0.05$). The pH values of breast and thigh meats significantly decreased with the administration of 200 and 300 mg/kg grapefruit essential oil to the diet (respectively, $P<0.05$, $P<0.01$). Besides, drip loss of breast meat was affected by the addition of grapefruit essential oil to the diet ($P<0.05$). Thigh meat drip loss was considerably increased with the addition of 300 mg/kg grapefruit essential oil to the diet, compared the control group ($P<0.05$). According to the results obtained from this study, the addition of 200 mg/kg grapefruit essential oil to the diet was effective in reducing meat pH without affecting performance parameters and slaughtering characteristics.

Keywords: Quail, meat quality, grapefruit essential oil, slaughtering characteristics, performance

INTRODUCTION

Citrus fruit, which includes fruits such as orange, lemon, and grapefruit, is a genus of shrubs/trees from the *Rutaceae* family. The expansion of citrus cultivation areas in the worldwide led to an increase in industrial sectors such as fruit juice production factories. Studies on citrus peel and pulp, which are considered as waste in juice factories and constitute approximately 25% of the original product, demonstrated that these by-products is rich in terms of crude protein, metabolic energy, phenolic compounds, ascorbic acid, pectin, coumarin, and flavonoids (Bampidis and Robinson, 2006). Moreover, these by-products can be obtained at low cost in most seasons in some countries such as Turkey. One of these citrus fruits is grapefruit.

Grapefruit is used in traditional medicine as a natural antibacterial, antifungal and antiviral preparate and is a fruit containing significant amounts of carotene, lycopene, organic acids, flavonoids, and furanocoumarins (Krajewska-Kulak et al., 2001). Besides grapefruit is a rich source of vitamin C, and these active ingredients and vitamin C act synergistically, thus

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improving the antioxidant capacity of grapefruit. The active ingredients are found in the pulp, peel, and seeds of grapefruit. The main active ingredients in grapefruit are nobiletin, scutellarein, tangeretin, sinensetin, heptamethoxyflavone, naringenin, hesperidin, neohesperidin, and poncirin (Galanty and Janeczka, 2003; Berdzik and Janeczka, 2001; Rypula et al., 2003).

These active substances contained in grapefruit by-products provide the potential to be used in animal nutrition as a good natural additive in terms of antimicrobial and antioxidant properties. However, the number of studies on the use of grapefruit in animal nutrition is limited compared to other citrus species. In one of these studies, it was determined that the addition of grapefruit pulp (2%) to broiler diets negatively affected performance, but reduced oxidation in meat (TBARs) and changed the microorganism population in the digestive system opposed to pathogens (Vlaicu et al., 2020). Vlaicu et al. (2021), similarly, stated that the grapefruit pulp improved the meat quality of broilers and it was an important source of antioxidants. In addition, Gabriel Junior et al. (2009) reported that the highest performance was obtained with the addition of grapefruit extract at the level of 124 mg/kg to broiler diets.

The number of studies examining the effects of grapefruit essential oil on the meat quality of poultry is quite scarce. With the increase in the number of studies, the usage area of this by-product would increase and it could contribute to the economy. Therefore, the aim of this study is to investigate the effects of adding grapefruit essential oil to the diet on performance, slaughtering characteristics, and meat quality in male quails.

MATERIAL AND METHODS

Criteria specified by European policy for the protection of animals (EPCEU, 2010) were followed during experimental period. Criteria specified by the National Institute of Health Guide for the Care and Use of Laboratory Animals were followed during the study period.The animal care practices were used in the experiment in consistency with animal welfare rules stated in Article 9 in government law in Turkey (No. 5996).

Material

A total of 96 male Japanese quails (*Coturnix coturnix japonica*) aged 91 days and weighing 215.8±8.2 g were used in the 30-day study. The quails were randomly assigned to 4 treatment groups with 6 replicates consisting of the addition of grapefruit essential oil at the levels of 0, 100, 200, or 300 mg/kg bound with zeolite to the basal diet containing the nutrients recommended by NRC (1994) (Table 1). The quails were placed in 30 cm x 45 cm cages. The study was carried out in a well-ventilated coop with a 16-hour lighting program and a temperature of 20±2.0°C. The quails were provided with feed and water *ad-libitum*.

Table 1. Basal diet and calculated nutrient content (as fed)

Ingredients	%	Nutrient contents	%
Corn	53.70	Metabolizable energy, kcal/kg	2899
Soybean meal	40.00	Crude protein	24.03
Soybean oil	2.50	Calcium	1.00
Limestone	1.06	Available phosphorus	0.50
Dicalcium phosphate	1.90	Lysine	1.31
Salt	0.35	Methionine	0.52
Premix[1]	0.25	Cystine	0.44
DL-methionine	0.24	Methionine + cystine	0.96
Total	100.00		

[1]Premix is supplied that per kg of diet; Manganese: 80 mg, Iron: 60 mg, Copper: 5 mg, Iodine: 1 mg, Selenium: 0.15 mg, Vitamin A: 8.800 IU, Vitamin D_3: 2.200 IU, Vitamin E: 11 mg, Nicotine acid: 44 mg, Cal-D-Pan: 8.8 mg, Riboflavin: 4.4 mg, Thiamine: 2.5 mg, Vitamin B_{12}: 6.6 mg, Folic acid: 1 mg, Biotin: 0.11 mg, Choline: 220 mg.

Methods

Determination of performance

The body weight of the quails used in the experiment was determined as g by weighing the birds at the beginning and end of the trial, and the body weight change was calculated as g from these data. The feed was given by weighing to the replicates and at the end of the experiment, the remaining feeds were weighed and the feed intake was calculated as g/day/quail.

Determination of carcass and organ weights

On the 30th day of the trial (121 days of age), 2 quails (48 in total) from each replicate were euthanized by cervical dislocation. In order to determine the relative weights, carcass, breast, thigh+drumstick, liver, heart, pancreas, abdominal fat, and testis were weighed with a 0.01 g precision scale. Carcass and organ weights were calculated as % of body weight, and breast and thigh+drumstick as % of carcass.

Determination of drip loss

A sample with 2 cm width was collected from the breasts and thighs and weighed and then, it was suspended in a polyethylene plastic bag on a steel hook at 4°C for 24 hours. After 24 hours, the sample was reweighed and the drip loss was calculated as % with the *((initial*

weight of sample – final weight of sample) / initial weight of sample) x 100 formula (Castellini et al., 2002).

Determination of cooking loss

Each sample from the breast and thigh was carefully weighed before cooking. The sample in a polyethylene roasting bag was immersed in an 80°C water bath until it reached an internal temperature of 75°C. After cooking, the sample was cooled to room temperature and blotted for remove excess water, then immediately weighed. Cooking loss was obtained from the formula *((raw weight – cooked weight) / raw weight) × 100* (Castellini et al., 2002).

Measurements of meat pH

Final pH values of the breast and thigh muscles were measured 45 minutes after slaughter using a portable pH meter (IQ150, IQ Scientific Instruments Inc., Carlsbad, CA) equipped with an insertion glass electrode. Before measurement, the pH electrode was calibrated using three buffers with pH values of 4.01, 7.00 and 9.01. Samples were measured in triplicate at the always same location (AOAC, 2000).

Statistical analysis

The One-Way ANOVA was used to determine the effects of experimental diets on performance, slaughtering traits and meat characteristics in male quails. The differences between means of the groups were determined by the Duncan (Duncan, 1955) multiple comparison test. Statistical differences were defined as $P<0.05$. All statistical analysis were performed using SPSS Package 23.

RESULTS AND DISCUSSION

The effect of grapefruit essential oil addition to the diet on the performance of male quails was demonstrated in Table 2.

Table 2. The effect of adding different levels of grapefruit essential oil to the diet on performance parameters in male quails

Parameters	Grapefruit essential oil, mg/kg					*P*-value
	0	100	200	300	SEM*	
Initial body weight, g	220.52	218.92	210.17	210.42	5.152	0.402
Final body weight, g	216.85	219.00	208.50	213.25	7.113	0.748
Body weight change, g	-3.67	0.08	-1.67	2.83	3.745	0.699
Feed intake, g/day/quail	20.71	21.39	21.19	20.81	0.499	0.759

*****Standard error means

Body weight and feed intake of quails were not statistically affected by the trial diets ($P>0.05$). These results disagree with the research of Vlaicu et al. (2020, 2021) who reported

that the administration of 2% grapefruit pulp to broiler diets negatively affected performance parameters. Citrus pulps could adversely affect the performance of poultry due to antinutritional factors such as oxalate, saponin, tannin, and phytate (Oluremi et al., 2006), but did not observed adverse effect because these antinutritional factors were removed during the extraction of fat process in the current study. In a study conducted to determine the effect of grapefruit extract on broilers (Gabriel Junior et al., 2009), it was reported that the addition of 124 mg/kg grapefruit extract to the diet was sufficient to obtain the highest performance. In the literature, it did not found the research with the addition of grapefruit essential oil. However, similar to the current study, Jaramillo et al. (2021) stated that the addition of a citrus essential oil mixture (500 mg/kg), including grapefruit oil, to the diet did not affect performance in broilers.

The effect of grapefruit essential oil supplementation to the diet on the slaughtering characteristics of male quails was given in Table 3. The relative weights of carcass, breast, thigh+drumstick, liver, heart, abdominal fat, and testis in male quails did not statistically affected by grapefruit essential oil addition to the diet (P>0.05). In a study on the issue, it was reported that the administration of 2% grapefruit pulp to the diet of broilers decreased carcass yield and liver rates (Vlaicu et al., 2021).

Table 3. The effect of adding different levels of grapefruit essential oil to the diet on carcass and some organ weights in male quails

Parameters [1]	Grapefruit essential oil, mg/kg					*P*-value
	0	100	200	300	SEM*	
Carcass	61.34	61.83	62.00	61.29	0.673	0.846
Breast	54.70	56.94	54.95	54.04	0.544	0.355
Thigh+drumstick	35.84	34.83	34.86	35.90	0.773	0.081
Liver	1.291	1.428	1.278	1.354	0.075	0.512
Heart	0.928	0.989	0.951	0.915	0.044	0.691
Abdominal fat	2.235	1.208	1.747	2.334	0.389	0.199
Testis	2.686	2.907	3.156	2.764	0.285	0.684

*****Standard error means

The effects of the treatments on pH, drip loss and cooking loss in breast and thigh meats were demonstrated in Table 4.

Table 4. The effect of adding different levels of grapefruit oil to the diet on some meat quality parameters in male quails

Parameters	Grapefruit essential oil, mg/kg				SEM*	*P*-value
	0	100	200	300		
Breast						
pH	6.94^{a}	6.76^{ab}	6.71^{b}	6.68^{b}	0.059	0.050
Drip loss	11.01^{ab}	10.54^{b}	12.90^{a}	12.30^{ab}	0.519	0.019
Cooking loss	14.11	14.18	11.27	13.48	1.455	0.469
Thigh						
pH	7.58^{A}	7.52^{A}	7.29^{B}	7.30^{B}	0.045	0.001
Drip loss	11.72^{b}	12.03^{ab}	12.39^{ab}	15.09^{a}	0.825	0.039
Cooking loss	10.80	14.03	11.48	14.06	1.346	0.258

*****Standard error means

$^{A, B}$: The difference between the values expressed with different letters on the same row is significant at the P<0.01 level.

$^{a, b}$: The difference between the values expressed with different letters on the same row is significant at the P<0.05 level.

The addition of grapefruit essential oil to the diet statistically affected neither breast nor thigh meat cooking loss (P>0.05). The pH and drip loss parameters of breast and thigh muscles were considerably affected by the supplementation of grapefruit essential oil to the diet. The administration of 200 or 300 mg/kg grapefruit essential oil to the diet significantly reduced the pH of breast and thigh meats compared the control group (respectively, P<0.05; P<0.01). In the experiment, the difference in breast meat drip loss was observed to be significant in the groups to which 100 and 200 mg/kg grapefruit essential oil was added (P<0.05) and it was found to be considerably higher in the group that added 200 mg/kg grapefruit essential oil to the diet compared to the group that supplemented of 100 mg/kg. With the addition of incremental levels of grapefruit essential oil to the diet, thigh drip loss increased and this increase was significant in the group with grapefruit essential oil added at 300 mg/kg compared to the control group. In previous researches, it was claimed that grapefruit by-products in the diet were effective in maintaining meat quality by reducing oxidation in poultry meat (Vlaicu et al., 2020, 2021). Kang et al. (2017), on the other hand, reported that the cooking loss and water holding capacity of meat treated with grapefruit extract could not be affected by the treatments, but the pH increased with the addition of grapefruit extract. Also, Jaramillo et al. (2021) expressed that the supplementation of 500 mg/kg citrus essential oil mixture to the broiler diets did not affect the pH and cooking loss of breast and thigh meats.

CONCLUSIONS

Besides there are differences in the active ingredients (essential oil) of citrus species, the number of studies with grapefruit essential oil is extremely limited. Therefore, it is obvious that there is a required for more studies on the evaluation of citrus waste products such as grapefruit grown abundantly in our country and has a great potential. According to the results obtained from this study, it was observed that the use of diets with grapefruit oil in the feeding of male quails did not affect the performance and slaughtering characteristics, but it was effective in reducing the pH that provided the better and faster maturation of meat.

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EFFECT OF RED BEET POWDER ADDITION TO THE DIET ON PERFORMANCE, CARCASS AND MEAT QUALITY OF MALE QUAILS

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ABSTRACT

This study was carried out to determine the effect of diets containing red beet powder on performance, carcass traits, and meat quality of male quails that are not used as breeders. In the study, a total of 96 male Japanese quails at the age of 43 days were randomly allocated to 4 treatment groups with 6 replicates. In the experiment, male quails were fed with trial diets added red beet powder at the levels of 0, 1, 2, or 3 g/kg to the basal diet for 30 days. The administration of red beet powder to the diet did not affect the body weight ($P>0.05$), but feed intake linearly increased with the addition of red beet powder to the diet ($P<0.01$). Relative breast meat weight linearly decreased ($P<0.05$), but other carcass and organ parameters were not affected by the addition of beet powder to the diet ($P>0.05$). The effect of supplementation of red beet powder to the diet on L*, a* and b* values of breast and thigh meats was found to be insignificant ($P>0.05$). According to the results of this study, it was determined that the addition of red beet powder to male quail diets affected the meat quality after slaughter negatively, but increased the appetite.

Keywords: Male quail, meat quality, red beet powder, performance

INTRODUCTION

The increasing demand for healthy and functional food is closely related to the livestock sector, especially poultry industry. With the draw a negative reaction to synthetic additives, natural colorants or antioxidant products are attract attention. One of these natural products is red beet with its rich color and antioxidant content. Beet (*Beta vulgaris L.*), is a plant of the *Chenopodiaceae* family and, it is widely grown in the northern hemisphere and it is a good source of betalain, the water-soluble pigments that give roots their distinctive color (Kowalski and Szadzinska, 2014). Similar to flavonoids and anthocyanins, betalains are nitrogenous, water-soluble substances that give beets their yellow to deep red color. In addition, phenolic compounds and beta-aldehydes have antioxidant properties that prevent lipid peroxidation (Attia et al., 2013; Chandran et al., 2014; Guldiken et al., 2016). Moreover, beets contain minute quantity of other compounds, such as ascorbic acid that can further increase their total antioxidant capacity. Red beet, which is higher in the antioxidant Vulgaxanthin I, vulgaxanthin II, indicaxanthin, betanin, prebetanin, isabetinin and neobetanin-containing betalain (Kujala et al., 2000), has the potential to be used as a source of antioxidants in both human and animal nutrition.

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In studies on the use of beet in poultry nutrition, mostly sugar beet was used and it was added to the diet as an alternative energy source at high levels such as 15% (Ashour et al., 2016) to 20% (Emam and Abdel Wahed, 2020). The number of studies on the use of red beet as a feed additive in poultry nutrition is quite limited. The majority of these studies focus on enhancing egg yolk color (Kopriva et al., 2014; Şengül, 2021).

Therefore, the aim of the present research was to determine the effect on the performance, carcass, and post-slaughtering meat quality of male quails fed with diets supplemented with red beet powder as a potential natural additive.

MATERIAL AND METHODS

Criteria specified by European policy for the protection of animals (EPCEU, 2010) were followed during experimental period. Criteria specified by the National Institute of Health Guide for the Care and Use of Laboratory Animals were followed during the study period. The animal care practices were used in the experiment in consistency with animal welfare rules stated in Article 9 in government law in Turkey (No. 5996).

Material

A total of 96 male Japanese quails (*Coturnix coturnix japonica*) aged 43 days and weighing 183.8±5.2 g were used in the 30-day study. The quails were randomly assigned to 4 treatment groups with 6 replicates consisting of the addition of grapefruit essential oil at the levels of 0, 1, 2, or 3 g/kg red beet powder to the basal diet containing the nutrients recommended by NRC (1994) (Table 1). The quails were placed in 30 cm x 45 cm cages. The study was carried out in a well-ventilated coop with a 16-hour lighting program and a temperature of 20±2.0°C. The quails were provided with feed and water *ad-libitum*.

Table 1. Basal diet and its calculated nutrient contents

Ingredients	g/kg	Nutrients	g/kg
Corn	537.0	Metabolizable energy, kcal/kg	2899
Soybean meal	400.0	Crude protein	240.33
Soybean oil	25.0	Calcium	10.00
Limestone	10.6	Available phosphorus	5.01
Dicalcium phosphate	19.0	Lysine	13.15
Salt	3.5	Methionine	5.25
Premix[1]	2.5	Cystine	4.39
DL-methionine	2.4	Methionine+cystine	9.64
Total	1000.0		

[1]Premix is supplied that per kg of diet; Manganese: 80 mg, Iron: 60 mg, Copper: 5 mg, Iodine: 1 mg, Selenium: 0.15 mg, Vitamin A: 8.800 IU, Vitamin D_3: 2.200 IU, Vitamin E: 11 mg, Nicotine acid: 44 mg, Cal-D-Pan: 8.8 mg, Riboflavin: 4.4 mg, Thiamine: 2.5 mg, Vitamin B_{12}: 6.6 mg, Folic acid: 1 mg, Biotin: 0.11 mg, Choline: 220 mg.

Methods

Determination of performance

The body weight of the quails used in the experiment was determined as g by weighing the birds at the beginning and end of the trial, and the body weight change was calculated as g from these data. The feed was given by weighing to the replicates and at the end of the experiment, the remaining feeds were weighed and the feed intake was calculated as g/day/quail.

Determination of carcass and organ weights

On the 30th day of the trial (73 days of age), 1 quails (24 in total) from each replicate were euthanized by cervical dislocation. In order to determine the relative weights, carcass, breast, thigh+drumstick, liver, heart, pancreas, abdominal fat, and testis were weighed with a 0.01 g precision scale. Carcass and organ weights were calculated as % of body weight, and breast and thigh+drumstick as % of carcass.

Determination of drip loss

A sample with 2 cm width was collected from the breasts and thighs and weighed and then, it was suspended in a polyethylene plastic bag on a steel hook at 4°C for 24 hours. After 24 hours, the sample was reweighed and the drip loss was calculated as % with the *((initial weight of sample – final weight of sample) / initial weight of sample) x 100* formula (Castellini et al., 2002).

Determination of cooking loss

Each sample from the breast and thigh was carefully weighed before cooking. The sample in a polyethylene roasting bag was immersed in an 80°C water bath until it reached an internal temperature of 75°C. After cooking, the sample was cooled to room temperature and blotted for remove excess water, then immediately weighed. Cooking loss was obtained from the formula *((raw weight – cooked weight) / raw weight) × 100* (Castellini et al., 2002).

Measurements of meat pH

Final pH values of the breast and thigh muscles were measured 45 minutes after slaughter using a portable pH meter (IQ150, IQ Scientific Instruments Inc., Carlsbad, CA) equipped with an insertion glass electrode. Before measurement, the pH electrode was calibrated using three buffers with pH values of 4.01, 7.00 and 9.01. Samples were measured in triplicate at the always same location (AOAC, 2000).

Measurements of meat color

The breast and thigh meat color (L*, a*, and b*) was measured in 3 replicates from the same location using a colorimeter device (CR 400, Minolta, Osaka, Japan). The colorimeter was calibrated using a standard white ceramic tile before measuring each sample.

Statistical analysis

Data were analysed in the SPSS 22.0 software package (IBM SPSS Statistics) with model of one-way ANOVA, using the group mean as an experimental unit. A probability value of $P<0.05$ was considered statistically significant. Orthogonal polynomial contrasts were used to evaluate the significance of linear and quadratic models to determine the response of the dependent variable to an increasing red beet powder level.

RESULTS AND DISCUSSION

The effect of red beet powder addition to male quail diets on body weight and feed intake was demonstrated in Table 2.

Table 2. The effect of red beet powder addition to the diet on the performance of non-breeding male quails

Parameters	Red beet powder level, g/kg				SEM[1]	*P*- value	
	0	1	2	3		Linear	Quadratic
Initial body weight, g	186.33	184.42	183.42	181.17	2.098	0.101	0.939
Final body weight, g	201.92	205.33	200.17	206.17	4.667	0.727	0.790
Body weight change, g	15.58	20.92	16.75	25.00	4.016	0.214	0.732
Feed intake, g/period	630.58	643.70	644.06	702.80	14.214	*0.004*	0.145

[1] Pooled Standard error of mean, * Data represent means based on six replicates per treatment, four quails per replicate

Body weight and body weight change did neither linearly nor quadratically affected by rationed red beet powder ($P>0.05$). Feed intake of male quails increased linearly with the supplementation of red beet powder to the diet and it reached up maximum with 3 g/kg level ($P<0.01$). This outcome was similar to the results of Pettersson and Razdan (1993) who reported that broiler diets including beet pulp at amounts ranging from 2-3% to 9.2% boosted feed intake. Similar results were stated by Ashour et al. (2016) in quails. The administration of 5 or 10 ml/kg of sugar beet extract to the diet throughout the 24-42 days of broilers decreased feed intake, according to AbouSekken et al. (2013). Gonzalez-Alvarado et al. (2010) also reported findings that were similar. According to Alagawany and Attia (2015) in quail and Souza et al. (2019) in hens, the addition of sugar beet did not affect the feed intake. In the current study, phenolic chemicals present in red beet, such as betalaine and beta-aldehydes, could be the cause of the increase in feed intake. Additionally, it was evident from the findings of this research that red beet powder has an appetite-enhancing effect in quails.

The effect of red beet powder supplementation to male quail diets on carcass traits and relative organ weights was given in Table 3. The addition of red beet powder to the diet did not statistically affect carcass parameters and relative organ weights except for breast weight ($P>0.05$). Breast weight decreased linearly with red beet powder in the diet and it reached the lowest point with the level of 3 g/kg ($P<0.05$). In previous years, it was declared that the administration of beet and by-products to the poultry diets did not affect the carcass and especially the breast meat ratio (Ashour et al., 2016; Grace et al., 2020; Salehi et al., 2021). These reports disagree with the results of the current study.

Table 3. The effect of red beet powder addition to the diet on carcass characteristics and some organ weights of non-breeding male quails

Parameters	Red beet powder level, g/kg				SEM[3]	*P*- value	
	0	1	2	3		Linear	Quadratic
Carcass[1]	59.62	59.32	58.98	57.74	0.686	0.071	0.510
Breast[2]	57.38	54.97	55.95	54.45	0.697	*0.025*	0.541
Thigh + Drumstick[2]	34.16	35.90	34.49	35.78	0.606	0.220	0.714
Liver[1]	1.60	1.52	1.48	1.77	0.138	0.467	0.201
Heart[1]	0.898	0.886	0.850	0.930	0.034	0.709	0.208
Pancreas[1]	0.209	0.196	0.207	0.207	0.0172	0.938	0.720
Testis[1]	3.11	3.32	3.31	3.33	0.172	0.419	0.628
Abdominal fat[1]	1.04	1.43	1.14	1.25	0.243	0.516	0.811

[1]% of body weight, [2] % of carcass weight, [3] Pooled Standard error of mean, * Data represent means based on six replicates per treatment, four quails per replicate

The effect of red beet powder administration to male quail diets on quality of breast and thigh meats was shown in Table 4 and Table 5.

Table 4. The effect of red beet powder addition to the diet on breast meat quality of non-breeding male quails

Parameters	Red beet powder, g/kg				SEM[1]	*P*- value	
	0	1	2	3		Linear	Quadratic
pH	5.74	5.70	5.53	5.52	0.044	*0.002*	0.597
Drip loss, %	14.08	12.82	13.87	12.02	0.706	0.122	0.683
Cooking loss, %	10.17	9.26	9.36	10.08	0.468	0.954	0.105
L*	37.92	38.73	37.09	38.16	0.428	0.631	0.774
a*	8.59	8.12	8.07	9.11	0.446	0.471	0.117
b*	14.99	14.83	14.43	14.82	0.505	0.699	0.596

[1]Pooled Standard error of mean, * Data represent means based on six replicates per treatment, four quails per replicate

Drip loss, breast meat cooking loss, and color (L*, a* and b*) properties were not affected linearly or quadratically by the treatments (P>0.05). However, the supplementation of

red beet powder to the diet linearly affected the pH values of breast and thigh meats and the cooking loss of thigh meat (P<0.01). With the addition of red beet powder to the diet at incremental levels, the pH values of breast and thigh meat decreased linearly and were minimum with the 3 g/kg level (P<0.01). In the literature, there was not found a research that examined the effects of beet, including red beet, addition to the poultry diets on the meat pH and cooking properties. However, betaine is abundant in beet, and it was claimed that the addition of betaine to the diet reduced (Fu et al., 2016) or did not affect (Chen et al., 2020; Wen et al., 2019; 2021) the pH of fresh breast meat. Thigh meat cooking loss increased linearly with the addition of red beet powder to the diet and it reached maximum at the level of 3 g/kg. However, it was reported that the addition of betaine presented abundant in beet to the diet did not affect cooking loss of breast meat in broilers (Chen et al., 2020; Nutautaite et al., 2020; Wen et al. 2021). Beet is rich in betalaine, a good antioxidant. Further studies are required to evaluate the possible effects of betaine as well as betalaine on meat quality.

Table 5. The effect of red beet powder addition to the diet on the quality of thigh meat of non-breeding male quails

Parameters	Red beet powder level, g/kg				SEM[1]	*P*- value	
	0	1	2	3		Linear	Quadratic
pH	6.37	6.32	6.21	6.09	0.046	*0.001*	0.123
Drip loss, %	13.45	14.70	13.47	13.48	0.506	0.627	0.245
Cooking loss, %	6.06	9.22	9.31	14.75	0.959	*<0.001*	0.275
L*	36.93	37.35	36.48	36.67	0.489	0.471	0.818
a*	6.66	6.12	7.69	7.11	0.490	0.209	0.966
b*	12.34	12.35	12.23	12.55	0.424	0.796	0.723

[1]Pooled standard error of mean, *Data represent means based on six replicates per treatment, four quails per replicate

CONCLUSIONS

According to the findings, it can be said that red beet powder addition to male quail diets at the concentrations used in this study increased feed intake but negatively affected meat quality. Also, it can be said that further studies are required to determine the effect of high level addition of red beet powder to the diet and its effect on meat storage.

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THE USES OF THE ELECTROPOLYMERIZED FILMS AS IMMOBILIZATION MEDIUM FOR CHOLESTROL BIOSENSOR DESIGN

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ABSTRACT

In this study, we aimed to use various electropolymerized films for the creation of cholesterol biosensors. Chemical and enzymatic methods can be used to measure blood cholesterol levels. The enzymatic reaction between cholesterol and cholesterol oxidase, which is measured at 0.6–0.7 V against Ag/AgCl in enzymatic methods, produces hydrogen peroxide. In this study, polyindoline and poly(4-methoxyphenol), two different polymeric materials made electrochemically, were used to immobilize cholesterol oxidase. Utilizing variables like pH, applied potential, and cycle count, the effects of the immobilization of the enzyme were investigated. We asserted that the aforementioned polymers could serve as a medium for the immobilization of cholesterol oxidase.

Keywords: Enzyme immobilization, cholesterol oxidase, polymer matrix, biosensor.

INTRODUCTION

Blood cholesterol concentration is a clinically important parameter for the diagnosis and treatment of some clinical diseases such as cardiovascular disease, hypertension, and atherosclerosis (Lang, 1990; Trettnak et al., 1993; Charpentier, et al., 1995; Tan, et al., 2005) Various chemical or enzymatic methods have been used to determine the level of cholesterol in human blood. Chemical reactions for cholesterol determination are associated with certain difficulties, including lack of specificity and selectivity due to interfering reactions and the use of unstable and corrosive reagents.

Enzymatic methods for measuring total cholesterol using cholesterol ester hydrolase (CEH) and cholesterol oxidase (COx) appear to be a real improvement over the chemical method, but both techniques are still time-consuming (Pioch, et al., 1992). However, the enzymatic method has some advantages, such as high selectivity, short reaction time, small electrodes, low cost, and high reproducibility.

Immobilization of enzymes on the electrode surface is a crucial step in the construction of amperometric biosensors (Guilbault and Kauffmann, 1987; Bokoch, et al., 2004). In recent years, mediators and conducting polymers have been used as immobilization media.

The enzymatic reactions in the use of cholesterol oxidase (COx) are as follows: The enzymatic reactions in the use of cholesterol oxidase (COx) are as follows:

Cholesterol + O_2 → Cholest-4-en-3-one + H_2O_2 (Brahim, et al., 2001; Kumar, et al., 2001)

$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$ (Bongiovanni, et al, 2001; Ekinci, et al., 1996)

Amperometric detection of hydrogen peroxide is usually performed anodically (e.g., oxidation at +0.7 V with a Pt working electrode), but is strongly influenced by many readily oxidizable interfering substances present in real samples (Ekinci, et al., 1998). We have used various polymeric materials prepared by electrochemical methods using polyindoline and poly(4-methoxyphenol).

In this study, polymeric materials were used as support materials for the immobilization of cholesterol oxidase. Immobilization was achieved by electrochemical polymerization, which served as an electron acceptor for ChOx. Electrochemical detection of cholesterol was performed using these electron mediators.

MATERIAL AND METHOD

Cholesterol oxidase (COx) (E.C.1.1.3.6. Pseudomonas sp. = *E.coli*) with a specific activity of 1.5 U/mg solids was obtained from MP Biomedicals. Indoline, 4-methoxyphenol and all other chemicals such as 2-propanol, KCl, NaCl, Na_2HPO_4 used for PBS (phosphate buffer salts) and Triton X-100 were purchased from Merck (www.merck-chemicals.com). Hexane, KH_2PO_4 for PBS, and cholesterol as reagent were purchased from Riedel-De Haen (www.riedeldehaen.com), Carlo Erba (www.carloerbareagenti.com), and Sigma (www.sigmaaldrich.com), respectively.

All aqueous solutions were prepared with deionized and double distilled water. The nitrogen gas used for purging and blanketing during electropolymerization was of high purity.

Cyclic voltammetry (CV), Bulk electrolysis (BE), and steady-state amperometric response (time-based; TB) were performed with an electrochemical analyzer, BAS 100W (Bioanalytical Systems, Inc., West Lafayette, IN, USA). BAS 100W used the standard 3-electrode system consisting of a Pt disk (BAS, MF-2013, 1.98 mm2) as the working electrode and an Ag/AgCl reference electrode together with a Pt wire coil as the auxiliary electrode. The pH was measured using a Jenway 3010 pH meter.

At the beginning of the electrochemical polymerization, the Pt disc working electrode was cleaned according to the standard procedure (Ekinci, 1999) and polished to 0.05 µL with aqueous alumina slurry. The monomers were electropolymerized in an unstirred deaerated solution containing 0.10 M monomer and 0.05 M KCl. In the preparation of the biosensor, we tried two different methods. One of them was prepared with adsorption for this purpose. A different amount (1-10 µl) of cholesterol oxidase was dropped on the polymer electrode and waited for 2 hours at +4 °C for immobilization. Second, 0.10 M monomer and different amounts of cholesterol oxidase were mixed in the same solution, and the biosensor was prepared via CV or BE.

For the amperometric experiments, the PBS solution was aerated for approximately 15 minutes and kept at room temperature. A potential of +0.75 V was applied to the cell system because of the H_2O_2 generated by the enzymatic reaction between cholesterol and cholesterol oxidase. The biosensor responses were registered as a current signal (nA-µA) by tracking the oxygen consumption at +0.75 V with respect to the Ag/AgCl electrode due to the enzymatic activity. When the constant background current value was reached, the substrate was added to the reaction cell. The buffer was refreshed after each measurement.

In the presence of surfactants, cholesterol is soluble in water. The literature (Vasudevan, et al., 1997; Li, et al., 2003; Li, et al., 2005; Srisawasdi, et al., 2005; Arya, et al., 2006; López, et al., 2006) contains numerous procedures for creating cholesterol standard solutions. In the literature, various solvents including alcohol, hexane, and triton were employed to prepare standard solutions of cholesterol. A bare platinum electrode was used to examine the effects of the solvents (Figure 1). To achieve this, steady-state amperometric behavior (TB) was assessed at +0.75 V in 0.1 M PBS. For additional research, hexane was chosen. The cholesterol standard solution was made by combining 20 mg of cholesterol with 10 mL of hexane (Fletourıs, et al., 1998).

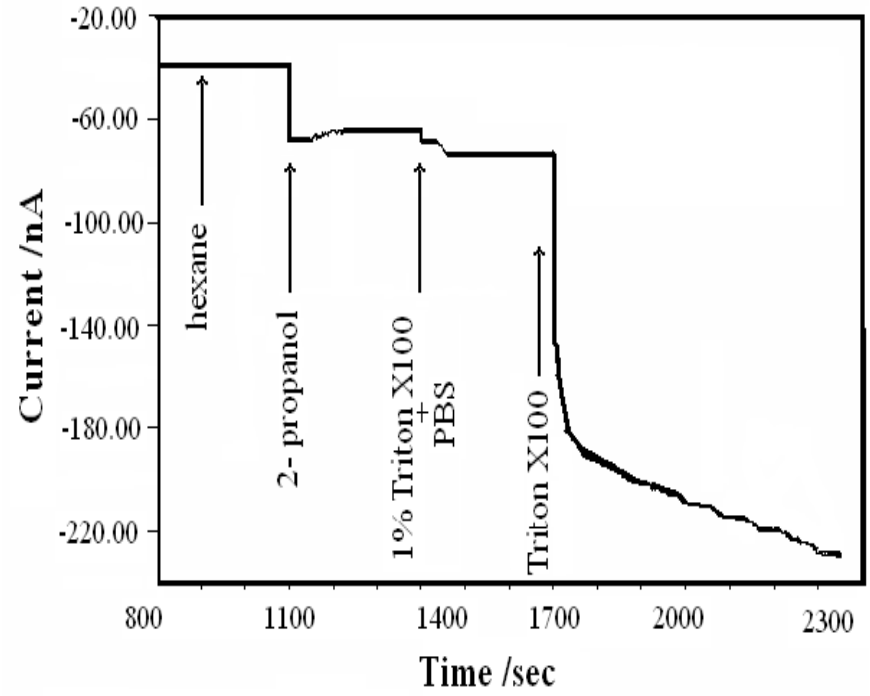


Figure 1. Effect of different solvents on bare Pt electrode: 900. s hexane; 1100.s 2-propanole; 1400.s %1 Triton X-100 + 0,1 M PBS; 1700.s Triton X-100.

RESULTS AND DISCUSSION

Indoline, also known as 2,3-dihydroindole, was polymerized electrochemically by CV and BE methods. It was found that the films grown with CV were more homogeneous and stable. The indoline concentration in 100 mM KCl was adjusted to 100 mM, and polymer films (polyindoline) of different thicknesses were prepared at 0-1200 mV and 10^{-5} sensitivity. It was decided that 24 cycles of these films were suitable. The CV for indoline is shown in Figure 2.

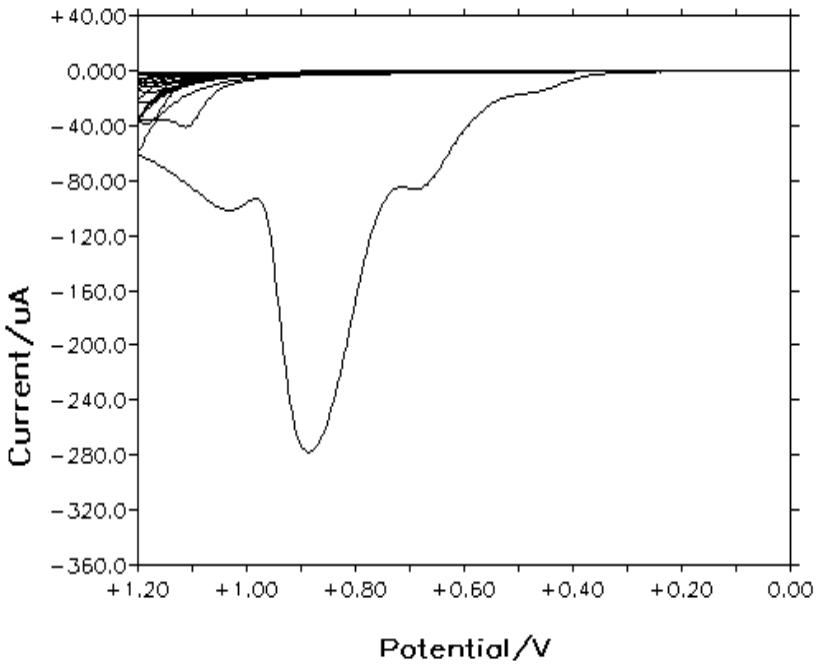


Figure 2. CV of Indolin (potential range of 0-1200 mV, scan rate of 50 mV/s, 24 cycles)

The COx (enzyme) solution was dropped onto the prepared polymer films and dried. The electrochemical behavior of the as-prepared enzyme electrode against the cholesterol solution was investigated using TB (Figure 4).

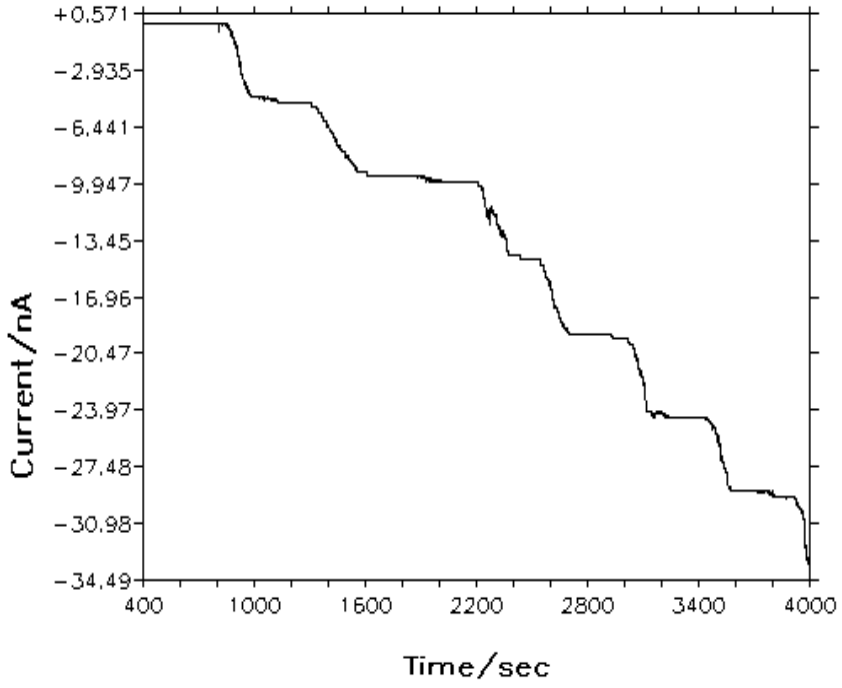


Figure 4. polyindoline COx electrode responses to cholesterol injections: 600, 1100, 1600, 2100, 2600, 3100, 3600 s (750 mV).

After detecting the expected electrochemical responses, the potential (550, 650, 750, 850 mV) and pH (5, 7, 9, 11) of the polyindoline cox electrode were determined to smooth the recorded current responses, as shown in Figures 5 and 6.

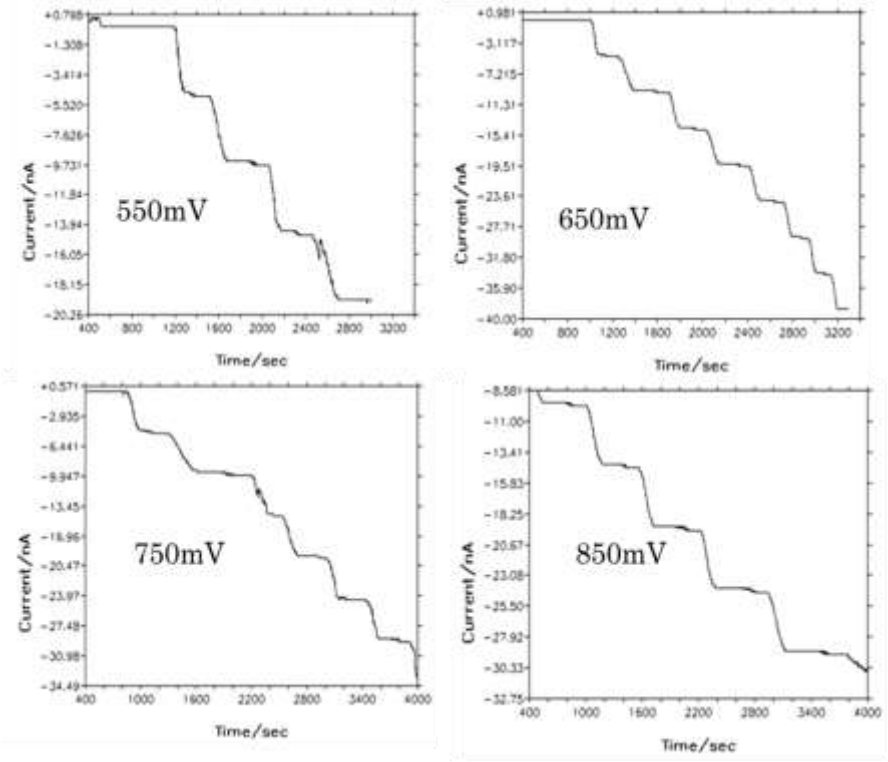


Figure 5. Responses of polyindoline COx electrodes to injections of cholesterol at 500, 1000, 1500, 2000, and 2500 s (at different potentials).

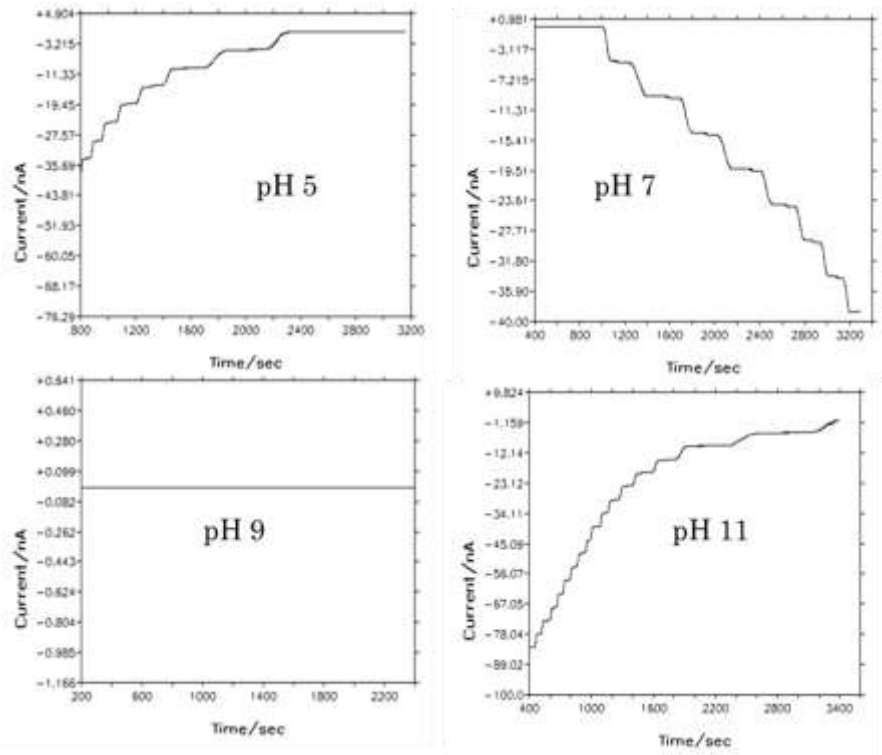
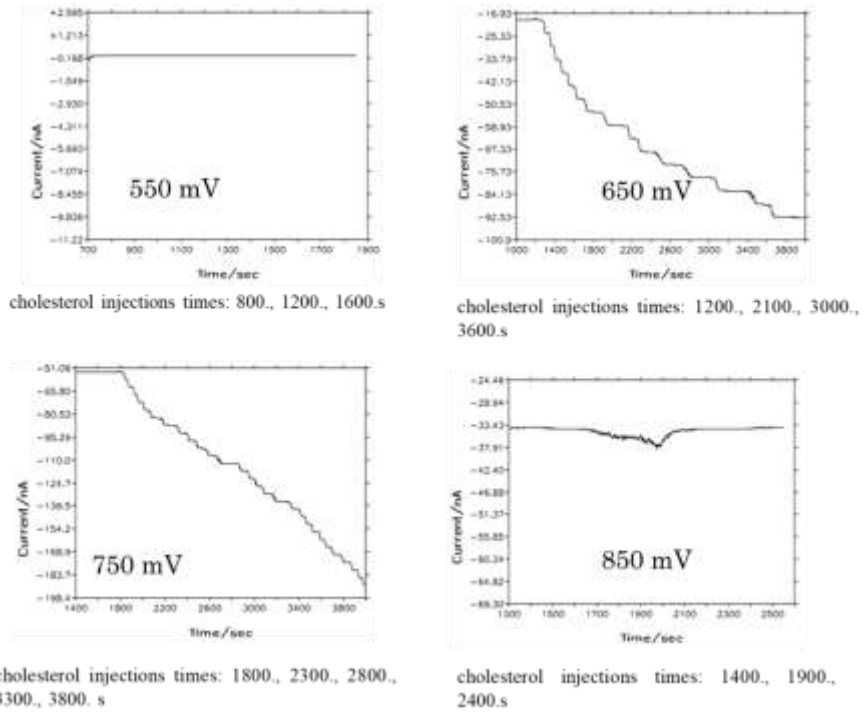


Figure 6. Responses of polyindoline COx electrodes to injections of cholesterol at 600, 1200, 1800, 2400, and 3000 seconds (at different pH values).

For poly(indolin), it was decided that 5 µL of COx, PBS solution at neutral pH and 750 mV was more suitable for cholesterol measurement.

Poly(4-methoxyphenol) films of different thicknesses were obtained by BE at 600 mV for 4-methoxyphenol prepared at a concentration of 100 mM in a 0.1 M KCl solution. The stepwise responses are due to the long measurement time of cholesterol. Therefore, the effects of applied potential (550, 650, 750, 850, 950 mV), pH (5, 7, 9, 11), and amount of enzyme dropped (1-4 µL) were systematically studied to make these reactions as linear as possible. They are shown in Figures 7, 8 and 9.



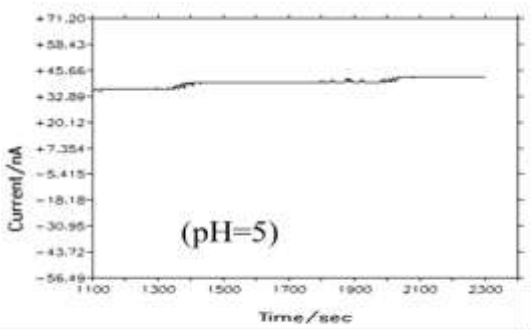
cholesterol injections times: 800., 1200., 1600.s

cholesterol injections times: 1200., 2100., 3000., 3600.s

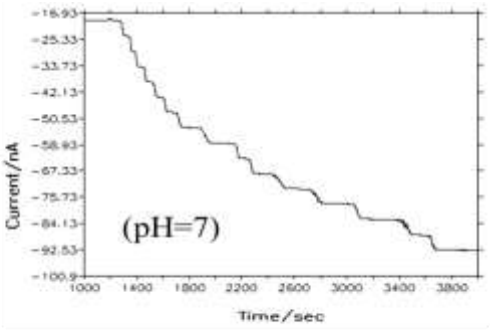
cholesterol injections times: 1800., 2300., 2800., 3300., 3800. s

cholesterol injections times: 1400., 1900., 2400.s

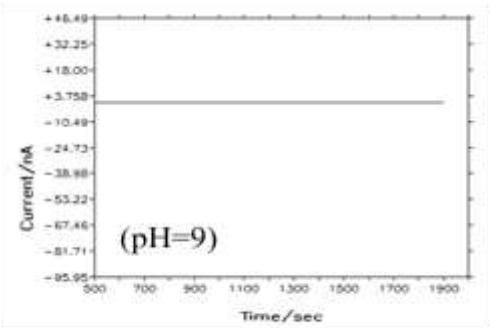
Figure 7. Responses of poly(4-methoxyphenol)-2 µL COx electrode to cholesterol injection



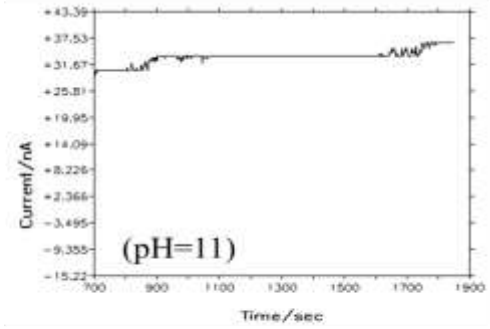
cholesterol injections times : 1200., 1500., 1800., 2100.s



cholesterol injections times: 1200., 2100., 3000., 3600.s

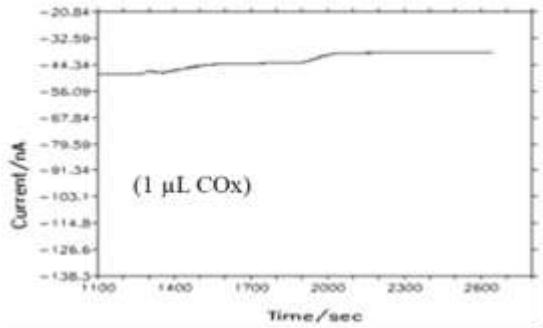


cholesterol injections times: 800., 1100., 1400., 1700.s

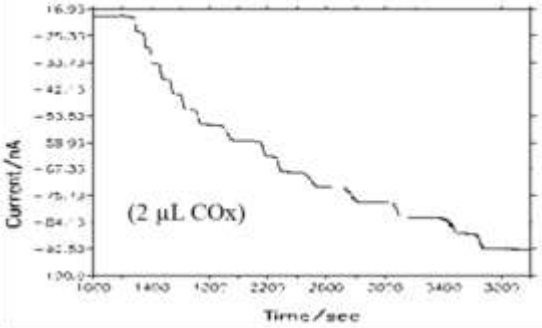


cholesterol injections times 800., 1100., 1400., 1700.s

Figure 8. Responses of poly(4-methoxyphenol)-2 µL COx electrode to cholesterol injection



cholesterol injections times: 1300., 1600., 1900., 2200., 2500.s



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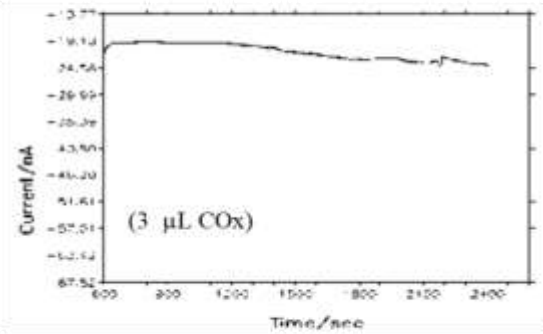
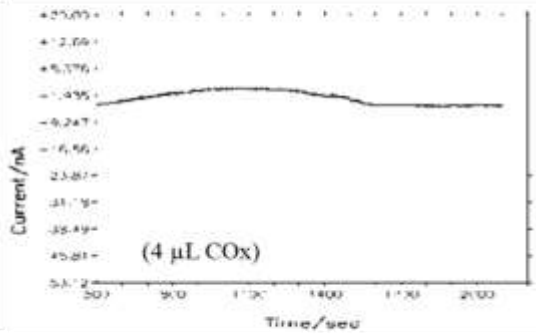


cholesterol injections times :., 1100., 1400., 1700., 2000., 2300.s



cholesterol injections times : 800., 1100., 1500., 1900., 2300.s

Figure 9. Responses of poly(4-methoxyphenol)- COx electrode to cholesterol injection

For poly(4-methoxyphenol), it was decided that 2 µL of COx, PBS solution at neutral pH and 750 mV was more suitable for cholesterol measurement.

CONCLUSIONS

Of the polymeric matrices prepared by the electropolymerization method, the enzyme immobilization studies performed with polyindoline, poly(o-toluidine), and poly(4-methoxyphenol) found that the appropriate response was obtained at neutral pH, and this finding was consistent with the literature. Charpentier and Murr chose 7 as the optimum pH in their study. It has been shown that polyinddol and poly(4-methoxyphonol) prepared by electrochemical polymerization can be used as a suitable immobilization medium for cholesterol oxidase.

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DETERMINATION OF RESISTANCE TO ALS INHIBITOR HERBICID IN WILD MUSTARD IS A PROBLEM IN WHEAT FIELDS IN AMASYA AND CORUM PROVINCES

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ABSTRACT

The method commonly used to solve the weed problem in wheat production areas in our country is the chemical control method. Herbicides, which are increasingly used for weed control in wheat fields, can cause many problems besides the benefits they provide.The most important of these problems is the herbicide resistance, which is defined as the reduction or disappearance of the herbicide's ability to control the population of the target weed species. Amasya and Çorum provinces in the Middle Black Sea Region in terms of both cultivation area and production area are the provinces where wheat agriculture is concentrated in the region. Wild Mustard (*Sinapis arvensis* L.) is important and common among weeds encountered in wheat production areas. In the fight against Wild Mustard in wheat production areas, chemical control is intensely carried out with herbicides that inhibit ALS (Acetolactate synthase) enzyme. It is known that resistance to this group of herbicides which are used extensively can develop in a short time. In this study, resistance status of the piercer against ALS enzyme inhibitor herbicides, which are used extensively in wheat production areas of Amasya and Çorum provinces, was determined between 2015-2020. As a result of the field studies carried out for this purpose; In Amasya and Çorum Provinces, 92 insane populations collected from wheat production areas were collected and the coordinates of the seed fields were determined. F2 offspring were obtained by planting the collected crazy populations in the greenhouse. The F2 populations obtained were subjected to N (dose) screening test with herbicides containing mesosulfuron-methyl+iodosulfuron-methyl sodium active substances under greenhouse conditions. Dose response trials with 8 different doses were established under greenhouse conditions in order to determine the resistance status of the populations with suspicion of resistance as a result of the screening test. As a result of the greenhouse works; mesosulfuron-methyl+iodosulfuron-methyl sodium in 16 populations.

Keywords: Wild Mustard (*Sinapis arvensis* L.), herbicide resistance, ALS, wheat

INTRODUCTION

Wheat, one of the most important plants used in human nutrition, is a strategic plant among the most important cereals consumed as a basic food source both in the world and in our country. Looking at the global world market, China comes first among the countries that produce the most wheat. Turkey wheat cultivation area constitutes 3.2% of the world wheat cultivation area as of 2020/21 production season. This area also constitutes 44% of the total cultivated grain area in Turkey. According to 2020 USDA data, Turkey ranks 10th in world

wheat exports (Anonim, 2021). In the Central Black Sea region, wheat production is carried out intensively in Samsun, Amasya and Çorum provinces. As of 2019, a total of 19,000,000 tons of wheat has been produced on 68,463,271 decares in these provinces where we have been working (Anonim, 2020).

Plant protection factors are at the forefront of the factors that negatively affect the agriculture of wheat and cause significant losses. Weeds, one of the plant protection factors, are the most important factor affecting the yield in wheat, and weed control has an important place in wheat production (Kaya Altop et al., 2017). Yield loss caused by weeds in cereals is around 20-40% (Güncan, 2010). There are many weed species in wheat cultivation areas. Mechanical, cultural, biological and chemical control methods are used in the fight against weeds in wheat cultivation areas. However, since wheat is not a hoe, it is not possible to apply mechanical methods to minimize weed damage. Cultural methods are insufficient for weed control in wheat cultivation areas, especially after emergence. For this reason, chemical control is undoubtedly one of the most preferred methods in the fight against weeds. It is the most preferred method of struggle because there are not many alternatives to chemical control, its applicability is easy, results are obtained in a short time, it is not affected much by ecological conditions and its cost is low (Kropff and Walter, 2000). However; Many problems come with it due to the fact that chemical control is not applied consciously. At the beginning of the problems; In addition to adversely affecting human, animal and environmental health, residue problem, phytotoxicity to the next crop plant, damage to non-target plants and herbicide resistance are other side effects that may occur (Gürbüz, 2016).

The chemical struggle against broadleaf weeds, which are a problem in wheat, with herbicides with 2,4 D and MCPA active substances in Turkey in the 1960s has increased rapidly every year. This situation causes the emergence of resistance to herbicides in weeds, which is an important issue in the control of weeds. Herbicide resistance is the ability of a plant to resist herbicides from different chemical classes, thanks to genetic characteristics. ALS inhibitor herbicides should be used in very small amounts per unit area, can be applied in many cultivated plants, being effective on both broad-leaved and narrow-leaved weeds, have a wide application time, it has a very high level of product safety and low permanence. Due to these features, it has spread very quickly (Mazur and Falco,1989; Prado et al., 2004).

With this study; Wild mustard resistance status was determined against herbicide, which is an ALS enzyme inhibitor, which is used extensively in the control of wild mustard in wheat production areas of Amasya and Çorum provinces.

MATERIAL AND METHOD

The main material of the study consisted of *Sinapis arvensis* seeds, which were collected from wheat fields in the provinces of Amasya and Çorum, where wheat cultivation is intense, and from untreated areas for comparison purposes. In addition, greenhouse, soil, peat, perlite, paper bags, plastic bags, sieves of different sizes, plastic pots, back sprayer, refrigerator, GPS and plant protection product with 6% iodosulfuron-metyl sodium + 3% mesosulfuron active ingredient formed the other materials used in the study.

Survey Studies

Field studies of the project were carried out between 16-30 June 2016 in 20 districts covering the study area of Amasya and Çorum provinces. Ripe seeds of wild mustard plants were taken by stopping the first wheat field, which is found every 5-6 km on the selected routes. The samples were collected in a way to represent the production area, before the wheat was

harvested and the wild mustard plants were collected at least 10 m from the field edge, circling in a V shape, paying attention to the seed maturity, and the plant samples were placed in paper bags. Data such as sample number, sampling location, GSP record, date were recorded on the sampling tag.

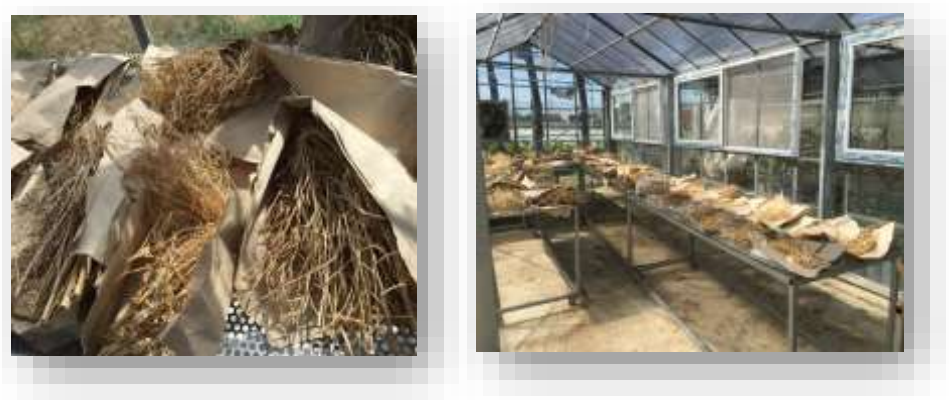

Figure 1. Wild mustard seeds collected from Amasya and Çorum

Determination of Resistance by Bioassay Method

Wild mustard seeds, whose need for cooling was met, were planted in 0.5 lt pots prepared with a planting medium consisting of soil, peat and perlite mixture (1:1:1) according to a randomized plot design with 4 replications. When they start to germinate, it is diluted to leave 2 plants in each pot. When the plants reached the 2-6 leaf stage, the herbicide N (application) dose with 3% Mesosulfuron-methyl + 6% Iodosulfuron-methyl sodium active substance was applied. The sprayed populations were kept in controlled greenhouses at 26±2°C for 28 days, and daily irrigation and maintenance of the populations were carried out during this period. The plants in the pots were watered 24 hours after the herbicide application. Observations were taken on the 28th day after the application and according to the 0-100% scale, Moss et al. (1999), developed by the endurance screening test.

Dose Effect Trials

In line with the dose-effect study, the trials were repeated twice, with 4 replications and 2 plants in each pot, as in the preliminary endurance study for each suspected population. Within the scope of the study, 8 different doses of herbicides were applied against resistant populations. For this purpose, N/4, N/2, N, 2N, 4N, 8N, 16N and 0 (control) administration doses were determined based on the license doses of the drugs considered. The studies were carried out under greenhouse conditions. In the drug-free controls, only water was applied. Spraying was done with a back sprayer and 30 liter/da spray norm water calculation. The trials were terminated at the end of the 28th day, the period when the recommended dose of herbicides showed the highest level of effectiveness in resistant populations.

Figure 2. Dosing applications

Dry weight data

On the 28th day after the herbicide application, the effect rates on weeds were determined as percent, and the dry biomass values were obtained by cutting from the root neck and drying at 70 0C for 48 hours.

Evaluation of Results

With the obtained dry weight values, dose-effect curves for each biotype and as a result, ED50 and b parameters for each biotype were obtained by means of the following 4-parameter logistic regression developed by Streibig (1988).

$$y = C + \frac{D - C}{1 + e^{[b\,(\ln(x) - \ln(ED_{50}))]}}$$

In this formula;

X: The herbicide dose applied, Y: parameter level (mean plant dry weight) obtained when X dose was applied, D: upper limit of the dose-effect curve (average weight formed by unmedicated control plants, where D=100 as the % value), C: The lower end of the dose-effect curve (usually the weight % level obtained from the highest herbicide doses), ED50: Herbicide dose that causes 50% weight reduction when applied.

RESULTS AND DISCUSSION

Survey Studies

2016 survey studies are conducted in Merkez, Merzifon, Taşova, Gümüşhacıköy, Suluova, Hamamözü and Göynücek districts of Amasya province and Merkez, Laçin, Dodurga, Oğuzlar, Ortaköy, İskilip, Sungurlu, Osmancık, Bayat, Bozkale, Uğurludağ, Kargı and Mecitözü in Çorum province. conducted in the districts. Wild mustard samples were collected from 95 locations in Amasya and Çorum provinces and districts.

Determination of Resistant by Biosass Methods

In the dose screening test performed with collected wild mustard seeds, as a result of the dose screening tests performed against the licensed dose (N dose) of herbicide with 3% Mesosulfuron-methyl + 6% Iodosulfuron-metyl sodium active substance of populations representing the field; Suspicion of resistance was found in a total of 34 populations, 11 populations in Amasya provinces and districts, and 23 populations in Çorum provinces and districts.

Dose Effect Trials

As a result of the screening test, new generation (F2) biotypes were obtained from a total of 34 wild mustard populations with suspected resistance in greenhouse conditions. The 8-character dose-effect test results of the herbicide with Mesosulfuron-methyl+idosulfuron sodium methyl active substance on f2 biotypes are given in table23.

In Amasya, 6 suspicious populations (Ams-9, Ams-10, Ams-11, Ams-17, Ams-24 and Ams-27) showed resistance to 3% Mesosulfuron-methyl + 6% Iodosulfuron-methyl sodium.

When the ED90 values and resistance rates of the populations resistant to 3% Mesosulfuron-methyl + 6% Iodosulfuron-methyl sodium were examined, it was determined that the coefficient changed between 1.26-4.13 (Ams-35/Ams-24) (Table 1). In the study they carried out to determine the resistance of B.radians, which is a problem in wheat, to ALS enzyme inhibitor herbicides, resistance was determined in two of 31 populations against 3% Mesosulfuron-methyl + 6% Iodosulfuron-metyl sodium of *B.radians* in Amasya provinces and districts. (Kaya et al., 2017).

3% Mesosulfuron-methyl + 6% Iodosulfuron-methyl sodium sulfonylurea is a herbicide licensed for wild mustard in our country. Different doses of the active substance and the distribution of its recurrences in the dose-effect curve when applied to wild mustard are given in Figure 3.

Table 1. ED90 values of populations resistant to 3% iodosulfuron-metyl sodium + 6% mesodulfuron-metyl of wild mustard collected from Amasya Province and its districts

Biyotip	Etkili Doz (ED)	Dayanıklılık katsayısı
	ED 90	
Ams-1	2,12±0,30	1,79
Ams-9	4,72±0,90	3,98
Ams-10	4,84±1,70	4,09
Ams-11	3,64±0,72	3,07
Ams-17	2,14±0,31	1,81
Ams-19*	1,18±0,12	1
Ams-20	1,62±0,20	1,59
Ams-24	4,90±0,78	4,13
Ams-27	2,57±0,53	2,17
Ams-33	1,74±0,25	1,47
Ams-35	1,49±0,18	1,26

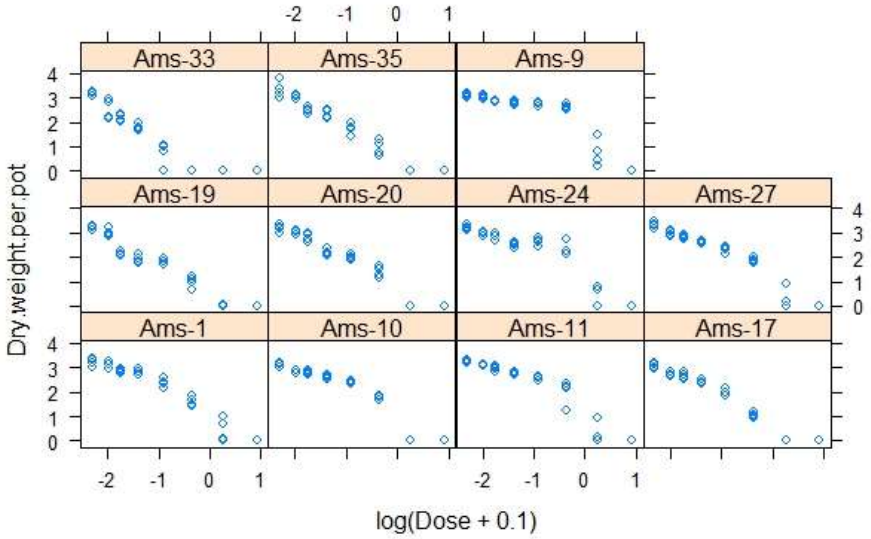

Figure 3. Distribution of different doses of 3% Mesosulfuron-methyl + 6% iodosulfuron-methyl sodium doses applied to different populations of wild mustard collected from Amasya provinces and districts in dose-effect studies.

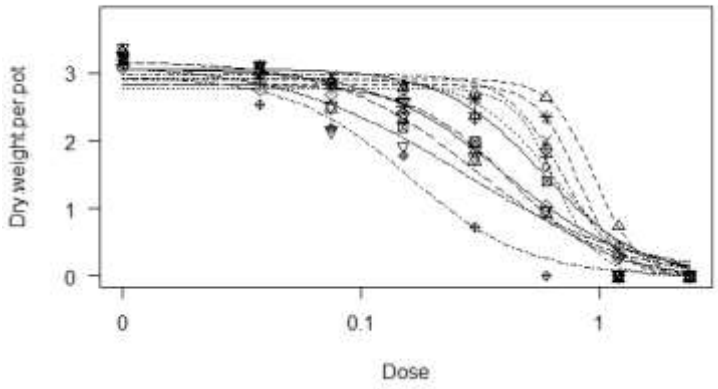

Figure 4. The dose-effect curve of different doses of 3% Mesosulfuron-methyl + 6% Iodosulfuron-methyl sodium doses applied to different populations of wild mustard collected from Amasya provinces and districts.

When Table 2 is examined, it is seen that 10 populations (Çor-8, Çor-13, Çor-16, Çor-17, Çor-21, Çor-25, Çor-35, Çor-36, Çor-39 and Çor-50) were determined as a result of dose effect trials from 23 populations collected from wheat cultivation areas in Çorum province. Show resistance to 3% Mesosulfuron-methyl + 6% Iodosulfuron-metyl sodium.When the ED90 values and resistance rates of the populations resistant to 3% Mesosulfuron-methyl + 6% Iodosulfuron-methyl sodium were examined, it was determined that the coefficient changed between 1.26-2.88 (Çor-40/ Çor-8). In the study carried out by Sarıhan in the provinces and districts of Çorum in 2013, it was investigated whether A. fatua, which is a problem in wheat cultivation areas, has resistance to herbicides. Resistance to mesosulfuron methyl+iodosulfuron methyl sodium was detected in 8 of 16 populations with suspected resistance.

Table 2. ED90 values of populations resistant to 3% iodosulfuron-metyl sodium + 6% mesodulfuron-metyl of wild mustard collected from Çorum Province and its districts

Biyotip	Etkili Doz (ED)	Dayanıklılık Katsayısı
	ED 90	
Cor-1	1.46±0.57	1,63
Cor-2	1.46±0.54	1,62
Cor-3	1.06±0.46	1,18
Cor-8	2.58±0.53	2,88
Cor-9	1.09±0.38	1,21
Cor-12	1.46±0.46	1,62
Cor-13	1.74±0.84	1,93
Cor-16	1.74±0.61	1,94
Cor-17	1.66±0.62	1,85
Cor-18	1.23±0.44	1,37
Cor-21	2.17±1.11	2,41
Cor-25	2.02±0.96	2,25
Cor-27	1.40±0.57	1,56
Cor-31	1.40±0.57	1,56
Cor-35	2.31±1.01	2,57
Cor-36	2.19±1.23	2,43
Cor-37	1.26±0.40	1,40
Cor-39	1.71±0.78	1,91
Cor-40	1.13±0.37	1,26
Cor-41*	0,89±0.26	1
Cor-49	1.25±0.42	1,39
Cor-50	2.19±1.18	2,43
Cor-51	1.33±0.5	1,48

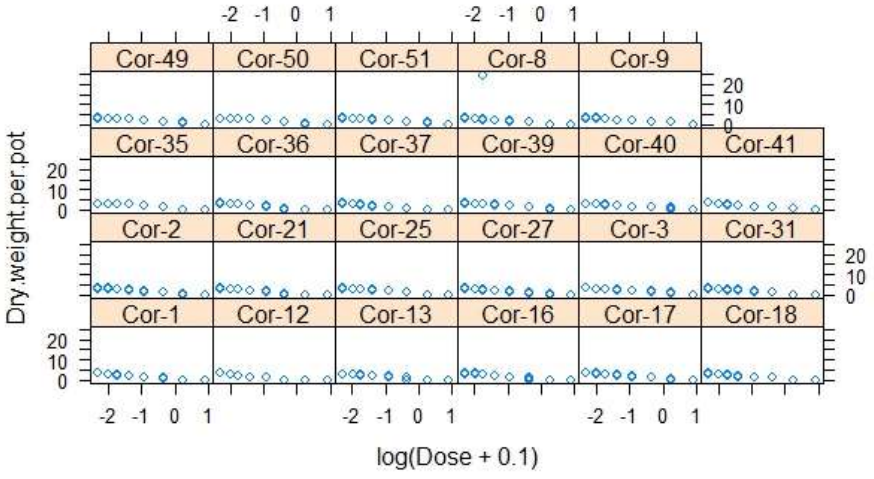

Figure 5. Distribution of different doses of 3% Mesosulfuron-methyl + 6% Iodosulfuron-methyl sodium doses applied to different populations of wild mustard collected from Çorum province and its districts in dose-effect studies.

When Figure 6 is examined, it is seen that the distribution of different doses of 3% Mesosulfuron-methyl + 6% Iodosulfuron-metyl sodium applied in the dose-effect trials established in 23 suspicious populations in Çorum provinces and districts is homogeneous.

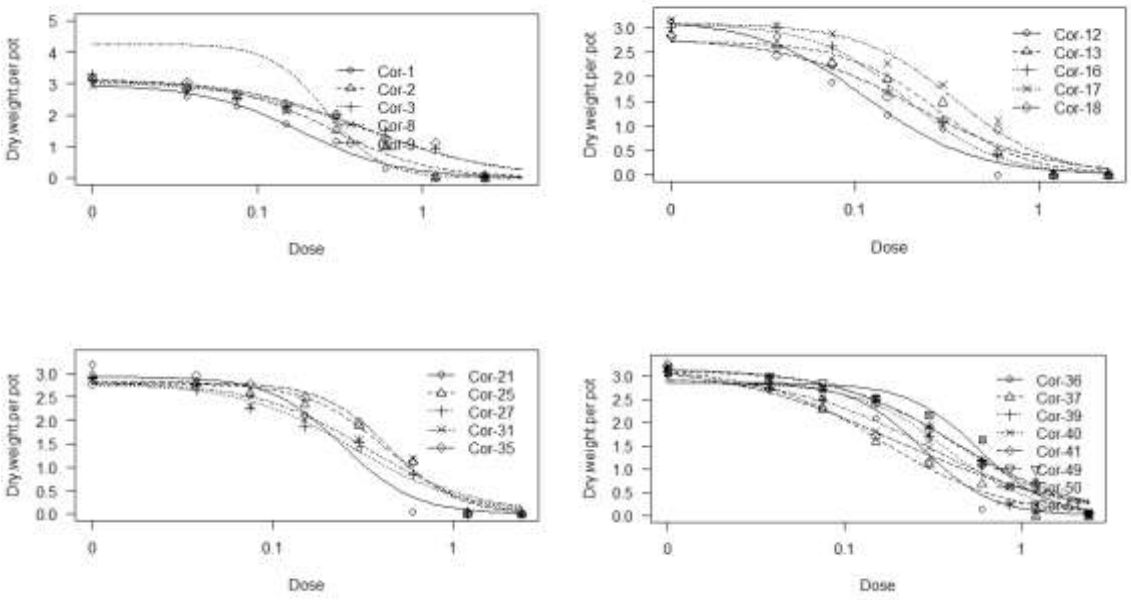

Figure 6. The dose-effect curve formed by different doses of 3% Mesosulfuron-methyl + 6% Iodosulfuron-methyl sodium doses applied to different populations of wild mustard collected from Çorum province and its districts.

CONCLUSIONS

In 92 wild mustard populations collected from the wheat cultivation areas of Amasya and Çorum Provinces, as a result of the screening tests performed with a double license dose of mesosulfuron-methyl + iodosulfuron-methyl sodium active substances from the ALS group, suspected resistance was found in the population of 47 fields. New generation (F2) seeds of suspicious populations were obtained. As a result of dose-effect trials with new generation (F2) seeds representing 47 fields from wheat production areas; It was determined that 6 biotypes in Amasya and 10 biotypes in Çorum showed different levels of resistance to mesosulfuron-methyl + iodosulfuron-methyl sodium.

Establishing and managing resistance control strategies in areas where resistance cases are seen, it is important that sustainable agriculture can be carried out under good agricultural practices.

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MOLECULAR FACTORS AFFECTING CHICKEN MEAT QUALITY AND FLAVOR

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ABSTRACT

Intensive poultry production is agricultural sub-sector that has gained popularity since the late 1940s and is growing the fastest today, with the intense industrialization of food production. Following this rapid development, the consumers have more interested in chicken meat products. These products are mostly derived from industrial poultry production, which is characterized by high growth rate and high breast meat yield. On the other hand, in recent years, many quality flaws (white striping, wooden breast, etc.) have appeared that damage the integrity and composition of the muscles, particularly the fillet. Several solutions have been explored to alleviate these faults, but they have proven insufficient. It is believed that the most effective strategy is to switch to slow-growing breeds with poor meat output. In recent years, customers have prioritized animal health and welfare, free-range poultry (including organic production systems), and later slaughter age. Moreover, they have been tended to the chicken meat products has unique taste such as geographical indication products, local delicacies etc. that contain natural flavor. These alterations in consumer demands directs the breeders to alternative poultry production systems. In this context, it is aimed to highlight the importance of some chicken breeds by determining and improving their meat quality characteristics, especially in developed countries. In line, many studies have been conducted to determine the biological pathways that regulate the meat quality and flavor of chickens. Some molecular investigations on genes involved in chicken meat quality problems and taste are summarized in this review.

Keywords: Chicken, Meat Quality and Flavor, Molecular Mechanisms

INTRODUCTION

Until the 1950s, farm animals in industrialized countries were raised using highly traditional methods that relied on labor for routine work. These methods were the feeding, remove of manure and often involved keeping animal's outdoors for at least part of the time. After the Second World War, a new generation of "intensive" production was emerged that kept animals in special enclosed environments and used equipment and automation instead of labor for many routine tasks (Fraser, 2001). Especially in poultry production, confinement methods have become more common in industrialized countries or species that feed heavily on grains and other concentrates (Fraser, 2008a). As a result, poultry production has shifted towards concentrated (intensive) production in more limited units (Fraser, 2008b). This concentrate system accompanied by large increases in poultry production. Intensive poultry production gained popularity from the late 1940s. World's poultry meat production increased rapidly between 1961 and 2001 (Table 1). In fact, these recent increases were roughly proportional to

the growth rate of the human population, which nearly doubled in the same 40 years. With the intense industrialization of food production, poultry production has become the fastest growing agricultural sub-sector today (Escobedo del Bosque et al., 2020).

Table 1. World production, world exports and percentage exported of four categories of meat, 1961 – 2001 (Fraser, 2008b).

Source of Poultry Meat	1961	1971	1981	1991	2001
World production (1 000 tonnes/year)	8.91	15.657	27.386	42.939	71.414
World exports (1 000 tonnes/year)	303	594	1.900	2.923	9.359
Percentage exported	3.4	3.8	6.9	6.8	13.1

Note: The data are from "Animal welfare and the intensifcation of animal production." by Fraser, D. (2008b).

In the last few decades, the demand for poultry meat has increased rapidly due to its ease of cooking and processing, nutritional value and low price. Breeders had to reduce their costs and increase their production in order to this the demand of poultry meat with increasing world population. Therefore, breeders have produced more yielding layers and broilers chickens by applying genetic selection and crossbreeding to their flocks (Pampouille et al., 2018). Thanks to genetic selection, an approximately 85% to 90% change occured in broiler growth rate (Havenstein et al., 2003a). Especially in nearly decade years, it has made a significant contribution to meat yield of chicken breast, which is currently more than one-fifth of the weight of the bird (Havenstein et al., 2003b; Petracci et al., 2015). However, all these developments have increased the incidence of broiler breast muscle abnormalities in the poultry industry in many countries (Pampouille et al., 2018). The most important of these muscle defects in chicken are "White Striping" (WS), "Wooden Breast" (WB) and "Spaghetti Muscle" (SM) (Figure 1).

Figure 1. Some quality defects of chicken breast muscles (Baéza et al., 2021).

On the other hand, intensive poultry production has increased consumers' concerns about animal welfare, especially in recent years (Escobedo del Bosque et al., 2020) and this has led consumers to poultry products obtained from free-raising systems (Fanatico, 2006; Tong et al., 2014). In particular, consumer demands for poultry meat products (local chicken meat products, geographical indication products) with a rich aroma, good taste (palatable) and texture has increased. As a result, consumers' increased awareness of poultry health and nutrition and the search for different flavors have encouraged breeders to create a market for poultry meat products produced using more acceptable rearing systems (Blair, 2018). On the other hand, researchers focused on investigating the mechanisms underlying breast muscle defects and on

improving the meat quality of chicken (Velleamn et al., 2015; Zambonelli et al., 2016; Vignale et al., 2016; Wold et al., 2017; Papah et al., 2018; Hubert et al., 2018; Soglia et al., 2020). In this review, some molecular studies on chicken meat defects and flavor, and suggested strategies are summarized.

QUALITY DEFICIENCIES IN CHICKEN BREAST MUSCLES

In the past few decades, there has been a rapid increase in demand for chicken meat, in particular. Therefore, to meet the growing demand for poultry meat, breeders focused on the selection for high-growth rate and breast-yielding chicken hybrids. However, despite an improvement in body weight gain, genetic selection has led to an increased incidence of various muscle abnormalities (Soglia et al., 2016).

Selection practices, to achieve rapid growth rates by increasing muscle growth of modern chicken hybrids, have also affected the anabolic and catabolic processes of muscle proteins. One of them, Myocardin is a protein that plays a role in the differentiation of smooth muscle cell line and vascularization, and also a decreasing in muscle vascularization has been observed when its expression level is decreased (Berri et al., 2001, 2007; Le Bihan-Duval et al., 2001; Pampouille et al., 2019; Tixier-Boichard, 2020).Myocardin expression level is also decreased in fast-growing hybrids with a decrease in heme pigment content compared to slow-growing lines (Goll et al., 1992; Dransfield and Sosnicki, 1999; Soglia et al, 2021). In addition, some alterations in muscle structure and biochemistry (as indicated by increased plasma activity of the intracellular enzyme creatine kinase) have been observed in broilers selected for their production performance (Rémignon et al., 1996; Dransfield and Sosnicki, 1999; Sandercock et al., 2006; Berri et al., 2007). The release of creatine kinase into the blood, for example, may be regarded a direct outcome of muscle damage (Sandercock et al., 2006). This situation has been thought to cause a disruption in the regulation of cell cations (i.e., increased sodium and calcium content along with an overall reduction in magnesium and potassium) in fast-growing birds and thus the initiation of degenerative processes in skeletal muscles affected by spontaneous abnormalities (Sandercock and Mitchell, 2004). Besides these alterations in muscle structure, selection progression has also been reported to affect the energetic metabolism of *Pectoralis major*. Selection for mass accretion for muscle growth in chickens has been associated with a shift towards type IIB fibers that use carbohydrates as their main energy source. In addition to all these, a negative genetic correlation was found between within tissue glycogen reserves and breast muscle development in fast-growing chickens (Berri et al., 2001, 2007). Concurrently, it has been established that there is a decrease in glycogen utilization and that genes involved in glycogen metabolism have lower expression levels (Pampouille et al., 2019).

Chicken breast muscle abnormalities, especially WS, WB and SM defects have increased incidance in recent years (Soglia et al., 2016). WS is defined as appearance of white striations parallel to muscle fibers. WB is defined as visually hard, swollen and pale areas on the ventral surface of the *Pectoralis major* muscles (Baéza et al., 2021). Although histological studies have revealed the presence of degenerative and atrophic fibers with lipidosis and fibrosis (Soglia et al., 2016b; Velleman & Clark, 2015), it has been reported that there is no health hazard associated with the consumption of WS and WB meat (Soglia et al., 2016a). SM is defined as a general impaired integrity of the muscle in which the fiber bundles tend to separate from each other (Soglia et al., 2021). These muscle abnormalities are not only a visually poor, but also significantly affect the quality characteristics and functional properties of meat. In particular, since the water holding capacity, composition and processebilty to protein of the meat are significantly impaired, this typies of meat is used in the production of finely chopped products

(for example, nuggets) and is considered to be significant economic losses for the poultry meat industry (Soglia et al., 2016b).

Abnormal chicken breast muscle has causes trimming/cropping of whole breast and carcass, decreasing yield and value, increased need for manual sorting on the deboning line and rejection by consumers due to undesirable sensory properties. The appearance, texture and flavor of chicken meat are the quality characteristics most noted by consumers. It has been found that muscle tissue degeneration in the myopathies studied has a direct effect on sensory properties (ie color and texture) and is a direct result of the histological features of myopathies (as in WS, WB and SM). This increase in breast meat abnormalities in recent years has led consumers to products other than standard chicken. Consumers' growing concerns about animal welfare, and the search for healty and tasty products have increased the interest in local chicken breeds. This has caused breeders to turn to local breeds and researchers to focus on meat quality characteristics of hybrids and local breeds (Petracci et al., 2019).

Researchers have conducted studies attempting to characterize features associated with the occurrence of WS, WB, and SM and to identify eventual abnormalities that may play a role in muscle structure or its components (Petracci et al., 2019). Histological investigations revealed that, whereas these three anomalies have distinct phenotypes, they have similar histological characteristics (Soglia et al., 2019a). These include deeply impaired muscular architecture, hypercontracted fibers having rounded profile, nuclei internalization, and the occurrence of multifocal myofibril degeneration (up to necrosis). Myofibril degeneration occurs with occasional regeneration, splitting, and fragmentation of the myofibers (Baldi et al., 2021). Along with these similar histological characteristics, muscles affected by WS, WB, and SM have different microscopic properties that may explain their phenotypes. While, an abnormal deposition of adipose tissue occurs in WS muscle, proliferation and thickening of the connective tissue is observed in WB muscle, and SM has a progressive rarefaction of the connective tissue (Baldi et al., 2021). On the other hand, several genetic studies have been carried out using different technologies (such as quantitative Real Time-PCR, RNA-sequencing, microarray) to determine the presence of genes affecting all these abnormalities (Mutryn et al., 2015; Velleman and Clark, 2015; Alnahhas et al., 2016; Zambonelli et al., 2016; Papah et al., 2018; Pampouille et al., 2018, 2019; Brothers et al., 2019; Marchesi et al., 2019; Papah and Abasht, 2019; Soglia et al., 2020).

SOME MOLECULAR STUDIES ON CHICKEN BREAST MUSCLES QUALITY DEFECTS

White Striping

White striping (WS) is a defect that adversely effects on sensory (tenderness and juiciness), technological (pH, water holding capacity etc.) and nutritional qualities (IMF content, fatty acid profile etc.) of broiler breast meat. This defect prevents the efficient use of the most expensive part of the carcass (fillet) and thus has led to negative economic consequences. For these reasons, studies investigating the mechanisms inducing WS have been conducted to reduce or even eliminate the incidence of WS in modern commercial broilers and suggested and discussed effective strategies.

Alnahhas et al. (2016) have stated that the genetic determinism of the WS defect is poorly understood for now. For this reason, they aimed to estimate the genetic parameters of WS in relation to other economically important characteristics such as body weight, carcass composition and technological meat quality by comparing experimental two population. For this purpose, two different chicken lines were selected for the high (pHu + line) or low (pHu-

line) final pH (pHu) of the *Pectoralis major* muscle. According to the findings, pHu + broilers had a greater prevalence of moderate to severe WS, whereas the lines tested have a significant genetic determinism for WS. In addition, it has been reported WS significantly genetically related to body weight and breast meat yield regardless of the line. A significant regression was observed between WS and various meat quality traits (breast and thigh pHu, and breast cooking loss). The intramuscular fat content of the *Pectoralis major* muscle was also revealed to be highly genetically associated to WS. In conclusion, this study emphasized the significance of genetics as the key factor of WS.

Pamppuelli et al. (2018) aimed to identify the first quantitative trait loci for WS, as well as breast muscle yield and meat quality traits, using a genome-wide association study (GWAS). Two different lines of chickens were selected for meat quality through *Pectoralis major* ultimate pH (pHu) and which exhibit the muscular defect. According to the findings, forty-two single nucleotide polymorphisms (SNPs) associated with WS and other meat quality traits and 18 QTL regions located on 13 chromosomes have been identified. 132 SNPs associated with molecular phenotypes were detected. In addition, 21 eQTL regions located on 16 chromosomes were identified. It has been reported that the *MYH15, MYH1E, MYH1B, MYH1F, MYH13, MYOCD*, selected candidate genes, are involved in muscle fiber regeneration and repair while *PDGFRα* gene is involved in adiposis and fibrosis. It is stated that *COL6A3, FN1, SGCB* genes are involved in the composition of the extracellular matrix or sarcolemma, *PNPLA7* gene is involved in muscle metabolism. The Myosin Heavy chain 15 gene (MYH15) located on GGA1 has been reported to be involved in the contraction, development and regeneration of avian skeletal muscles. It has been determined that the most important SNP associated with WS is located within this gene. Another selected candidate gene in this study is *SGCB* which a member of the sarcoglycan complexis. Previously, it has been shown that transgenic beta-sarcoglycan-deficient mice exhibited progressive muscular dystrophy with extensive degeneration and regeneration of muscle fibers (Araishi et al., 1999). Therefore, it is suggested that *SGCB* an interesting positional and functional candidate gene for the QTL3 region which a pleiotropic effect on WS and BMY was claimed. As a result, the presence of a large effect gene was not detected in this study, but it was emphasized that the defect and other meat quality traits examined were polygenic traits.

Vignale et al., (2016) were investigated that the effect of WS on protein turnover and expression of genes related to protein degradation and fatty acid synthesis in broiler breast muscle. In this study carried out on randomly selected 17 broilers (at day 60; 16 infused, 1 control), breast muscle tissues were used for gene expression analysis of *MuRF1, atrogin-1, IGF-1, insulin receptor (IR), fatty acid synthetase*, and *acetyl CoA carboxylase (ACC)* genes. Of the breast samples taken, totally 10 breast muscle tissue samples were selected for analysis as 5 normal and 5 severe WS. According to the findings, there was no significant variation in fractional synthesis rate between SEV WS, NORM, and breast flesh sample locations. However, the fractional breakdown rate was found to be significantly higher in birds with SEV WS compared to NORM. It was stated that birds with SEV WS compared with NORM birds showed significantly higher relative expression of *MuRF1* gene and slightly higher relative expression of atrogin-1 gene. Furthermore, these birds were shown to have lower relative expression of *IGF-1* than NORM birds.

Wooden Breast

Wooden breast (WB) is a muscle defect that adversely affects breast meat quality, which is classified as breast myopathy, causes increasing hardness of tissue and decreasing meat quality (Hubert et al., 2018). The incidence of WB has increase in recently years, so it has

globally affected consumer preferences linked to economic losses. WB has increased especially in the last decade and has been reported to affect more than 50% of commercial flocks (Abasht et al., 2016; Sihvo et al., 2017). WB differs from other pectoral myopathies in that it appears as moderate to severe degenerative necrosis with varying degrees of interstitial fibrosis (Hubert et al., 2018). While some of these features are observed separately in other myopathies, localized pectoral myopathy and the appearance of fibrosis and striations are unique to WB. Despite histopathological and serological studies, dietary treatments, and gene expression studies on WB (Mutryn et al., 2015; Abasht et al., 2016; Papah et al., 2017; Cruz et al., 2017; Radaelli et al., 2017; Griffin et al., 2018), the causative factors of WB are still unknown. In the light of this information, Hubert et al. (2018) were purposed to investigation of molecular basis of WB by using comparative analyses. They performed comparative transcriptomic pathway analyzes of WB samples against various genotypes/phenotypes. Gene expression levels in the *Pectoralis major* muscle tissue from fast- and slow-growth broilers were compared. Twenty genes have been proposed for the WB breast and emphasized that it has a polygenic basis. It was determined that WB shows an age-dependent gene expression pattern. Results of the pathway analysis showed enrichment of glycolysis, cell differentiation, tumor suppression.

Papah et al. (2018) were aimed to characterize the molecular profile associated with the onset and progression of WB defect during the early growth period (up to 4 weeks). With this purpose, they studied on muscle biopsy samples from *Pectoralis major* muscles between affected and unaffected birds belonging to the high-breast-muscle-yield, purebred broiler line. The results were revealed various biological processes and pathways (dysregulation of energy metabolism, response to inflammation, vascular disease, and remodeling of extracellular matrix etc.) associated with the onset and progression of WB defect. It has been stated that presence of molecular perturbations involving the vasculature, extracellular matrix and metabolism were pertinent to the onset and early pathogenesis of WB defect in commercial meat-type chickens.

Lake et al. (2021) performed a genome-wide association analysis using a commercial crossbreed broiler population (n = 1193) to examine the genetic basis of the WB defect. They stated that heritability was estimated that 0.5 for WB with high genetic correlation (0.88). According to the results of GWAS, 28 QTL were identified on five chromosomes for WB. As a result of in this study, top candidate genes were stated that related to WB defect include *CDKN1C, CTSD, KCNQ1, LSP1, SLC22A18* and *USH1C* on GGA5 and *DNM2* on GGA30.

Praud et al., (2020) reported in a study that to date no solution has been identified that would significantly reduce the incidence of WB and WS defects without affecting the growth performance of broilers. Therefore, it was aimed to follow the evolution of molecular phenotypes related to both fast growth rate and occurrence of defects to identify potential biomarkers that can be used for diagnosis using histological and molecular tools that allow precise quantification of the different lesions found in WS or WB affected muscles. In this study, histological and transcriptional approaches were applied using breast muscles in a slow and fast-growing line, affected or unaffected by WS and WB. Results showed that fast-growing muscles produced more reactive oxygen species (ROS) than slow-growing ones, independent of WS and WB formation. Despite higher mitochondria density, fast-growing muscles affected by WS or WB defects did not show higher cytochrome oxidase activity (COX) activity, suggesting altered mitochondrial function. According to the study, immunohistochemistry staining of *FN1, NCAM,* and *MYH15* was greater in slow-growing muscles than in fast-growing muscles. It was also found that the amounts of these biomarkers increased linearly with the presence and severity of WS and WB defects. It has been reported that these could be potential biomarkers. Muscle expression of genes such as *TGFB1* and *CTGF* or *Twist1* were also positively correlated with the presence and severity of WB and WS defects. Finally, it has been

suggested from the obtained findings that a balance between *TGFB1* and *PPARG* genes would be necessary for the induction of fibrosis or adiposis and thus to determine the WS and WB phenotypes.

Spaghetti Muscle

Spaghetti meat (SM) defect is a disease that appears long, thin, solid and cylindrical, like popular pasta, due to the unraveling and splitting of muscle fibers (Petracci et al., 2019; Che et al., 2022). SM is defined as the separation of muscle fiber bundles mainly in the cranial region of the pectoral muscle. Because these myopathies are not caused by infectious pathogens, they do not pose a public health risk. However, the changed appearance of severely impacted fillets can have a detrimental impact on customer approval, resulting in fresh meat rejection or depreciation due to poor quality. (Petracci et al., 2019). Moreover, these myopathies have been associated with decreased nutritional and organoleptic qualities, such as higher fat and lower protein content compared to normal fillets (Kuttappan et al., 2012; Mudalal et al., 2015; Wold et al., 2017; Baldi et a., 2019). Meat processability issues, such as poor texture, higher drip and cooking losses as well as lower marinade uptake, have also been associated with these myopathies (Che et al., 2022). Because of this image, the consumption acceptability of meat is decreasing and producers either discard the fillet instead of selling it fresh or use it to produce more processed products (Tasoniero et al., 2020). In the first studies on SM, poor meat quality characteristics were detected in the affected fillets and this deterioration observed in the superficial part of the muscle was demonstrated (Baldi et al., 2018, Baldi et al., 2019).

In order to obtain more information about SM, Tasoniero et al. (2020) examined and evaluated the features of breast fillet such as mineral profile, salt-induced water uptake, cook loss, final yield, myofibrillar and sarcoplasmic protein solubility, and emulsifying properties. For this purpose, the effects of SM myopathy and sampling location on the physical properties, composition and protein functionality of chicken breast meat were investigated on 30 normal and 30 SM boneless fillets. The water holding capacity, approximate composition, mineral profile, myofibrillar, SDS-PAGE, and sarcoplasmic protein solubility and emulsifying properties were evaluated in both the superficial and deep layers of the breasts. Based on the findings, it was revealed that the superficial portion of the SM fillets contained the lowest protein and ash, on the other hand having the highest moisture and fat contents. When the deep part of normal and SM fillets was compared with their superficial parts, it was determined that the total and soluble collagen amounts for both were higher in the superficial part. It has been reported that the highest calcium and sodium levels were detected in the superficial portion of SM fillets. In general, it was noted that the myopathy showed minor effects protein profiles, while a more noticeable effect on the muscle layer. SM fillets were found to have a higher pH but exhibit a lower myofibrillar protein solubility. In addition, it was determined that there is a potential impairment of water-holding capacity in the SM-affected meat. Considering the importance of protein quality in the meat processing process, they emphasized that the next step in understanding this myopathy is to investigate the intrinsic protein characteristics (Tasoniero et al., 2020).

Sanden et al. (2021) emphasizing that connective tissue is affected by both WB and SM, investigated the amount and structure of collagen in these two myopathies. The collagen structure in Normal, WB and SM fillets were compared using histological methods and Fourier Transform Infrared (FTIR) microspectroscopy and imaging. As a result of histological analysis, an increase in the amount of connective tissue was reported in chicken abnormalities, especially in the perimysium. It was determined that WB exhibited a mixture of thin and thick collagen fibers, while collagen fibers in SM were thinner, fewer, and shorter. According to FTIR data, it

was determined that SM fillets expressed the lowest mature collagen fibers compared to normal and WB fillets and it had fewer β-sheets structures than WB fillets.

The Z discs are the smallest functional units in striated muscle that line the lateral borders of sarcomeres. Ultrastructural studies carried out in the previous years revealed that vimentin (VIM) and desmin (DES) play an important role in the preservation of sarcomere cytoarchitecture by forming a three-dimensional scaffold around the Z-disk (Granger and Lazarides, 1979; Tokuyasu et al., 1985; Soglia et al., 2020). In other studies, it has been reported that the increased abundance of VIM and DES is a proof of muscular dystrophy and can be used as reliable markers for regenerative processes in muscle tissue (Bornemann and Schmalbruch, 1992; Gallanti et al., 1992; Fröhlich et al., 2016). Soglia et al. (2020) investigated the distribution of VIM and DES proteins, as well as the expression status of corresponding genes, in *Pectoralis major* muscles with WS, WB, and SM anomalies and those with a macroscopically normal appearance. For this purpose, immunohistochemistry, immunoblotting and gene expression analyzes were performed in the same fast growing broiler flock (*Pectoralis major* tissue from 20 broilers). The SM had significantly lower content of VIM and DES compared to the others (WS, WB, and NORM), while higher amounts of DES were found in WS. This is due to the advancement of regeneration mechanisms that need its production to retain the structural organization of growing WB muscle fibers. According to the results of gene expression, VIM mRNA levels were higher in SM samples than in NORM samples, and the highest expression was detected in WB samples relative to NORM samples. In the findings obtained, it was determined that intense regenerative processes took place in both WB and WS muscles. Intriguingly, the highest level of VIM mRNA detected in SM muscles was associated with significantly lower content of relative protein. In conclusion, it was emphasized that the mechanisms and processes underlying VIM transcription and translation should be investigated in more depth.

MOLECULAR STUDIES ON THE FLAVOR OF CHICKEN MEAT

Advances in genetics area have significantly increased the production of fast-growing broilers. However, it has been reported that this increase has some negative effects on the flavor quality of broiler meat. It has been found fast-growing broilerst have worse meat quality, more degenerate muscle fibers, and higher plasma creatine kinase concentrations than slow-growing chickens (Velleman, 2019). This situation has raised concerns of both the welfare of animals reared in intensive breeding system and human health (Escobedo del Bosque et al., 2020) and tended consumers to poultry products obtained from free-raising systems. Thanks to the changing consumer profile, poultry products obtained from breeding systems that increase mobility with free-range areas, from organic farming or geographical indication products have become popular food sources (Fanatico, 2006; Tong et al., 2014). In this context, many strategies have been developed to increase the meat quality and to reduce muscle defects of fast-growing chickens and to increase the quality/yield capacity of local chicken breeds which have unique flavor and meat quality characteristics.

Meat quality is one of the most comprehensively evaluated economic characteristics in poultry production (Huang et al., 2022a). In recent years, consumers' search for more delicious chicken products has caused breeders to focus not only on meat yield, but also on meat quality and flavor. Most of the studies on improving meat quality have been carried out to investigate the production and storage processes of some biochemical compounds in muscle tissues (Yan et al., 2018; Kubota et al., 2019). Studies have revealed that the umami taste in meat is closely related to the content of amino acids (especially glutamic acid), inosine monophosphate (IMP)

and intramuscular fat (IMF), and IMP content can be considered as an index of flavor in meat (Huang et al., 2022a).

Meat flavor, which is considered to be the most important factor affecting meat flavor, is characterized by umami taste and aroma properties. It has been reported that umami taste is more determined by IMP than sodium glutamate (40-fold) (Huang et al., 2022b). For this reason, IMP content is expressed as an important indicator of meat quality and freshness (Blonde and Spector, 2017; Gabriel et al., 2018). It has been determined that de novo biosynthesis of IMP takes place in 10 steps (Yan et al., 2018), and many genes involved in different steps in IMP biosynthesis have been identified in studies conducted to date. Among these genes, *ADSL, AMPD1, AK1, ENTPD8, ATIC* genes have been reported to play important roles directly or indirectly in IMP biosynthesis (Yan et al., 2018; Zhang et al., 2020).

Yan et al. (2018) supplemented the diet with IMP to improve the taste of chicken meat. Expressions of genes involved in IMP synthesis were investigated in leg and breast muscle tissues collected from China's native yellow feathered chickens at 52 days of age. It has been reported that the use of 0.2% IMP in the diet causes the AMPD1 gene to be expressed at the highest level in the breast muscle tissue.

It has been observed that IMP, which is regarded as a major predictor of meat palatability, plays a vital role in the development of high-quality chicken breeds. Therefore, the need of investigating the molecular processes involved in the storage of IMP in muscle tissues was emphasized. Huang et al. (2022b) identified key proteins regulating IMP storage in different muscle groups of 180-day-old Jingyuan chickens. The IMP content in the tissue samples collected from the leg and breast muscles of chickens was determined, the structures of the isolated proteins were examined and the expression levels of some genes encoding these proteins were determined by RT-qPCR analysis. According to the analysis results, it was determined that the breast muscle tissue of 180-day-old Jingyuan chickens contained more IMP compared to the leg muscle. It has been shown that the *AMPD1* gene, which has been linked to IMP storage, is higher expressed in breast muscle tissue than in leg muscle tissue. *AMPD1* protein retrieved via Western blot analysis has been reported to confirm other findings. Interaction analyzes of key proteins regulating IMP deposition in breast and leg muscles of Jingyuan chicken breed were performed by bioinformatic analyses. In the findings obtained, it was reported that the AMPD1 gene is directly related to the AK1 and ATIC genes (Figure 2). As a result, it has been stated that chicken breeds with high meat quality can be developed with studies on genes (such as *ATIC, AK1, PGM1*) that play an important role in IMP storage, such as *AMPD1*, and that more delicious meat products can be reached by regulating the IMP storage process in the muscles.

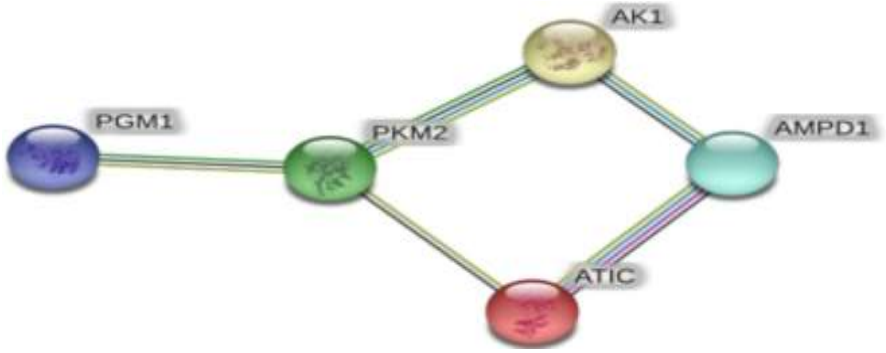

Figure 2. Interaction analysis of key proteins regulating specific accumulation of IMP in breast and leg muscles of Jingyuan chicken (Huang et al., 2022b).

Huang et al. (2022b) investigated the expression levels of IMP storage and IMP metabolism-related proteins in the muscle tissues of 180-day-old Jingyuan chickens. According to the findings, *AK1* expression was much greater in the breast muscles than in the leg muscles and was directly related to the quantity of IMP in the muscles. In conclusion, it was emphasized that the *AK1* gene directly or indirectly regulates IMP storage through purine metabolism.

According to the findings of a study carried in black boned Lueyang chickens from China, the expression of the *purH* gene (*ATIC*) in the leg muscles of free-raised chickens rose on the 60th day, and this was directly linked to the high IMP concentration in the muscle tissue (Zhang et al., 2018).

CONCLUSIONS

Commercial hybrids dominate the poultry industry now as a result of crossbreeding and intensive selection procedures used to maximize output per animal with the influence of heavy industrialization in commercial poultry production. Heterosis applied in the production of hybrid chicken breeds over the years and some feed additives and feeding managements used in the intensive system have caused the hybrids to gain weight very quickly in a very short time and thus to reach the slaughter age in a shorter time. This rapid growth has caused some defects in the economically valuable muscles (especially fillet) of chickens (such as WB, WS, SM). These defects have caused producers to discard meat that should be sold fresh or to convert it into products with lower economic value. Consumers, on the other hand, have sought for alternative tastes as a result of changing living conditions, as well as worries about the welfare of animals bred in this intense production system and human health. All of these phenomena have sparked the interest of breeders and academics. On the one hand, research have been conducted to decrease muscular deformities in fast-growing chickens; on the other hand, studies have been conducted to improve the meat flavor and quality of local chicken breeds that are distinctive to the region where they live. The processes governing muscle growth and meat quality have not yet been fully understood as a consequence of the investigations, and it has been discovered that these qualities are influenced by several genes. However, further research is needed to discover genes with large effects, disclose gene-gene interactions, and find genes and polymorphisms that may be employed as molecular markers in both slow and fast-growing broiler lines.

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EXAMINATION OF STUDIES RELATED TO COVID 19 EFFECTS ON AGRICULTURE SECTOR BY BIBLIOMETRIC ANALYSIS METHOD

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ABSTRACT

In this research, it was aimed to reveal the impacts of Covid-19 pandemic, which has started in 2020, on the agricultural sector, in a systematic and comprehensive way. To achieve this aim, scientific publications including the purpose of determining the pandemic effects were examined by bibliometric analysis method. The database consisted of scientific publications from Scopus or Web of Science. The data, which were obtained by examination of mentioned publication database were illustrated with the visual mapping technique. Therefore, the research provides an opportunity to evaluate the impact of Covid-19 on the agricultural sector from a general perspective. Visual network analysis was performed using the VOSviewer 1.6.15 package program as a research method.

Keywords: Agriculture, Bibliometric analysis, Covid-19, Visual mapping, VOSviewer

INTRODUCTION

Agriculture, which is the oldest economic activity in the history of humanity, is one of the sectors that always must be protected. Agricultural sector is carried out depending on natural conditions, it is the sector where risks and uncertainties are encountered the most. And this is the sector which human beings owe their vital existence. Therefore, there is a need for deep-rooted and protective policies in agriculture in order to meet the food, shelter and clothing needs of the rapidly increasing population of the world.

Gradual decrease in cultivable land area, precipitation regime changes, drought, global climate crisis and pandemic diseases are global problems that agriculture is facing today.

COVID-19, which started in Wuhan, China in December 2019, spread all over the world in a short period of time and was declared an epidemic by the World Health Organization (WHO), affected all societies in terms of health, education, nutrition, medicine, trade, economy, unemployment and social aspects (Ongan et al., 2020).

From the perspective of the agricultural sector, with the effects of the Covid-19 global epidemic; concerns about food access is increasing rapidly due inadequacies and disconnections on food supply chain following the decrease on incomes for working class and small businesses, demand and supply changes and uncertainties on food prices. (Torun, Kayabaşı, 2020).

The 5% decrease in real GDP at the global level due to the coronavirus epidemic, followed by unemployment, income imbalance and uncertainty will cause a decrease in the demand for agricultural products (Yavuz, 2020). On the other hand, the decrease in demand will narrow the supply, and depending on the contraction in supply and demand, negative economic consequences such as price imbalances, low income and unemployment will occur.

In the studies that focused on the effects of the pandemic, we come across the conclusion that it has caused more negative effects. During the pandemic; governments have first started to take measures on individual and public health. The expectations of decision makers in the economy has affected negatively due to Covid 19's effects of global supply chain and global economy. (Ceylan and Özkan, 2020)

It is important to conduct scientific research for creating functional policies and action plans especially on agricultural sector to prevent the world from possible effects of pandemics may spread on the future.

In this research, the effect of coronavirus on agriculture was made by examining the world and Turkish literature. It has been seen that the researches focus on supply, demand, product prices, supply chain and agricultural employment.

MATERIAL AND METHOD

In this research, it was aimed to make a bibliometric network analysis of scientific publications containing the terms "covid-19" and "agriculture" in the topics section from 2020 to the present. It has been tried to visualize the literature in a mapped way with the program. In this study, a package program called VOSviewer (1.6.15) was used to visualize the bibliometric analysis method. 781 scientific publications were found when the Web of Science database was searched on the subject. The Web of Science database was preferred over Scopus because fewer scientific publications on the subject are scanned from Scopus.

RESULTS AND DISCUSSION

The researches on Agriculture and Covid 19 subjects were obtained from the Web of Science database. Researches have been made in the "topic" field with keywords "Agriculture" and "Covid 19" from the year "2020" to "present". Total of 781 scientific publications were obtained from this search. 75.27% of these publications are review articles, 10.63% are research articles, 6.21% are early access, 4.06% are editorial content, 2.75% are papers, 0.60% are letters and 0.24% are book chapters and also 0.24% are in the editing phase.

The distribution of the mentioned scientific publications by years from 2020 to the present is given in Table 1. As it seen form the Table 1, the scientific publications on Covid 19 and Agriculture subjects continue to increase. Although only the first half of 2022 is over, the number of publications is about this particular subjects are 182 and more publications expected to be released until the end of the year.

Table 1. The Distribution of Scientific Publications on Covid 19 and Agriculture by Years

Year	2020	2021	2022
Number of Publications	180	420	182

The geoFigureical distribution of researchers contributing to the Agriculture and Covid 19 literature is given in Figure 1. There are 121 countries contributing to the literature and the first 15 countries are shown in the figure. America ranks first in the number of publications with the percentage of 14.04% and it is followed by India and People's Republic of China with 8.18% and 5.02%, respectively. Turkey takes 15th place among 121 countries.

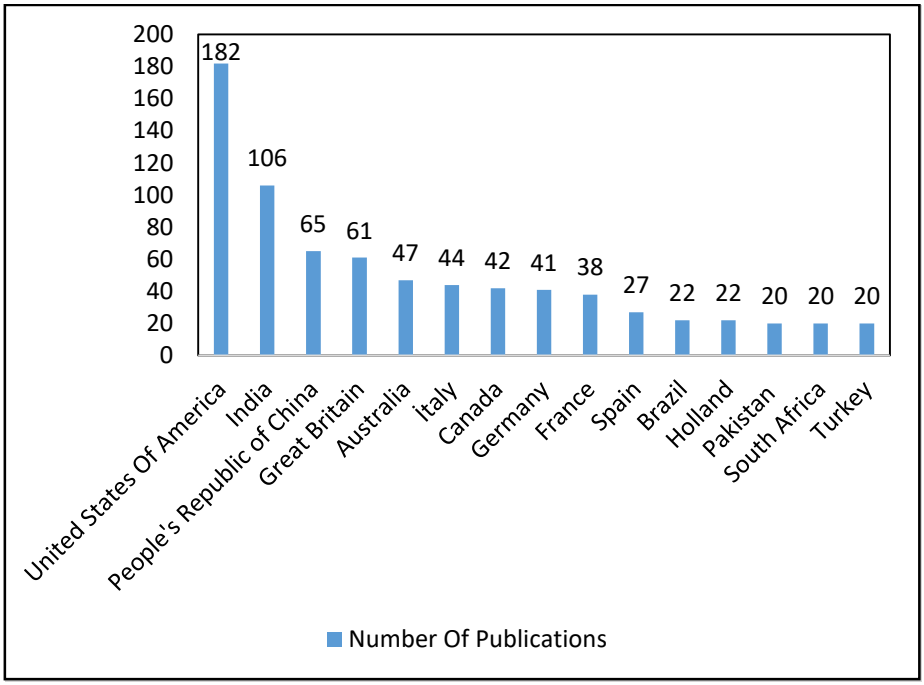

Figure 1. The Distribution of Scientific Publications on Covid 19 and Agriculture by Countries

The colored visual network analysis created with the VOSviewer program is examined in Figure 2. In this figure numbers of scientific publications according to the countries has shown with colored circles. The circle size is determined by the number of publications in the specified country. The larger the circles refers more publications. America, India and the People's Republic of China are important countries in terms of the number of publications by looking at the size of the circle. The same circle colors indicate whether the studies conducted in the countries refer to each other, and the lines between the circles indicate that the countries are related to each other in terms of scientific publications (Şenbabaoğlu and Parıltı, 2019).

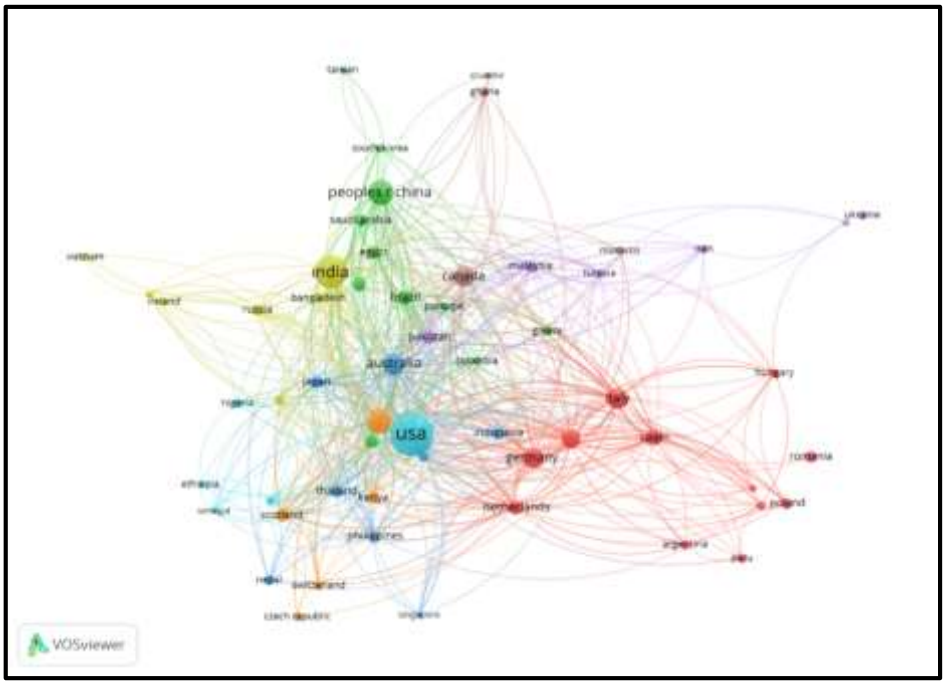

Figure 2. Bibliometric Network Analysis of Scientific Publications on Covid 19 and Agriculture by Countries

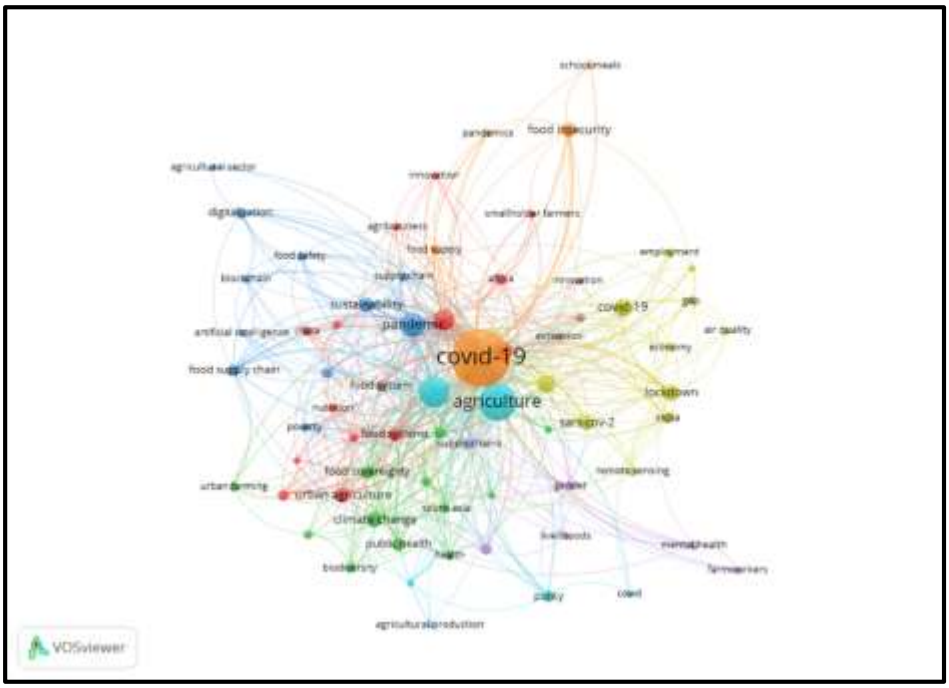

Figure 3. Bibliometric Network Analysis of Keywords Used in Scientific Publications on Covid 19 and Agriculture

The keywords used in the scientific publications analyzed in Figure 3. Cluster sizes shows the frequent usage of keywords, cluster colors shows that they are used together with keywords, and the lines between the clusters shows that they are in a relationship with each other.

According to the results of bibliometric network analysis; food security, pandemic, resilience, coronavirus, climate change, sustainability, quarantine, food industry, food supply chain, food sovereignty are the frequently used keywords.

CONCLUSIONS

In this study, it is aimed to examine the scientific researches on the effects of the Covid-19 pandemic, on the agricultural sector, using the bibliometric research method.

When the national and international literature was examined, 781 studies were found to be investigating the effects of the pandemic on the agricultural sector in 2020 to present. It was seen that 75.0% of the studies are composed of reviews, while 2.75% were composed of presentations. This situation is estimated to be sourced from the fact that the topic is quite new and the effect of the pandemic still continues all around the world. The concurrent statistical data for agricultural sectors may be announced in the early months of the following year. Therefore, studies related to assessment Covid 19 impacts on agricultural sector are expected to increase in the future since more data will be available in time.

According to FAO data, USA, China and India are among the top five countries in world total agricultural production. Also, the total population of these three countries is more than 35% of the total world population. China is the starting point of the pandemic and is also the most populated country of the world. Considering the geoFigureical locations of these countries, it can be said that they are the countries that making the most effort to ensure food supply security. Therefore, 27.4% of 781 scientific publications were made by researchers in these three countries. Another noteworthy point is that the percentage of countries contribution to researches has developed a proportional correlation with their production. For example, while Turkey ranks 11th in world total wheat production, it is ranked 15th in total publications on the topic.

Covid-19 has taken the world by storm with an unprecedented speed. It has effected growth rates of countries. Many sectors closed or stopped working for periods of time due to quarantine processes. During pandemic, governments taken economic measures in general and agriculture sector has been one of the most critical sectors following the health care sector. Access to food, one of the most basic needs of humanity, has become more important than ever. Thus food security, sustainability, food industry, food supply chain, food sovereignty are the most frequently used keywords on the studies related to Covid 19 and agriculture.

Researchers from countries with similar geoFigureical and economic conditions tended to collaborate more frequently with each other. This situation is clearly revealed with the proximity of the countries which are marked with red in Figure 2. Netherlands, Germany, Spain, Italy, Romania and Hungary are all EU members and are neighboring countries to each other. In future studies, effects of pandemic on agriculture and aspects of the studies on this topic will be investigated in more detail.

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EXPERIMENTAL DESIGNS FOR TRIPLOID INDUCTION METHODS IN NILE TILAPIA (*Oreochromis niloticus*)

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ABSTRACT

Polyploidy is not just commonly used in plant agriculture; it could also be used less commonly in fish culture. It has been shown that some cultured fish species such as trout, salmon, and tilapia have adaptation ability to survive as triploid. Triploid individuals are desirable due to some advantages such as fast-growing and minimizing problems originated from sextual maturity. Moreover, triploid tilapia individuals prevent overpopulation in the culture of tilapia which could be occurred owing to inherently prolific ability of diploid tilapia. Some methods such as high pressure, thermal shocks, electric shock, chemicals, and different pH treatments are applied to a fertilized egg at a certain period after fertilization to obtain triploid individuals. These methods should also include preservation of gametes from collection to use for fertilization. Even though these methods have been applied in many studies, experimental designs of them are not described comprehensively. Since success in triploid individual rate is highly correlated to appropriate application of these methods, detailed definitions of them will be helpful for standardization of obtaining high triploid rates. In this study, experimental setups and implementation conditions of these methods were depicted and discussed.

Keywords: Tilapia, Triploid, Triploidy induction methods

INTRODUCTION

Polyploidy referring to having more than a pair of sets of homologous chromosomes in a cell is a phenomenon observed in plants and some specific groups of fish and amphibians (Woodhouse et al., 2009). Polyploid possessing three sets of chromosomes is named as triploid. Triploid individuals (3n) have some differences from diploid (i.e. normal) individuals (2n). Some of these differences could be used as advantages in culture condition. For instance, triploid plants have larger biomass and stronger stress resistance when compared to their diploid counterparts. Moreover, triploidy condition has effects on sterility of organisms (Wang et al., 2016).

Obtaining triploids in fish is mainly owing to their oogenesis progress. Eggs completed their second meiotic division immediately after fertilization (Tyler and Sumpter, 1996). A mature egg which is ready for fertilization has two maternal chromosome sets. When a spermatozoon enters an egg, newly fertilized egg will have three (two maternal and one paternal) chromosome sets. Normally, one of maternal chromosome sets is ejected from egg as the completion of the second meiotic division. If the second meiotic division is inhibited by a triploid method, the fertilized egg keeps three chromosome sets, and maintains its triploid condition (Johnstone, 1992). Since triploid condition does not interfere in mitotic division, growth of triploid embryo continues. Triploid individuals have larger cells, comparing to haploid individuals. On the other hand, meiotic division could not be carried out, consequently triploid individuals could not produce functional gametes during their lifespan. Especially

triploid females become sterile. Such properties could make triploid individuals desirable for aquaculture (Thorgaard and Gall, 1979; Tiwary et al., 2004).

Triploidization methods are applied to a fertilized egg at a certain period after fertilization. Among these methods, using high pressure is preferred for the commercial scale production of triploid individuals (Piferrer et al., 2009). Other methods could be used under basic laboratory conditions readily. These methods should also include preservation and transportation of gametes and fertilized eggs from collection to use for fertilization. Even though these methods have been applied in many studies, experimental designs of them are not described comprehensively. Since success in triploid individual rate is highly correlated to appropriate application of these methods in terms of duration and timing of initiation of applications, detailed definitions of them will be helpful for standardization of obtaining high triploid rates. In this study, experimental setups and implementation conditions of the methods used under basic laboratory conditions were depicted and discussed.

MATERIAL AND METHOD

In terms of the experimental setups, the triploid production methods could be classified as high hydrostatic pressure, thermal shock, electric shock, and chemical treatments (Figure 1). In this study, experimental setup for high hydrostatic pressure is not described. In the experimental setups and implementation conditions for other methods, a water bath (NB 20 Nüve, Ankara, Turkey), a power supply, (Thermo Scientific, EC 300XL, USA) a single cell gel electrophoresis assay tank (CSL-COM20, Cleaver Scientific, UK), and an oven (FN 500, Nüve, Turkey) were employed. Oxygen supply in the experiments was provided by an aquarium air pump (Air pump 1000, Eheim, Germany). Eggs from Nile tilapia (*Oreochromis niloticus*) fixed with % 70 ethanol were used for demonstrations. Eggs were placed to bottom part of an egg tumbler (Figure 2). This egg tumbler is also suitable for incubation of fertilized tilapia eggs. During the experiments, eggs were transferred with a syringe with plastic hose and plastic Pasteur pipettes when necessary (Figure 2). The diameter of plastic hose should be at least 4 mm while tips of Pasteur pipettes were cut to allow passing eggs through.

Figure 1. The classification of triploid production methods in terms of experimental setups

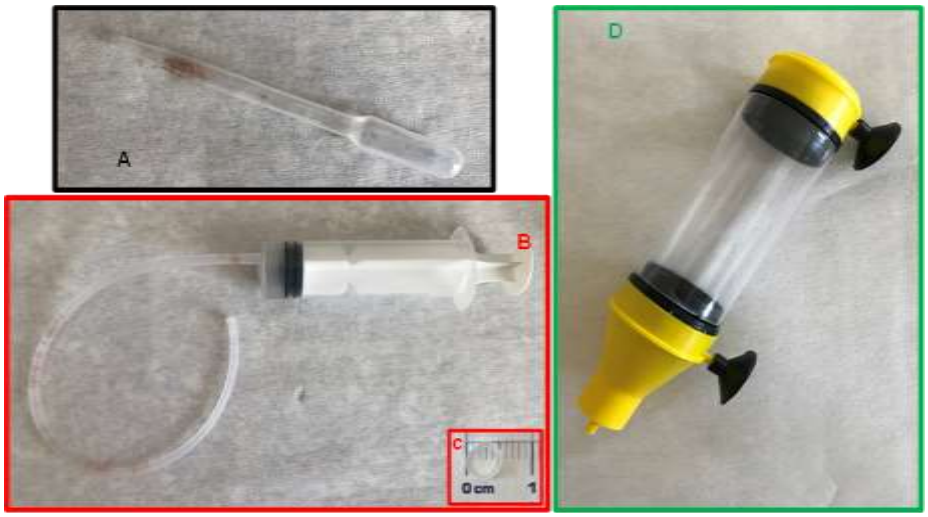

Figure 2. Plastic Pasteur pipettes (A), syringe with plastic hose (B, C), and egg tumbler (D) used for transportation and placing of eggs during the experiments

RESULTS AND DISCUSSION

Triploidy in fish provides important advantages for controlling of overpopulation and improving growth in juveniles and mature fish. Triploid fish consume their energy to somatic growth rather than sexual maturation (Tiwary et al., 2004). Therefore, these individuals are desirable in aquaculture. Triploidization procedures have been applied to different farmed fish species, particularly salmonid and tilapia species (Wootton and Smith, 2015). The general approach of triploidization methods is the inhibition of the second meiotic division of (fertilized) egg at a defined time after fertilization with an application of a shock (Pradeep et al., 2013; 2014).

One of the commonly used triploidization methods is thermal shock. Thermal shock is divided as cold and heat shocks (Tiwary et al., 2004). Heat shock is more commonly used than cold shock due to obtaining higher triploid induction ratios. An optimum degree of temperature to induce triploidy depends on living condition of fish species. For instance, Gamal et al. (1999) suggested that 40-41 °C of exposure for 5 minutes starting at 4-6 minutes after fertilization induced triploidy at different ratios for Nile tilapia. After this application, fertilized eggs transferred to egg tumbler kept in an in aquarium at 28 °C until hatching. The images of experimental system for the heat shock method were presented Figure 3. Two different ways of aeration were also shown (Figure 4).

Another method could be used for triploid production is electric shock. The electric shock is less commonly used when compared to the other methods. In other words, the electric shock (named also as electroporation) is a newer method than the other methods mentioned in this study. Hassan et al. (2018) have shown that triploidization in red hybrid tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) was induced if fertilized eggs 4 minutes after fertilization kept in an electric field of 12 V provided by batteries in an aquarium tank for 10 minutes. On the other hand, in this study, it has been suggested that an experimental system with a single cell gel electrophoresis assay tank could be useful for the electric shock method. The electroporation process could be controlled by a power supply (Figure 5). The egg tumbler loaded by eggs could be placed to the tank via plastic ventouses. Also, oxygen supply in the electrophoresis tank was provided by aquarium air pump with a small airstone (Figure 6). This experimental system needed to be tested.

Figure 3. General (A) and operating state (B) images of experimental system for the heat shock method

Figure 4. Direct (A) and airstone (B) aeration supplies in experimental system for the heat shock method

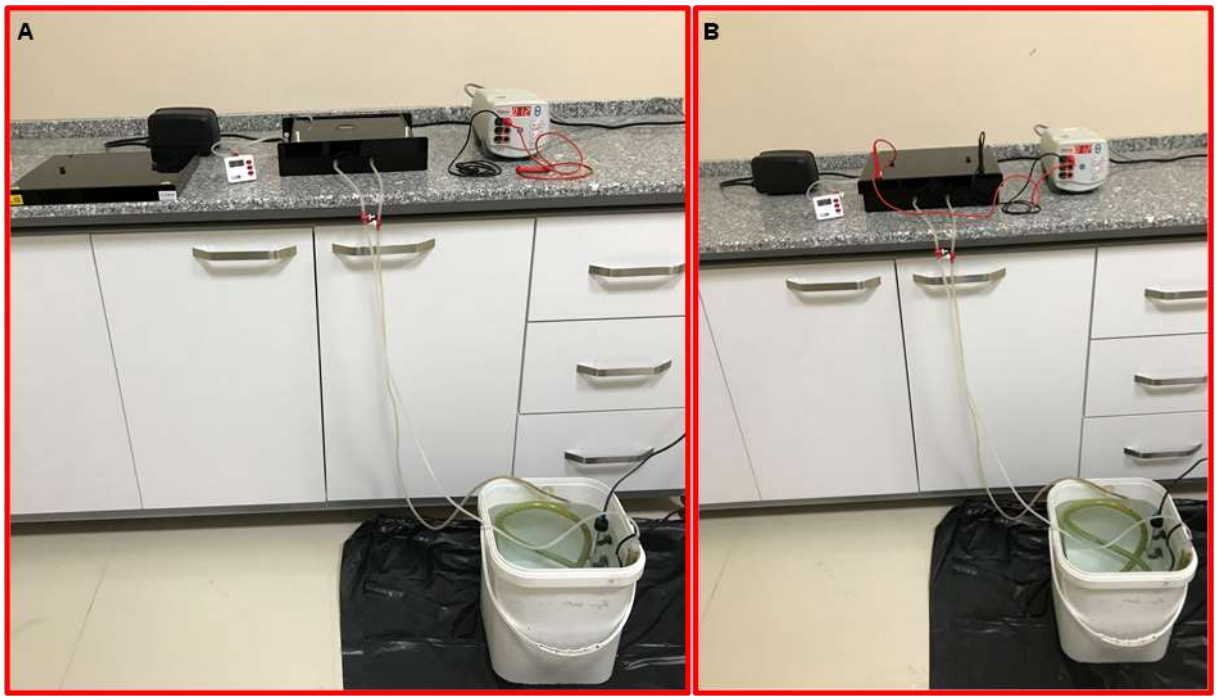

Figure 5. General (A) and operating state (B) images of experimental system for the electric shock method

Figure 6. Placing egg tumbler to the electrophoresis tank (A) with aeration supply and recirculation system (B) to keep the tank temperature stable

Triploidization can also be carried out using with some chemicals such as cytochalasin A, B, D, and colchicine, additionally high pH levels and calcium could initiate triploidization. It should be noted also that some anaesthetic gases like nitrous oxide could be used for initiation of triploidization (Johnstone et al., 1989). Research articles on the chemical treatments for triploidization of Nile tilapia are limited. In salmonid species, it has been shown that 10 µg/ml of cytochalasin B and 0.01% of colchicine solutions could be useful to induce triploidy (Refstie et al., 1977; Smith et al., 1979). Timing and duration of treatments of these kinds of chemicals should be tested for each species. After a certain time of fertilization, toxic effects of these chemical could cause very high death rates (Refstie et al., 1977). Moreover, Ueda et al., (1988)

reported that fertilized eggs of rainbow trout 10 minutes after fertilization were immersed in an ionic solution (780 mg NaCl, 28 mg KCl, and 1.11 mg $CaCl_2$ dissolved in 100 ml distilled water, pH 10), and kept for 20 minutes to obtain triploid embryos. In this study, the suggested experimental system for the chemical treatments method was presented in Figure 7. Different sizes of beakers were placed to an oven, and aeration support was provided with aquarium air pump and different sizes of airstone (Figure 8). Temperature of water with relevant substances in beakers was stabilized by oven temperature and this temperature should be checked before the treatment. Different sizes of beakers allow different volume, even in small quantities, of water.

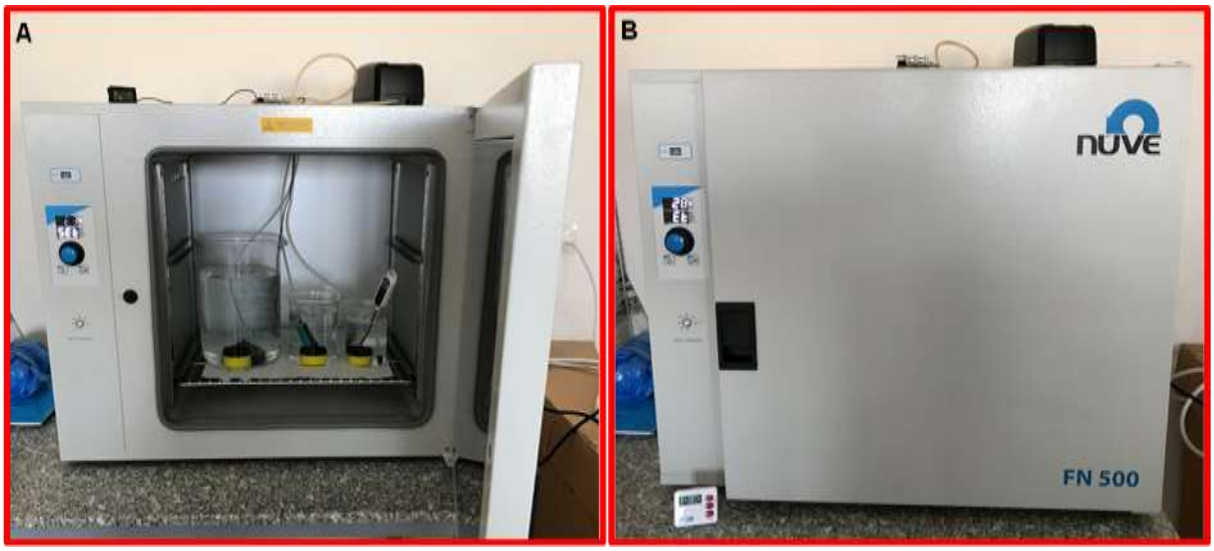

Figure 7. General (A) and operating state (B) images of experimental system for the chemical treatments method

Figure 8. Placing egg tumbler to different sizes of beakers in the oven (A) and aeration supply in the beakers (B).

CONCLUSIONS

This study attempts to visualize the experimental systems for the triploidization methods used under basic laboratory conditions readily. Thus, it was aimed to reveal positive and negative properties of the experimental setups and implementation conditions, making them to be discussed more clearly. Based on the experience in this study, the followings should be paid attention when preparing these kinds of experimental systems:

-All shocks should be kept under control exactly. And the experimental systems are set before the application to stabilize oxygen and temperature. Shocks which could interfere with other shocks should be prevented. For instance, when the electric shock is applying to fertilized eggs, temperature of environment should be under control.

-Fertilized eggs and gametes are susceptible to light. Dark environmental conditions should be provided during the application.

-To ensure an exact time of fertilization, gametes should be kept without losing their quality. In that point, sperm samples could be prepared before eggs due to preservation of spermatozoa are more efficient than eggs (İnanan, 2020).

ACKNOWLEDGEMENTS

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EFFECT OF HAWTHORN (*Crataegus* spp) ROOTSTOCK ON FRUIT QUALITY IN LOQUAT (*Eriobotrya japonica* Lindl.)

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ABSTRACT

The aim of the study is to determine the effects of hawthorn rootstocks on fruit quality of the Hafif Çukurgöbek(HÇG) loquat cultivar. In the study, loquat seedling was also used as a control rootstock. Fruit pomological analysis and yield characteristics of the cultivar/rootstock combinations in the study were determined. In order to determine the fruit quality, 50 fruits were randomly picked from each cultivar/rootstock combination, and physical measurements and chemical analyzes were carried out with five replicates. Hawthorn rootstock provided two days earliness in fruit harvest (May 17) compared to loquat seedling rootstock. Fruit weight and fruit dimensions were 16.89 g, 32.13 mm and 34.62 mm, respectively, in hawthorn rootstock. They were determined as 18.32 g, 31.72 mm and 33.67 mm in loquat seedling rootstock. Seed number and seed weight were lower in hawthorn rootstock (3.40 and 4.40 g, respectively) than those in loquat seedling rootstock (4.02 and 5.25 g, respectively). In terms of flesh/seed ratio, a higher value was obtained from hawthorn rootstock (2.84) than from loquat seedling rootstock (2.50). The total soluble solid value was determined as 9.40% in fruits grown on hawthorn rootstock and 11.30% in fruits grown on loquat seedling rootstock. Titratable acidity was calculated as 0.34% in hawthorn rootstock and 0.44% in loquat rootstock.

Keywords: Dwarf rootstock, hawthorn, high density, fruit quality, yield

INTRODUCTION

Hawthorn plants are highly drought resistant and are recommended for water-saving horticultural practices (Nas et al., 2012). Hawthorn, which is more durable than quince, can be used as rootstock for medlar, quince and pear (Phipps et al. 2003). As a matter of fact, hawthorn seedlings are used as dwarf rootstock and resistant to environmental stress in some regions of Iran (Lombard and Westwood, 1987; Qurunfleh, 1993; Hummer and Janick, 2009). Hawthorn, which grows naturally in different regions of Turkey, is transformed into pears and sometimes apples by turning grafts. Hawthorn is a good rootstock for pears to be grown in shallow, dry, sandy and stony soils. Pears grafted on hawthorn rootstock do not grow much and remain stunted (Özbek 1978).

On the other hand, although hawthorn is not very common, it is reported to be used as rootstock for quince (Ghasemi et al. 2013; Gharaghani et al. 2016; Valipour et al., 2018; Tataria

et al., 2020). It is of great importance that hawthorn is used as rootstock in loquat as well as other pome fruit species in terms of making cultivation sustainable in arid climate conditions due to global warming, due to its resistance to arid conditions and stunted growth.

Although it is stated in some sources (Demir, 1987; Polat, 1995; Polat and Kaska, 1992a) that hawthorn can be used as rootstock for loquats, no research has been found examining the effects of this species on fruit quality in loquats. For this reason, it is of great importance to investigate the possibilities of using hawthorn as rootstock in loquats and to determine the effects of this rootstock on fruit quality characteristics of grafted loquat varieties. As a matter of fact, in this study, the effects of HÇG loquat variety grafted on hawthorn rootstock on pomological characteristics were determined.

MATERIAL AND METHOD

The experiment was conducted in Hatay, Turkey, located at 36º52′ N latitude, 36º12′E longitude, with an elevation of 80 m. Hatay is located on the Mediterranean coast of Turkey and is completely under the influence of a Mediterranean climate. Long-term average temperature for Hatay is 18.3^0C and total annual precipitation is 1168 mm. In the study, a loquat orchard established with a 2-year-old HÇG loquat cultivar grafted on loquat and hawthorn seedling rootstocks was used. The spacing of plants with hawthorn rootstock was 1.0 m x 0.5 m, while the spacing of plants with loquat seedling rootstock was 1x1 m. Trial material plants were trained with the Goble system and irrigated with a drip irrigation system. In addition, annual technical and cultural maintenance operations were carried out regularly.

To determine fruit quality, 50 fruits (10 fruits from each replicate) were randomly sampled from each cultivar/rootstock combination and physical and chemical measurements and analyzes were carried out including fruit weight (g), fruit dimensions (mm), seed weight (g), number of seeds (pieces), flesh seed^{-1} ratio (%), total soluble solids (TSS) (%), titratable total acidity (TA) (%), pH.

The experiment was arranged according to a completely randomized design with 5 replications and 6 plants were used in each replicate. The variance analyzes of the data obtained from the experiment were performed in the SPSS computer package program, according to the "Completely Randomised Design" (Steel and Torrie, 1980). The differences between the rootstock means were compared with the LSD test.

RESULTS AND DISCUSSION

Fruit Quality

The fruit quality characteristics of loquat cv HÇG on hawthorn and loquat seedling rootstocks are given in Table 1 and Figure 1.

Table 1. The effects of different rootstocks on fruit quality characteristics of loquat cv. HÇG.

Rootstock	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Seed weight (g)	Seed number per fruit	Flesh/seed ratio
Hawthorn	16.89	32.13	34.62	4.40	3.40	2.84
Loquat seedling	18.32	31.72	33.67	5.25	4.02	2.50
$LSD_{\%5}$	NS[x]	NS	NS	NS	NS	NS

[x]:*Not significant*

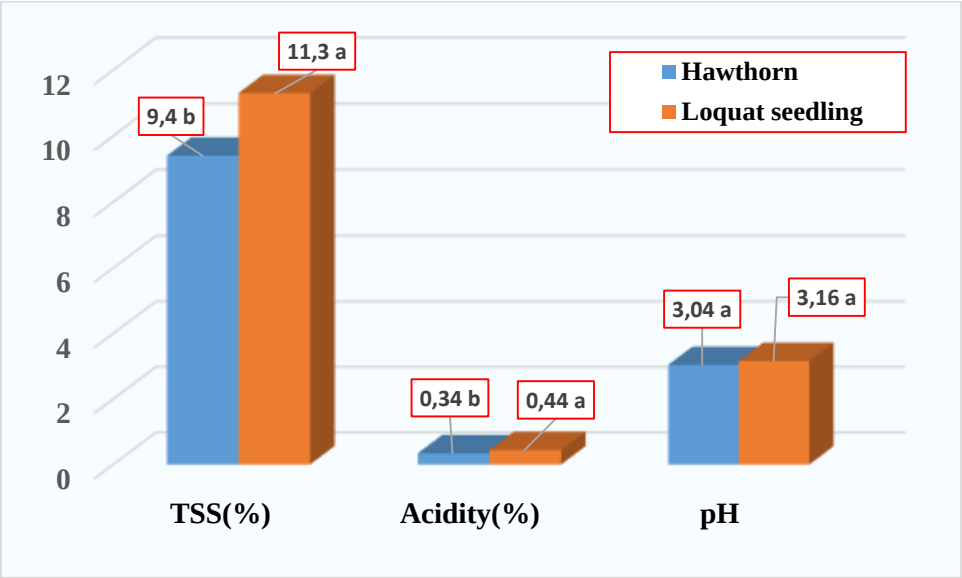

Figure 1. The effects of rootstocks on TSS, acidity and pH properties of HÇG loquat cultivar.

Although fruit weight value (16.89 g) of plants grafted on hawthorn rootstock was slightly lower than the relevant value of those grown on loquat seedling rootstock (18.32 g), fruit sizes were higher. In addition, while the number of seeds and seed weight were lower in hawthorn rootstock (3.40 and 4.40 g, respectively) than those in loquat seedling rootstock (4.02 and 5.25 g, respectively), in terms of fruit flesh/seed ratio, a higher value was obtained from hawthorn rootstock (2.84) than from seedling rootstock (2.50). All three characteristics are very important in terms of fruit quality in loquat cultivation and affect consumer preferences. In loquat fruits, it is desirable that the number and size of seeds be low and the fruit flesh/seed ratio should be high. The findings of this study show that the effect of hawthorn rootstock on the specified fruit quality characteristics is positive in terms of consumer preferences. However, since these findings are the first data on hawthorn rootstock, it is thought that it would not be correct to generalize them at this stage.

In our study, while total soluble solid content was determined as 11.30% in fruits grown on loquat seedling rootstock and 9.40% in fruits grown on hawthorn rootstock, titratable acidity value was calculated as 0.34% in hawthorn rootstock and 0.44% in loquat rootstock. Our findings are consistent with the findings of previous studies.

In the literature, it was not possible to compare our data, since published sources on the use of hawthorn rootstock in loquat cultivation could not be reached. On the other hand, a research result was reached in which hawthorn was used as rootstock for quince. A study by Tartari et al. (2020) was carried out in Iran, and the effect of four quince rootstocks including BA29, A, B, and C, along with quince and hawthorn seedlings, on some qualitative and quantitative traits of 'Isfahan' quince cultivar was investigated during 2013–2017. Characteristics of the fruit were affected by rootstock, so that the hawthorn, BA29, and QA rootstocks induced higher fruit weight, total soluble solid/total acidity, and fruit firmness to the scion. According to the results, for the establishment of 'Isfahan' quince orchard, three rootstocks including the hawthorn (low growth), QA (medium growth), and BA29 (relatively vigorous) are recommended.

CONCLUSION

Although some researchers have stated that hawthorn can be used as rootstock for loquats, there is no data on the use of hawthorn rootstock both in Turkey and in other countries. In this study, the effects of HÇG grafted on hawthorn rootstock on fruit quality were studied for the first time. For this purpose, hawthorn rootstock was compared with loquat seedling rootstock. Considering the lack of available studies on the use of hawthorn rootstock in loquat in the literature, the findings of our study are very important and valuable as they are the first findings in terms of the use of hawthorn rootstock in loquats. When the findings of our study are evaluated in general, it is seen that hawthorn rootstock gives superior results compared to loquat rootstock. However, it is necessary to continue the studies for a while in order to obtain more precise results in such studies.

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INFLUENCE OF QUINCE ROOTSTOCKS ON VEGETATIVE GROWTH, PHENOLOGICAL CHARACTERISTICS AND FRUIT SET RATIOS IN LOQUAT (*Eriobotrya Japonica* Lindl.)

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ABSTRACT

The aim of the study is to investigate the vegetative growth and phenological characteristics of the loquat cultivar grafted on some quince rootstocks. In the experiment, 'Hafif Çukurgöbek (HÇG)' loquat cultivar grafted on three Quince rootstocks (BA 29, Quince A = MA, Quince C = MC) was used. The experiment was laid out in a completely randomized design with 5 replications and 6 plants were used in each replicate. In the study, vegetative growth parameters such as annual shoot length, trunk diameter of stock and scion and also structure canopy, phenological observations such as the flowering periods (beginning of flowering, first flowering, full bloom, end of flowering and fruit set dates), and fruit set ratios (percent blossom ratios, % initial fruit set, and % final fruit set) of the cultivar/rootstock combinations were determined. Quince-C rootstock gave higher values in terms of annual shoot length, scion and rootstock diameter compared to Quince-A and BA-29 rootstocks. The differences between the rootstocks in terms of vegetative parameters were found to be statistically significant at 1% level. In terms of first flowering, fruit set and fruit ripening, BA-29 rootstock was found earlier than the other two rootstocks. According to the two-year average, the flowering rate was found the highest in BA-29 rootstock, the lowest in MA rootstock. MA rootstock yielded the lowest value in the ratio of fruit harvested, while MC and BA-29 gave close values to each other. The data shows that BA-29 and MC give better results than the MA rootstock.

Keywords: Loquat, quince rootstocks, flowering, fruit set

INTRODUCTION

Some researchers state that when quince is used as rootstock for loquats, the trees show a dwarf growth, fruiting early and ripening earlier, the fruits are larger and of higher quality (Demir, 1987; Polat and Kaşka, 1992a; Polat 1995). In some studies, it has been determined that quince rootstock provides 20-25% dwarfing in loquat varieties compared to loquat seedling rootstock (Polat and Kaşka, 1992a,b; Polat, 1995). Polat et al. (2004) reported 3 to 4 times higher yield from high density planting compared with using standard planting distances. They also stated that it would be appropriate to evaluate the effect of quince rootstocks, especially BA-29 rootstock, in high density planting trials. There are no study on effects of quince rootstocks on the flower phenology and fruit set in the loquat were examined detailed. The present study evaluated the vegetative growth, phenological properties, flowering and fruit set of loquat cv. 'HCG' budded on MA, MC and BA 29 quince rootstocks. Here, the first results were presented.

MATERIAL AND METHOD

This research was carried out during 2018-2019 at experimental plot of Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University, Antakya, Hatay, Turkey. In the study, two-year-old plants of HÇG loquat cultivar budded on BA-29, MA and MC quince rootstocks were used. The experimental plants were planted at high density with planting spaces of 1.0 m x 0.5 m in January 2017. The experiment was arranged according to a completely randomized design with 5 replications and 6 plants were used in each replicate. In the study, the effects of the rootstocks on the flowering periods, the inflorescence characteristics, flowering and fruit set rates of the cultivar were evaluated. Data were obtain on date of first blossoming, full bloom, end of blossoming and harvest time. We considered the beginning of flowering as the date when 5% of the flowers were open; 70% as full bloom and 90% petal drop as the end of blossoming. To determine the flowering periods and the fruit set rate of the cultivar according to rootstocks, a branch was selected randomly from the four sides of the canopy and they were tagged accordingly. All flower buds were counted at the preblossom phase at each of these selected branches to carry out phenological observations. The flowers of each tagged branch were counted and % blossom rate was calculated by taking the proportion of the flower amount to bud amount during the observations made between the phase when blossoming starts and ends. 10 days after the end of blossoming, the number of initial fruits was found and by dividing this number by flower amount, % initial fruit set was determined. Percentage final fruit set was determined by taking the proportion of the number of fruit during the maturing period to the number of flowers. In addition, the productivity of these cultivars was calculated by taking the proportion of the number of harvested fruit to the total number of flowers.

In order to determine the vegetative growth of the experimental plants, the following parameters were measured at three-month intervals as of February 2018 during the study, but only February-May measurements are given in the article.

Annual shoot length (cm): Four shoots from each plant were measured from 4 sides of the plants. *Trunk diameter (mm):* Scion and rootstock trunk diameters (5 cm below and above of bud union) were measured in all plants with a digital caliper sensitive to 0.01 mm. *Bud union-first branching (cm)*: The distance between the bud union and the first branching on the scion trunk was measured. *First branching - longest shoot (cm)*: The distance between the first branching and the top of the longest shoot on the scion trunk was measured. *Bud union-longest shoot (cm):* The distance between the bud union and the top of the longest shoot on the scion trunk was measured.

The percent values were transformed ($\sqrt{\arcsin}$) to increase normality. The variance analysis of the data obtained from the experiment was performed according to the 'Completely Randomized Factorial Design' with SPSS statistical software. The means of significant variation sources were compared according to the "LSD Test" at the 0.01 or 0.05 level (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

In the research, the findings related to the effects of some quince rootstocks on the vegetative growth, phenological characteristics and fruit set rates of the densely planted HÇG loquat variety are examined under separate headings below.

Flowering Periods

The findings of 2018-2019 regarding the effects of quince rootstocks on the flowering properties of the HCG variety are presented in Table 1.

The first flowering and fruit set were recorded on the plants with BA-29 rootstock (11 December and 13 February, respectively), and the latest in plants with Quince-C rootstock (15 December and 17 February, respectively). Fruit ripening occurred the earliest on plants with BA-29 rootstock (May 11), followed by Quince-C (May 18) and Quince-A (May 19) (Table 1).

Table 1. The effects of quince rootstocks on the flowering properties of the HCG variety (2018-2019 average).

Rootstock	Beginning of lossoming	Date of first flowering	Full Bloom	End of blossoming	Fruit Set	Date of fruit ripening
MA	23 Nov.	14 Dec	18 Jan	31 Jan	17 Feb	19 May
MC	21 Nov.	15 Dec	10 Jan	23 Jan	17 Feb	18 May
BA-29	22 Nov.	11 Dec	11 Jan	25 Jan	13 Feb	11 May

Flowering and Fruit Set Rates

The findings regarding the effects of quince rootstocks in the experiment on flowering and fruit set rates of HÇG loquat variety are given in Table 2.

Table 2. The effects of quince rootstocks on inflorescence characteristics, flowering and fruit set rates in loquats (2019-2020 years)

Rootstock	Years	lo. of flower buds er/plant	No. of flowers per plant	Flowering rate (%)	Initial fruit se (%)	Small fruit set (%	Final set(%)	Frui
MA	2018	191,30	171,80	90,00	21,19	12,49	1,77	
	2019	470,30	359,30	76,42	10,90	5,15	3,62	
MC	2018	413,60	352,80	85,28	14,54	9,74	6,98	
	2019	861,80	816,60	94,79	10,14	5,10	4,38	
BA-29	2018	246,10	244,20	100,42	9,91	6,62	5,43	
	2019	807,70	711,80	88,18	13,44	6,86	5,07	
Average. Rootstock	MA	330,80 c [x]	265,55 c	83,21 c	16,04 a	8,82 a	2,69 b	
	MC	637,70 a	584,70 a	90,04 b	12,34 b	7,42 ab	5,68 a	
	BA-29	526,90 b	478,00 b	94,30 a	11,67 b	6,74 b	5,25 a	
Average Year	2018	283,67 b	256,27 b	91,90 a	15,21 a	9,62 a	4,72 a	
	2019	713,27 a	629,23 a	86,46 b	11,49 b	5,70 b	4,36 a	
LSD(year[a])		**	*	**	**	**	NS[y]	
LSD(rootstock[b])		**	**	**	**	**	**	
LSD(a x b)		**	**	**	**	**	NS	

[X] Different letters within columns indicate significant difference by LSD test at $P < 0.05$ or $P < 0.01$.

**: Significant at $P < 0.01$; *: Significant at $P < 0.05$; [y] NS: Not significant

Year, rootstock and year x rootstock interaction effects were found to be statistically significant in terms of all parameters examined except the final fruit set rate.
In terms of the number of flower buds and blooming flowers per plant, higher values were obtained in 2019; in terms of flowering and fruit set rates, higher values were obtained in 2018.

According to the two-year average, the highest values in terms of the number of flower buds and blooming flowers per plant were obtained from the plants grafted on MC rootstock (Table 2). These differences between rootstocks were found to be statistically significant. The highest blossoming rate (94.30%) was recorded in plants grafted on BA-29 rootstock and the lowest rate (83.21%) was in the plants grafted on the MA rootstock. While MA rootstock gave the highest values in terms of initial fruit and small fruit set ratios in the cluster, this rootstock gave the lowest value in terms of fruit harvested. These differences between rootstocks were found to be statistically significant at $P < 0.05$ level. In terms of all three parameters, BA-29 and MC gave close values (Table 2).

In the study conducted by Polat (2007) on four different loquat cultivars, the flowering rate was 92.54% and 95.93%. The initial fruit set rate was determined as 6.98% and 12.39%, and the final fruit set rate was determined as 5.66% and 11.03%. Polat (2015) determined the flowering rate as 56.69%, the small fruit set rate as 2.16% and the final fruit set rate as 1.19% in the HÇG cultivar grafted on loquat seedling rootstock. In our study, the ratios of flowering, small fruit set and final fruit set determined in both years were found to be lower than the findings of Polat (2007), but according to the findings of Polat (2015), excluding final fruit set, they were found to be significantly higher. It is expected that the findings obtained from different studies will differ, since, as Polat (2007) stated, fruit set rates of loquats vary according to variety, year and environmental conditions.

Year effect was found to be statistically significant in terms of all parameters examined. The values of 2019 were found to be higher than the values of 2018. The differences of rootstocks in terms of vegetative parameters except for the distance between the budding union and the first branching of the stem were found to be statistically significant at 1% level. The Quince-A rootstock had lower values than the other two rootstocks. In terms of the distance between the budding union and the first branching of the trunk, BA-29 gave the highest value, followed by QA, and Quince-C gave the lowest value (Table 2). Year x rootstock interaction effects were found to be statistically significant in terms of all parameters examined except the annual shoot length and rootstock diameter.

In previous studies, Polat and Kaşka (1992a) reported the shoot growth as an average of 23.4 cm in buddings on the Quince-A rootstock. Polat (1995), in the measurements made between 1993 and 1995, measured the scion diameter in the loquat (cvs. Akko-XIII and Armut Şekilli) saplings whose rootstocks were Quince – A as 18.24 mm, 30.15 mm and 36.39 mm and the sapling height as 74.3 cm, 120.2 cm and 124.4 cm, respectively. The values obtained from our study were lower than the values measured by Polat and Kaşka (1992a) and Polat (1995). It is thought that this is due to the effect of the difference in the cultivar and rootstocks of the trial material plants as well as the age difference.

Vegetative Growth

In terms of annual shoot length, scion and rootstock diameter, MC rootstock showed stronger growth and gave higher values than MA and BA-29 rootstocks (Table 2).

Table 2. The effects of quince rootstocks on annual shoot length, scion and rootstock trunk diameters and some vegetative growth parameters of loquat cv 'HÇG' (2018-2019 years).

Rootstock	Years	Annual shoot length (cm)	Rootstock diameter (mm)	Scion diameter (mm)	Bud union-first branching (cm)	First branching-longest shoot (cm)	Bud union-longest shoot (cm)
MA	2018	15,33	17,67	13,86	12,63	37,14	49,77
	2019	50,78	26,25	21,30	29,30	66,28	95,58
MC	2018	41,73	23,72	20,00	17,10	50,30	67,40
	2019	90,18	37,05	31,60	19,03	107,30	126,33
BA-29	2018	34,29	20,64	16,47	21,78	34,09	55,87
	2019	76,61	35,61	29,10	23,70	101,40	125,10
Average. Rootstock	MA	33,06 c[x]	21,96 c	17,58 c	20,97	51,71 c	72,68 c
	MC	65,96 a	30,38 a	25,80 a	18,07	78,80 a	96,87 a
	BA-29	55,45 b	28,13 b	22,79 b	22,74	67,74 b	90,49 b
Average Year	2018	30,45 b	20,68 b	16,78 b	17,17 b	40,51 b	57,68 b
	2019	72,52 a	32,97 a	27,33 a	24,01 a	91,66 a	115,67 a
LSD(year[a])		**	**	**	**	**	**
LSD(rootstock[b])		**	**	**	NS	**	**
LSD(a x b)		NS[y]	NS	*	**	**	*

[X] Different letters within columns indicate significant difference by LSD test at P < 0.05 or P < 0.01.
**: Significant at P < 0.01; *: Significant at P < 0.05; [y] NS: Not significant

CONCLUSION

Reducing vegetative growth is very important in loquat cultivation due to its very high tree size. The most effective method for this is the use of dwarfing rootstocks such as quince (*Cydonia oblonga*). In this study, the effect of Quince-A, Quince-C and BA 29 quince clone rootstocks on phenological properties, flowering and fruit set rates of the HCG variety was studied in detail for the first time. Results obtained from this research revealed that dwarf quince rootstocks such as Quince-A, Quince-C and BA-29 can be used in intensive loquat cultivation. However, it is of great benefit to continue the further work to make a definitive judgment about the possibilities of using BA-29, Quince-A and Quince-C rootstocks as new dwarfing rootstocks. Therefore, researches need to be continued, especially to determine all the characteristics regarding yield and fruit quality.

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ANTIOXIDANT ACTIVITY OF BUFFALO CHEESES

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ABSTRACT

Milk proteins are accepted as the most important source of bioactive peptides, which are defined as specific protein fragments that have a positive impact on body functions and may effect human health. These bioactive peptides are encrypted within the amino acid sequence of the protein and can be released through enzymatic hydrolysis. Lately, large number of bioactive peptides with antioxidant, antimicrobial, antihypertensive, antithrombotic, hypocholesterolemic, immunomodulatory, opioid and mineral binding activities have been identified and isolated from dairy products like milk protein hydrolysates, fermented dairy products and cheese. Among them antioxidative peptides are of special interest due to their important function in controlling the oxidative processes in the human body, and most of them are formed during cheese production. Since buffalo milk and cheese draw attention with their high antioxidant content, and are increasingly becoming the subject of research, this presentation focuses on the antioxidant activity of buffalo cheeses.

Key Words: bioactivity, antioxidant, buffalo, cheese

INTRODUCTION

Buffalo is an important farm animal with high economic value. It is more advantageous than cattle in terms of being a raw material for meat and dairy products, and has good resistance to animal diseases and easy care conditions. Buffalo milk is ranked second after cow milk, with 15% of the total worldwide milk production (FAO, 2022). It is considered to be a potential substitute for cow milk due to its nutritional benefits. Buffalo milk is richer in vitamins A and E, and draws attention with its high antioxidant content. Since the protein ratio and fat content of buffalo milk are high, a significant yield in cheese production is achieved. The higher content of κ-casein compared to cow milk favour the production of cheese, accelerating the enzymatic phase of the coagulation, shortening the coagulation time with rennet, and reducing the necessary amounts of chymosin (Islam et al., 2014). Additionally, the high calcium and phosphorus content of buffalo cheese affects the fresh cheese yield and rennet coagulation time also. The cheese is accepted as aromatic and delicious.

Lately, there has been a wide research about bioactive compounds in milk and dairy products that have beneficial physiological, nutritional and biochemical functions on human well-being (Park, 2009). The major bioactive functional compounds of milk are enzymes, lactoferrin, vitamins, growth factors and cytokines, lactose, oligosaccharides, lipids, immunoglobulins and peptides formed from proteins. For example, casomorphins are derived from α-casein and β-casein have opioid agonist activity. Isracidin and casocidin (from α_{s1}- and α_{s2}-casein respectively) have antimicrobial activities. Lactoferrin has antioxidant, antimicrobial and immunomodulatory activities. Among all of these bioactive compounds of milk, bioactive peptides are recognized as noteworthy. Bioactive peptides are defined as peptides that bind to

specific receptors on target cells, and thereby modulate physiological functions (FitzGerald and Murray, 2006). Milk proteins are accepted to be the main sources of bioactive peptides with antioxidant, antihypertensive, antithrombotic, antidiabetic, opioid, immunomodulatory, antimicrobial, mineral binding and hypocholesterolemic activities (Claire and Swaisgood, 2000; Silva and Malcata, 2005; Korhonen and Pihlanto, 2006; Korhonen, 2009).

Bioactive peptides are encrypted within the amino acid sequences of the protein structure (Clare and Swaisgood, 2000; Park, 2009). They contain 3-20 amino acid residues, and their bioactivity depends on amino acid composition and sequence. Many peptides are known to have more than one function, like hormone-like or drug-like activity (Clare and Swaisgood, 2000; FitzGerald and Murray, 2006; Korhonen, 2009). They may be released by gastrointestinal enzymes like pepsin and trypsin, or proteolytic enzymes of microorganisms, or they may be formed during food processing (Gobbetti et al., 2002; Fitzgerald et al., 2004; Silva and Malcata, 2005; FitzGerald and Murray, 2006; Korhonen and Pihlanto, 2006; Korhonen, 2009). Cheese a rich source of bioactive peptides and during cheese making and ripening stages, various bioactive peptides are released from milk proteins by the enzymes of milk origin (plasmin), rennet (chymosin and pepsin), or enzymes from microorganisms (Gobbetti et al., 2002; Ong and Shah, 2008; Hernández-Ledesma et al., 2011; Barać et al., 2016; Fialho et al., 2018). These proteolytic microorganisms are either the starter culture, or the non-starter lactic acid bacteria from milk, especially in traditional cheese making (Sahingil et al., 2014). Bioactive peptide formation in cheeses depends on the type of milk, heat treatment, starter culture, ripening time and conditions (temperature, relative humidity, packaging etc.) (Korhonen and Pihlanto, 2006; Gomez-Ruiz et al., 2007; Gagnaire et al., 2011; Sahingil et al., 2014). The concentration of bioactive peptides increases with secondary proteolysis during cheese ripening (Ryhänen et al., 2001; Mushtaq et al., 2015; Mushtaq et al., 2016). However, it has been determined that the bioactive peptides may decrease in the later stages of ripening in some researches (Smacci and Gobbetti, 2000; Gomez-Ruiz et al., 2002; Choi et al., 2012). This decrease is attributed to the breakdown of the peptides due to advanced proteolysis (Smacchi and Gobbetti, 2000; Gupta et al., 2009; Pritchard et al., 2010; Meira et al., 2012).

ANTIOXIDANT ACTIVITY

As a result of oxidative metabolism of the body, free radicals and reactive oxygen species (ROS) are formed (Pihlanto, 2006). Normally, the ROS are blocked by specific enzyme sytems (superoxide dismutase and catalase) and some compounds such as uric acid and tocopherol (Lichtenthaler et al., 2003). An antioxidant is defined as a substance that blocks or highly postpones the oxidation of easily oxidized materials, even in small amounts (MacDonald-Wicks et al., 2006). When there is an imbalance between formation of the ROSs and their elimination, they influence macromolecules like DNA, proteins and lipids and cause oxidative stress which cause health problems such as aging, inflammation, diabetes, atherosclerosis, and cancer (Suetsuna et al., 2000; Pihlanto, 2006; Gupta et al., 2009). Involving food components into the diet with antioxidant activity such as antioxidant peptides, helps to reduce oxidative stress related diseases. In addition, lipid peroxidation in foods reduces food quality, causing sensory changes like rancid flavor and shortening the shelf life. Since artificial antioxidants such as butylated hydroxyanisole has potential risks *in vivo*, natural antioxidants are preferred as food additives (Liu et al, 2005).

The antioxidant activity of is determined by the radical scavenging assays such as Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), cupric reducing antioxidant capacity (CUPRAC), 2,2-diphenyl-1-picrylhydrazil (DPPH), ferric reducing antioxidant power (FRAP), and total phenolics assay. These methods rely on the

electron transfer or hydrogen donation from the antioxidant compound to a free radical (Pihlanto, 2006). The TEAC assay is based on 2,2-azinobis(3-ethylbenzothiazollin-6-sulfonic acid, ABTS) radical reduction, and the antioxidant activity is measured as the concentration of Trolox (vitamin E analogue) (Re et al., 1999). The ORAC assay is based on the determination of the scavenging function of induced 2,2'-azobis(2-amidinopropane) dihydrochloride, against peroxy radicals (Číž et al., 2010). Fluorescein is used as a radiation probe and the reduction in this radiation gives information about the degree of degradation of fluorescein. In CUPRAC method the reduction ability of the copper (II)-neocuproin complex (Cu(II)-Nc) to copper (I)-neocuproin (Cu(I)-Nc) chelate is measured (Apak et al., 2004). In DPPH assay, the decrease in colour has been correlated to a dose response curve with a standard antioxidant as in the TEAC assay (Arnao, 2000). When the DPPH solution is mixed with an antioxidant substance, the reduced form of DPPH losses the dark violet color. Total phenolics assay with Folin-Ciocalteu reagent is based on electron transfer from phenolics and reducing compounds to molybdenum (Singleton et al., 1999). Blue complex formed is measured at a spectrometer, and results are given as gallic acid equivalents (GAE mg/L).

Some food proteins such as milk caseins have radical scavenging activities towards the oxidative agents (Cervato et al., 1999). In recent years, many bioactive peptides with antioxidant activity have been identified and isolated from milk proteins (Pihlanto, 2006). For example, a hexapeptide from pepsin treated α_{s1}-casein showed potent radical scavenging activity (Suetsuna et al., 2000). β-casein fragments have been discovered to have antioxidant activity according to DPPH assay (Pihlanto, 2006). Milk derived antioxidant peptides have 5-11 amino acids in their sequence like histidine, proline, tryptophan, or tyrosine (Pihlanto, 2006; Gupta et al., 2009; Hernández-Galán et al., 2017). It is well defined that the antioxidative properties of these peptides are based on composition, hydrophobicity, and structure (Chen et al., 1998) and molecular weight (Li et al., 2008).

Several studies have reported the antioxidant activity of bioactive peptides found in different types of cheeses; Cottage (Abadía- García et al., 2013), Cheddar (Gupta et al., 2009; Pritchard et al., 2010; Lee et al., 2016, Huma et al., 2018), Kashar (Oner and Saridag, 2018), Coalho (Silva et al., 2012), Parmigiano-Reggiano (Bottesini et al., 2013), Feta (Meira et al., 2012), Roquefort (Meira et al., 2012; Apostolidis et al., 2007), Pecorino (Meira et al., 2012), Burgos (Timón et al., 2014), White cheese (Erkaya and Sengul, 2015; Barac et al., 2016), Tulum (Ozturk and Akin, 2017), Akawi (Gandhi and Shah, 2016) and Kalari (Mushtaq et al., 2015).

ANTIOXIDANT ACTIVITY OF BUFFALO CHEESES

Despite the several studies made on different types of cheeses, a small number of research has been done on the antioxidant activity of buffalo cheeses. The activities of fresh cheese, Cheddar, Kalari, Ricotta and Mozzarella made with buffalo milk have been investigated so far.

The antioxidant activity of the water soluble peptide (WSP) extracts of fresh buffalo cheeses was evaluated by ABTS and DPPH assays (da Silva et al., 2019). The antioxidant activity increased with the concentration of WSP, 63.27 ± 0.18% in ABTS assay and 80 ± 0.15% in DPPH assay for 20 mg/mL. Moreover, the effect of basil fortification on the total phenolic content (TPC) of fresh buffalo cheeses was studied (Ribas et al., 2019). The TPC of cheeses with basil increased compared to control, with an increase in basil concentration. Also DPPH radical scavenging activity was higher.

WSP extracts of buffalo Cheddar cheeses were screened for their antioxidant potential with ABTS radical scavenging assay (Huma et al., 2018). Additionally, WSP effect on the human colon adenocarcinoma Caco-2 cell viability and production of ROS were investigated.

The antioxidant activity of buffalo Cheddar cheese increased remarkebly after 90 days till the end of the ripening period. Intracellular ROS production decreased significantly till 150th day. Moreover, WSP extracts' antioxidant activity was found to be dose-dependent, with higher activity at 15 mg/mL concentration. In another study, buffalo Cheddar cheese was evaluated for determining the effect of accelerated ripening at a higher temperature (Batool et al., 2018). Fresh and ripened buffalo Cheddars' total antioxidant capacity were higher than cow Cheddars, and increased during the 120 days of ripening period. Higher sulphur containing amino acids and vitamin E content, and catalase activity of buffalo cheeses may be the explanation of this high activity. There was a 14.39% difference in total antioxidant capacity between the ripened buffalo Cheddars, on the behalf of accelerated ripened one. Also, the DPPH radical scavenging activities of buffalo Cheddars were greater compared to cow, and higher in accelerated ripening till the 80th day. Cheddar cheeses were manufactured with black pepper, clove and cumin added buffalo milk to determine the effect of spices on the antioxidant activity (Shaukat et al., 2021). The extract of black pepper added buffalo Cheddar had the highest total antioxidant activity (6122.8 ± 205.4 µg TE/g), followed by clove (5897.5 ± 23.29 µg TE/g) and cumin (4739 ± 153.02 µg TE/g) after in vitro digestion of ripened cheeses. The antioxidative potential of buffalo Cheddars increased with spice addition due to the antioxidant activities of the spices used. The TPC of the cheeses increased after in vitro digestion, but there were no differences among the spices. After 9 months of ripening, the TPCs of clove added buffalo Cheddar (2982.1 ± 4.74 µg GAE/g) and cumin added one (15269 ± 1.16 µg GAE/g) reached maximum. Cumin added cheese extract exhibited the highest DPPH value (1525.8 ± 1.43 µmol TE/mL). These results obtained from above studies claim that extracts of buffalo Cheddars may protect cells from oxidative damage, and they may be used in food preservation as an antioxidant agent.

The antioxidant activities of water soluble extracts (WSEs) of Kalari, which is a Himalayan cheese made from buffalo milk, were investigated with DPPH and ABTS assays (Mushtaq et al., 2015). Kalari cheese WSEs had lower antioxidant activity compared to cheeses made by enzymatic coagulation. There was a significant increase in ABTS radical scavenging activity of the Kalari cheese during storage, however DPPH activity observed was not significantly changed. It was concluded that the antioxidant potential of Kalari cheese would be useful in preventing oxidative stress related diseases like atherosclerosis, diabetes and cancer. In a similar study, where Kalari cheese was made with the addition of probiotic cultures, the antioxidant activity was enhanced (Mushtaq et al., 2016). The higher activity could be attributed to the proteolytic action of probiotic bacteria, the formed peptides with strong antioxidant activity amino acids like lysine and proline (Sah et al., 2014), and the antioxidant enzymes produced by probiotic bacteria such as superoxide dismutase and catalase (Zhang and Li, 2013).

In another study, the ROS formation was measured for the investigation of antioxidant potential of buffalo Ricotta cheese against oxidative stress induced by hydrogen peroxide in IEC-6 cells (Pepe et al., 2019). The gastrointestinal digests of the Ricotta cheese decreased ROS formation in a dose-dependent way. The peptide BRP (β-lactoglobulin f(60-72)) from the digest of buffalo Ricotta cheese, had antioxidant activity (Basilicata et al., 2018) and reduced ROS formation in IEC-6 cells.

Mozzarella is a fresh pasta filata Italian cheese, made from buffalo milk. In a research, TEAC value of the buffalo Mozzarrella extract (7.86 ± 1.35 nmol/mg protein) was found to be higher than bovine cheese extract (5.88 ± 1.88 nmol/mg protein) (Balestrieri et al., 2002). Mozzarella cheese consumption increases in summer season and there may be some difficulties in reaching the fresh buffalo milk, thus freezing the milk or curd could be useful alternative. However, freezing induces changes such as oxidation of proteins and lipids or degradation of

antioxidants (Vidal-Valverde et al., 1993). This determination was supported with a few studies. In a research where the effect of using frozen milk or curd in buffalo Mozzarella cheese on the antioxidant activity was evaluated, large quantities of frozen curd usage in production lowered the antioxidant activity of Mozzarella cheese (Tripaldi et al., 2022). Additionally, the decrease in DPPH antioxidant activity of Mozzarella cheese was significant with the increase in frozen curd, with a 1.27 mmol TE/100 g difference in 50% frozen curd added samples (Rinaldi et al., 2021). The antioxidant activity of fresh curd (10.3 mmol TE/100 g) is higher than the frozen curd (9.4 mmol TE/100 g).

CONCLUSION

Although the production and consumption of buffalo cheeses are constantly increasing, there is still not much research done. Taking into account the antioxidant activities of buffalo cheeses put forward in this review, it can be assumed that buffalo cheese consumption may be beneficial in protection from oxidative stress and related health issues. However, in order to generalize and accept this assumption, more verified results of antioxidative studies should be obtained on buffalo cheeses. Therefore, further research is needed to shape around isolation and identification of these antioxidant peptides from buffalo cheeses, optimization of the cheese making and ripening stages they are released, and defining their tolerance to gastrointestinal digestion. Animal studies and human clinical trials for the confirmation of bioavailability *in vivo* should be conducted also.

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OVERVIEW OF DIFFERENT TYPES OF ARTIFICIAL NEST BOXES USING BY LESSSER KESTREL IN EUROPE

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ABSTRACT

Lesser Kestrel (*Falco naumanni*, Fleischer, 1818) is one of the smallest falcons found in Europe. In Europe, declines equivalent to 46% in each decade since 1950 have occurred and on the wintering grounds in South Africa, there have been declines equivalent to 25% in each decade since 1971. In the breeding range, problems include demolition of older buildings where the birds nested, loss of habitat through afforestation, intensification of agriculture, and urbanization, pesticide poisoning, human persecution, and interspecific competition. The most serious problem today is the critically low number of populations and isolation, which do not allow the species to recover naturally. Due to the drastic reduction of natural habitats, the placement of artificial nest boxes provides reliable nesting sites with a low risk of predation. The research show that the use artificial nest boxes of Lesser Kestrel is common practice in Europe. Countries like Bulgaria, Greece, Italy, Spain, Portugal and etc. use them for recovery as a breeding species and strengthening existing colonies. The artificial nest boxes are constructed by different materials made to be strong, reliable and to protect birds, eggs and young chicks. The surveys also, conducted that have different types of artificial nest boxes used for Lesser Kestrel. Some of them are classical wall nest box, cavity wall nest box, under-roof nest box, classical nest boxes directly on the floor and etc. which are specially designed for the Lesser Kestrel and ecology of the species. Modern architecture considers providing suitable nesting places for important species like Lesser Kestrel. All studies show that Lesser Kestrel adapts extremely successfully to artificial nest boxes and this is a major way to conserve the species as well as increase its numbers.

Keywords: Conservation, *Falco naumanni*, Rare Birds, Recovery

INTRODUCTION

Lesser Kestrel (*Falco naumanni*, Fleischer, 1818) is one of the smallest falcons found in Europe. The species nests in Southern Europe, North Africa, Asia Minor and the Middle East, Southern Russia, the Caucasus, Central Asia east to China. Most birds breeding in western Europe winter in Africa south of the Sahara, although some individuals remain in Spain (Negro et al., 1991), southern Turkey (Cade, 1982). Some of these birds may be early migrants, depending on climatic conditions and food availability (Global Raptor Information Network, 2022).

Lesser Kestrel often nests in urban areas, as they provide nesting sites and the level of threat of predation in the nest is lowland are usually surrounded by agricultural areas or open uncultivated areas providing food resources.

In Europe, declines equivalent to 46% in each decade since 1950 have occurred and on the wintering grounds in South Africa, there have been declines equivalent to 25% in each decade since 1971 (BirdLife International, 2004).

In the breeding range, problems include demolition of older buildings where the birds nested, loss of habitat through afforestation, intensification of agriculture, and urbanization, pesticide poisoning, human persecution, and interspecific competition (Biber, 1996). The principal threats in South Africa are the loss of grassland habitat to overgrazing and pesticide effects, particularly when the birds are attracted to outbreaks of locusts or crickets, which are sprayed by farmers (Pepler 2000).

The most serious problem today is the critically low number of populations and isolation, which do not allow the species to recover naturally. To preserve and ensure the sustainable existence of the recovered colony are necessary additional conservation efforts. Due to the drastic reduction of natural habitats, the placement of artificial nest boxes provides reliable nesting sites with a low risk of predation.

MATERIAL AND METHOD

Peer-reviewed literature analysis was done. We performed a Web of Science search in order to provide information about the distribution of scientific knowledge with the following keywords: Lesser Kestrel, *Falco naumanni*, artificial, nest boxes, nesting place, nesting sites, breeding, conservation, recovery. We exclusively focused on the term “artificial nest boxes” in Title and Topics fields, in order to capture only those papers that describe the placement and use of artificial nest boxes in the recovery of Lesser Kestrel in country of Europe.

The information about artificial nest boxes was obtained from organizations and project with personal contacts. Organisations that were known to, or had the potential to, coordinate, fund and support nest boxes installation, were contacted by email and asked to provide details about any projects with which they were involved. The materials and reports were sent by e-mail. A project study was also carried out which their main activities are the recovery of Lesser Kestrel and the creation of suitable nesting place.

Additional information about the countries and projects that work with the species we used from European colour-ring Birding (Fig.1).

Figure 1. The online platform European colour-ring Birding

RESULTS AND DISCUSSION

The research show that the use of artificial nest boxes for Lesser Kestrel is common practice in Europe. Countries like Bulgaria, Croatia, France, Greece, Italy, Spain, Portugal uses them for recovery as a breeding species and strengthening existing colonies.

As a result of the survey, it was found that most countries in Europe are from leading beneficiaries of the EU's LIFE programme. Among them are projects:

- Better Life for Lesser Kestrel in South-East Balkans LIFE19 NAT/BG/001017
- "LIFE- ZEPAURBAN - Management of Urban SPAs in Extremadura for the conservation of Lesser kestrel (Falco naumanni)" LIFE15 NAT/ES/001016
- "LIFE FALKON - LIFE FALKON – Fostering the breeding rAnge expansion of central-eastern Mediterranean Lesser Kestrel pOpulatioNs" LIFE17 NAT/IT/000586
- "Un falco per amico" - LIFE11 NAT/IT/068

The nest boxes are made from materials to be strong, reliable and to protect birds, eggs and young chicks. The material and the structure have to be impermeable to water to avoid flooding of the nest box, which would lead to drowning of eggs or chicks. Several small holes should be opened in the floor of the nest box to allow drainage of water when necessary. Such openings should be provided in the higher parts of all walls to allow ventilation inside the nest. It is recommended to cover the floor of the nest box with fine sand, to enable the birds form egg-laying cavities and secure mechanical protection of the eggs, since Lesser Kestrels do not build typical nests and there is a risk for the eggs to roll over the smooth flat surface of the nest bottom. The measurements of the entrance hole should be to avoid penetration of other bird species, competing with Lesser Kestrels for breeding sites. It is good to put a door on one of the walls, in order to allow the implementation of manipulations or examinations when needed. In the bottom of the nest box have to be with an extra hole and a shutter, allowing easy cleaning of the nest box after the end of every breeding season. The door for inspection and the shutter of the opening for cleaning the nest box was securely fixed and tightened, so that they cannot be opened by martens, domestic cats, rats or other predators.

The surveys also, conducted that have different types of artificial nest boxes used for Lesser Kestrel. Some of them are classical wall nest box, cavity wall nest box, under-roof nest box, classical nest boxes directly on the floor and etc. which are specially designed for the Lesser Kestrel and ecology of the species.

Figure 2. Lesser Kestrel in front of cavity wall nest box. /Photo credit: "Green Balkans – Stara Zagora" NGO/

Figure 3. Lesser Kestrel and classical wall nest box /Photo credit: "Green Balkans – Stara Zagora" NGO/

Figure 4. Lesser Kestrels and under the roof nest box. /Photo credit: "Green Balkans – Stara Zagora" NGO/

In Italy the wooden nest boxes are effective in mitigating for the loss of traditionally used nesting sites of Lesser Kestrels and that they can be used successfully when nest sites are lost due to building renovation. (Bux et al., 2008).

In France artificially increasing nest availability with the use of nest boxes in order to augment the degree of philopatry or the yearling survival, could be an effective management strategy (Prugnolle et al., 2003).

In Bulgaria about 25 pairs of the national population which is more than 60 % of the population occupied manufactured artificial nest boxes, with special design for the Lesser Kestrel (Gradev et al., 2021).

Urban bird species have an environmental, cultural, touristic, and even educational value, and play a key role for enhancing environmental awareness among city dwellers. They should not be neglected or overlooked in a modern world where urban areas increase along with the proportion of the human population inhabiting them. In the case of the Lesser Kestrel, it has been predicted that the western European population will soon depend on artificial nest sites (Catry et al. 2009).

CONCLUSIONS

The artificial nest boxes are constructed by different materials made to be strong, reliable and to protect birds, eggs and young chicks. The surveys also, conducted that have different types of artificial nest boxes used for Lesser Kestrel. Some of them are classical wall nest box, cavity wall nest box, under-roof nest box, classical nest boxes directly on the floor and etc. which are specially designed for the Lesser Kestrel and ecology of the species.Modern architecture considers providing suitable nesting places for important species like Lesser Kestrel. All studies show that Lesser Kestrel adapts extremely successfully to artificial nest boxes and this is a major way to conserve the species as well as increase its numbers.

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MOLECULAR CHARACTERISATION OF SOME PLANT-DERIVED RETROTRANSPOSONS BY IRAP-PCR METHOD IN BAFRA LAMBS AT DIFFERENT DEVELOPMENTAL STAGES

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ABSTRACT

Bafra sheep obtained by crossbreeding Chios and Karayaka breeds is one of the prolific sheep genotypes. Retrotransposons are mobile genetic elements, replicating themselves via copy-paste mechanism. It is known that 20% of the sheep genome consists of retrotransposons. In this study, the polymorphism ratios of barley-specific *Nikita* and *Sukkula*, tomato-specific *Copia like*, and soybean-specific *SIRE1* retrotransposons were examined in Bafra lambs at the age of 30th and 60th days after weaning by using IRAP (Inter-Retrotransposon Amplification Polymorphism) molecular marker method. *Nikita*'s polymorphism rates were 0-63% on the 30th day and 0-57% in the 60th day group. *Sukkula* showed 0-11% and 6-50% polymorphism ratios in 30th day and 60th day groups, respectively. The results of *Copia like* retrotransposon were determined as 0-18% for the 30th day and 0-33% for the 60th day group. Moreover, *SIRE1* showed 0-13% and 0-50% polymorphism rates in 30th day and 60th day groups, respectively. Retrotransposons belonging to plant species indicated different polymorphism rates in different developmental periods. Whether the cause of this difference is due to dietary genome interactions should be investigated by controlled feeding studies.

Keywords: Epigenetics, mobile genetic elements, nutrigenomics, polymorphism, IRAP-PCR

INTRODUCTION

The earliest archaeological evidence of sheep domestication has been found in the Taurus Mountains of Southeastern Anatolia, Turkey, and evidence suggests it took place 10,500 years ago (Her et al., 2022). The sheep (*Ovis aries*) is one of the most economically, culturally, and socially important domestic animals, bred primarily for producing meat, milk, wool, and fur (Gebreselassie et al., 2019).

Small ruminants are important components of rural life, and they play an essential role in the life of livestock breeders. Sheep production in Turkey within the livestock sector depending on the climate and geoFigurey of the country has gained an important place due to being suitable for social, cultural and economic structures. Turkey has more than 42 million sheep, mainly fat-tailed breeds and a small number of hybrid breeds with foreign genotypes. Akkaraman,

Morkaraman, Karayaka, Awassi, Malya and Bafra sheep which include about 80% of domestic sheep breeds in Turkey are primarily suitable for various difficult geoFigureical and environmental conditions (Şen et al., 2021).

Bafra sheep was obtained by crossbreeding between 25% meat-type Karayaka and 75% milk-type Chios (Güngör and Ünal, 2020). Although there are many sheep breeds throughout the country, their production has not reached the desired levels. While meat production is mainly targeted in sheep farming in Turkey, unfortunately, studies are neglected to improve milk and wool production (Kırıkçı et al., 2021).

The genome size of sheep is estimated to be 2.6 Gb. It is known that transposons constitute 20% of the sheep genome and 19.44% of them are non-LTR retrotransposons, 0.54% are LTR retrotransposons and 0.02% are DNA transposons (Mustafa, 2018). Transposable elements (TEs) are classified as Class I and II according to the transposition mechanisms. Class I elements (RNA transposons or retrotransposons) are viral RNAs that integrate into DNA using reverse transcription mechanism. Endogenous retroviruses (ERVs) consist of open reading frames (ORFs) to encode viral proteins surrounded by two long terminal repeats (LTRs). Class II elements (DNA transposons) encode a transposase which is essential for excision and insertion in cut and paste mechanism. Autonomous transposons use their own transposase for transposition while non-autonomous transposons because of lacking internal domains require another transposons for movement. Similar to LTR sequences found in retroransposons, ORF encoded the transposase is surrounded by two inverted terminal repeats (ITRs) in DNA transposons (Fueyo et al., 2022).

Nutrigenomics investigates the relationships between nutrition and gene expressions and/or gen expression regulations. The study aimed to analyse identify retrotransposons belonging to different plants (barley-specific *Nikita* and *Sukkula*, tomato-specific *Copia like* and soybean-specific *SIRE1* retrotransposons) and even evaluate the polymorphism ratios of them in Bafra sheep at two developmental stages (30^{th} and 60^{th} days after weaning) by using IRAP molecular marker method.

MATERIAL AND METHOD

Genomic DNA isolation

Genomic DNAs of eight different lambs at the age of 30^{th} and 60^{th} days after weaning were isolated by the salting out method (Miller et al., 1988). Qualitative and quantitative measurements of gDNAs were analysed with 1.5% agarose gel and a spectrophotometer (NanoDrop®, Thermo, USA), respectively.

Copia like, Nikita, SIRE1 and *Sukkula* IRAP-PCR Analyses

IRAP-PCR analysis were performed according to Kalendar and Schulman (2006). PCR reactions were performed on the T100 Thermal Cycler (BIO-RAD, USA) with a final volume of 20 µL containing 10 µL of PCR master mixture (ABT 2X PCR MasterMix), 4 µL of ultrapure sterile water, 2 µL of primer (10 mM/µL) and 4 µL of template genomic DNA (4 µM/µL). Primer sequences were F 5'GGGGCTTGGTTCGAAAGGTTT3' for *Copia like* F and 5'-

TCTGAGGCAAGACGTTCCTT3' for *Copia like* R, 5'-ACCCCTCTAGGCGACATCC-3' for *Nikita* (Leigh et al., 2003), 5'-CAGTTATGCAAGTGGGATCAGCA-3' for *SIRE1* (Chesnay et al., 2007) and 5'-GGAACGTCGGCATCGGGCTG-3' for *Sukkula* (Leigh et al., 2003). PCR conditions were as follows: 95°C for 5 min, followed by an initial denaturation at 95°C for 30 seconds at 35 cycle of denaturation, *Copia like* at 40°C, *Nikita* to 52 C°, for *SIRE1* 47°C and for *Sukkula* at 50°C annealing for 30 seconds and 72°C. for 1 minute extension. IRAP-PCR products and a molecular weight marker (Bioptic DNA Ladder Mix, C109301) were resolved in 1,5% agarose gel electrophoresis at 80 V for 60 min in 1× TAE buffer (90 mmol/L Tris, 20 mmol/L acetic acid, 1 mmol/L EDTA, pH 8.0). After running, the gels were visualised on a UV transilluminator. Homomorphic and polymorphic bands clearly observed in samples were counted to calculate polymorphism ratios via Jaccard Similarity Coefficient (Jaccard, 1908).

RESULTS AND DISCUSSION

Transposable elements could play role in sheep's evolvement, particularly in early developmental periods. In addition to the maternal effects of transposons (including preimplantation stage and extra-embryonic development during pregnancy), *PEG11* (RTL1) is an alternative effector candidate as a paternal effect. *PEG11* is a *Ty3-Gypsy* retrotransposon-like element and conserved in placental mammals. It exists on the 18th chromosome in the sheep genome. It has a gag-pol-like retroviral structure with a long ORF encoding 1,333 amino acids (Xu et al., 2015).

There are effects of transposable elements in the developmental stages of animals through nutrition. Some studies have shown changes in fur color in the offspring of agouti mice as a result of the mother's supplemental feeding including the presence of methyl donors such as betaine, colin and folic acid (Waterland and Jirtle, 2003). DNA methylation of a transposable element was found in the upper part of the agouti gene. Along with this, the relationships between nutrition and epigenetic factors has gained much attention (Pappalardo et al., 2021). In this direction, presence and also the movements of transposable element were investigated in the samples obtained on 30^{th} and 60^{th} days after weaning of Bafra lambs via IRAP moleculer marker method.

Copia like retrotransposons showed both homomorphic and polymorphic band profiles for eight biological replicates belonging to at the age of 30^{th} and 60^{th} days after weaning. Samples displayed a band profile in the range of 50-1000 bp (Figure 1).

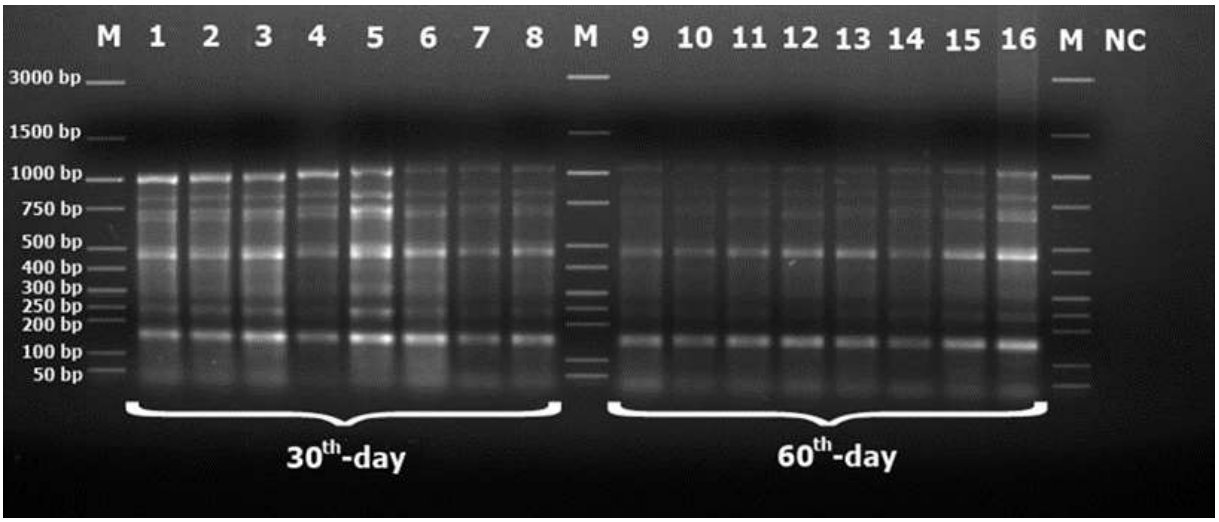

Figure 1. IRAP-PCR result for *Copia like* retrotransposon in Bafra lamb. M, marker; 1-8: 30th day and 9-16: 60th day groups of Bafra lamb, NC, negative control.

Polymorphism rates for *Copia like* retrotransposon were 0-18% for 30th day group and 0-33% for 60th day group. When compared to groups, we observed 0-33% polymorphism rates (Table 1).

Table 1. Polymorphism rates of Bafra sheep for *Copia like* retrotransposon in 30th and 60th day groups.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0															
2	0	0														
3	10	10	0													
4	0	0	10	0												
5	18	18	9	18	0											
6	18	18	9	18	0	0										
7	0	0	10	0	18	18	0									
8	0	0	10	0	18	18	0	0								
9	0	0	10	0	18	18	0	0	0							
10	0	0	10	0	18	18	0	0	0	0						
11	0	0	10	0	18	18	0	0	0	0	0					
12	20	20	27	20	33	33	20	20	20	20	20	0				
13	11	11	20	11	27	27	11	11	11	11	11	11	0			
14	11	11	20	11	27	27	11	11	11	11	11	11	0	0		
15	25	25	17	25	8	8	25	25	25	25	25	25	33	33	0	
16	18	18	9	18	17	17	18	18	18	18	18	18	27	27	8	0

Similar to *Copia like*, barley-specific retrotrotransposon, *Nikita*, indicated polymorphic and homomorphic band profiles among samples. Eight biological replicates showed a band profile of 400-1600 bp in length (Figure 2).

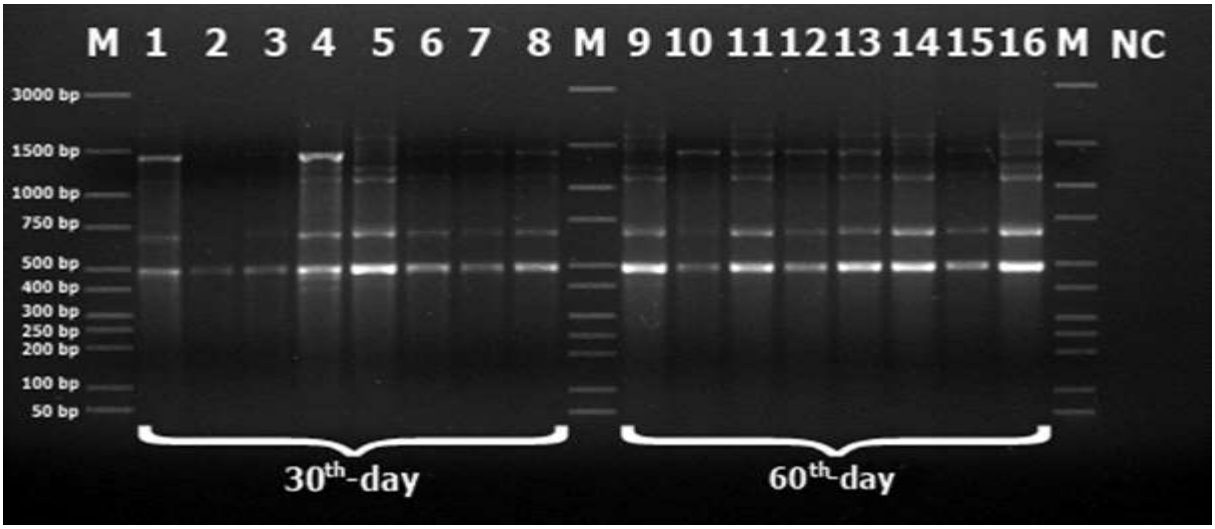

Figure 2. IRAP-PCR result for *Nikita* retrotransposon in Bafra lamb. M, marker; NC, negative control; 1-8: 30th day and 9-16: 60th day groups of Bafra lamb.

The intrapopulation polymorphism rates for the *Nikita* retrotransposon were 0-63% in 30th day group and 0-57% in 60th day group. The interpopulation polymorphism was 0-63% (Table 2).

Band profiles of soya bean-specific *SIRE1* retrotransposons ranged from 300-3000 bp (Figure 3). Polymorphism rates in the *SIRE1* retrotransposon 30th and 60th day samples were 0-13% and 0-50%, respectively. In the comparison between groups, the rate was observed as 0-38% (Table 3).

Table 2. Polymorphism rates of Bafra sheep for *Nikita* retrotransposon in 30th and 60th day groups

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0															
2	50	0														
3	33	25	0													
4	25	63	50	0												
5	38	57	43	13	0											
6	33	25	0	50	43	0										
7	33	25	0	50	43	0	0									
8	43	40	20	38	29	20	20	0								
9	38	57	43	13	0	43	43	29	0							
10	50	0	25	63	57	25	25	40	57	0						
11	50	50	33	25	14	33	33	17	14	50	0					
12	50	50	33	25	14	33	33	17	14	50	0	0				
13	50	50	33	25	14	33	33	17	14	50	0	0	0			
14	38	57	43	13	0	43	43	29	0	57	14	14	14	0		
15	38	57	43	13	0	43	43	29	0	57	14	14	14	0	0	
16	50	50	33	25	14	33	33	17	14	50	0	0	0	14	14	0

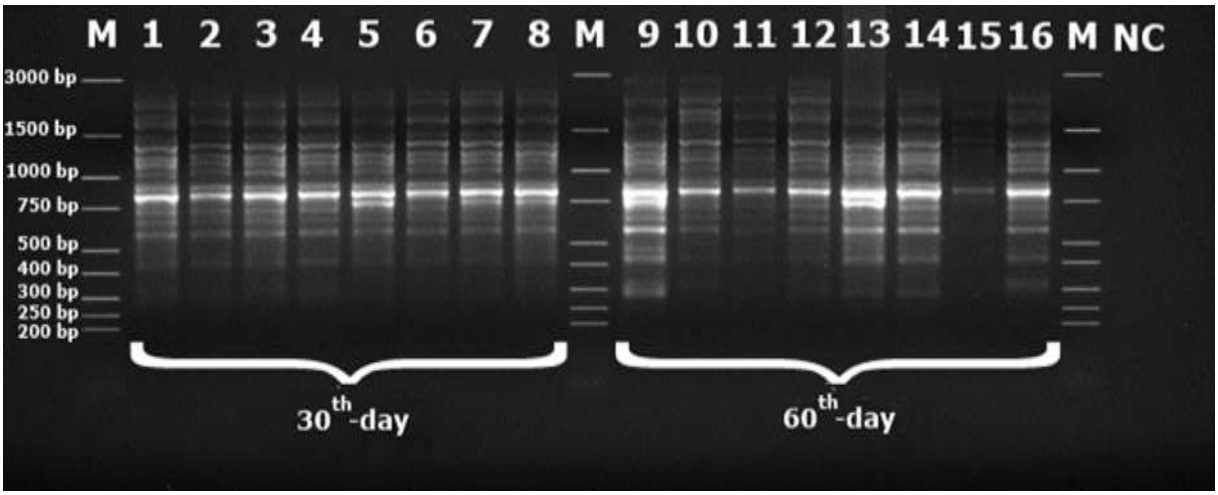

Figure 3. IRAP-PCR result for *SIRE1* retrotransposon in Bafra lamb. M, marker; NC, negative control; 1-8: 30th day and 9-16: 60th day groups of Bafra lamb.

Table 3. Polymorphism rates of Bafra sheep for *SIRE1* retrotransposon in 30th and 60th-day groups.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0															
2	0	0														
3	0	0	0													
4	0	0	0	0												
5	12	12	12	12	0											
6	12	12	12	12	13	0										
7	12	12	12	12	0	13	0									
8	12	12	12	12	0	10	0	0								
9	26	26	26	26	18	28	18	18	0							
10	17	17	17	17	28	18	28	28	32	0						
11	12	12	12	12	24	13	24	24	37	6	0					
12	12	12	12	12	24	13	24	24	37	6	0	0				
13	18	18	18	18	19	19	19	19	24	33	29	29	0			
14	17	17	17	17	18	6	18	18	22	22	18	18	24	0		
15	35	35	35	35	27	38	27	27	41	50	47	47	33	41	0	
16	12	12	12	12	13	0	13	13	28	18	13	13	19	6	38	0

For *Sukkula* retrotransposon, band profiles of 300-3000 bp were observed in the 30th and 60th day groups of Bafra lambs (Figure 4).

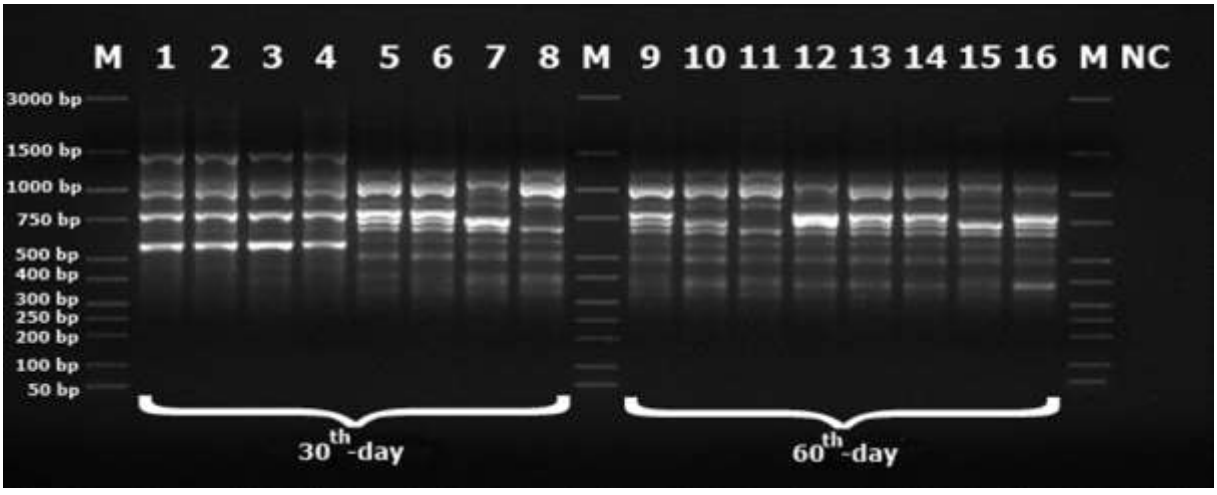

Figure 4. IRAP-PCR result for *Sukkula* retrotransposon in Bafra lamb. M, marker; NC, negative control; 1-8: 30th day and 9-16: 60th day groups of Bafra lamb.

When the interpopulation rates for *Sukkula* retrotransposon were examined, it was 0-11% in the 30th day group and 6-50% in the 60th day group in eight biological samples. The rate of polymorphism among the general populations was 0-50% (Table 4). Moreover, we observed 0-41% polymorphism rates when compared to two groups.

Table 4. Polymorphism rates of Bafra sheep for Sukkula retrotransposon in 30th and 60th-day groups.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0															
2	0	0														
3	6	6	0													
4	0	0	6	0												
5	6	6	0	6	0											
6	6	6	0	6	0	0										
7	6	6	0	6	0	0	0									
8	11	11	6	11	6	6	6	0								
9	21	21	17	21	17	17	17	11	0							
10	6	6	11	6	11	11	11	16	16	0						
11	6	6	12	6	12	12	12	17	26	11	0					
12	0	0	6	0	6	6	6	11	21	6	6	0				
13	12	12	6	12	6	6	6	12	12	17	18	12	0			
14	6	6	0	6	0	0	0	6	17	11	12	6	6	0		
15	41	41	38	41	38	38	38	41	50	44	47	41	44	38	0	
16	11	11	17	11	17	17	17	11	11	16	17	11	12	17	50	0

Transposons may be transferred via vectors such as viruses or ticks and these transitions are named as horizontal transposon transfer (HTT). For instance, it has been claimed that *BovB* (*Bovine-B*) was transferred from squamata to ruminants through HTT. It was detected that sheep and goats carry six copies of *BovB*, cattle have three copies of *BovB* and other vertebrate species do not carry any copies of *BovB* in inflammatory bottom disease (IBH) region (Pan et al., 2019).

The presence of retrotransposons in an existing plant genome can be found in other plant species. Marakli (2018) stated that *Nikita* and *Sukkula* retrotransposons are found in aniseed plant but showed only homomorphic band profiles. In a different study, these two retrotransposons were identified in *Pinus nigra* var. *pyramidata* and *Seneriana* variants. In the cultivar Pyramidata, the polymorphism rate of the *Nikita* retrotransposon was 0-56% and the

polymorphism rate of the *Sukkula* retrotransposon was 0-76%. In Seneriana cultivar, no polymorphism rate was detected in *Nikita* and *Sukkula* retrotransposons (Marakli et al., 2019).

Transposon systems have also been used for gene transfer. Bai et al. (2017) used the PiggyBac (PB) transposon system to produce green fluorescent protein (EGFP) and transgenic Cashmere goat fetal fibroblasts (GFFs) in cashmere goat (*Capra hircus*) in their study. As a result, the PB transposon system was found to be beneficial in transgenic technology.

Investigated the relationship between the motility and development of barley-specific *Nikita* and *Sukkula* retrotransposons in three different Caucasian honey bee (*A. mellifera caucasica*) colonies. Moreover, *Hordeum vulgare* L., *Triticum turgidum* L., *Bombus terrestris* and *Apis mellifera* L. transposon sequences belonging to the genus were examined and their evolutionary relationships were also investigated (Mercan et al., 2022b). One queen bee, five worker bees, and five larvae were sampled from each colony, and polymorphism rates were calculated. Polymorphism rates were 0-100% for *Nikita* and 0-67% for *Sukkula* in all samples from three colonies. In this direction, the close relationship between transposons in plant-insect genomes was confirmed. The presence of plant retrotransposons was found in the honeybee genome for the first time. In addition, the presence of barley-specific (*Nikita* and *Sukkula*) retrotransposons was detected for the first time in the Turkish native breed Gerze chicken genome, and polymorphism rates were calculated. While the *Nikita* retrotransposon polymorphism rate was found to be 0-60% while no polymorphism was observed in the *Sukkula* (Mercan et al., 2022a).

CONCLUSION

Plant-specific retrotransposons were identified and also polymorhism rates were evaluated in the samples collected on the 30th and 60th days after weaning Bafra lambs. Results could give valuable information related to nutrition-genome interaction for HTT. The presence of *Copia like*, *Nikita*, *SIRE1* and *Sukkula* retrotransposons in the Bafra sheep genome supported the ideas about the HTT, and even diet genome interactions in previous studies (Mercan et al., 2022a,b). However, more studies are needed to determine the relationships among gene expression profiles, retrotransposons and developmental variations depending on nutrition differences and developmental periods.

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THE CHARACTERIZATION AND INDUSTRIAL APPLICATIONS OF THERMOPHILIC B-XYLOSIDASE OF THE GENUS *GEOBACILLUS*

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ABSTRACT

β-xylosidase enzymes hydrolyze the β-1,4-D-xylan and xylobiose to produce the D-xylose from nonreducing end. They are classified in various glycoside hydrolase (GH) families (GH1, GH3, GH5, GH30, GH39, GH43, GH51, GH52, GH54, GH116 and GH120) according to the similarities of their sequence and the structures of catalytic clefts. The β-xylosidase enzymes synergistically act on hemicellulose, which is the second largest portion of the lignocellulosic material after cellulose, for its complete degradation with the other hemicellulase enzymes. β-xylosidases are of great interest for many industrial processes including biobleaching process of paper pulp, the increasing of animal feed digestibility, fruit juice clarification, the production of xylooligosaccharides and the production of fermentable sugar from lignocellulosic material. Since thermophilic β-xylosidase enzymes have high activity and stability at high temperatures, they are favourable for those industrial applications. Thermophilic β-xylosidase enzymes are produced especially by bacteria and fungi. Among these, the genus *Geobacillus*, which thrives in extreme area (e.g. hot springs, volcano, geothermal region) having high temperature, is an important microbial source of thermophilic hydrolase enzymes including β-xylosidase. In the present work, the production, characterization and industrial applications of thermophilic β-xylosidase enzymes from *Geobacillus* species have been examined.

Keywords: β-xylosidase, *Geobacillus*, thermophilic enzymes

INTRODUCTION

The lignocellulose structure in plant cell walls consists of cellulose, hemicellulose and lignin. Hemicellulose, the second most common polysaccharide, represents about 20-35% of lignocellulosic biomass (Saha 2003). Complete degradation of hemicellulose requires different enzymes including β-xylosidases (Madeira et al. 2017). In recent years, hemicellulases such as β-xylosidase have received much attention for their potential industrial use such as pulp biobleaching, the bioconversion of lignocellulose into fermentative products, and the improvement of digestion in animals (Zafar et al. 2022; Le and Yang 2019; Terrasan et al. 2013; Evangelista et al. 2022).

The genus *Geobacillus* is one of the most important sources of thermophilic β-xylosidase enzymes, and they can live especially in geothermal soils, waters, oil fields and hot springs with high temperatures. The ability of *Geobacillus* species to survive at high temperatures makes them attractive as a source of thermostable enzymes including β-xylosidase enzymes (Khaswal et al. 2022).

In this study, the sources, production, characterization and biotechnological application of thermophilic β-xylosidase enzymes from the *Geobacillus* genus have been considered.

PROPERTIES AND CLASSIFICATIONS OF THERMOPHILIC β-XYLOSIDASES

Lignocellulosic materials consist of cellulose, hemicellulose and lignin. Hemicellulose, the second most common polysaccharide in plants, represents about 20-35% of lignocellulosic biomass (Saha 2003). Complete hydrolysis of xylan, which is the main structure of hemicellulose, is taken place via cooperative action of xylanolytic enzymes including α-L arabinofuranosidases, α-D glucuronidases, acetyl xylan esterases, xylanase and β-xylosidase (Madeira et al. 2017). β-xylosidases (E.C. 3.2.1.37) are exotype glycosidases that can hydrolyze short-chain xylooligosaccharides into single xylose units (Manju et al. 2011). These enzymes are categorized into 11 glycoside hydrolase (GH) family from CAZy classification based on amino acid sequence and structural similarities of the active site. These classes are GH1, GH3, GH5, GH30, GH39, GH43, GH51, GH52, GH54, GH116 and GH120 (http://www.cazy.org/). Currently, it has been reported that β-xylosidases are used in a wide range of industrial processes such as pulp biobleaching, increasing of the monogastric animal feed digestiblity, biofuel production and prebiotics synthesis (Zafar et al. 2022; Le and Yang 2019; Terrasan et al. 2013; Evangelista et al. 2022; Saha 2003). Because industrial processes generally require high temperatures (minimum 60 °C) to eliminate the risk of contamination and to ensure a more fluid environment (therefore better dissolution of the substrates) (Morozkina et al. 2010), thermophilic β-xylosidase enzymes are more preferred in this processes.

THE GENUS GEOBACILLUS: A β-XYLOSIDASE ENZYME SOURCE

β-xylosidases are produced by bacteria, yeast, algae, protozoans, snails, crustaceans, insects and plants (Sunna and Antranikian 1997), and enzymes from thermophilic microorganisms (especially bacteria and fungi) are more preferred in industrial processes. Recently, β-xylosidase enzyme identifications from thermophilic microorganisms have been performed (Zafar et al. 2022; Le and Yang 2019; Terrasan et al. 2013; Evangelista et al. 2022).

Geobacillus genus is one of the most important source of thermophilic β-xylosidase. These bacteria have a wide range of habitats and their main habitats are geothermal soils, waters, oil fields and hot springs. *Geobacillus* species are rod-shaped, aerobic or facultatively anaerobic, and endospore-forming bacteria. These are obligate thermophilic bacteria and can be live in a wide temperature range. Depending on the *Geobacillus* species, the growth temperatures can be in the range of 35°C-80°C. However, temperatures of about 45 °C to 70°C are required for most isolates. The ability of these species to survive at high temperatures makes them attractive as a source of thermostable enzymes including β-xylosidase enzymes (Khaswal et al., 2022; Nazina et al., 2001).

PRODUCTION OF THERMOPHILIC β-XYLOSIDASES FROM *GEOBACILLUS* GENUS

Several works have been performed on the biochemical characterization of the thermostable β-xylosidase enzyme of *Geobacillus* species from different regions. Some of these studies included high production of these enzymes in a different host by the recombinant route. In literature, recombinant production of the β-xylosidase enzyme has been carried out in the same strain (*E. coli* BL21 (λDE3)) (Bhalla et al. 2014; Huang et al. 2014; Ratnadewi et al. 2013). However, different vector systems have been used for this purpose. For example, pET30a expression vector system has been used for the production of β-xylosidase enzyme of *Geobacillus thermoleovorans* IT-08 isolated from Gunung Pancar hot spring, Bogor, West Java, Indonesia, and pRham N-His SUMO vector system has been utilized for the production of β-xylosidase from *Geobacillus sp*. WSUCF1 isolated from The Compost Facility at Washington State University. The use of different vector systems for the enzyme production was resulted in different purification strategies. Accordingly, β-xylosidase produced by pET30a and pRham N-His SUMO vector systems was purified via nickel affinity chromatoFigurey, which enabled the production of histidine-tagged protein (Bhalla et al. 2014; Puspaningsih 2008; Ratnadewi et al., 2013). On the other hand, the pGEX-6P vector system was used for the production of β-xylosidase from *Geobacillus stearothermophilus* from Marine Culture Collection, deep-sea, and was purified via glutathione-S-transferase (GST)-labeled xylosidase. (Bhalla et al. 2014; Puspaningsih 2008; Ratnadewi et al., 2013). Unlike GST-labelled β-xylosidase was purified by affinity chromatoFigurey technique through glutathione sepharose resin (Huang et al. 2014). Histidine-labeled enzymes yielded a purer protein than the GST-labeled ones. In addition to the recombinant production of thermostable β-xylosidase enzymes from *Geobacillus* genus, some studies have been reported on the β-xylosidase production by direct use of its own natural source (Anand et al. 2013; Galanopoulou et al. 2012; Quintero et al. 2007). Sources of β-xylosidase enzymes produced in these studies were *Geobacillus thermodenitrificans* TSAA1 (Anand et al. 2013) isolated from Saga high-temperature compost plant located near Fukuoka, Japan, *Geobacillus sp.* isolated from Santorini volcanic area, Aegean Sea, Greece (Galanopoulou et al. 2012) and *Geobacillus pallidus* isolated from spring waters at Las Trincheras (Venezuela) (Quintero et al. 2007). Special media containing xylan have been used for he production of β-xylosidase enzymes, inducing its production in the natural strain. Because they are usually extracellular enzymes, the supernatants after the centrifugation of the culture have been used either directly (Anand et al. 2013) or after exposure to additional purification steps (Galanopoulou et al. 2012; Quintero et al.,2007).

CHARACTERIZATION OF THERMOPHILIC β-XYLOSIDASES FROM *GEOBACILLUS* GENUS

Characterization studies of β-xylosidase enzymes from *Geobacillus* species have indicated that these enzymes optimally work in the pH range of 5.5-8.0, which are between weak acidic and weak basic pH condition (Table 1). Some of these enzymes are active over a wide pH range. For example, the relative activity of β-xylosidase enzymes from *Geobacillus sp.* strain WSUCF1, *Geobacillus thermodenitrificans* TSAA1 and *Geobacillus pallidus* has been shown to be at least 20% in the pH range of 4.0-9.0 (Anand et al., 2013; Bhalla et al. 2014;

Quintero et al. 2007). On the other hand, β-xylosidase enzymes from *Geobacillus stearothermophilus* and *Geobacillus thermoleovorans* IT-08 have a relatively narrow working pH range. Regarding this, they have a relative activity of over 15% in the pH 4.5-7.5 range (Huang et al. 2014; Ratnadewi et al. 2013).

Optimum temperatures of β-xylosidase enzymes from *Geobacillus* species have been in a range of 60-70^{o}C (Anand et al. 2013; Bhalla et al. 2014; Galanopoulou et al. 2012; Huang et al. 2014; Quintero et al. 2007; Ratnadewi et al. 2013; Shallom et al. 2005). The temperature operating range for some of these enzymes has been found as 30-80^{o}C (Anand et al. 2013; Huang et al. 2014; Ratnadewi et al. 2013). The β-xylosidase enzyme from *Geobacillus pallidus* was active in a wider temperature range (30-90^{o}C) (Quintero et al. 2007). In addition, the thermal stability of β-xylosidase enzymes from *Geobacillus* species differ greatly from each other. For example, at 70^{o}C, β-xylosidase from *Geobacillus sp.* WSUCF1 retained 50% of its activity after 9 days (Bhalla et al. 2014), while the enzyme from *Geobacillus stearothermophilus* almost completely lost its activity after 60 minutes (Huang et al. 2014). On the other hand, *Geobacillus sp.* WSUCF1 β-xylosidase lost 60% of its activity after 250 minutes at 70^{o}C (Anand et al. 2013), whereas the enzyme from *Geobacillus pallidus* completely lost its activity after approximately 50 hours at the same temperature (Quintero et al. 2007).

In literature, β-xylosidase enzymes from *Geobacillus* species are mostly GH43 (Galanopoulou et al. 2012; Ratnadewi et al. 2013; Shallom et al. 2005) and GH52 family (Anand et al. 2013; Huang et al. 2014; Quintero et al. 2007). Only β-xylosidase of *Geobacillus sp.* WSUCF1 belonged to the GH39 family (Bhalla et al. 2014).

Table 1. β-xylosidase enzymes, their characteristics and β-xylosidase-producer *Geobacillus* species

β-xylosidase-producer Geobacillus species	The habitat of Geobacillus species	GH sınıfı	Optimum pH and temperatures	Reference
Geobacillus pallidus	Spring waters at Las Trincherasi Venezuela	GH52	pH 8.0, 70°C	Quintero et al. 2007
Geobacillus sp.	Santorini volcanic area, Aegean Sea, Greece	GH43	pH 7.0, 65°C	Galanopoulou et al. 2012
Geobacillus sp. WSUCF1	The Compost Facility at Washington State University (WSU), Pullman, WA	GH39	pH 6.5, 70°C	Bhalla et al. 2014
Geobacillus thermoleovorans IT-08	Gunung Pancar hot spring, Bogor, West Java, Indonesia	GH43	pH 6.0, 60°C	Puspaningsih 2008; Ratnadewi et al. 2013
Geobacillus stearothermophilus		GH52	pH 5.5, 70°C	Huang et al. 2014
Geobacillus stearothermophilus T-6		GH43	pH 6.5, 65°C	Shallom et al. 2005
Geobacillus thermodenitrificans TSAA1	Saga high-temperature compost plant located near Fukuoka, Japan	GH52	pH 7.0, 60°C	Anand et al. 2013

BIOTECHNOLOGICAL APPLICATIONS OF THERMOPHILIC β-XYLOSIDASES FROM *GEOBACILLUS* GENUS

β-xylosidase enzymes cooperatively work with other hemicellulosic enzymes in various agro-industrial processes. These industrial processes include production of second-generation biofuels such as bioethanol (Zafar et al. 2022), prebiotic production of short xylooligosaccharides (Le and Yang 2019), pulp biobleaching (Terrasan et al. 2013), monogastric animal feed digestibility and addition to animal feeds to increase feed utilization

(Evangelista et al. 2022) and fruit juice enrichment to increase the quality and quantity features of fruit juices (Suryawanshi et al. 2019).

To the best of our knowledge, there have been no studies in the literature on the use of β-xylosidase enzymes from *Geobacillus* species in biotechnological processes, except for biofuel production. Enzymes synthesized by the genus *Geobacillus*, including β-xylosidase, have a versatile catabolism such as degrading hemicellulose and starch, and it is known that

Their importance of the enzymes have gradually increased of for the production of second generation biofuels (Hussein et al. 2015). It has been reported only one study on the potential use of β-xylosidase enzymes from the genus *Geobacillus* for biofuel production.

In this study, complete conversion of oat-spelt xylan, beechwood xylan and birchwood xylan into xylose and arabinofuranose monosaccharides by cocktails containing β-xylosidase from *Geobacillus thermodenitrificans* NG80-2 and other xylanolytic enzymes has been carried out. This study concluded that these cocktails containing the enzymes β-xylosidase, xylanase and α-L-arabinofuranosidase from the genus *Geobacillus* have a great potential for the conversion of plant biomass to biofuels (Huang et al. 2017).

CONCLUSION

To conclude, β-xylosidase enzymes create a synergistic effect with other xylanolytic enzymes. These enzymes are used in a number of agro-industrial processes, including increasing animal digestibility, pulp bleaching, second generation biofuel production, and enrichment of fruit juices. The fact that *Geobacillus* species have a wide range of habitats including geothermal soils, waters, oil fields and hot springs and they are obligatory thermophiles make these bacteria and their enzymes attractive to be used in agro-industrial processes requiring high temperatures. Supporting this, β-xylosidase enzymes obtained from *Geobacillus* species optimally work at high temperatures (60-70^{o}C) and have a very high thermal stability. The suitability of β-xylosidase enzymes to many agro-industry process conditions will make them more attractive for the next investigations.

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CLINICAL PATHOLOGICAL FINDINGS IN DOGS WITH LEISHMANIASIS

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ABSTRACT

Canine leishmaniasis is a serious zoonotic disease caused by the protozoan parasite *Leishmania infantum* (syn, *L chagasi*) where dogs are the main reservoir. In this study, 12 dogs of different ages were analyzed. Dogs owners have noticed progressive weakness of their pet, loss of hair in the eye area and in some parts of the limbs, loss of the appetite and loss of vitality. To evaluate the presence or absence of Leishmania, the rapid tests idexx leishmania snap test were used. Defining the diagnosis of the disease is also supported by the cytological examination. Hematological and biochemical parameters were analyzed in all positive samples. Erythrocytes (4.97 ± 1.53), hemoglobin (11.35 ± 4.11), hematocrit, the number of lymphocytes and monocytes are lower than the norm compared to the reference values, while the MCHC value was higher than the norm. The value of BUN (42 ± 29.82) and globulin (4.71 ± 1.11) were higher compared to the norm, while the value of albumin (2.25 ± 0.24) was lower than the norm compared to the reference values.

Key words: Leishmaniasis, cytological examination, hematological, biochemical parameters

INTRODUCTION

Canine leishmaniasis is a serious zoonotic disease caused by the protozoan parasite Leishmania infantum (syn, L chagasi) where dogs are the main reservoir. Since the discovery of canine leishmaniasis in Tunisia, by Nicolle and Comte (1908), the dog has been implicated as the main reservoir of the etiological agent of canine leishmaniasis, playing a major role in the transmission of the disease (R. R. Ribeiro, et al., 2018). The manifestation of the disease, which is quite complex, depends on several factors, especially genetic factors and the immune response. In susceptible animals, infection may spread to multiple sites (for example, skin, lymphatic and hematopoietic organs). In advanced stages, various organs and systems may be affected (for example, kidneys, liver, eyes, joints, gastrointestinal tract). Leishmaniasis is included in the group of diseases transmitted by vectors. The causative agents are obligate intracellular protozoan parasites of the genus Leishmania. The manifestation of the disease depends on both the immune status of the host and the type of the infecting parasite. Clinical manifestation ranges from curable skin lesions to fatal visceral disease.

Canine leishmaniasis (CanL) caused by Leishmania infantum is a globally distributed zoonosis with lethal potential for humans and dogs, which constitute the main reservoir and

source of infection in humans (Gramiccia M, Gradoni L, 2005). According to Herwaldt, BL., 1999 leishmaniasis is endemic in 88 countries affecting tropical and subtropical regions, and other areas including deserts, rural and suburban areas. According to Baneth G, et al., 2008 leishmaniasis is endemic in more than 70 countries in the world. Its presence is reported in the regions of Southern Europe, Africa, Asia, South and Central America (Baneth G, et al 2008) and it has also been reported in the United States of America (Petersen CA and Barr SC., 2009). Currently, it is estimated that 350 million people are at risk of infection and disease and of the 1.5-2 million new cases each year, 70,000 are fatal (Reithinger, R., J. et al., 2007). Canine Leishmaniasis is endemic in Asia, Southern Europe, Northern Africa and Central and South America (Lluis Ferrer., 2013; Levy, S., 2002). Data suggest that it is expanding (Levy, SA, et al., 2010).

This pathology is also a significant concern in non-endemic countries where diseased or infected dogs are imported and pose a veterinary and public health problem (Shaw SE, et al., 2009).

About 70 species of mammals, including humans, are considered vertebrate hosts of various species of Leishmania worldwide, and some of them are reservoirs of the parasite in nature (World Health Organization, "Control of the leishmaniasis," Report of the meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, Switzerland, 2010, Vol. 9492010). Determining the precise role played by each host in the transmission cycle remains a challenge.

Several hypotheses can be considered to explain the mode of transmission. The distribution of canine leishmaniasis is highly related to the spread or the presence of appropriate transmission vectors (Torsten J Naucke, et al., 2016). According to the data of Solano-Gallego et al., 2009, there is no clear data on the existence of canine leishmaniasis in Albania. While according to EFSA J. 2015 in Europe, canine leishmaniasis is considered an endemic disease in the countries around the Mediterranean Basin, where Albania is included among several countries.

Leishmanias are able to avoid the non-specific defenses of the organism they infect (phagocytosis) and multiply in macrophages. The progress of the infection depends on the efficiency of the immune response of the organism which is affected by these protozoa. There are two effective mechanisms of the organism's defense that participate against *Leishmania spp* parasites: the first is the release of superoxide (O2 -) from neutrophils and macrophages through the NADPH oxidase complex (Bogdan, C., Röllinghoff, M., 1998), and the the second is the killing effect of parasites by the action of nitric oxide (NO), mediated by interferon (IFN-γ) and tumor necrosis factor (TNF-α) released by macrophages (Solano-Gallego, et al., 2009; Bogdan, C., Röllinghoff, M., 1998; Loría-Cervera, EN, Andrade-Narváez, FJ, 2014; Pinelli, E., et al., 1994; Pinelli, E., et al., 1999; do Nascimento, PRP, et al., 2013). The opposite effect is susceptibility to disease which is manifested by decreased cell-mediated immunity and a mixed response of Th1 and Th2 cytokines (Solano - Gallego L., et al., 2009; Bogdan, C., Röllinghoff, M., 1998). These cytokines are transforming growth factor (TGF-β), IL-10 (Bogdan, C.,

Röllinghoff, M., 1998) and iron regulatory protein 2 (Pinelli, E., et al., 1999; do Nascimento, et al., 2013).

Objectives of the study

- Diagnosis of Leishmaniasis in dogs
- Clinicopathological findings of Leishmaniasis disease in dogs
- Investigation of hematological and biochemical indicators in dogs naturally infected with Leishmania

MATERIAL AND METHODOLOGY

For the realization of this study, dogs of different ages presented at the "Vet Hospital" were randomly analyzed. The complaints presented by the owners are: progressive weakening of the dog, loss of hair in the area of the eyes and in the limbs, loss of appetite and loss of vitality. To assess the presence or absence of Leishmania, the idexx leishmania snap test was used. Establishing the diagnosis of the disease is also supported by the cytological examination. Hematological and biochemical parameters were analyzed in all positive samples.

RESULTS AND DISCUSSIONS

In this study, 12 dogs of different ages were analyzed. The owners have noticed a progressive weakness of the dog, loss of hair in the eye area and in some parts of the limbs, loss of appetite and loss of vitality. These were the clinical signs that worried the owner as well as the reason why they appeared near the "Vet Hospital". After the general check-up in the hospital, the rapid idexx leishmania snap test was performed to examine leishmaniasis. Dogs that tested positive to the rapid test showed clinical signs characteristic of the cutaneous form of leishmaniasis. The formation of scales has been observed in certain areas of the skin, mainly around the eyes and on the limbs. Clinical signs generally characteristic of visceral leishmaniasis have also been found, where the dog is in poor body condition and with coarse hair.

Skin lesions are the most obvious clinical manifestation of canine leishmaniasis (Solano-Gallego et al., 2009) which includes alopecia and exfoliative dermatitis, ulcerative, nodular, pustular and papular dermatitis (Ferrer et al., 1988a). Such lesions were also found in the dogs included in this study. These lesions are shown in photo number 1.

The clinical manifestation of canine leishmaniasis includes a wide spectrum of clinical symptoms, which in dogs varies from the absence of symptoms to generalized disorders that can result in death: fever, anemia, lymphadenopathy, weight loss, emaciation, hepatosplenomegaly, conjunctivitis, renal alterations, keratitis and skin lesions (Solano-Gallego, L., et al., 2009; Dias, EL, et al., 2008; Sakamoto, KP, et al., 2013; Sanches, FP, et al., 2014).

Fig. 1. Different manifestations of the cutaneous form of leishmaniasis

In the samples that proved positive to the rapid test, material was taken to perform cytological analysis on the presence of microorganisms in the cytoplasm of macrophages. In the samples analyzed during cytopathological examination, the presence of leishmanic protozoa in the cytoplasm of macrophages was found.

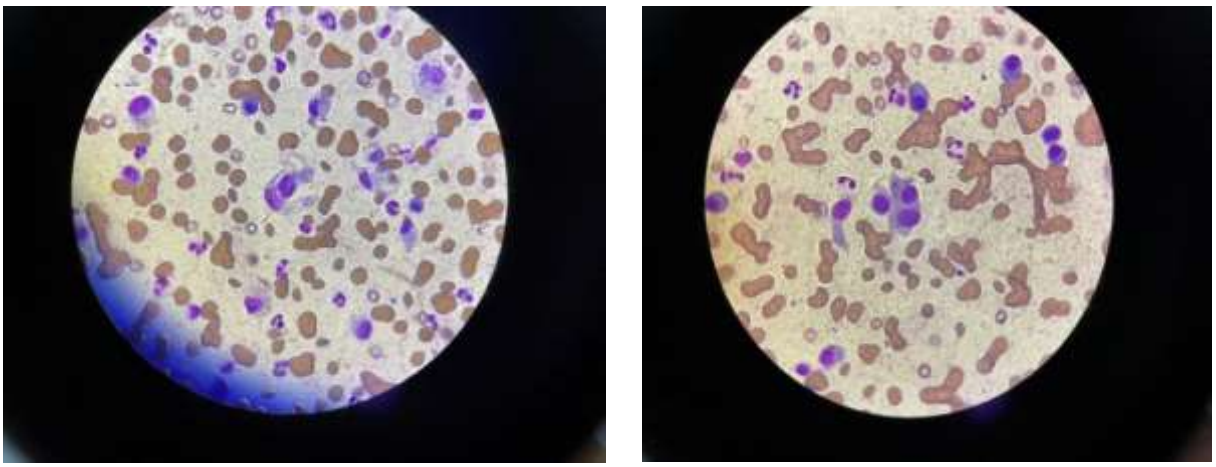

Fig. 2. Cytological picture showing the presence of leishmanial protozoa in the cytoplasm of macrophages

Hematological and biochemical analyzes were carried out for each analyzed sample.

Table 1. Hematological indicators in dogs tested positive for leishmaniasis

Hematological parameters	Average and Dev. Stand.	Min	Max	Reference values
Leukocytes (10 3 /µL)	11.63 ± 2.42	8.8	15.2	6.0 - 17.0
Erythrocytes (10 6 /µL)	4.97 ± 1.53 ↓	3.02	7.35	5.50 - 8.50
Hemoglobin	11.35 ± 4.11 ↓	7.7	18.7	12.0 - 18.0
Hematocrit	28.8 ± 10.43 ↓	18.6	47.7	37.0 - 55.0
MCV	61.65 ± 1.83	59.9	64.9	60.0 - 77.0
MCH	23.93 ± 1.49	22.1	25.5	19.5 - 24.5
MCHC	39.1 ± 1.67 ↑	36.9	41.4	32.0 - 36.0
Platelets (10 3 /µL)	222.83 ± 112.02	70	345	200 - 500
Lymphocytes (%)	10.73 ± 7.25 ↓	5.5	24.9	12.0 - 30.0
Monocytes (%)	2.78 ± 2.01 ↓	1.8	6.8	3.0 - 10.0
Eosinophils (%)	3.15 ± 2.13	0.8	5.3	2.0 - 10.0
Granulocytes (%)	80.48 ± 9.31 ↑	67.5	90.2	60.0 - 80.0
RDW	11.93 ± 0.81	11.6	12.9	12.0 - 16.0
PCT	0.106 ± 0.04	0.07	0.18	0.00 - 2.90
MPV	5.2 ± 0.10	5.1	5.3	6.7 - 11.1
PDW	18.3 ± 0.97	17.2	19.4	0.0 - 50.0

The panel of hematological parameters is presented in table number 1. Comparing the values of this study with the reference values, it results that the number of erythrocytes, the level of hemoglobin, the hematocrit, the number of lymphocytes and the number of monocytes are below the norm, while the value of MCHC and the number of granulocytes results above normal values.

Leishmaniasis is a disease with a very variable and complex clinical manifestation involving several factors. Visceral leishmaniasis is a chronic infectious disease that can be characterized by the development of symptomatic or asymptomatic infection accompanied by the appearance of various typical clinical signs (José Cláudio Carneiro de Freitas, et al., 2012). The hematological and biochemical parameters of the serum, although limited in the diagnosis of canine visceral leishmaniasis, are very useful in evaluating the clinical status of the animal and the degree of lesions and can give indications on the prognosis of the animal (Ikeda FA, et al., 2003; Reis AB, et al., 2006).

The value of anemia in dogs infected with leishmanic protozoa is also reported by other authors such as Raul Rio Ribeiro, et al., 2018; Lluis Ferrer, 2013. Igor Ulchar, et al., 2015 also reports low values of erythrocyte count, hemoglobin level and hematocrit.

Table 2. Biochemical indicators in dogs tested positive for leishmaniasis

Biochemical indicators	Average and Dev. Stand.	Min	Max	Reference values
Glucose (mg/dL)	86.16 ± 7.73	81	101	74 -143
Creatinine (mg/dL)	0.85 ± 0.46	-.--	1.3	0.5 - 1.8
BUN (mg/dL)	42 ± 29.82 ↑	21	97	7 - 27
BUN/Creatinine	21.5 ± 14.03	-.--	39	
TP (g/dL)	7.01 ± 0.95	6.0	8.1	5.2 - 8.2
Albumin (g/dL)	2.25 ± 0.24 ↓	2.0	2.5	2.3 - 4.0
Globulin (g/dL)	4.71 ± 1.11 ↑	3.5	5.9	2.5 - 4.5
Alb/Glo	0.53 ± 0.12	0.4	0.7	
ALT (U/L)	49.83 ± 22.85	21	76	10.0 - 125
ALKP (U/L)	92.5 ± 79.72	36	242	23 - 212

The non-regenerative anemia that accompanies canine leishmaniasis is usually explained as a consequence of chronic kidney damage, but there are also findings that suggest that the decrease in erythrocytes in the peripheral blood may be the result of direct erythrocyte damage caused by the parasite (Dias, EL, et al., 2008) or result from bone marrow dysfunction (Dias, EL, et al., 2008; Nicolato, R.deC., et al., 2013).

Lluis Ferrer, 2013 reports on hypoalbuminen in dogs infected with leishmaniasis as it resulted in our study. Even in the study of Igor Ulchar, et al., 2015, below-normal values of albumin are reported.

In our study, the value of urea was above the norm. The same difference is presented by Igor Ulchar, et al., 2015. Uremia is considered as an indicator of a late, aggravated stage of

canine leishmaniasis, which corresponds to chronic kidney damage. Chronic kidney damage is caused by immune glomerulonephritis.

The resulting hyperglobulinemia in this study is consistent with the findings of other authors (Igor Ulchar, et al., 2015; Solano-Gallego, L., et al., 2009; Dias, EL, et al., 2008; Sanches, FP, et al., 2014; Kargin Kiral, F., et al., 2004). This hyperglobulinemia is probably caused by polyclonal beta or gamma hyperglobulinemia.

CONCLUSIONS

In Albania, canine leishmaniasis is considered an endemic disease according to the World Health Organization (WHO, 2010). The complexity of canine leishmaniasis and the wide range of clinical manifestations, from asymptomatic infection to severe disease, make the management of canine leishmaniasis challenging. Diagnosis is performed based on clinicopathological examination and upon confirmation of infection using mainly serological and molecular techniques. The hematological and biochemical parameters of the serum, although limited in the diagnosis of canine visceral leishmaniasis, are very useful in evaluating the clinical status of the animal and the degree of lesions and can give indications on the prognosis of the animal (Ikeda FA, et al., 2003; Reis AB, et al., 2006). The indicators that were most changed in this study are: red blood cells, hemoglobin, hematocrit, BUN, albumin and globulin.

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CULTURAL ECOSYSTEM SERVICES AS KEY ELEMENT IN HUMAN WELL-BEING IN STRANDJA NATURE PARK, BULGARIA

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ABSTRACT

The future sustainability of society in global level depends on the ecosystem services provided by natural ecosystems. Protecting natural, unmodified ecosystems helps to increase the diversity of life and resilience to the adverse effects of climate change, which in turn sustains and restores services. Nature and human well-being are interrelated. Each ecosystem provides many services. Human relationships with the environment and the importance they have are in accordance with the definition of cultural ecosystem services and well-being. Despite there are different systems for classifying ecosystem services (ESS), cultural ecosystem services (CES) are defined as "... the intangible benefits of ecosystems (aesthetic pleasure, recreation and tourism, inspiration for culture, art and spiritual experience) "(Millennium Ecosystem Assessment, 2005). Although the growing interest in the study and evaluation of ESS is not yet clearly defined and there are few attempts to evaluate it. The difficulty comes from the fact that it has to be assessed non-material benefits, with material means. The territory of the Park is large and the ecosystem services are very diverse. The aim of the current study is to review the cultural ecosystem services in the Strandja Nature Park, which are in the preservation of the culture of traditions and local communities. Based on a review of the literature - scientific journals, publications of Bulgarian and foreign authors, on-line (electronic) editions, as well as author's research in Strandja Nature Park, the following domains of well-being of CES can be identified: Heritage, Existence and Spirituality, Sense of Place and Identity, Knowledge /Education/ and Biodiversity. Regardless of material ecosystem services are most recognizable by local communities (mushrooms, herbs, wood etc.). Cultural ecosystem services are essence of overall well-being. The promotion of cultural ecosystem services in Strandja Nature Park play key role for the conservation and sustainable use of natural resources. In addition rural development is supported.

Key words: Biodiversity Ecosystem services, , Nature, Strandja.

INTRODUCTION

Each ecosystem provides a range of services that are fundamental to human well-being, health, livelihoods and survival (Costanza et al., 1997; MA, 2005; TEEB, 2010). Different ways of defining ecosystem services have been developed. They can be described as the benefits that people derive from ecosystems (MA, 2005) or as the direct and indirect contributions of ecosystems to human well-being (TEEB, 2010). More recent publications define ecosystem

services (ES) as the contribution of ecosystem structure and function to human well-being (Burkhard et al., 2012; Burkhard B. & Maes J. Eds., 2017). Human relationships with the environment and the importance they have are in accordance with the definition of cultural ecosystem services. Despite different systems for classifying ecosystem services (ESS), cultural ecosystem services (CES) are defined as "... the intangible benefits of ecosystems (aesthetic pleasure, recreation and tourism, inspiration for culture, art and spiritual experience) "(Millennium Ecosystem Assessment, 2005). Although the growing interest in the study and evaluation of ESS is not yet clearly defined and there are few attempts to evaluate it. The difficulty comes from the fact that it has to be assessed non-material benefits, with material means.

An ecosystem cannot provide any benefits to people without the presence of people, their communities and their environment. Man and nature are interconnected and his negative impact continues to increase leading to loss of habitats and biodiversity.

It has been established that in the border regions of a number of countries falling on both sides of the so-called "Iron Curtain" from the recent past, there are huge territories with preserved nature and biodiversity. After restrictions on access to these areas, the strict control, and not infrequently, the geoFigureical isolation of the border territories, they have preserved key places, rare and protected species and unique landscapes. These areas are united in the European Green Belt initiative. It forms a 12,500 km long Pan-European ecological corridor along the former Iron Curtain from the Barents Sea in the north to the Adriatic and Black Seas in southeastern Europe. The Balkan Green Belt covers the southern part of the low Green Belt, which extends over the territory of 9 countries, including the border territories of Bulgaria and Turkey. Some of the most significant NATURA 2000 areas in Bulgaria have been identified in these cross-border areas, such as Strandja, Dervent Heights, Sakar, Maritsa, Tundja and many others preserving unique biodiversity. On the other side of the border, in Turkey, experts have identified a number of potential areas that would be included in NATURA 2000 if Turkey were to join the EU. These are the territories in the Turkish part of the Strandja Mountain, the area of lakes and forests around Ineada, the Dupnitsa cave and others.

Strandja is a mountain with a thousand-year history that has preserved the heritage of several civilizations in its lands and spiritual spaces (https://www.strandja.bg/).

The aim of the current study is to review the cultural ecosystem services in the Strandja Nature Park, which are in the preservation of the culture of traditions and local communities.

MATERIAL AND METHOD

Study area

Strandja Nature Park was declared in 1995. It's the largest Bulgarian protected area (116 136,2 ha), which occupies 1% of the country's territory.

The Park with its own nature protection status includes: 5 reserves, 12 protected areas and 17 natural landmarks. About 80% of the territory is a forest fund, which is managed by 5 state forestry departments. The territory of the Park covers the hills of Strandja Mountain, including the rocky coast of the Black Sea. Strandja mountain is located in the eastern part of the historical-geoFigureic region of Thrace between the Burgas lowland, the Black Sea and the Eastern Thrace plain. Strandja has three well-defined ridges - the main southern ridge in Turkey, the bordering Rezov ridge and the Bosna hill, located entirely on Bulgarian territory. The southern ridge, located in the northwest-southeast direction, is entirely in Turkish territory and on it is the highest peak of the mountain - Mahiada, 1031 m high. The border ridge is surrounded by the valleys of Rezovska Reka and Veleka, and its highest point is Golyamoto Gradishte peak (710 m) – the highest peak in the Park and in the Bulgarian part of the mountain. To the east

and northeast, within the geoFigureical limits of the Strandja mountain, is the Bosna hill, with the highest peak Papia (501 m) with the branched Rosen bair and Medni hill extending beyond the Park's borders.

Among the invertebrates in the Park there are 164 species of conservation importance. There are 34 relict species, 4 of the endemic species are local and 80 - Bulgarian. The World Red Data Book has included 16 of these species, while the European has listed 10 of them. The Habitats Directive protects the following butterflies - *Lycaena dispar*, *Euphydryas aurinia*, and *Callimorpha quadripunctaria*, and of the beetles –*Lucanus cervus*, the capricorn beetle, and others.

Strandja Nature Park is the Bulgarian protected territory with the richest vertebrate fauna – 404 species. The park ranks among the first in Europe by virtue of its fishing resources. The interior of Strandja hosts 41 species of freshwater and passage fish, and another 70 species inhabit the coastal waters. The number of relict species is high – 9 Ponto-Caspian (preserved since the time of the ancient Sarmatian Sea) and 5 boreal relicts (from Latin - Borealis – northern, species that originated from the northern regions and reached our latitudes during the Quaternary frosts).

There are 6 species endemic for the Black Sea basin – e.g. *Neogobius melanostomus, Mesogobius batrachocephalus*, and others. Two types of gobies and the Sander marina are endemic for the Black Sea basin and the Caspian basin. The World Red Data Book includes 12 species of freshwater fish, and the National Red Book – 8 species.

Ten species of amphibians have been identified on the territory of the Nature Park, which is more than half of the total of 16 Bulgarian species.

Twenty-four species of reptiles, which represent 70% of the richness of the Bulgarian herpetofauna have been found in Strandja Nature Park.

269 species of birds can be found within the boundaries of the Park, which represent two thirds of the entire Bulgarian ornithofauna.

The second largest migratory route of the birds in Europe (*Via Pontica*) passes over Strandja – it brings together the flocks of Northern and Eastern Europe, and Siberia. The migration front narrows over the Nature Park and in peak days visitors can see enormous flocks of white storks – up to 40 000 a day, as well as tens of thousands of pelicans, perns, buzzards, and spotted eagles. The importance of the Park is further reinforced by the bays and estuaries along the coast of the Black Sea, which are used by the waterfowl species not only for rest, but also for hibernation.

Sixty-six species of mammals breed within the boundaries of the Park. Individual representatives of the population of the monachus sporadically visit the underwater caves near Silistar.

The bats are represented with 25 species, 13 of which are listed in the World Red Data Book (*Myotis bechsteinii, Myotis capaccinii,* and *Myotis emarginatus, Rhinolophus euryale and Rhinolophus hipposideros, Barbastella barbastellus,* and others).

Today, there are 21 settlements - two cities and 19 villages with a population of about 8,000 people - on the territory of the "Strandja Nature Park. The entire municipality of Malko Tarnovo, most of the municipality of Tsarevo and a very small part of the municipality of Primorsko fall within the boundaries of the protected territory.

The Strandja region is inhabited by three ethnoFigureic groups - Rups, Tronkas and Zagorci. For centuries they live in direct cultural and domestic contact.

Based on a review of the literature - scientific journals, publications of Bulgarian and foreign authors, on-line (electronic) editions, as well as author's research in Strandja Nature Park, to define domains of well-being of CES were used. The survey was designed for two focus groups and includes face-to-face interviews with 15 random local people and 15 academics (researchers, biodiversity experts, ecologists) on The Festival of Rhododendron which take place in village Brodilovo in May 2022.

RESULTS AND DISCUSSION

A discussion with different stakeholders from focus groups helps us to determine the most relevant domains of well-being of CES: Recreation and tourism, Heritage, Existence and Spirituality, Sense of Place and Identity, Knowledge /Education/ and Biodiversity.

Some of the CES domains are easy recognized by people because they are a part of their lifestyle. The other CES are abstract and non-popular so there is a difficulty to understand and define.

Only 10 % of people became familiar with CES concepts and can define it, they belong to academics group. More than 80 % of respondents recognize the Recreation and tourism, Sense of Place and Identity as most important CES. Heritage, Existence and Spirituality is most significant predominantly for 75 % of local people. The CES domain - Knowledge /Education/ and Biodiversity is prevailing relevant for 60 % of ecologist and experts.

Human-nature connections are strongly recognized from local communities and reflected in various festivals which organized in each village (Festival of Rhododendron, folk festivals, food festivals etc).

Each of the three ethnoFigureic groups in Strandja (Rups, Tronkas and Zagorci) carries and preserves various customs and traditions, from which the cultural ecosystem services originate and are transmitted from generation to generation.

One of the emblematic customs is Nestinari. From time immemorial, in the wilds of the mystical Strandzha Mountain, the extraordinary tribe of Nestinari lives - the "children" of St. Constantin and Helen, those who do not burn in the fire, who see into the future...Nestinar is typicaland registered only in Strandja of Bulgaria.

Carried over from ancient times, today nestinari is preserved only in some Strandzhan villages and Eastern Thrace, from where After the Balkan War of 1913, the Bulgarian population from South Strandzha, which remained within the borders of Turkey, was forced to move to its Bulgarian part. The researchers outline a probable Nestinarian land in the shape of a triangle, whose vertices are the cities of Tsarevo (with the old name Vasiliko) in present-day Bulgaria, Midia and Lüleburgas in European Turkey. It is believed that in the past there were about thirty Nestinar settlements, the most famous among them being Bulgari (formerly Urgari or Vurgari), Kosti, Brodilovo, Slivarovo, Gramatikovo, Kondolovo, etc. Zaberno, Vizitsa, Stoilovo, Kolibite, Kurudere, Yana, etc. are also mentioned The name "nestinari" comes from the Greek "anastenaria" and literally means "in the hearth, in the fire", i.e. nestinars are people who play with fire. Indeed, ritual play in the embers is the most emblematic feature of firework. The dance in the embers is only the most visible and spectacular part, but more essential are its deeply spiritual aspects. The so-called entrapment is the special state of trance and connection with the sacred in which the firebenders reside before entering the embers and during the fireplay itself. Then some of them also receive oracular insights (https://nestinari.eu).

Figure 1. Typical Nestinars – ritual starts at day and continued in the night with mystic dance on the embers. (*Photo credit https://nestinari.eu)*

Community centers as a cultural institutions are keepers of the knowledge of local communities which contribute rural sustainability through transition it to next generation. CES are key elements of human well-being.

Biodiversity is in the base of training of student, scientific studies ect. In the NP Strandja every year there is summer training of students from different Universities of Plovdiv and Sofia.

Figure 2. Festival of *Rhododendron ponticum (Photo credit: Green Balkans)*

Figure 3. Celebrating Midsummer day (24.06) (*Photo credit: NP Strandja Directorate)*.

Sense of Place and Identity are strongly recognized CES domain from local communities (fig.4).


Figure 4. An typical old house and Church in Kosti village. (*Photo credit:https://www.tsarevo.info/za-obshtinata/gradove-i-sela/selo-kosti/)*

Biodiversity is the basic resource of ecotourism and recreation (figs.5 and 6). The forests in Strandja**,** which occupy around 3/4 of the territory of the Nature Park, are remnant of the tertiary vegetation, preserved due to its remoteness from the Quaternary glaciations, mild winter, high rainfall and air humidity. A characteristic feature of the Strandja forests is the inversion, a process in which mesophilic beech-tree forests with rhododendron ponticum undergrowth take parts of the valley slopes and oak forests extend over them. The communities of Mediterranean and sub-Mediterranean type also have considerable presence amidst the vegetation of the Nature Park: thermophilic oak forests with undergrowth of evergreen sclerophyllous shrubs such as *Erica arborea*, *Ruscus aculeatus* or grass cover of *Hypericum calycinum*, as well as undergrowth of *Phillyrea latifolia*, *Cistus incanus*, *Cistus salvifolius* and others.

Strandja is rich of water resources (rivers, springs and sea) and also preserved anthropogenic tourist resources - tourist routes, mystical places associated with legends (the Bastet goddess for example) or treatment of diseases – chapels and healing springs etc. Tourism goes hand in hand with folk beliefs.

From a bioclimatic point of view, the region of Strandja Mountain and the Strandja coast offer significant opportunities for recreation and ecotourism. The healing qualities of the climate in the coastal area combined with the beaches and the sea are the reason for the recognition in 1963 of Sinemorets as a climatic sea resort of local importance, and in 1972 Ahtopol was declared a climatic sea resort of national importance. /Strandja park/ In-depth studies of the climate in the region of Malko Tarnovo have been carried out, which provide a basis for the development of the tradition of climate treatment of lung and allergic diseases. Already in the 1930s, Malko tarnovo became a favorite place to spend the summer months for the residents of Burgas, where, at that time, apart from the usual air pollution for big cities, malaria was also prevalent. Malko Tarnovo was declared in 1981 as a mountain climatic resort of local importance (www.strandja.bg/en/info).

The tourist service in Strandja takes three main forms: mass sea tourism in Tsarevo and Primorsko municipalities, alternative tourism, developing mainly in the actual forested part of the mountain, as well as hunting tourism (Nikolov, 2018).

Figure 5. Ecotourism in Strandja Mountain *Photo credit: Nadezhda Petkova*

Figure 6. Seaside protected area **(***Photo credit: NP Strandja Directorate***)**

CONCLUSIONS

We found that the most relevant CES domains in Strandja are Recreation and tourism, Heritage, Existence and Spirituality,

For future understanding and clarifying the CES collaboration between academics and local people is needed. Our findings are in synchrony with Balazsi et al., 2021 that suggest the need of interdisciplinary research for CES policy and management.

Cultural ecosystem services are essence of overall well-being. The promotion of cultural ecosystem services in Strandja Nature Park play key role for the conservation and sustainable use of natural resources. In addition reginal rural development is supported.

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POPULATION DENSITY OF APHIDS ON SOME HYBRID CULTIVARS OF SUNFLOWER (*HELIANTHUS ANNUS* L.) AT DIFFERENT DEGREE OF WEEDING

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ABSTRACT

The cultivated sunflower is one of the main oil-bearing crops in the world and it is determined in Bulgaria as the main strategic crop. The aphids of sunflower are one of the factors that reduce the yield and quality of the production. The investigations were carried out in 2020-2021 in training trial field Vrazhdebna of the Forestry University, Sofia (42°70'76.1"N, 23°43'73.1"E). The following hybrid cultivars 'Dracaris CLP', 'Coloris CL', 'Accordis CLP' and 'In Sun CLP' were tested. Studies were carried out under field conditions by observing and reporting the natural infestation of experimental plant material by existing local populations of aphids. The species composition of aphids was determined, with the dominant species *Aphis fabae* subsp. *evonymi* Fabricius и *Sipha (Rungsia) maydis* Passerini. The data collected for the observation period showed different degrees of attack (0 – 7). The studied hybrid cultivars 'Dracaris CLP' and 'Coloris CL', were strongly attacked by aphids, with the highest degree reported in (7.0 ± 0.1 and 6.7± 0.1 resp.) and the lowest (4.1 ± 0.3) in the 'Accordis CLP'. Aphid infestation was not reported in the hybrid 'In Sun CLP'. The relationship between degree of weeding and the attack by aphids was strong (R= 0.61– 0.74). The obtained results give reason to conclude that the hybrid cultivars have different host suitability to aphids and their degree of attack may be influenced by the degree of weeding.

Keywords: Sunflower, Aphids; Host Suitability; Weeding

INTRODUCTION

The sunflower (Helianthus annus L.) is a plant that is grown in different regions of the world. In Bulgaria, it is the main oilseed crop. In the last few years, there has been a massive multiplication of several insect pest on sunflower. The aphids on sunflower are one of the factors that reduce the yield and quality of the production in Europe (Lerin and Badenhausserq 1995; Duca and Clapco, 2021). Besides direct damage, aphids also cause indirect damage as vectors of viral diseases on sunflowers (Singh et al., 2005; Bhat and Reddy, 2016). It has been suggested that changes in climate may affect their bioecological features, the resistance of cultivars and hybrids, and host-pest relationships (Coakley et al., 1999; Castex et al., 2018). In this context, obtaining information about possible changes in the appearance and development of aphids on sunflowers and finding new effective methods of control and management of the populations is essential. Conducting research to establish the resistance of hybrids to biotic stress factors and the reasons determining their specific response implies the accumulation of knowledge to develop plant protection programs responding to changing environmental conditions.

The aim of the present research was to evaluate different sunflower hybrids for their suitability as hosts for aphids under different weeding levels.

MATERIAL AND METHOD

The investigations were carried out in 2020-2021 in training trial field Vrazhdebna of the Forestry University, Sofia (42°70'76.1"N, 23°43'73.1"E), on an area of 432 m^2.

The study was conducted with the following hybrid cultivars 'Dracaris CLP', 'Coloris CL', 'Accordis CLP' and 'In Sun CLP', which are distinguished by high yield, excellent adaptability to the soil and climatic conditions of Bulgaria, with high oil content and tolerance of diseases.

The experiment included 4 blocks of 4 randomised plots. The test plots (each plot was 25 m^2) with the various hybrids were near each other, allowing the aphids to choose between them. The population density of aphids was observed by direct visual methods after the growth stage BBCH 30-39, evaluations were carried out every 10 days. The number of aphids in the experimental plots was counted on 10 randomly selected and marked plants, based on that an average was calculated per plant. A modified approximate scale from 0 to 7 was used to determine the degrees of attack (Schnelle, 1995), with 0 = no aphids and 7 = 750 average aphid count. For the exact identification of the species, microscopic preparations were made according to Hille Ris Lambers (1950) and the determination books by Taylor and Robert (1984) and Blackman and Eastop (2000) were used.

The degree of weeding was determined by removing weeds on a 4-point scale, each unit of the scale corresponding to 25 % area covered by weed plants (Dimitrova et al., 2004). The different coverage of plots with weeds was obtained by periodic weeding of individual plots.

The mathematical processing of the results was performed with the statistical computer program SYSTAT® 10.2. Analysis of variance (ANOVA) was used to determine the influence of the tested factors. Means were compared by Tukey's test. The degree of dependence between two parameters was expressed by the correlation coefficient.

RESULTS AND DISCUSSION

The following species of aphids were found:

Kingdom: Animalia
Subkingdom: Eumetazoa
Phylum: Arthropoda
Subphylum: Hexapoda
Class:Insecta
Order: Hemiptera -
Suborder: Sternorrhyncha
Superfamily: Aphidoidea
Family: Aphididae
Aphis fabae subsp. evonymi Fabricius, 1775
Sipha (Rungsia) maydis Passerini, 1860
Brachycaudus helichrysi (Kaltenbach, 1843)
Myzus (Nectarosiphon) persicae Sulzer, 1776

The percentage ratio of the 4 found aphid species (*Aphis fabae subsp. evonymi Fabricius*, 1775, *Sipha (Rungsia) maydis* Passerini, 1860, *Brachycaudus helichrysi* (Kaltenbach, 1843), *Myzus (Nectarosiphon) persicae* Sulzer, 1776) was relatively constant during the observation period. Aphids of the species *A. fabae* subsp. evonymi and S. maydis had the highest percentage of presence. The species *S. maydis* in 2020 occurred 31.3% and 19.3% in 2021, while *A. fabae subsp. evonymi* dominated in both research years – 60.2% (2020)

resp. 74.5% (2021). The species *B. helichrysi* was of limited occurrence – 9.5% in 2020 and 6.2% in 2021. Only single individuals of the species *M. persicae* were also found. The data were obtained from the average number of aphids/plant counted during the growth stage BBCH 30÷81, averaged for both years of research. The highest number of aphids was observed during the growth stage BBCH 51÷69.

The soil cover by weeds in the tested variants was from 10% to 85%, which corresponds to a degree of weeding from 0 to 4. In weed communities, 15 weed species from different biological groups have been recorded, mainly *Convolvulus arvensis* L., *Cirsium arvense* L., *Sonchus arvensis* L. and *Amaranthus* spp. and other.

Mean aphid density at different degree of weeding in the tested hybrid cultivars are presented in Figure. 1. The data obtained show different degree of aphid attack in the tested hybrids and degrees of weeding. Statistically significant differences were found between the population density values of the tested sunflower hybrids depending on the genotype of the tested plants (different hybrids) and depending on the degrees of weeding.

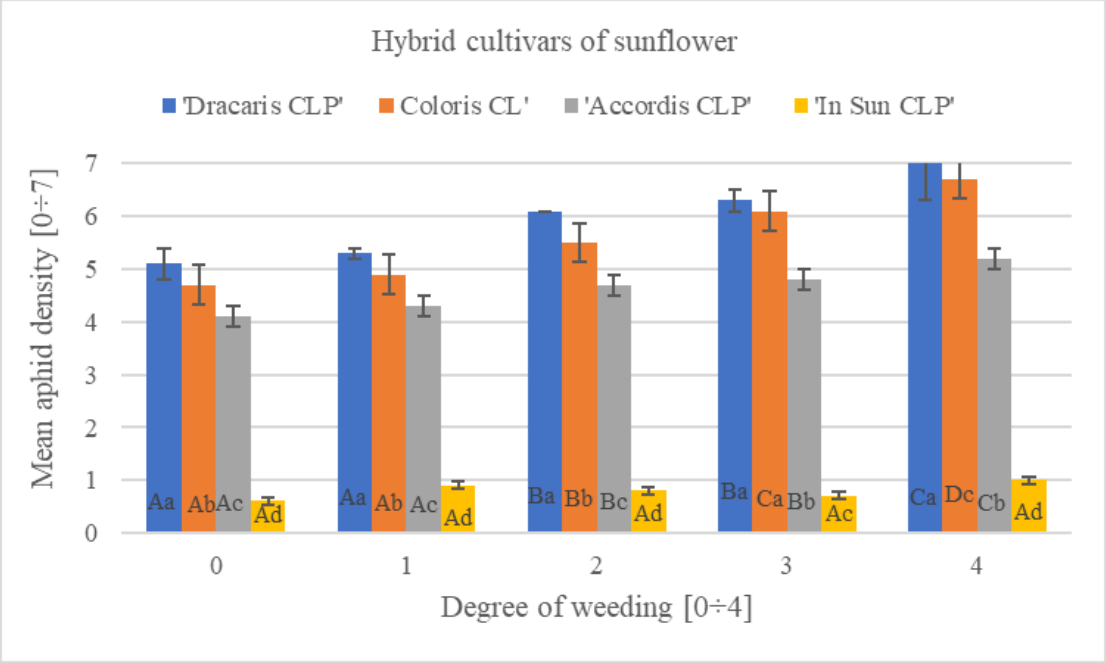

Figure 1. Population density of aphids at different degree of weeding
(mean ± SE; different letters = significant differences; capital letters indicate significant difference of degree of weeding, small letters indicate significant difference of genotype; $p < 0.05$; Tukey test)

The hybrids 'Dracaris CLP' and 'Coloris CL' were more strongly attacked by aphids at weeding with index 3 and 4. The highest aphid density was recorded for 'Dracaris CLP' (7.0± 0.1) at weeding index 4, and the lowest for the hybrid 'Accordis CLP' (4.1±0,3) in the variants without weeds (index 0). Aphid infestation in the hybrid 'In Sun CLP' was very low (□ 1) and hybrid 'Coloris CL' occupied an intermediate position.

The influence of the two variable factors, namely genotype (hybrid cultivars) and degree of weeding, was tested using a two-way analysis of variance (ANOVA). In the Table 1 shows the results of the statistical processing, which prove the existence of a lasting effect of the influence of the individual factors, but do not confirm their joint effect.

Table 1. Two-factor dispersion analysis (ANOVA) of the effect of weeding degree and of genotype on the aphid population density in 2020-2021

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
weeding degree	5.474	3	2.737	11.599	0.000
genotype	3.597	3	1.199	5.082	0.002
weeding degree * genotype	1.472	7	0.245	1.039	0.400
Error	53.802	228	0.236		
Total					

*** p ≤0, 001, **p ≤0,01

The investigated sunflower hybrids showed a different suitability as hosts for aphids. A different genotypic reaction of sunflower hybrids to the aphid *Aphis gossypii* Glov was also reported by Misbah-ul-Haq et al. (2003) and Ahmed et al. (2013). Research results show that aphid population density on sunflower plants increases with increasing weed density. Although the literature reports on the effect of weediness on aphid population density are divergent. *Aphis fabae* aphids on organic faba beans were reported to be more numerous in weeded than in unweeded plots (Patriquin, et al., 1988). Pobożniak (2005) also found that the number of aphids *Aphis fabae* and the number of colonised red beet plants decreased with greater weed cover, with the lowest aphid numbers in plots where, the weeds have not been removed. Significant correlations were found between the results of the t attack by aphids and the degree of weeding (Table 2).

Table 2. Correlations between population density of aphids index [0-7] and degree of weeding [0-4], r = correlation coefficient

Analysed dependencies	Hybrid cultivars of sunflower			
	'Dracaris CLP'	'Coloris CL'	'Accordis CLP'	'In Sun CLP'
Population density of aphids/Degree of weeding	Correlation coefficient r			
	0.74**	0.67*	0.71*	0.61*

It is necessary to note that the attack of aphids and their density were reported under field dynamic conditions. It is known that aphids can actively spread and be selective choose their hosts. The differences found here between the tested hybrid cultivars can be explained by antixenosis, which under field conditions expresses strongly (Tomchev and Markova, 2013). In this aspect, further research is needed, since abiotic and biotic stress factors have a temporary influence on plant development and their host suitability.

CONCLUSIONS

The following species of aphids *A. fabae subsp. evonymi, S. maydis, B. helichrysi* and *M. persicae* were found on the tested sunflower hybrids. The species *A. fabae subsp. evonymi* and *S. maydis were* dominant.

The density of aphids was very high in the variants with a high level of weed infestation

The population density of aphids was very high in the variant with hybrid cultivar 'Dracaris CLP'(7.0 ± 0.1) and a high level of weed infestation; the lowest in the variant without weeds and hybrid cultivar 'Accordis CLP' (4.1 ± 0.3). Aphid infestation in the hybrid 'In Sun CLP' was very low (< 1).

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A REVIEW ON THE CUTTING-EDGE BIOTECHNOLOGICAL METHODS WITH THEIR UTILIZATION FOR IMPROVED FRUIT CULTIVARS IN TURKIYE

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ABSTRACT

In the human diet, fruits are a significant source of vitamins, beneficial nutrients, fiber, and antioxidants, as well as a key source of foreign exchange earnings. Turkiye has a rich scope of plant genetic resources and a wide range of fruit diversity, making it one of the most significant in the world. More than 80 fruit species are cultivated. Among them are temperate, tropical, and subtropical fruits such as apples, figs, pears, citrus (lemons, mandarins, and oranges), bananas, avocados, hazelnuts, Prunus (apricots, plums, peaches, cherries, almonds, and nectarines), and kiwis, etc. But in recent years, the abrupt increase in population, climate change and diverse adversities, mainly biotic and abiotic stresses, are hampering fruit productivity in Turkiye and globally. To counter the above-mentioned constraints and to better understand the genetic resources and germplasm conservation of Turkish fruits, various biotech solutions are being considered. For instance, modern molecular biotechnology tools such as tissue culture, genetic markers, DNA-based molecular markers, sequencing, omics approaches, and genome editing are being used by researchers. In the same way, transgenes have been effectively used to enhance fruit crops, mainly based on improved tolerance to biotic and abiotic stress, increased fruit yield, minimizing generation time, high nutrition content, and better pre-and post-harvest features. In this note, we provide and report the latest studies being conducted by Turkish breeders and scientists.

Keywords: Climate change, Pomology, Molecular characterization, Genome-editing. Plant biotechnology,

INTRODUCTION

Turkiye sits in the region where Asia, Africa, and Europe, the three continents of the old world, are nearby, and straddles the meeting point between Europe and Asia. Because of its geoFigureic location, Turkiye has an influential geopolitical status. In the north, it is surrounded by the Black Sea, in the south, by the Mediterranean Sea, and in the west, by the Aegean Sea. Turkiye indeed has a relatively temperate climate, but the varied nature of the landscape, particularly the mountains running parallel to the coastlines, contributes to significant variations in climatic conditions between regions. A hot summer and a cold winter characterize the inland Anatolia plateau, whereas the coastal areas experience milder climates. Despite the growth of the population, the world does not have enough food to feed its population adequately. All varieties of grain, vegetables, fruit, and forestry products can be produced in great abundance in Turkiye due to the vastness of its land resources and its climatic characteristics. The most significant crops in Turkiye are cereals, particularly wheat and barley (Öktem, 2004). Fruit science is an important agricultural sector, as the demand for fruits is increasing gradually with the growing world population. The improvement of fruit crop varities, being an essential part of a healthy diet and a considerable source of nutrients, has attracted the researcher's interest. According to 2020 data, Turkey's fruit production is 23 million 585 thousand 768 tons. Considering the production amounts of important products among fruits, apricots decreased by 4%, grapes by 12.8%, and cherries by 4.8%, apples increased by 4.5%, strawberries by 22.4% and olives by 32.1% compared to 2019. Tangerines from citrus fruits increased by 14.7%, oranges by 30.6%, lemons by 30.4%, and hazelnuts from hard-shelled fruits increased by 2.9%, while pistachios decreased by 59.7%. While banana production increased by 21.3%, fig production remained unchanged at 320 thousand tons (TUIK, 2022).

Biotechnology is an interdisciplinary field of science that encapsulates a broad range of disciplines such as microbiology, human and plant genetics, agriculture/plant, virology, immunology and genetic engineering. It plays an important role in the health sector, seed and crop management, yield improvement, soil management, cellular processes, ecology and bio-statistics (Dundar and Akbarova, 2011). Therefore, in this article, we compiled and reported the latest studies being conducted by Turkish and world biotechnologists to counter the above-mentioned constraints and to better understand the genetic resources and germplasm conservation of Turkish fruits.

Advanced DNA markers used for improved fruit cultivar in Turkiye/world

In recent years, it has been observed that biotechnology studies have been adapted to breeding studies carried out on many plant species. Especially by adapting molecular techniques to breeding processes, it is possible to shorten the process and make a selection in terms of some characteristics in the early period (Simsek et al., 2017). With the use of molecular techniques, which have been rapidly developing in recent years, in plant breeding, many benefits can be achieved. Molecular markers used in QTL (Quantitative Trait Loci) analyses, genetic mapping, identification of cultivars, protection of newly developed varieties, determination of genetic relatedness, purity analysis in seed breeding, characterization of gene sources, understanding the structure of the genetic source, identification of duplicate genotypes, reorganization of genetic source and breeding (YORGANCILAR et al., 2015). Molecular markers are important tools in plant breeding and can be divided into two main groups: markers based on hybridization RFLP - Restriction fragment length polymorphism), and markers based on polymerase chain reaction (PCR) AFLP- Amplified fragment length polymorphism, RAPD - Random amplification of polymorphic DNA, SRAP- Sequence-related amplified

polymorphism, ISSR- Inter simple sequence repeat, CAPS- Cleaved Amplified Polymorphic Sequences, SSR- Simple sequence repeat, SSCP- Single-stranded conformation polymorphism, SNP- Single nucleotide polymorphism.

Among these molecular techniques, SSR markers with a high frequency of use stand out. SSR markers are composed of 1-6 bp nucleotide groups that are sequentially repeated on the chromosomes of higher organisms. In the SSR, regions of the genome with repeated base sequences are replicated. Polymorphism occurs according to the number of repeats, and each band representing a different number of repeats indicates a different allele. The strands to the right and left of the repeated DNA are specific to that sequence, that is, specific (Şimşek et al., 2018). RAPD markers are defined as the amplification of randomly scattered regions of genomic DNA using simple, short oligonucleotide primers (Williams et al., 1990; Vos et al., 1995) developed the AFLP method by utilizing the principles of the RAPD-PCR method. The reproducibility and polymorphism level of the AFLP technique is higher than those of RAPD-PCR method. The ISSR method is based on the random distribution of nucleotide units such as 2, 3, 4, and 5 repeating in eukaryotic genomes in a locus-independent manner but is much more sensitive and reproducible than the RAPD method.
Some molecular marker studies carried out in fruits in the last 10 years are presented in (Table 1).

Table 1. Some molecular marker studies carried out on fruits in the last 10 years

Fruit name	Techniques	Aim	References
Mrytle	SSR	Development of SSR marker by RNA-seq	(Şimşek et al., 2022)
Strawberry	ISSR	Genetic diversity of hybrids and their parents	(Saridas et al., 2021)
Citrus	ISSR, SSR, SSCP	Genetic diversity of mutant mandarin and lemon varieties	(Sülü et al., 2020)
Apple	SSR	Genetic diversity	(Mažeikienė et al., 2019)
Grape	SSR and SNP	Genetic diversity	(Ebadi et al., 2019)
Kiwifruit	SSR	Genetic diversity	(Liao et al., 2019)
Grape	SSR	Genetic relationships	(Dong et al., 2018)
Cactus pear	RAPD	Molecular characterization	(TÜTÜNCÜ et al., 2016)
Citrus	SSCP	Genetic relationships	(Kacar et al., 2014)
Strawberry	SSR	Linkage map	(van Dijk et al., 2014)
Apple	SSR	Genetic diversity	(Patzak et al., 2012)

Tissue culture/ Genetic Engineering used for improved fruit cultivar in Tukiye/world

Genetic engineering has offered new solutions for the development of new varieties and rootstocks, the selection of desired traits and the creation of genetic diversity in plants. Thanks to plant genetic engineering applications, it has been possible to transfer a foreign DNA segment encoding specific genetic information from donor organisms to recipient plant species.

Gene transfer is made to plants for the improvement of nutritional quality and plant breeding (provide resistance to diseases Inhibiting or revealing some functions by silencing genes, obtaining cultivars tolerant to abiotic stress factors, obtaining varieties that are tolerant to herbicides, insects and viruses. Gene transfer methods to plants are divided into two catagories: direct and indirect gene transfers. Direct gene transfer methods include Biolistic, Electroporation, Sonication, Desiccation, and Gene transfer via Pollen Tube. Indirect gene transfer is carried out by *A. tumefaciens* or *A. rhizogenes.* The production success of transgenic plants depends on several factors; i) stable transfer of foreign DNA into the plant cell genome, ii) direct or indirect method of plant regeneration from transformed cells, iii) detection of transgenes from a population of non-transformed individuals (Somers et al., 2003).

Agrobacterium-mediated transformation is an effective and universal tool for broad-spectrum genetic modification of plant species. The Ti (tumor-promoting) plasmid of *A. tumefaciens* is responsible for crown tumor growth in suitable host plants. The T-DNA region of the Ti plasmid is transferred into the nucleus of the host plant and stably integrated into the nuclear genome (AKASAKA et al., 1998). The T-DNA transfer process begins when *Agrobacterium* detects certain phenolic and sugar compounds from injured plant cells. These phenolic compounds serve as stimulators of bacterial vir genes. Thus, *Agrobacterium* overwhelms some of the plant's defenses and uses these compounds to signal the presence of potentially susceptible plants (Gelvin, 2000). After the discovery of the Agrobacterium mechanism, gene transfer studies have been carried out in many fruit species. Some recent studies on gene transfer to fruits by *Agrobacterium* are presented in (Table 2).

Table 2. Some recent studies on gene transfer to fruits by *agrobacterium*

Fruit name	Aim	References
Citrus	Improving the gen transfer procedure	(Li et al., 2022)
Apple	Development of pathogen tolerant plants	(Dong et al., 2022)
Citrus	Development of salt-tolerant plants	(Abou and El-Banna, 2021)
Apricot	Improving the gen transfer procedure	(Pérez-Caselles et al., 2021)
Strawberry	Development of pathogen tolerant plants	(Ma et al., 2021)
Grape	Improving the gen transfer procedure	(Nakajima et al., 2020)
Citrus	Development of abiotic stress tolerant plants	(Hasan et al., 2019)
Sweet cherry	Improving the gen transfer procedure	(Zong et al., 2019)
Banana	Development of oxidative stress tolerant plants	(Tiwari, 2019)
Apricot and plum rootstock	Improving the gene transfer procedure	(Mourenets et al., 2018)

Most of the fruit species are hard to investigate at the molecular level because of their perennial nature, which makes the development of linkage maps and mapping populations a difficult task. Therefore, the genome sequence is a fundamental prerequisite for completely understanding the gene functions in genomics-based systems biological approaches,and effectively using induced and inherited genetic diversities of plant species (Sattar et al., 2021). The recent advancement of high-throughput sequencing techniques has revealed important genomic information on various traits of fruit crops. Furthermore, sequencing of many plant

species leads to the understanding of physiological processes including juvenility, ripening, blooming, shelf life and molecular mechanisms of many plant genomes. Initially, plants with smaller genome sizes were sequenced because of the limited techniques available to scientists (Sharma et al., 2016). The emergence of technological developments and the advancement of bioinformatics tools and sequencing approaches in the last decade have led the focus of biologists beyond the limits. This paradigm has been changed completely with the advent of next-generation sequencing (NGS) approaches. Next generation sequencing (NGS) was first used for genome sequencing of Arabidopsis thaliana in 2000, which laid the foundation for an expansion in genomic research and utilization of annotated genes for exploring orthologous genes of other plants. Though genomic studies of fruit plants are complex however, with the availability of NGS techniques such as Illumina SILiD Helicose and FLX-454, a huge resource of fruit plant genomes is generated (Barabaschi et al., 2012; Quail et al., 2012). Although next-generation sequencing (NGS) is a cost-efficient method when compared with first-generation sequencing, certain sequencing errors may still occur in homopolymer regions on some NGS platforms involving short sequence read length and Ion Torrent PGM leading to imprecise sequencing (Grada and Weinbrecht, 2013; Quail et al., 2012). Regardless of these limitations fruit plant breeders are suggested to utilize wide genomic information fruit varietal development programs in multidisciplinary fields of bioinformatics and molecular biology.

NGS and its promising aspects

During the last 20 years, whole genome sequence drafts of various plant species were characterized through NGS techniques, thus laying the foundation of genomics. Advances in sequencing technologies enable decoding important genomic information of many complex genomes of plant species such as barley, cotton, maize, wheat, sugarcane, etc. with less time and expense. NGS also helps in the exploration of biological niche, species abundance and minor species that are crucial for food system security. Plant scientists can access this genomic information to find new possibilities for solving existing problems and for developing more resistant varieties of cultivated crops (De Coster et al., 2021; Marx, 2021; Tedersoo et al., 2021). NGS can generate huge amount of data to discover new genes for expression profiling in several plant parts for inducing specified improvements. Analysis of expression patterns helps to uncover many regulatory processes at the molecular level under environmental stress conditions (Barabaschi et al., 2012). Thus, NGS technologies pave the way for exploring new genetic resources, characterization of desired genes, improving genome editing, and designing SNPs, SSRs and EST markers in plant species for sustainable production of crops (Ashraf et al., 2022).

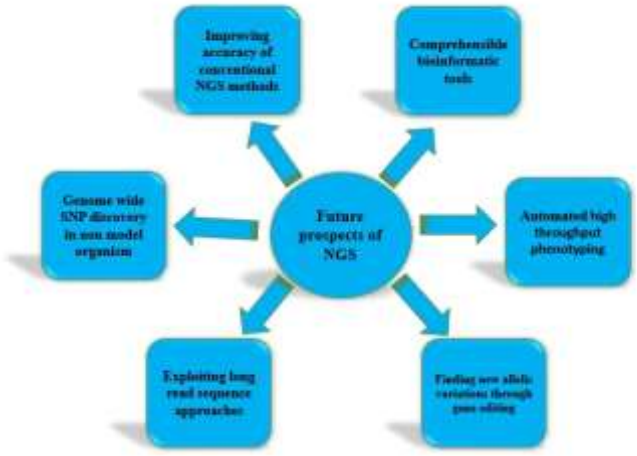


Figure 1. Future Prospects of NGS.

Applications of NGS in Genetics

Transcriptome profiling

The initial step in determining functional genes in a species is to sequence a species' transcriptome. Transcriptome sequencing, whether by first-generation Sanger sequencing or high throughput NGS techniques, provides information on gene expression in a specific tissue or during various growth processes of a species. A huge volume of sequence information is a valuable resource for identifying sequence variants for the construction of different markers, facilitating the localization of candidate genes/QTLs for required characteristics (Shan et al., 2013). A large number of comparative and functional genomics investigations have employed sequence-based molecular markers for t their favorable traits, such as genome-wide distribution, the specific position of chromosomes-dominance and reliability. Large amounts of information are generated by high-throughput NGS methods, proving this to be an ideal technique for understanding many sequence variants in the genome or transcriptome of crop plants. For instance, various programs for SNP identification including Atlas (Shen et al., 2010), GATK (De Pristo et al., 2011), SOAPsnp (Li et al., 2009) have been applied frequently. Sim et al (Sim et al., 2012) has developed an SNP genotyping array on large scale utilizing 8784 SNPs based on the transcriptome sequence of six genotypes obtained via the NGS technique. Barchi et al (Barchi et al., 2012) performed mapping of anthocyanin-associated QTLs in eggplant with the help of intraspecific and interspecific linkage maps, using restriction site associated DNA strategy in combination with Illumina sequencing for generating SNPs. In another study NGS SOLID, 5500 was applied for developing intron targeting markers to derive tetraploid potato transcripts and tested them on other genotypes of potato in Solanaceae (Ahmadvand et al., 2014).

Epigenomics during the age of NGS technologies

Even though traditional breeding programs have increased food production, still plant breeding heavily relies on molecular breeding techniques for crop improvement and to generate cultivars that are climate robust, resistant to biotic stress, highly nutritive and productive. Advancements in NGS techniques facilitate the variable phenotypic studies and epigenetic modifications for the improvement of crop breeding strategies. Conrad H. Waddington used the word "epigenotype" to describe a collection of interconnected developmental pathways that allow one genome to generate several epigenomes, and subsequently, a variety of cell types that make up the entire organism. The word "epigenetics" now refers to a wide range of genetic changes that do not occurs with DNA modifications in sequences but through chemical alterations to the DNA (cytosine methylation) or to histones (e.g., acetylation, methylation) that affect chromatin structure and function (Law and Jacobsen, 2010). Nowadays small RNA are playing a key role in the regulation of epigenetic modifications across the whole plant genome technologies now provides insight into millions of SNPs from the whole genome and transcriptome sequencing data, as a result, many high-throughput genotyping systems were created for the large-scale genotyping of SNPs on a large scale, some of them includes SNPStream (Bell et al., 2002), GoldenGate Genotyping Technology, Illumina (Oliphant et al., 2002), KASPar, and GeneChip (Nijman et al., 2008).

NGS and Translational research

Transcriptome studies in crops of the Solanaceae family revealed certain transcription factors linked with the development of fruits. 632 linkage-specific genes were identified, among which 343 are linked to potatoes and 289 are specific for asteroids (Massa et al., 2011). In

tomato, NGS techniques differentiate many SNPs in beef type tomato and cherry type tomato showing the relationship of cherry type tomato with a wild type S. *pimpinellifolium* because of similarities in SNPs in both species. Many SNPs present in a chromosomal region that can alter the QTLs related to fruit size, color, shape, etc. can also help in exploring other fruit characteristics (Silva et al., 2014). Pungency is an economically important trait of fruits specifically present in pepper and was extensively studied and explored with NGS technology providing detailed insight about associated genes with pungency. Transcriptome profiling reveals three genes dihydroxy acid dehydratase, prephenate aminotransferase and Thr deaminase which are involved in biosynthetic pathways in capsaicinoid (Lejeune et al., 2003). Comparative analysis of capsaicinoid potato and tomato showed different expression of pathway genes at adult stages 16 DPA, 25DPA of pepper fruit, however, the orthologous genes in tomato and potato are not fully expressed (Kim et al., 2014).

Single-nucleotide variations (SNVs) for potato virus Y and pepper mottle virus were found in resistant and susceptible pepper populations using NGS technology. The SNVs were aligned with tomato syntenic region/loci using comparative genomic techniques. Subsequently, to identify the potyvirus resistant 4 (pvr4) locus, the SNVs were transformed into PCR-based CAPS (cleaved amplified polymorphic site) markers. Substantial Marker Assisted Selection (MAS) algorithms can utilize these molecular markers (Devran et al., 2015). Comprehensive transcriptome analysis of tomato seedlings treated with exogenous ABA confirmed the presence of several genes involved in diverse responses to stress (Devran et al., 2015). It involves several transcription factors, pathogenic resistance pathways and heat shock proteins. Besides that, exogenous ABA increased the activity of the ethylene, jasmonate, and salicylic acid signaling pathways. Furthermore, the Sly-miR169c, a candidate of the miR169 family, was overexpressed in the tomato transgenic lines. Decreased stomatal opening, a slower rate of transpiration, less water loss, and improved drought tolerance were all characteristics of the transgenic plants (Zhang et al., 2011).

Genome sequence status in fruit crops

*Grapefruit (*Vitis vinifera) is the first fruit crop whose genome was sequenced by an international grape genome project (IGGP) (Barabaschi et al., 2012; Quail et al., 2012). The genome size of Vitis vinifera was estimated to be 504.6 Mb and 447.1Mb of corresponding genome sequences were accumulated in 2093 metacontigs, whereas 435.1 Mb were assembled in 19 linkage groups. The predicted gene number was 29585, among these 96.1% were related to linkage groups (Velasco et al., 2007). About 1,751,176 SNPs are mapped on the chromosome of them few were recognized in 86.7% of anchored genes. A detailed description of grapevine genome sequence opens new prospects for molecular breeding programs making it feasible to introduce required traits and resistance genes to the existing fruit cultivars (Sharma et al., 2016).

Papaya *(Carica papaya)* is the second fruit crop sequenced through the Hawaii papaya genome project and is an exceptional system for exploring genomes of tropical fruits. Genome size is relatively small consisting of 372 megabases. A female plant of SunUP which is a transgenic cultivar generated through Sunset transformation was reported to contain 2.8 million WGS reads. Genome sequencing of papaya makes this crop the best transgenic fruit crop among the characterized commercial crop varieties. Since the assembled genome contains 92.1 percent of the unigenes and 92.4 percent of the mapped genetic markers, the number of anticipated genes in the papaya genome might be 7.9% greater, or 24,746, and around 11-20% smaller than Arabidopsis. In papaya, sex determination is controlled by pair of sex chromosomes having a smaller male-specific Y chromosomal region 8. The number of genes involved in ethylene synthesis is similar as in Arabidopsis (Ming et al., 2008).

The cultivated apple *(Malus domestica)* is the primary fruit crop in temperate regions around the world. Velasco et al., 2010 showed that a recent (>50 million years ago) genome-wide duplication (GWD) in the Pyreae resulted in the shift from nine ancestral chromosomes to 17 chromosomes. A phylogenetic analysis of Pyreae and the genus Malus concerning key Rosaceae taxa indicated *M. sieversii* as the parent of the cultivated apple. A three-way sequence alignment performed between expected gene space in apple (84 Mb) and experimentally derived EST data from pear (14.9 Mb) and peach (18 Mb) suggests that the genetic distance between members of Rosaceae increases from apple to pear to peach, based on DNA sequence divergence per base pair. A comparison of estimated values of gene spaces in apple and pear fruit cultivars gives a value of 96.35 percent nucleotide similarity was calculated for these two species of the tribe Pyreae. Whereas the nucleotide similarity of grapes with apple and pear fruits was estimated to be 85.21% (Sharma et al., 2016).

Woodland strawberry *(Fragaria vesca) is an experimental plant having a small genome of 240 Mb sharing genetic identity with cultivated strawberry and some other fruit plants of family roasacea. 34,809 genes were identified through gene prediction and most are suitable for transcriptome mapping (Shulaev et al., 2011).*

Pear *(Pyrus bretschneideri Rehd)* is a member of the subfamily Pomoideae and is the third most important fruit crop in the temperate region after grapes and apples. The majority of the cultivated pear fruit cultivar are diploid (2n=34). The genome size estimated through sequencing is 512.0 Mb which corresponds to 97.1% of the genome size of heterozygous species. Protein coding genes of pear are 42,812 and approximately 28.5% of the genomes is encoding various isoforms. Repetitive sequences of this fruit are 271.9 Mb long and account for 53.1% of the total genome size (Wu et al., 2013).

Sweet oranges *(Citrus sinensis)*, a significant nutritional source and an economically important fruit cultivar are identified as a diploid plant with 9 chromosomal pairs having a genome size of 367Mb. The total estimated assembled sequence of orange is 87.3% which is fairly compact, whereas 20% of the genome contains repetitive sequences. Genome sequencing data identifies 1.06 million SNPs and 176,953 indels of which 80% are reported to be found on 9 pseudochromosomes. The predicted number of protein-coding genes is 29,445. Genome sequencing of orange cultivars proved to be a valuable resource for the improvement and understanding of other citrus varieties (Xu et al., 2013).

Date palm *(Phoenix dactylifera L.)* is another cultivated fruit plant with great nutritive and economical value. There are only a limited number of genomic studies conducted on this fruit cultivar until now. Recently, a report on assembled genome based on Illumina GAII sequencing data is generated by researchers. The estimated genome size of the date palm is 658Mb, the predicted gene number is 25,059 and the total assembled genome is 58% (Al-Dous et al., 2011). Another transcriptomic study was performed through pyrosequencing data from the Roche GS-FLX Titanium platform on the mesocarp of the fruit (Bourgis et al., 2011). Genomic and transcriptomic studies on large scale open the way for genomic studies of all fruit cultivars in the family Arecaceae.

Peach *(Prunus Persia)* a genetically characterized fruit plant species has been under breeding practice for more than 4000 years. The genome of peach is of high importance in research studies of both fruit and forest trees. Verde et al., 2013 have described the genome sequence of peach fruit in homozygous peach genotype obtained through the Sanger whole genome sequencing shotgun method. They presented High-quality whole genome assembly by the shotgun method in peach (2n=2x=16) and estimation of genome size which is 265 Mb.

27,852 protein-coding genes and 28,689 protein-coding transcripts were reported. Among these genes, 24,423 were homologous to Arabidopsis of which 18,822 possess Swissport homologs and 26,731 are having TrEMBL homologs. Phylogenetic studies on annotated gene families of peach cultivars allow the identifications of similar genes in other sequenced fruit species involved in metabolic processes such as metabolism, transport, aroma and comparison with other species in the rosacea family. Homologous subgenomes in peach fruit and grapes are derived through similar paleohexaploid procedures and these events occur before the arrival of rosaceae.

Pear (Pyrus) is the oldest fruit crop commonly grown in China and Europe since antiquity. Genome assembly draft of European pear was presented by Chagné et al., 2014. Genome assembly was performed by applying second-generation technology Roche 454. It covers 577.3 Mb of the total genome representing the whole 600Mb genome of Pyrus. The predicted gene number is higher than many plant species because of duplication of the whole genome in pyreae. Proteomes of all 13 species contain 5,350 protein clusters and 14,348 were predicted as European pear fruit proteins.

Mango *(Mangifera indica)* an important fruit crop of tropical regions belonging to the family Anacardiaceae is admired worldwide for its exceptional nutritional value and taste. Next-generation sequencing plays a significant part in gene expression analysis of mango fruit and in generating sequencing datasets through sequencing platforms like Illumina HiSeq 2500, Illumina NextSeq 500, Illumina HiSeq 2000, etc. Genomic information obtained through these NGS techniques will help in the selection of desired fruit traits in mango cultivar including taste, huge production, and variability (Zahid et al., 2022). It is an allotetraploid (2n=40) fruit having a small genome size of 450 M bp size. Recently, a genome assembly draft containing 412,728 contigs was presented which covers around 95% of the complete genome. The number of predicted genes is 63,130 with 711 bp length. Furthermore, an assembled draft of 1, 96,362 SSRs are also presented (Singh et al., 2014).

Table 3. Next generation sequencing status in fruit cultivars

Fruit cultivar	Genome size (Mb)	Estimated number of genes	Sequencing techniques	References
Grapevine *(Vitis vinifera)*	504.96	29,585	Highly efficient sequencing through synthesis and Sanger shotgun sequencing.	(Velasco et al., 2007)
Papaya *(Carica papaya)*	372 Mbp	28,629	Whole genome shotgun sequencing	(Ming et al., 2008)
Apple *(Malus domestica)*	742.3 Mbp	57,386	Whole genome shotgun sequencing	(Tedersoo et al., 2021)
Woodland strawberry *(Fragaria vesca)*	240 Mbp	34, 809	Illumina, SOLiD and Roche/454 Platforms.	(Shulaev et al., 2011)
Pear *(Pyrus bretschneideri Rehd)*	512 Mb	42,812	NGS and BAC by BAC	(Velasco et al., 2010)
Sweet Orange *(Citrus sinensis)*	320.5 Mb	29,445	Illumina Gall platform, Whole Genome shotgun method, paired-end tag sequence read.	(Wu et al., 2013)
Peach *(Prunus Persia)*	265 Mbp	27,852	Sanger Whole genome shotgun sequencing	(Verde et al., 2013)
Datepalm *(Phoenix dactylifera L.)*	605.4 Mb	41, 660	BAC end sequencing, SOLiD, Roche/454 and Illumina platforms	(Al-Mssallem et al., 2013)
Mango *(Mangifera indica)*	450 Mbp	63,130	PACBio sequencing	(Singh et al., 2014)

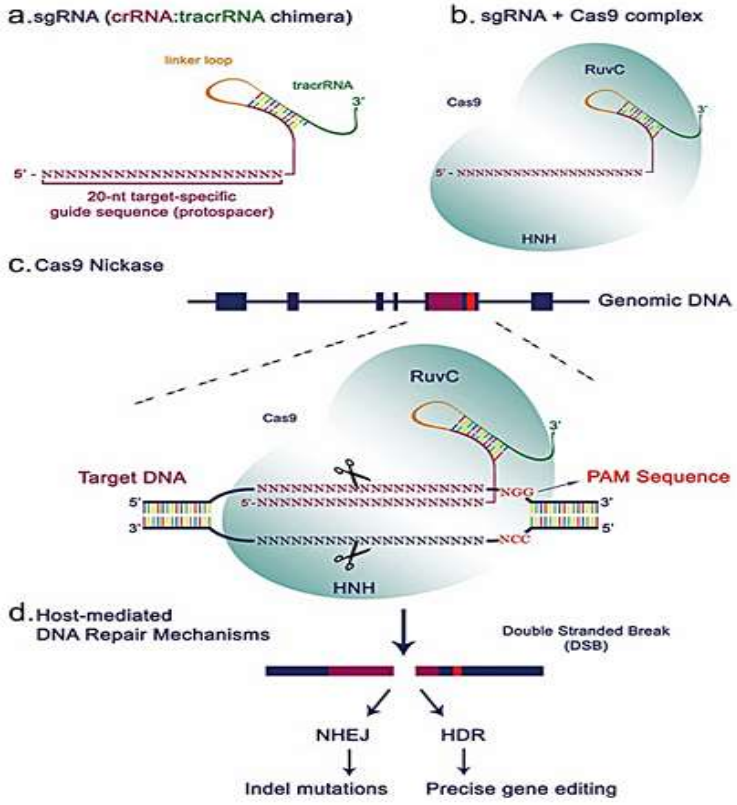
Gene Editing and CRISPR/Cas9 used for improved fruit cultivars

Gene editing system is a breakthrough approach with high potential for species where classical plant breeding techniques have not been thriving or for species and varieties which are recalcitrant.

CRISPR-Cas9 System

Prokaryotic bacteria and archaea have been found to possess the clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) system which is known as an adaptive immune system, which can fight off invading nucleic acids. The CRISPR/Cas system-mediated genome editing has been commonly used in agricultural production owing to its simplicity, adaptability, and high efficiency. A Cas9 effector protein and an easily modified guide RNA (gRNA) are the main two components of the successful CRISPR/Cas9 system (Cao et al., 2021). The CRISPR array and Cas9 nucleases are the two arms of this system. CRISPR array is a sequence made up of 50 bp repetitions and similar-length unique spaces. Moreover, Cas9 is the nuclease protein linked with CRISPR which aids in the mechanisms that degrade foreign DNA (Tyagi et al., 2021). The development of CRISPR/Cas technology has already had a significant impact on the life sciences, particularly in the fields of medicine and

biotechnology. It also provides a cutting-edge, potent complement (or alternative) to the traditional method of plant breeding for trait improvement. It is not surprising that there has been interest in using various CRISPR/Cas technologies in recent years to create plants which are durable and suitable for resistance of given plant viruses or viral pathogens which cause plant devastating and losses of crop yield (Açar and Kaçar, 2021). Cas endonucleases can be utilized to specifically target viral DNA or RNA, inactivate host susceptibility genes, and develop plant resistance to viruses (Kalinina et al., 2020).



Source: (Demirci, Zhang, & Unver, 2018)

Figure 2. The CRISPR-Cas9-mediated gene editing diagram.

Delivery systems for CRISPR/Cas9 reagents

Furthermore, for delivery into plant cells, CRISPR/Cas9 cargo can be packaged in various shapes such as Cas mRNA which is provided with a separate sgRNA, and DNA plasmids that encode both the sgRNA and Cas protein, and ribonucleoproteins (RNP). Additionally, protoplast transfection, biolistic delivery, *Agrobacterium*-mediated gene transfer, usage of nanoparticle platforms, and plant viral vector-mediated delivery are some of the current techniques to introduce CRISPR/Cas reagents into plant cells (Kalinina et al., 2020). Moreover, the mutation efficiencies are depending on the GC content of the sgRNA, Cas9 protein expression levels, and the number of transformed cells obtained in transgenic cells. For instance, the range of 80–100% has been reported as CRISPR-Cas9 efficiency in rice or *Arabidopsis* species and in *Cucurbitaceae* species it ranged from 20–100%, and the level of frequency in mutation efficiency of banana was 59-100% (Erpen-Dalla Corte et al., 2019), clearly diploid and octoploid strawberry effected in albino phenotype at a high frequency of 50-88% (Wilson et al., 2019), sweet orange 64.7% has been reported (Peng et al., 2017). In general, the application and optimization of CRISPR/Cas9 have been considered to invest the larger

effort in *Arabidopsis* or rice compared to the fruit crops, and cucurbit plants, then the CRISPR/Cas9 efficiency in fruits can be known as durable, suitable, and acceptable (Hooghvorst and Nogués, 2020).

Application of CRISPR/Cas9 in fruits

The CRISPR-Cas9 system has been successfully applied for gene editing in various fruit crops such as citrus, cocoa, apples, bananas, grapes, pears, and kiwi. Distinct approaches have been assessed for optimizing the CRISPR/Cas9 approach for its application in fruit varieties (Erpen-Dalla Corte et al., 2019). The CRISPR-Cas9 tool is frequently applied to investigate complete genetic modification and gene functions. Current studies have initiated the newest advancement of the CRISPR/Cas9 technique in fruit development for example fruit quality and the modification of targeted gent. For instance, the CRISPR/Cas9-mediated targeted mutagenesis of fruit ripening inhibitor gene (RIN), SlMYB12, pectate lyases (PL), lycopene desaturase (PDS), and CLAVATA3 (CLV3) can affect fruit size, fruit bioactive compounds, fruit coloration, fruit texture, and fruit ripening, (Xu et al., 2020).

Gene editing application for fruit bio-active compounds and fruit ripening

The flesh fruit goes through a developing process and results in an irreversible maturation stage. Many physiological, structural, and biochemical changes have observed during the ripening process of fruit, and it may attract much more seed spreaders (Gapper et al., 2013). Generally, when the fruit reaches the optimum edible stage, it slowly starts deterioration process begins, and the fruit quality reduced. Therefore, the regulation of fruit ripening has become the focus of many scientists (Martín-Pizarro and Posé, 2018). Anthocyanin, lycopene, carotenoid, and gamma-aminobutyric acid (GABA) are the main functional factors of fresh fruits bioactive compounds. Hence, the increase of bioactive substances in fruit has been focused on by several studies (Patel et al., 2015). For instance, lycopene desaturase (PDS) is an essential enzyme for the accumulation of lycopene and carotenoids (Bai et al., 2016). Furthermore, the successful mutations PDS1 and PDS2 of banana cv. Rasthali is produced by the CRISPR/Cas9 by inserting a termination codon into the gene sequences. Authors reported that the mutants' total carotenoid and chlorophyll contents have decreased (Kaur et al., 2018).

CRISPR–Cas9 gene editing in grapevine and apple

Genomic sequences extracted from T0 apple shoots were examined for targeting the endogenous apple PDS gene using a single gRNA to confirm the altered sequences at the target loci in transformants caused by CRISPR/Cas9. The target site sequence in apple affected the effectiveness of genome editing. It should be highlighted that the apple plantlets had an almost flawless mutation in the first generation at 13.6%. The results of the sequence analysis showed that all apple PDS mutant alleles had insertion or deletion like +1 to -8 bases independent of the target site (Nishitani et al., 2016). Furthermore, the introduction of targeted mutations into significant agricultural plants, such as apple and grape, as well as the availability of whole genome sequencing from plant species have changed the ability to both examine genetic changes and add new capabilities. The study outlined the techniques for adapting the CRISPR-Cas9 system to apple and grapevine plants. The delivery of CRISPR-Cas9 ribonucleoproteins (RNPs) was made directly to apple and grapevine protoplasts in addition to employing plasmids for genome editing (Osakabe et al., 2018).

Currently, the target gene IdnDH (L-idonate dehydrogenase) controls the tartaric acid (TA) content accumulation in the cells of grapes, and it was chosen as the target gene to confirm the effectiveness of the CRISPR-Cas9 system in Chardonnay (*Vitis vinifera* L.) in the study

carried out by Ren et al. 2016, by using *A. tumefaciens* to transform Chardonnay suspension cells, two distinct expressions of sgRNA cassettes were HR-inserted into the dual pCACRISPR-Cas9 vector. The transformation level of 37.78% has been found in the cell mass (CMs) after the insertion of exogenous T-DNA was detected by PCR. Sanger sequencing and CEL I endonuclease assays demonstrated that the transgenic CMs and plant's target-editing was successfully directed by the sgRNA1. Targeting two different L-idonate dehydrogenase gene locations with single guide RNAs (sgRNAs) (IdnDH). The target site's indel mutations have been defined by CEL I endonuclease assay and sequencing, and the transgenic CM and matching regenerated plant that expressed sgRNA1/Cas9 both showed the frequency of mutation was 100%. Among the transgenic CM mutations that were found, 1-bp insertions and the deletions of 1- to 3-nucleotides predominated (Osakabe et al., 2018). Besides, *VvWRKY52* targeted gene for botrytis cinerea resistance character has been examined in grape by applying *Agrobacterium*-mediated somatic embryo transformation. Additionally, in apple varieties by utilization of the PEG-mediated protoplast transfection, the DIPM1, DIPM2, and DIPM4 genes were analyzed for resistance to fire bright disease. In bananas also PDS gene which is responsible for albino phenotype has been studied using two distinct methods of *Agrobacterium*-mediated suspension cells transformation and *Agrobacterium*-mediated embryogenic cell suspension cultures transformation. This targeted gene also has been evaluated in strawberries by applying *Agrobacterium*-mediated leaf and petiole transformation; in this plant also Auxin Response Factor 8 (FvARF8) and Auxin biosynthesis gene (FveTAA1) for auxin biosynthesis trait have been investigated by Agrobacterium-mediated transformation as delivery method (Malnoy et al., 2016).

CRISPR–Cas9 application in orange

For altering the PthA4 binding region in the CsLOB1 promoter of Wanjincheng orange, five CRISPR/Cas9 constructs have been created. Depending on the design employed, the mutation rate varied from 11.5 to 64.7%, and four of the altered lines demonstrated improved resistance to citrus canker. Using in vivo infiltration, the resistance of modified plants to canker citrus was further established. Up to 12 dpi, no pustules or canker signs were found in S2-6. Pustules and canker symptoms in S2-5 and S2-12 were much less common during the injection than in the wild type. Authors discovered that 42% of the mutant plants contained the required changes and 23.5% of those mutants displayed citrus canker resistance (Peng et al., 2017). The fortunate the utilization of Xcc-facilitated agroinfiltration to deliver Cas9, along with a synthetic sgRNA targeting the CsPDS gene, into sweet orange and the mutation level has been approximately 3.2 to 3.9% (Jia and Wang, 2014). Furthermore, the delivery method of *Agrobacterium*-mediated epicotyl transformation has been used to introduce the DMR6 resistance gene to huanglongbing in citrus (sweet orange).

Application of CRISPR–Cas9 gene editing in a pear

The improvement of CRISPR/Cas9 has been performed by using two distinct single guide RNAs (gRNAs) which have linked to Cas9 nuclease for knock-out the terminal flower 1 (TFL1) and phytoene desaturase (PDS) targeted genes in apple and pear. These gRNAs have been spaced under the control of U3 and U6 promoters of apple. The sequencing of target regions of pear, and apple CRISPR-TFL1.1 and CRISPR-PDS transgenic lines has demonstrated that the two gRNAs induced mutations but at variable frequencies. The chimeric biallelic edition profiles of the PDS and TFL1.1 genes were the most prevalent. Overall findings demonstrate that the CRISPR-Cas 9 system is a potent and exact tool for inducing mutagenesis which is targeted in the first generation of apple and pear transgenic cultivars, despite the frequent incidence of chimerism. The CRISPR/Cas9-mediated targeted mutation of the pear

gene (PcTFL1.1) with a level of elaboration of 9% of the obtained T0 plants has also been reported for the first time in this study. This mismatch may be the cause of the reduced rate of mutant phenotypes seen in pears (Charrier et al., 2019).

CRISPR/Cas9 system application in berry and citrus

In addition, to assess blueberry (*Vaccinium* spp.) genome editing using CRISPR/Cas9 has been performed. To improve the genetic transformation efficiency for 'Blue Muffin' and 'O'Neal' were firstly optimized for plant regeneration mechanism. Following a sequence analysis, it was discovered that CEN alleles had 1 to 2-bp insertions or deletions, with an average mutant allele ratio of 19% for gRNA1 and 22% for gRNA2, respectively. Two of the four gRNAs (gRNAs 3 and 4) did not result in mutations, indicating the importance of choosing the right gRNA sequences when editing the genome. The growth characteristics of the CEN-mutated lines suggest that the blueberry genome may contain a non-functional CEN allele that limits vegetative development. The outcomes discussed here support the usefulness of the CRISPR/Cas9 genome editing method for identifying the functions of blueberry genes (Omori et al., 2021). The work described how Cas9/sgRNA, a two-component system developed from CRISPR-Cas9, modified the citrus gene CsLOB1 in transgenic citrus with the help of the *Agrobacterium*-mediated epicotyl transformation process (Jia et al., 2019).

Application of CRISPR-Cas9 for abiotic stress response in the plant

Moreover, the authors produced the OST2 novel alleles, a proton pump in *Arabidopsis*, utilizing gene editing mediated by a truncated gRNA (tru-gRNA)-Cas9 combination with no off-target effects. Newly created mutations in CRISPR-Cas9 transgenic plants were found by monitoring the production of tru-gRNAs and Cas9 and 32.8% have been reported as high average levels of mutation. The mutation rate was lowered by decreasing nuclear localization signals in Cas9. Contrarily, the heritable mutation rate in *Arabidopsis* was boosted by tru-gRNA Cas9 cassettes driven by promoters specific to reproductive, and meristematic tissues, demonstrating high levels of expression in the germ line can result in bi-allelic mutations. Recently, stomatal closure in response to environmental factors was affected by the novel mutant alleles discovered for OST2 (Osakabe et al., 2016).

Current and future perspectives of various biotechnological approaches used for developed fruit cultivar

Biotechnological techniques such as genome editing techniques can introduce mutations of traits of interest into the plant genome (Osakabe et al., 2018). Consequently, high-throughput biotechnological methods such as genome editing, transcriptomic analysis, RNA interference, epigenomics, and virus-induced gene silencing approaches needed to be applied to Turkish fruit cultivars to expedite the progress of functional genomes for advanced breeding programs to increase fruit crop production. Classical fruit breeding technologies require a long time for production. However, advanced molecular breeding methods have assisted in developing and improving superior fruit cultivars for several traits, including disease resistance, fruit quality, quantity and resistance to abiotic stress factors. Molecular markers have been used in various fruit studies, all based on breeding studies as well as apricot (Gürcan et al., 2019; Gürcan et al., 2015; Özrenk et al., 2020; Pinar et al., 2013), apple (Aksu and Şahin-Çevik, 2022; Sevindik et al., 2019), orange (Juibary et al., 2021; Zanganeh and Sheidai, 2022), grapevine (Hizarci et al., 2013; Kaya et al., 2019; Kaya et al., 2016; F. Yılmaz et al., 2020; Yılmaz et al., 2022), strawberry (Çelik et al., 2017; Saridas et al., 2021). Furthermore, molecular markers have a greater contribution to fruit crop breeding as mentioned by various studies on breeding programs. For instance, GWAS has been applied to fruits (Zahid et al., 2022), and CRISPR

gene editing methods such as Cas9 also have been applied to various fruits (Dutt et al., 2020; Huang et al., 2020; Jia and Wang, 2014; Zhang et al., 2017). Nevertheless, Turkiye is a country where most fruits could be found, so further attempts should be assayed in Turkish species to achieve a higher genome editing efficiency, such as applying endogenous promoters of Cas9 and sgRNA expression to increase the ratio of fruit production. Furthermore, emerging genomic and biotechnological methods like functional genomics, marker-assisted breeding, genetic engineering, marker-assisted selection, and have been used to speed up the fruit breeding program because it requires a long period (32 weeks to provide transgenic embryos from a small-scale of embryogenic calli, during which somatic mutations constantly happen and abnormal plantlet are often created (Ren et al., 2016). Further studies are required to improve, develop, and increase fruit production in Turkiye to satisfy the population's demands and fulfill the gap in fruit breeding and production globally. There is a huge opportunity and need to enhance the width and impact of such an innovative breeding program. The genotyping-by-sequencing (GBS), next-generation sequencing (NGS), RNA sequencing, or transcriptomics technologies have resulted in significant breakthroughs in whole genome sequencing, which delivers ultra-high-throughput sequences that are altering plant modern breeding and genotyping. Additionally, the combination of molecular markers and high throughput sequencing approaches revealed more distinct applications to large-scale crop genomes such as fruits, vegetables, and wheat. The knowledge obtained by CRISPR/Cas9 system analysis for fruits will help to accelerate progress in fruit breeding studies to develop their production and obtain of new durable cultivars.

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STUDY ON DIFFERENT CULTIVATION DATES OF PEPPER AS EARLY FIELD PRODUCTION

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ABSTRACT

The optimization of agro-technical factors for managing the productivity of vegetable crops is importance. It is an indisputable fact that in recent decades global warming and global climate change have taken place. It is related to the adaptation of the terms for sowing and planting of vegetable crops in accordance with the changed ecological environment. Pepper is a vegetable species with high requirements for environmental conditions and especially for temperature and light conditions during its cultivation. Therefore, research is needed to optimize the timing of sowing and planting directly related to climate change. The main aim of the experimental work in this article is to study the different cultivation date in the production of early pepper in field condition. The experiments were carried out in the period 2019-2021, in the experimental field of the Department of Horticulture at the Agricultural University - Plovdiv. Sivria 600 variety was used. The experimental design was based on the block method Three variants of cultivation dates were tested. The results show that early sowing (February) contributes to the formation of better biometric indicators, justifying better vegetative development of plants and build an active photosynthetic leaf apparatus. In early sowing the plants develop under conditions closer to their biological requirements and there is a better synchronicity between their vegetative development, flowering and fruiting. Higher yield is obtained from plants with the early date of sowing. The difference with the yield from the second sowing date is 26.93%. The same plants also have a 35% higher yield than the first harvest.

Keywords: pepper, *Capsicum annum* L., early field production, productivity, yield,

INTRODUCTION

Capsicum annum is a valuable vegetable species of the Solanaceae family. The significant importance and distribution of this culture is due to its high biological value as human food and the great opportunities for its use in fresh and processed state. According to the recommendations for a balanced diet, the annual consumption of pepper per person in Bulgaria should be 20 kg (Cholakov, 2009). According to the Department of Agrostatistics of the Ministry of Agriculture (2019-2020), the open areas with this crop are 25000-30000 da/year. There are some differences between years. The total production varies from 63982 t for 2019 to 50992 t for 2020. Compared to 2019, the total production decreased by 20.3% and the harvested open areas by 15.1%. The average yields are relatively constant, respectively - 1827.6 kg/da for 2019 and 1822.1 kg/da. Basic production region is the South Central region with annual production, respectively 42359 t for 2019 and 32206 t for 2020.

Providing appropriate conditions for the growth and development of plants during the growing season helps to develop their maximum productive potential and is an important requirement for increasing the profitability of vegetable production. From the point of view of healthy and rational nutrition of the people, it is necessary to extend the period for supplying fresh produce and raw materials for processing. In this regard, the optimization of agro-technical factors for managing the productivity of vegetable crops is importance. It is an

indisputable fact that in recent decades global warming and global climate change have taken place. It is related to the adaptation of the terms for sowing and planting of vegetable crops in accordance with the changed ecological environment.

Pepper is a vegetable species with high requirements for environmental conditions and especially for temperature and light conditions during its cultivation. Therefore, research is needed to optimize the timing of sowing and planting directly related to climate change.

The scientific importance of the problem is outlined by the intensified research work in many countries (Erickson and Markhart, 2001; Turner and Wien, 1994; Ghovatia et al., 1993; Nahardani et al., 2013; Waterer, 1992; Islam et al., 2010; Zhang, et al., 2009; Dhaliwal et al., 2017; Mends-Cole et al., 2019; Sandhu et al., 2020). A review of research over the last 10 years clearly shows that the need to set appropriate timing for sowing and planting pepper is the subject of scientific interest from many leading scientists around the world. Similar studies have been conducted with other vegetable crops, such as Haque et al (2013) - potatoes; Dhaliwal et al (2017) - tomatoes, Prosanta et al (2013) - okra, Yaser et al. (2016) - garlic. In Bulgaria, research of the pepper is mainly aimed at breeding new varieties with high adaptability to changing environmental conditions. Technological research is limited.

The main aim of the experimental work in this article is to study the different cultivation date in the production of early pepper in field condition.

MATERIAL AND METHOD

The experiments were carried out in the period 2019-2021, in the experimental field of the Department of Horticulture at the Agricultural University - Plovdiv. Sivria 600 variety was used. It is typical representative of the varieties from the early pepper group and the main variety for the early field production.

The experimental design was based on the block method in 4 replicates with the size of the experimental plot of 10 m^2. The following variants were tested:

1. First date of sowing the seeds for seedling production - 14.02;
2. Second date of sowing the seeds for seedling production - 28.02;
3. Third date of sowing the seeds for seedling production - 21.03;

The plants were grown, using early field production technology, through seedlings (Cholakov, 2009).

In stages in May the planting was carried out. The cultivation scheme is 60 x 15 cm. For the optimal development of the plants, during the vegetation the necessary agro-technical practices, based on the applied technology, were carried out.

Phenological observations were carried out to determine the phases: beginning and mass: emergence (in days after sowing), appearance of: flower buds, flowering, fruit formation, fruiting. The beginning of the appearance of the corresponding phase was recorded when it occurred in 10% of the plants, and by mass - in 70%.

In the phase of mass fruiting, a morphological characterization of the plants was performed on 5 plants from each replicate, including: Plant height (cm); Weight of the vegetative organs of the whole plant (g); Number of branches; Stem dry mass (g); Weight of the stem (g); Number of leaves; Weight до leaves (g); Leaf dry mass (g); Leaf area (cm^2);

Productivity was determined based on the three harvests carried out, in the stage of technological maturity of the fruits.

The content of total chlorophyll was examined by measuring 5 well-developed leaves from three plants in each replicate at the beginning of fruiting phase with a SPAD-502 chlorophyll meter (Minolta, Japan).

The obtained results were processed mathematically by the method of variance analysis for one-factor field experiments (Dimova, D., E. Marinkov, 1999)

RESULTS AND DISCUSSION

Different seed sowing dates for the production of early pepper seedlings (Table 1) affect seed germination. For the longest time, the seeds sown in the earliest sowing date sprout - 20 days. With the improvement of the temperature conditions in the cultivation facilities used for the production of seedlings, the seeds of the second and third date germinate, respectively, in 14 and 11 days. The mass onset of the phase is observed 6-7 days after germination. The laying of the first generative organs of the seedling plants is between 48-46 days after mass germination. Together, without significant differences for the individual variants, mass flower buds also occur. The beginning of flowering for all plants is after 60 days from the mass germination, and about 10 days after the mass flowering the first fruits formed. Mass fruiting occurs after about 100 days.

Table 1. Phenological observations

Variant	Emergence		flower buds		Flowering		fruit formation		Fruiting	
	Beginning	mass	beginning	mass	beginning	mass	beginning	mass	beginning	mass
First data of sowing	20	26	47	57	63	71	71	79	97	101
Second data of sowing	14	20	46	56	61	70	70	79	95	103
Third date of sowing	11	18	48	55	60	70	67	79	92	106

The seedling period lasts 55-57 days. In this period, the plants develop their leaf assimilation apparatus, form their root system and set the first buds. Equal indicators of seedling plants can be explained by growing them in cultivation facilities, where the microclimate parameters are close to the biological requirements of the plants, and also the existing possibilities for applying differentiated care for them.

The main indicators characterizing the vegetative development of plants are monitored in two main phases of plant development: in the seedling phase - immediately before planting and in the phase of mass fruiting. These two phases provide information on the condition of the plants in the initial stage of their development and their ability to catch and adapt better in real field conditions. The good vegetative development of the plants in the phase of mass fruiting is a mandatory condition for the manifestation of the productivity of the plants – Flower buds, formation and growth of the fruits.

Regardless of the differences in sowing dates and the initial slow germination of the plants, at the end of the seedling period the plants of the different variants have similar stem parameters (Table 2).

Table 2. Morphological characteristics of pepper plants - stem, before transplanting

variant	weight of the stem (g)	Plant height (cm)	Number of branches	Stem dry mass (g)
First data of sowing	4.67	18.67	-	0.943
Second data of sowing	5.02	18.50	-	0.825
Third date of sowing	5.67	18.17	-	0.695

With very small differences in values are the mass and the height of the stem. It is being observed that in terms of dry mass of the stems, those of the plants from the first sowing date accumulated more biomass, compared to the plants of the other two variants.
A similar trend is observed in leaves and buds (Table 3). The number of leaves varies from 20.67 to 16.00, and their fresh weight varies from 5 to 9 g. The dry mass of the leaves is greatest in plants sown in March. Immediately before planting, the plants set their first buds.

Table 3. Morphological characteristics of pepper plants - leaves, before transplanting

Variant	Leaf weight (g)	Number of leaves	Leaf dry mass (g)	Number of buds
First data of sowing	5	16.00	1.043	1.67
Second data of sowing	7	19.30	1.082	1.00
Third date of sowing	9	20.67	1.117	1.00

According to biometric indicators, in all three variants of the experiment, seedling development was observed without deviations, so that standard plants were used for planting (Mihov et al., 2014). In the phase of mass fruiting, the effect of sowing date on plant height is insignificant (Table 4). Plants sown on 14.02 have the greatest height (67.50cm), followed by those sown on 28.02. The stem mass changes from 162.50 g for the first sowing date to 112.50 g for the third date plants. The plants from the first sowing date formed the highest number of branches (4.83). On the other two dates, the plants had 3.8 branches.

Table 4. Morphological characteristics of plants in the phase of mass fruiting

	Stem			Leaves			Fruits	
Variant	weight (g)	height (cm)	Number of branches	weight (g)	Number of leaves	Leaf area, cm^2	number	weight (g)
First data of sowing	162.50	67.50	4.83	220.83	201.61	6815.88	5.98	251.33
Second data of sowing	145.80	63.25	3.85	193.50	178.43	5743.65	4.18	201.00
Third date of sowing	112.50	61.50	3.83	151.67	150.28	4520.16	3.46	180.17

The highest number of leaves per plant (201.61) were formed by the plants on the first date of sowing, and the lowest (150.28) by those sown in March. Plants from the earliest sowing have

25% more leaves. Similar differences are reported in the total leaf area. Plants from the first date in February had over 33% more leaf area than March sown plants. The mass of the leaves follows the trend established for their number and leaf area. The fruits formed on one plant are the most when sown on 14.02 - 5.98 pieces, followed by plants sown on 28.02 - 4.18 pieces. The fewest fruits are formed in the plants sown in March - 3.46 pieces. Differences between the individual variants are also observed in terms of fruit mass. It is the highest in the first variant, followed by those in the second sowing date.
The chlorophyll content in the leaves is highest in the leaves of the plants with the latest sowing date in March (Fig. 1).

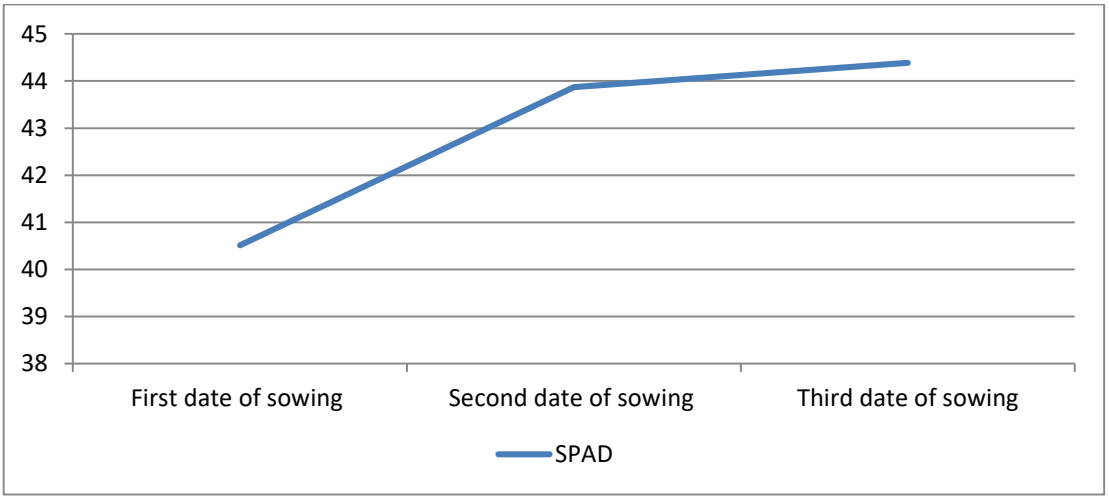

Figure 1. Chlorophyll content, mass fruiting phase.

It can be noted that the plants of the tested variants have normal vegetative development. There are no sharp deviations or lags in their development. The formed leaf assimilation surface and the high content of photosynthetic pigments are a prerequisite for the normal establishment of the generative organs and the formation of the economic yield.
Pepper sown on 14.02 emerged as the best in terms of higher number of fruits (8.73/plant) and maximum yield/plant (0.366 kg) and total yield (4073.959 kg/dka) compared to the best the late date (21.03) with a minimum number of fruits (5.75/plant) and fruit yield (0.299 kg/plant and 3322.190 kg/dka) (Table 5).

Table 5. Yield and yield components

variant	Weight of 1 fruit (g)	Number of fruits per plant	Yield from plant (kg)	Total yield (kg/dka)
First data of sowing	42	8.73	0.366	4073.959
Second data of sowing	48	6.25	0.300	3333.300
Third date of sowing	52	5.75	0.299	3322.19

The results show that sowing on 14.02 has the greatest positive impact on fruit yield/plant and is increased by 18.45% compared to the third date of planting (21.03) and 18.18% compared to the second date of sowing (28.02). The results obtained are similar to those published by Koner et al. (2015). The increase in yield with the sowing date of 14.02 may be

due to the presence of favorable environmental conditions for appropriate timing of flowering and subsequent transformation into fruits to achieve optimum yield (Nahardani et al., 2013). Oxley and Rivard (2014) also obtained the highest yield with early planting of pepper. The reduction in fruit number at later planting may be related to reduce concentrations of reducing sugars in flower buds and flowers, which may result in pollination inefficiency and cause flower and set drop under high temperature conditions (Wien et al., 1989; Erickson and Markhart, 2001). Taskovics et al. (2010) indicated that under high temperature conditions, fewer fruits develop and they remain malnourished. The obtained results of the experiment contradict this statement, because when sowing in March, the fruits have the highest mass of 52 g. Rather, our results fully correspond with the authors' conclusions, which substantiate these plant responses with the more suitable environmental conditions during fruit formation and growth.

At the first harvest, the largest amount of fruit was obtained from the first cultivation variant - 1131.338 kg, which is 27.77% of the total yield and more than 35% more than the realized yield from the variant with sowing on 28.02 (Fig. 2).

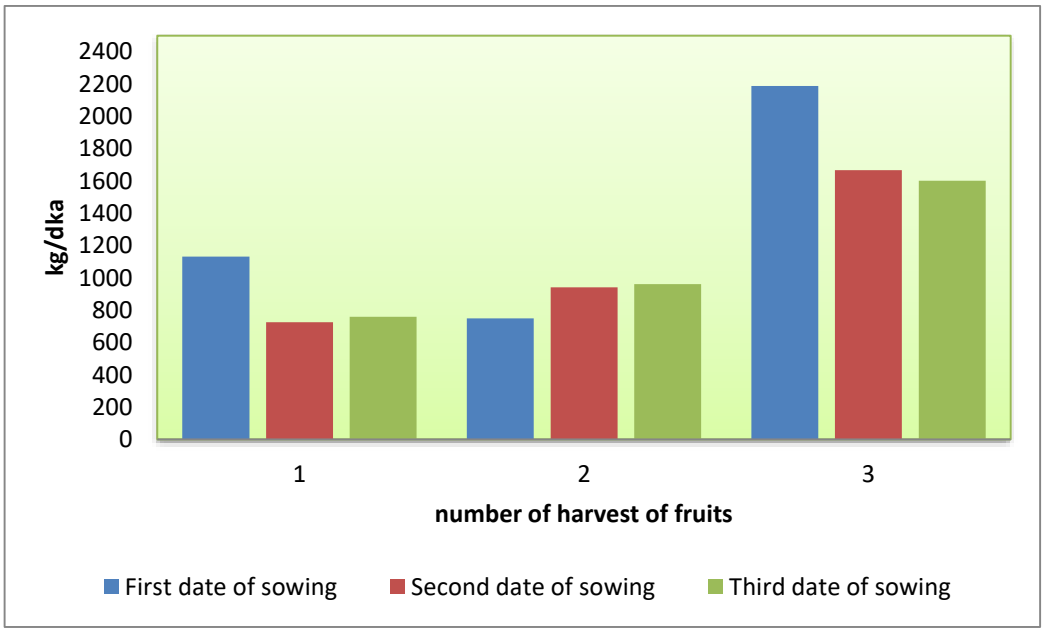

Figure 2. Fruiting dynamics

After the first harvest, in the best variant, a slight drop in yield was observed during the second harvesting of the plants. Then 18.40% of the total yield is realized for the variant with sowing on 14.02. In the other two variants, the yields at the second harvest were 941,324 kg and 960,777 kg, respectively. These are 28.24% and 28.92% of the total yield for these variants. The main part of the yield is realized during the third harvest. With small differences for the variants, about 50% of the total yield obtained for the growing season is realized. At the earliest sowing, this yield was 53.73%. It can be noted that for all the tested variants the yields are within the limits of the average yields for the variety Sivria 600 (Mihov et al., 2014).

CONCLUSIONS

As a result of the conducted researches on the production of early pepper at different terms of cultivation the following conclusions can be made: Early sowing (February) contributes to the formation of better biometric indicators, justifying better vegetative development of plants and build an active photosynthetic leaf apparatus. In early sowing the plants develop under conditions closer to their biological requirements and there is a better synchronicity between their vegetative development, flowering and fruiting. Higher yield is obtained from plants with the early date of sowing. The difference with the yield from the second sowing date is 26.93%. The same plants also have a 35% higher yield than the first harvest.

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LAMINITIS IN DAIRY COWS

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ABSTRACT

This study is completed in a farm with 120 dairy cows. In the beginning of June, the condition of laminitis in the farm with 120 lactating cows, resulted for 15 of them with clinical laminitis and formation of wounds. Within 10 days, the animals progressed to clinical metabolic acidosis and non-profuse watery stools, accompanied by rash and pain reflected by lameness. Because of this condition, an immediate intervention was made, especially in the food ration, which contains fiber, dry matter, concentrate, buffer solutions, and as a result, within 3-4 days, the condition was normalized. During summer until beginning of September, the number of cows with clinical laminitis increased, stimulated by thermal stress. In late August and early September, the number of heads with clinical laminitis reached to 30 heads. In all cases where we found signs of metabolic acidosis or redness of the hoof, we carried out general interventions by rebalancing the food ration and using substances with microelements and buffer solutions. As soon as superficial wounds appeared on the legs, the animal was treated individually. For prophylaxis and therapy in all animals, podalic 30% was used in spray form two times a week, as well as the bath for the hooves at the moment when they come out of milk two times a week with podalic 4%, which has given us very good results in farm especially in dairy cows.

Key words: farm, dairy cows, laminitis

INTRODUCTION

Laminitis due to hoof lesions is one of the most common diseases reported in modern dairy production (Bergsten and Herlin, 1996), and 90% of laminitis occurs due to hoof problems (Murray, et al., 1996). The predominant hoof problems that cause paleness in cows and reduced milk production are hoof sole ulcers, white line abscess, interdigital phlegmon, and interdigital dermatitis (Warnick et al., 2001; Hernandez et al., 2002; Amory et al., 2002).

Economic losses come from e.g. reduced milk production, fertility problems and increased collection rates and treatment costs. Usually costs are calculated separately for each issue (eg Souza et al., 2006), but some studies have calculated total costs (€224–320/individuals) (Guard, 2001; Ozsvari et al., 2007).

Various authors clearly show that cows with lameness (laminitis) produce less milk (Rajala-Schultz et al., 1999; Green et al., 2002; Hernandez et al., 2002; Hultgren et al., 2004; Hernandez et al., 2004; Amory et al., 2008; Bach et al., 2007). Costs calculated in different

studies range from 1.5 kg per day to 2.8 kg/day (Rajala-Schultz et al., 1999; Warnick et al., 2001; Green et al., 2002). In some studies, milk loss has been estimated at 270 – 440 kg/lactation depending on the stage of lactation (Coulon et al., 1996). Amory et al., (2008) found an average milk loss of 574 kg and 369 kg associated with sole ulcers and white line disease, respectively. High milk production is thought to be a risk factor for hoof problems (Enevoldsen et al., 1991; Barkema et al., 1994; Fleischer et al., 2001; Hultgren et al., 2004; Amory et al., 2004), although e.g. Dohoo et al., (1984) could not find such an effect.

Laminitis also plays a negative role on reproductive efficiency (reviewed by Fourichon et al., 2000 and Hultgren et al., 2004). Collick et al., (1989) and Barkema et al., (1994) have noted that laminitis extends open days from 11 to 40 days. When Hultgren et al., (2004) investigated reproduction and sole ulcers, regardless of any acute laminitis; they found a clear negative effect to exist.

Hoof lesions cause the majority of laminitis in dairy cows (Murray et al., 1996), and laminitis is also an important cause of culling (Collick et al., 1989; Barkema et al., 1994; Sprecher et al., 1994). However, Hultgren et al., (2004) found no relationship or association between sole ulcers and castration. Booth et al., (2004) discussed how the stage of lactation decreases; a cow with laminitis that produces or is accompanied (followed) by a sole ulcer in late lactation is more likely to be culled than a cow that exhibits it in early lactation.

Most hoof problems in the bovine species affect dairy cows. Apart from direct damage to the hoof, including punctures, ulcers, abscesses and cracks, laminitis is the most common non-infectious process in cattle. Laminitis can also cause many other problems in the hoof that will only occur from direct injury. Laminitis, as its name implies, is inflammation of the laminar layer. This disease, which affects all ruminants as well as horses, is called founder or primary when it becomes chronic. The laminar layer consists of sensitive connective tissue that holds the pedal bone to the hoof wall. When the tissue becomes inflamed (thumps), it puts pressure on the wall of the hoof and interrupts the blood flow in the blood vessels of the corium, as a result, the laminar layer is not supplied with oxygen and nutrients. As the tissue dies, the bond holding the pedal bone to the hoof wall weakens, and the bone begins to separate from the wall. With less support and a weaker attachment to the wall, the pedal bone begins to pull away from the deep flexor tendon and rotate into the hoof. The hooves (toe) where laminitis occurs also begin to grow at abnormal rates due to increased vascularization, changing the shape of the hoof and adding more discomfort to the animal. In cattle, laminitis itself is not as harmful as the side effects it produces. Although laminitis will produce lame animals (cows), other hoof problems that develop as side effects of laminitis are usually more severe. Laminitis in cattle results from dysfunction of the blood vessels supplying the lamina and from softening of the suspensory ligaments leading to rotation of the pedal bone and compression of adipose tissue. This causes bleeding in the sole area, as well as the formation of a horn (nail) of a lower quality in the hoof. When the bone begins to separate from the wall, it can cause the sole to separate from the hoof wall at the white line, causing a condition known as white line (line) disease. If the white line is pulled too far from the sole, it can open the hoof up to infections. Laminitis can also lead to problems such as plantar abscesses, and a condition known as heel spurs, which results from overuse of the sole.

In this study, the focus is on non-infectious diseases of the hoof, especially laminitis-related lesions that manifest as hemorrhage, white line disease, and sole ulcers.

RESULTS AND DISCUSSIONS

-Laminitis period (June-September)

In the farm *"IMPULS SH.P.K" farm* Fushë Krujë, about 280 heads of the Holstein breed, of which 160 are lactating cows, were been examined. Laminites are a fairly widespread problem in stable regimes because the degree of utilization of cows is quite high, which results in the fulfillment of nutritional and environmental needs, which become in some way difficult to impossible on the farm due to the position geoFigureical area of the terrain where it is located. In the farm where the study was carried out, problems with laminitis were evident as a result of feeding and breeding conditions. In the period of the beginning of June 2019, the condition of laminitis on the farm in 120 lactating cows, 15 of them resulted in clinical laminitis with the formation of wounds up to interdigital. As the temperature increased over time, which causes thermal stress in stall-breeding cows, the number of animals with laminitis increased. Plus the impact from the breakdown of food balances as a result of a mechanical defect in a food preparation equipment. Within 10 days the animals progressed to clinical metabolic acidosis and non-profuse watery stools, accompanied by rash and pain reflected by lameness of a ballerina-like gait on the tip of the hoof in the anterior ¼ of the hoof and vertebral bending of the ventral spine with arch. Because of this condition, an immediate intervention was made, especially in the food ration, which contains fiber, dry matter, concentrate, buffer solution, and as a result, within 3-4 days, the condition was normalized. Throughout the summer period until the beginning of September, cows with clinical laminitis increased, helped by thermal stress. In the period of late August and early September of 2019, the number of heads with clinical laminitis reached 30 heads. In addition, there were also cases in 14-24-month-old repair cows in 5-15 cows due to the food ration, environmental stress, poor breeding conditions and the high number of cows in a stable with low capacity.

CATEGORIES OF ANIMALS AFFECTED BY LAMINITIS

1. 70% of animals are first calving cows.

2. 30% are cows of the second-high calving.

- Reasons why heifers are affected:

1. have a negative energy balance 30-45 days after birth
2. the coincidence of the last change of teeth with the stress factor
3. changing the environment from the remont to the dairy cow stall
4. change of ruminal microflora from heifers to dairy cows

Part of the heifers affected by laminitis also had mild chronic metritis with not very profuse vaginal discharge. Whereas the heifers that went into ketosis were negligible. Regarding mastitis, there was an increase in their cases in both heifers and cows, which is related to the addition of soluble protein to the food ration to increase milk production. After the intervention in the food ration, mastitis suffered a drastic decrease.

- second category 30%

The cows affected by laminitis had more to do with the stable regime with balancing of the food ration, thermal stress and in some cases the destruction of the angle of the hoof shape from bad cutting by the technician or from the abnormal gait of the animal.

As for the heifers affected by laminitis, they passed it very quickly and easily during remounting.

TREATMENT OF LAMINITIS

In all cases where we found signs of metabolic acidosis or reddening of the hoof crowns of the cows as a whole, we carried out general interventions by rebalancing the food ration and using substances with microelements and buffer solutions. As soon as light wounds appeared on the legs, the animal was treated individually.

- The treatment was carried out as follows:

The part where the wound was created, the location of the inflammatory process of laminitis and the necrotic tissues are completely cleaned. It is disinfected with podalic 30% solution, then we apply herbal cream and the wound is hermetically bandaged.

After 5-7 days, the animal is rechecked and the wound is reopened to see how the condition of the wound is progressing.

If the wound is healed, it is left open and treated with bluspray or brandospray and rinsed twice a week with podalic. If the wound is not healed, it is re-dressed again.

In the most difficult cases when the wound has been very generalized and has affected the sole of one hoof, it is intervened by placing plastic heels in the form of a shoe to avoid contact of the wound with the ground, thus making it possible to eliminate the pain. The wound is treated as above.

Regarding injections with antibiotics, they are not used in dairy cows, except in some very serious cases when we have 75% damage to the hoof, we use ceftifur, a fourth generation antibiotic that does not leave residues in milk and is highly effective, but also very expensive. high for our economy. Residual antibiotics are never used in dairy cows. In heifers in difficult cases treated with laminitis, we use Amoxicillin and Tilosin, but with less of the latter.

For prophylaxis and therapy in all animals, podalic 30% was used in spray form 2 times a week, as well as the bath for the hooves at the moment when they come out of milk 2 times a week with podalic 4%, which has given us very good results in farm especially in dairy cows.

PHOTOS FROM THE PRACTICAL REALIZATION OF THIS STUDY

CONCLUSIONS

- According to the statistics of the farm where this study is realised, the biggest problems were in the period of summer and the beginning of autumn, related to the stress caused by high climatic temperatures and food raw materials without performing the relevant analyses.
- Animals of the first calving are more affected.
- During the summer period, the bacterial load in the stable premises is quite high.
- The use of fans and water cooling of cows with simultaneous systems during peak hours has given a very good effect in reducing thermal stress.
- The food ration should be very balanced, fresh and preferably prepared 2-3 times a day.

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EFFECTS OF DIETARY PROBIOTICS ON THE PRODUCTIVE PERFORMANCES AND BLOOD PARAMETERS IN WEANED PIGS

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ABSTRACT

The aim of this study was to investigate the effects of probiotic product (*Lactobacillus plantarum* and *Bacillus subtilis*) supplementation in diet on productive performances of weaned pigs. A total of 48 weaned pigs (Duroc x Largewhite x Landrace), consisting of 32 males and 16 females, were used in the experiment. The experiment design was a randomized completely blocked design with 4 treatments. Each treatment consisted of 4 blocks (body weight 10.0-11.0 kg, 9.0-10.0 kg, 8.0-9.0 kg and 6.5–8.0 kg). Treatment 1) Control diet (commercial diet); Treatment 2) Control diet + antibiotic; Treatment 3) Control diet + probiotic 0.1%; Treatment 4) Control diet + probiotic 0.2%. The experimental period was 27 days. Body weight and feed intakes were recorded every 2 weeks. At the end of the experiment, 4 pigs were randomly chosen from treatment 1, 2 and 4, then blood samples were taken for hematology analysis. The results showed that there were no significant differences in feed intake, weight gain, average daily gain, and feed efficiency among treatments ($P>0.05$). However, a tendency of higher weight gain and average daily gain was observed in pigs receiving diet with antibiotic ($P>0.05$) compared to the other groups. The pigs receiving diet with probiotic 0.2% had higher RBC ($P<0.05$) compared to control group. No significant difference in RBC were obtained between pigs receiving diet with probiotic 0.2% and pigs receiving diet with antibiotics. There were no significant differences in Hemoglobin, Hematocrit, Leukocyte, Neutrophils, Eosinophils, Lymphocytes and Monocytes among treatment. The results indicated that supplementation of *Lactobacillus plantarum* and *Bacillus subtilis* had positive effects on RBC.

Keywords: probiotic, weaned pig, productive performances, blood parameters

INTRODUCTION

Weaning period is known to be a critical time for swine production. In commercial swine production system, weaning occurs at a time when the gastrointestinal tract, as well as immune system, are still developing. The weaned pigs are faced with many stress factors such as separation from the sow, dietary changes from milk to solid feed, adapting to a new environment, mixing of pigs from different sources and histological changes in the small intestine. These may negatively affect the response of immune system and lead to an intestinal gut dysfunction (Rhouma *et al*. 2017) as well as digestive disorders in wened pigs. This commonly resulted in body weight loss and intestinal disturbances that lead to diarrhea, and

thus increase the need for antibiotics. This may lead to the development of resistant strains in the intestinal microbial of humans and animals (Hu, *et al*., 2014 ; Betancur *et al*., 2020).

Probiotics are preparations or products with defined and viable microorganisms sufficient to alter the intestinal microflora of the host and exert a beneficial health effect)Schrezenmeir and de Vrese, 2001; Chen et al., 2005(. It is reported that health benefits of probiotics include improving productive performance (Chen *et al*., 2005; Yan and Kim, 2012; Hu *et al*. 2014; Dlamini *et al*., 2017; Nguyen *et al*., 2019; Betancur *et al*., 2020), feed utilization (Yan and Kim, 2012; Blavi *et al*., 2019; Nguyen *et al*., 2019; Oliveira *et al*., 2019; Betancur *et al*. 2020), gut health (Chen et al., 2005; Yan and Kim, 2012; Hu *et al*. 2014; Nguyen *et al*., 2019) and stimulate the immune system)Dlamini *et al*., 2017) of weaned pigs. Therefore, it is suggested that the appropriate use of probiotic can be an alternative for antibiotics use in animals. However, the effects of these probiotic are variable, which are possibly associated with species or strain differences, dose level, diet composition, feeding strategy as well as housing and sanitary condition (Chen *et al*. 2005; Vondruskova *et al.*, 2010; Oliveira *et al*., 2019). The objective of this study was to investigate the effects of probiotic product (*Lactobacillus plantarum* and *Bacillus subtilis*) supplementation in diet on productive performances of weaned pigs.

MATERIALS AND METHODS

Experimental design, animal and diets

Fourty eight weaned pigs (Duroc x Largewhite x Landrace), consisting of 32 males and 16 females, were used in the experiment. The experiment design was a randomized completely blocked design with 4 treatments. Each treatment consisted of 4 blocks (body weight 10.0-11.0 kg, 9.0-10.0 kg, 8.0-9.0 kg and 6.5–8.0 kg). Dietary treatments included Treament 1) Control diet (commercial diet); Treatment 2) Control diet + antibiotic (Amoxicillin 50% 300 ppm+Halquinol 60% 240 ppm and Tiamulin 10% 200 ppm); Treatment 3) Control diet + probiotic 0.1%; Treatment 4) Control diet + probiotic 0.2%. The components of probiotic ptoduct are *Lactobacillus plantarum* $1X10^9$ cfu/kg and *Bacillus subtilis* $1X10^9$ cfu/kg. The pigs were allowed *ad libitum* access to feed and water through all the 27 days experimental period.

Sampling and measurements

Body weight and feed intakes were recorded every 2 weeks to monitor ADG, ADFI and feed/gain. At the end of the experiment, 4 pigs were randomly chosen from treatment 1, 2 and 4, then blood samples were taken for hematology analysis.

Statistical analysis

The data was analyzed as a randomized complete block design. Means were separated using Duncan's multiple range test and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows effect of dietary probiotic supplementation on productive performances in weaned pigs. The results showed that there were no significant differences in feed intake, weight gain, average daily gain, and feed efficiency among treatments ($P>0.05$). However, a tendency of higher weight gain and average daily gain was observed in pigs receiving diet with

antibiotic (P>0.05) compared to the other groups. The ADG obtained from this study was 477-528 g/head/d and FCR was 1.27-1.34.

Table 1. Effects of probiotics supplementation on the productive performances of weaned pigs

parameters	T1	T2	T3	T4	p-value
initial body weight (kg)	9.05±1.42	9.00±1.26	9.033±1.31	9.00±1.28	0.862
final body weight (kg)	22.52±2.14	23.27±2.07	21.84±2.25	22.33±2.38	0.143
weight gain (kg)	13.47±0.92	14.27±0.83	12.90±1.20	13.32±1.57	0.122
average daily gain (g)	499±33.98	529±30.83	478±44.44	493±58.13	0.122
Feed intake (g/head/d)	635±36.70	684±51.69	637±22.00	649.±32.93	0.180
FCR	1.28±0.04	1.29±0.05	1.34±0.09	1.32±0.10	0.449

T1 = Control diet (commercial diet); T2 = Control diet + antibiotic (Amoxicillin 50% 300 ppm + Halquinol 60% 240 ppm and Tiamulin 10% 200 ppm); T3 = Control diet + probiotic 0.1%; T4 = Control diet + probiotic 0.2%.

Several studies on probiotics have demonstrated that probiotics had beneficial effects on gut health, immunity status as well as nutrient utization in weaned pigs (Liao and Nyachoti, 2017; Hu *et al*. 2014). Betancur *et al* (2020) reported that the pigs supplement with *Lactobacillus plantarum* had higher live weights, ADG and better FCR compared to the pigs in control group ($p<0.05$) during 21-49 days of age. Furthermore, there were no significant differences in live weights, ADG and FCR between the pigs with *Lactobacillus plantarum* supplementation and the pigs with antibiotic supplementation. The positive effect of *Bacillus subtilis* supplementation on productive performances in weaned pigs has been reported by Hu *et al* (2014). It was reported that during the 28 days trial, ADG and FCR of weaned pigs supplemented with *Bacillus subtilis* at 2×10^9, 4×10^9 and 20×10^9 cfu/kg feed as well as pigs supplemented with antibiotic (neomycin sulfate) had better ADG and FCR compared to control. However, in the present study, no significant differences in productive performances were observed among treatments. These results are consistent with results of Jorgensen *et al*. (2016) who found no significant effects on productive performance of weaned pigs during the prestarter period (28–42d) when fed with 400 mg/kg of the *Bacillus*-based probiotic compared to control group. The similar results were also reported by Oliveira *et al*. (2019) who found that commercial feed with the addition 0.2 % probiotic containing a blend of microorganisms (*Lactobacillus plantarium, L . bulgaricus, L . acidophilus, L . rhamnosus , Bifidobacterium bifidum, Streptococcus thermophilu* and *Enterococcus faecium)* or commercial feed with the addition of 0.1% yeast-based probiotic *(Saccharomyces cerevisiae boulardii*) did not influence the growth performance parameters of weaned pigs (21-63 days of age). Vondruskova *et al*. (2010) suggested that the variation of the effectiveness of the probiotic on productive performances depend on the combination of selected bacterial genera, their doses, and feed composition. Furthermore, the housing and sanitary conditions possibly contribute to the significant effect of the probiotic on the productive performance of the pigs (Oliveira *et a*l., 2019). In the present study the optimal feedstuff quality of commercial feed, which fulfills the nutrient requirement of the weaned pigs, and the fact that they were kept in a pen with a low density, may have contributed to less effectiveness of probiotic supplementation on productive performances obtained from this study.

Table 2. Effects of probiotics supplementation on blood parameters of weaned pigs

Parameters	T1	T2	T4	p-value
Sample Number	4	4	4	
Erythrocytes (RBC, 10^6 cells/mm^3)	6.13^a	6.80^b	6.83^b	0.039
Hemoglobin (HB, g/dl)	10.55	11.70	11.73	0.111
Hematocrit (HC, %)	39.25	43.50	43.00	0.135
Leukocyte (W.B.C, cell/mm^3)	14,300	17,975	16,000	0.354
Neutrophils (%)	35.50	38.25	28.25	0.732
Eosinophils (%)	1.75	1.25	2.25	0.579
Lymphocytes (%)	61.25	58.75	70	0.676
Monocytes (%)	1.50	1.75	2.00	0.809

^{ab} values within a row with different superscripts means significant values .$p<0.05$

T1 = Control diet (commercial diet); T2 = Control diet + antibiotic (Amoxicillin 50% 300 ppm + Halquinol 60% 240 ppm and Tiamulin 10% 200 ppm); T3 = Control diet + probiotic 0.1%; T4 = Control diet + probiotic 0.2%.

The pigs receiving diet with probiotic 0.2% had higher RBC ($P<0.05$) compared to control group (Table 2). No significant difference in RBC were obtained between pigs receiving diet with probiotic 0.2% and pigs receiving diet with antibiotics. There were no significant differences in Hemoglobin, Hematocrit, Leukocyte, Neutrophils, Eosinophils, Lymphocytes and Monocytes among treatment. Betancur *et al*. (2020) reported that there were no significant differences in RBC, HB, HC, WBC, Lymphocytes, Monocytes, Eosinophils and obtained from weaned pigs with *Lactobacillus plantarum* supplementation compared to pigs with antibiotic supplementation and pigs from control group. The similar result was also reported by Chen *et al*. (2005) who suggested that supplementation of *Lactobacillus acidophilu*s, *Saccharomyces cerevisae* and *Bacillus subtilis* had no effect on histological value of weaned pigs. However, the results from the present study are consistent with results of Tymczyna *et al*. (2020), who found that supplementation of *Bacillus cereus* and vitamin C caused a significant increase in the RBC, but had no effect on *Leukocytes neutrophils* and *Lymphocytes*. Hematological parameters are affected by several factors including age, sex, nutritional and health status, breed, season, and stress. The value obtained in the present study was in a range of reference value reported by Cooper *et al*. (2014).

CONCLUSIONS

The results from this study indicated that supplementation of probiotic product contains *Lactobacillus plantarum* and *Bacillus subtilis* or antibiotic in diet of weaned pigs had no effect on productive performances. However, probiotic supplementation or antibiotic supplementation in diet improved RBC in weaned pigs.

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PRE-TREATABILITY OF TUNCELI ORGANIZED INDUSTRIAL ZONE WASTEWATERS BY COAGULATION/FLOCCULATION PROCESS

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ABSTRACT

In the present work, the pre-treatability of Tunceli (Turkey) Organized Industrial Zone (OIZ) wastewaters by coagulation and flocculation processes was investigated. For this purpose, iron (II) sulfate was used as coagulant and batch experiments were performed with a jar test setup. Response surface methodology (RSM) based on a central composite design (CCD) were applied to optimize the operating variables (i.e. coagulant dosage and pH). The optimal conditions were as follows: 141.37 mg/L of coagulant dosage and an initial pH equal to 4.72. Under these conditions, the removal efficiency of chemical oxygen demand (COD) was 51.44%. The results showed that the coagulation/flocculation process would be a useful process for the pre-treatment of Tunceli OIZ wastewaters.

Keywords: Central composite design, Coagulation/flocculation treatment, Chemical oxygen demand removal, Optimization

INTRODUCTION

In recent years, environmental pollution has become an important problem as a result of the rapid increase in population and industrialization efforts. Within the scope of industrial development; the environment is unconsciously polluted by the wastewater of industrial establishments, sometimes because factories take advantage of legal loopholes in the control of wastewater and sometimes because the necessary technology is not available and/or expensive in our country. Organized industrial zones (OIZ) are a model that aims to minimize and/or eliminate environmental problems arising from industry and to ensure balanced development between regions that differ economically. The establishment of OIZs in our country was shaped in 1960. Together, OIZs started to play a major role in Turkey's economic development (Üstün et al., 2014). The Tunceli OIZ was established in 2013. The distribution of 17 industrial facilities in Tunceli OIZ according to sectors is as textile industry, food industry, plastic industry and furniture manufacturing.

Physico-chemical methods that have proven their efficiency in the treatment of wastewater with high pollution load such as textile, food, paper recovery, which requires easy installation and less residence time in the industrial sense, and in the removal of dissolved, suspended and colloidal pollutants come to the fore. Physico-chemical methods can be applied both alone and in combination with mechanical, chemical and biological methods. In this method, suspended solids, colloidal substances and oily particles are rendered unstable and thus agglomerated. Because they agglomerate to form larger flocs, the density of the flocs is greater than the density of water, and thus the flocs are removed by settling. Coagulation-flocculation method is needed to remove such contaminants. In this method, where high removal efficiencies

can be achieved, chemical costs and difficulties in the disposal of sludge formed as secondary pollution are seen as disadvantages (Öztürk et al., 2021).

In the present work, the pre-treatability of Tunceli Organized Industrial Zone (OIZ) wastewaters by coagulation and flocculation processes was investigated.

MATERIAL AND METHOD

The wastewater used in the study was obtained from the OIZ in Tunceli. Approximately 65 m^3 of wastewater is generated daily in the OIZ. The pollution characteristics of the wastewater sample taken from the OIZ were determined and the obtained values are presented in Table 1. The wastewater sample was brought to the laboratory in a plastic container and stored at 4 °C. Coagulation/flocculation experiments were performed with the classical jar test device. The pH of the sample was adjusted to different pH values using 1.0 M H_2SO_4 or 1.0 M NaOH. Different doses of iron (II) sulfate ($FeSO_4.7H_2O$) were added to the flasks with 250 mL wastewater sample. In coagulation experiments, the samples were mixed rapidly at 150 rpm for 5 minutes, then mixed slowly at 40 rpm for 20 minutes and waited for 1 hour for the sample to settle. Samples were taken from the supernatant after precipitation and chemical oxygen demand (COD) and total solids (TS) analyzes were performed according to standard methods (APHA, 2005).

Table 1. Characteristic of OIZ wastewater

Parameter	Concentration
pH	4.78
COD	10,580
TS	4340

Design Expert (trial version 7.0, USA) software was used to optimize the coagulation process. For this purpose, the central composite design (CCD) under the response surface methodology (RSM) was used. Coagulant dosage and pH were chosen as independent parameters and their values are given in Table 2.

Table 2. Experimental factors and levels for COD removal

Factors	**Range and values**				
	-α (-1.414)	-1	0	+1	+α (+1.414)
A: Coagulant dosage (mg/L)	100	202.51	450	697.49	800
B: pH	4	4.73	6.50	8.27	9

RESULTS AND DISCUSSION

The effects of two important factors (coagulant dosage (A) and pH (B)) that affect COD removal by the coagulation-flocculation process were investigated by CCD consisting of 13 experimental sets. The central composite design matrix for the experimental design of the

process variables and the experimentally obtained COD removal efficiency values are given in Table 3.The empirical relationship (% removal) for COD removal is explained by a quadratic polynomial model based on the equation given below:

$$Y_{COD}(\%\ \text{removal}) = +42.53 - 1.20A + 2.63B + 7.94AB + 0.46A^2 - 0.62B^2 \quad (1)$$

Table 3. Experimental factors and COD removal results according to CCD

Run no	Factors		COD removal (%)
	A	***B***	
1	0.000	0.000	42.53
2	-1.000	-1.000	46.69
3	0.000	0.000	42.53
4	1.000	-1.000	27.22
5	0.000	1.414	44.99
6	-1.414	0.000	45.93
7	0.000	0.000	42.53
8	0.000	-1.414	40.83
9	0.000	0.000	42.53
10	0.000	0.000	42.53
11	1.414	0.000	44.23
12	1.000	1.000	50.66
13	-1.000	1.000	38.37

Equation (1) expresses the relationship between process variables (*A* and *B*) and COD removal efficiency (%). These factors can be used to predict and analyze COD removal. Each negative sign in the equation indicates the antagonistic effect of the variables, while a positive sign indicates the synergistic effect of the variables (Amari et al., 2008). Regression coefficients and statistical significance of the model and adequacy of the selected model were evaluated using analysis of variance (ANOVA) (Table 4). According to ANOVA, the model's *F* value of 13.03 and its low probability value ($P<0.005$) indicate the high importance of the model. The value of the correlation coefficient (R^2) is 0.9030, which indicates that 90.30% of the total variations can be explained by the model. Adjusted R^2 is an important factor showing the fit between the experimental and predicted removal efficiency for COD removal of the model. The adjusted R^2 value was found to be 0.8337. Adequate precision measuring the signal-to-noise ratio should be greater than 4. The signal-to-noise ratio for this study was 13.956. This indicates that the model has an adequate signal. Thus, the model is reliable and can be used to navigate the design space. The low value of coefficient variation (CV) (5.25) indicates the reliability of the applied experimental values.

Table 4. Analysis of variance (ANOVA)

Source	**Sum of**	**Degree of**	**Mean square**	**F- Value**	**P-value**
Model	323.59	5	64.72	13.03	0.0020
A	11.48	1	11.48	2.31	0.1722
B	55.14	1	55.14	11.10	0.0126
AB	252.17	1	252.17	50.77	0.0002
A^2	1.47	1	1.47	0.30	0.6031
B^2	2.72	1	2.72	0.55	0.4836
Model statistics					
Std. Dev	2.23				
Mean	42.43				
CV %	5.25				
R^2	0.9030				
Adjusted R^2	0.8337				
Adequate precision	13.956				

The interaction effects of independent variables on COD removal are shown in Figure 1 with a two-dimensional (2D) contour plot. Experimental results were optimized with Design-Expert software. Optimization results are shown in Table 5. The optimized conditions were chosen as follows: 141.37 mg/L coagulant dosage and pH 4.72. Under these conditions, the removal efficiency of COD was 51.44%. As a result of the control experiments to determine the accuracy of the selected values, a removal efficiency of 50.65% was obtained, which indicates the accuracy of the model in estimating the COD removal efficiency.

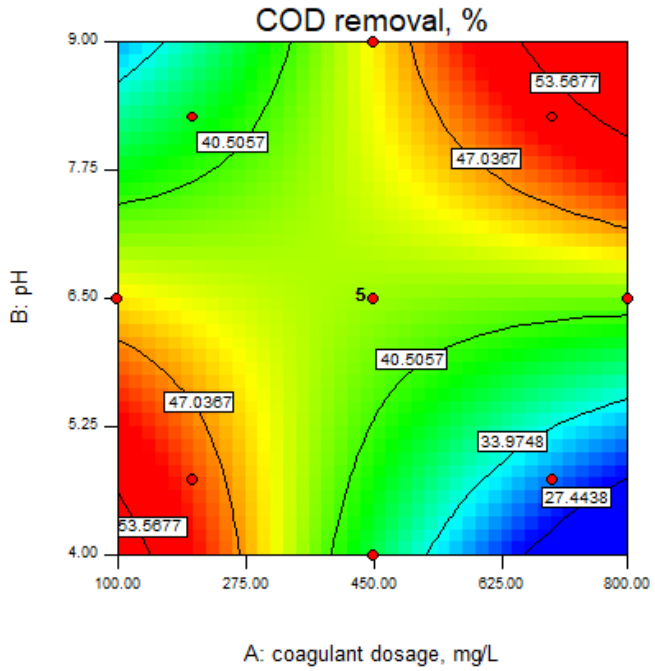

Figure 1. Effect of independent variables on COD removal efficiency.

Table 5. Numerical value of process factors for maximum COD removal efficiency (Desirability=1.000)

	Coagulant dosage (mg/L)	**pH**	**COD removal (%)**
Optimum value	141.37	4.72	51.44

The maximum COD removal efficiencies (%) obtained with the different wastewaters in previous studies were compared in Table 6.

Table 6. Comparison of COD removal efficiency (%) with different wastewater stated in literatures.

Wastewater	Coagulant	COD removal (%)	Reference
Cheese whey	$FeCl_3.6H_2O$	34	(Gürtekin et al., 2011)
Meat slaughterhouse	$Al_2(SO_4)_3.18H_2O$	36.40	(Özyonar and Karagözoğlu, 2011)
Citrus	$Al_2(SO_4)_3.18H_2O$	60.25	(Yeşiltaş and Ersü, 2021)
Pesticide production	$Al_2(SO_4)_3.18H_2O$	47	(Katip, 2019)
Tunceli OIZ	$FeSO_4.7H_2O$	51.44	This study

CONCLUSIONS

In this study, physicochemical treatment of Tunceli OIZ wastewater by coagulation/flocculation process was investigated and optimum values of operating conditions were determined. It has been observed that the treatment of Tunceli OIZ wastewater with the coagulation/flocculation process provides COD removal, but the treatment is not at a level that can be discharged to the receiving environment. It has been observed that higher removal efficiencies can be achieved if different advanced treatment methods are integrated in addition to the coagulation/flocculation process. If the wastewater generated in different industries is discharged, cleaning it to meet the standards is a very important issue in terms of protecting environmental pollution and water resources. It is very important for businesses to recover their wastewater and reuse it in the process, if possible, in terms of protecting our natural resources and contributing to our country's economy.

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EFFECTS OF DIFFERENT MAIZE STRAW MATERIAL APPLIED IN THE SOIL ON CORN YIELD PARAMETERS

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ABSTRACT

Adding an organic carbon source to soils not only improves the general quality of soils, but also positively affects yield parameters. In this study, 1, 2, 4 tons of ground corn straw (CS), corn green parts (CGP) and corn compost (CO) were applied to the soil as an organic carbon source. Cob sampling was done from each plot before harvesting from the corn plant that completed its vegetation. Length (cm), weight (g), diameter (mm) and number of grains (piece) were determined in the cob samples taken. When the results were examined, the maximum increase in the number of grains was obtained with the highest application of CGP. The differences in cob diameter size were statistically significant, and all doses of the applications increased significantly compared to the control. The increases in cob weight were the highest doses of the applications compared to the control, respectively, according to CS, CGP and CO; there was an increase of 91.72%, 109.82%, 134.02%. The difference in cob length measurements were statistically significant, but no big differences were found between the applications. The increase in cob length was determined at the highest dose of CO and was determined as 3.1% compared to the control. When the results are examined, the evaluation of these carbon source materials, which are disposed of in various ways and cannot be used in agriculture in any way, should be used widely both to increase soil and product quality and to sustainable soil health in our agricultural lands by bringing them into the soil without harming the environment.

Keywords: Corn yield parameters, Compost, Corn straw, Corn green part

INTRODUCTION

Grain products are widely grown in Turkey. Corn is among the main food sources with its increased production in recent years, which is in the grain group. It is widely used in various fields and is especially preferred in the food and livestock sector. It is also known as a strategic product (Li, 2017). According to the latest data, the production amount of grain products decreased by 14.3% in 2021 compared to the previous year and amounted to approximately 31.9 million tons. Compared to the previous year, wheat production decreased by 13.9% to approximately 17.7 million tons, barley production decreased by 30.7% to approximately 5.8 million tons, rye production decreased by 32.4% to 200 thousand tons, oat production decreased by 12%. It decreased by 0.2% to 276 thousand tons (TÜİK 2021). Over the years, corn production has increased, and while it was between 5.9 and 6.6 million da between 2014 and 2018, it increased by 8% in 2019 compared to the previous period and reached 6.4 million da. In 2020, it increased by 8.3% to 6.5 million tons. In 2021, it increased by 3.8% compared to the previous year and became approximately 6.8 million tons. When the province-based distribution in production is examined, it has been determined that the ranking is in the form of

Konya, Adana and Mardin (TÜİK 2020). If it is assumed that at least the same amounts of straw and straw are formed depending on our production, composting should be applied for their recovery and recycling. As a result of the composting process, which is an alternative method by abandoning the traditional methods, our straw-straw potential will be evaluated and it will play an active role in enriching the soil without harming the environment and improving its physical, chemical and biological properties. The use of materials such as compost, which are sources of organic matter, not only improves soil properties, but also improves product quality and yield. In this study, the effects of various organic carbon sources on yield parameters will be investigated.

MATERIAL AND METHOD

The research was grown in Selçuk University Faculty of Agriculture Research and Application located in Konya Sarıcalar region, where it has clay texture. Evaluation of compost as an organic resource, excerpts have been made. In the study, the doses of maize compost, maize green parts and ground maize stubble were applied in 4 repetitions at 1,2, and 4 tons per decare. Pack of 4 control plots for your difference with applications. The study was carried out in a total of 40 plots. As an experimental plant, DRACMA of the silage plant was grown on the application and a cob sample was taken from each park before harvesting. Weight (g), length (cm), diameter (mm) and number of grains (piece) were determined in the cob samples taken. Cob weight measurement: After drying in an oven at 70° for 48 hours, corn cob samples were weighed. Cob Length Measurement: It was measured by the corn cob with the help of a gauge. Cob Diameter Measurement: It was measured with the help of a caliper in the widest part of the corn cob sample. Grain Count Calculation: The number of grains of each cob was counted. After the measurements and calculations, the average values of each feature were subjected to the DUNCAN test by using the R STUDIO program in the computer environment.

RESULTS AND DISCUSSION

Organic matter content of Turkish soils is generally low (Eyüpoğlu 1998; Gezgin et al. 1999). It has been known for a long time that organic matter positively affects the physical, chemical and biological properties of the soil (Shirani et al. 2002). With the application of organic material to the soil, the amount of organic matter in the soil increases, and accordingly, it has a positive effect on the soil's aggregate stability, air-water balance, resistance to erosion and the uptake of plant nutrients in the soil. It is also reported that organic fertilizers improve soil properties and increase the yield of crops, with studies conducted in different parts of the world (Olsen et al. 1970; Sommerfieldth and Change 1985). According to the data obtained from the measurements and calculations determined after this study, which was carried out in field conditions in line with these researches, various organic carbon sources applied on the soil with a clayey and poor quality physical structure increased the yield potential of the soil. When the effect of the applications on the number of kernels per cob was examined, the number of cob kernels in the control soil, which was measured as 643.50, increased as the application doses increased and a statistically significant difference was obtained ($p<0.05$). It is observed that the highest increase occurred at the highest dose of green parts and compost application (Table 1). When the effect on the cob weight is examined, the highest doses of the applications

compared to the control, respectively, according to the corn stubble, green parts and compost; There was an increase of 91.72%, 109.82%, 134.02%. The difference in cob length measurements was statistically significant, but no big differences were found between the applications. The increase in cob length was determined at the highest dose of Compost and was determined as 3.1% compared to the control. The increase in cob diameter was the highest at the highest doses of applications compared to the control.

Table1: Effects of different carbon sources on yield parameters

Application	Number of Grains	Cob Diameter	Cob Weight	Cob Length
Control	643,50±59,70^{c}	39,33±2,49^{b}	83,88±15,86^{b}	17,88±0,65^{cd}
Compost %1	684,50±68,10^{abc}	44,55±2,39^{a}	163,10±43,70^{ab}	19,58±1,31^{abc}
Compost %2	679,00±59,60^{abc}	46,86±2,58^{a}	196,60±54,40^{a}	19,38±1,31^{abc}
Compost %4	764,00±24,00^{a}	45,55±1,81^{a}	193,70±51,50^{a}	20,98±1,09^{a}
Straw %1	736,00±15,23^{abc}	43,87±2,29^{ab}	143,50±39,90^{ab}	17,88±0,74^{cd}
Straw %2	754,30±32,50^{ab}	45,87±0,77^{a}	164,20±21,80^{ab}	18,53±0,42^{bcd}
Straw %4	749,50±29,00^{abc}	46,41±1,92^{a}	175,60±54,20^{ab}	19,70±0,56^{abc}
Green part %1	647,50±28,70^{bc}	43,78±1,37^{ab}	145,60±27,30^{ab}	17,15±0,52^{d}
Green part %2	727,00±10,50^{abc}	47,89±1,16^{a}	232,43±17,01^{a}	19,33±0,62^{abc}
Green part %4	775,30±79,60^{a}	47,48±2,75^{a}	217,90±46,80^{a}	20,23±0,38^{ab}

It has been determined that the yield potential has increased with the applications where the yield before the application is extremely low in the soils of Konya Sarıcalar region, which has low organic matter and weak structural features. It has been determined that the most important effect among the applications is corn compost (Figure 1, Figure 2, Figure 3, Figure 4). In fact, an increase in yield parameters was detected in all organic carbon applications, but it is thought that the effect will be long-term due to the fibrous and late decomposition of the corn stubble. In this context, corn compost should be used more widely due to its easy decomposition and rapid effect on improving soil physical properties.

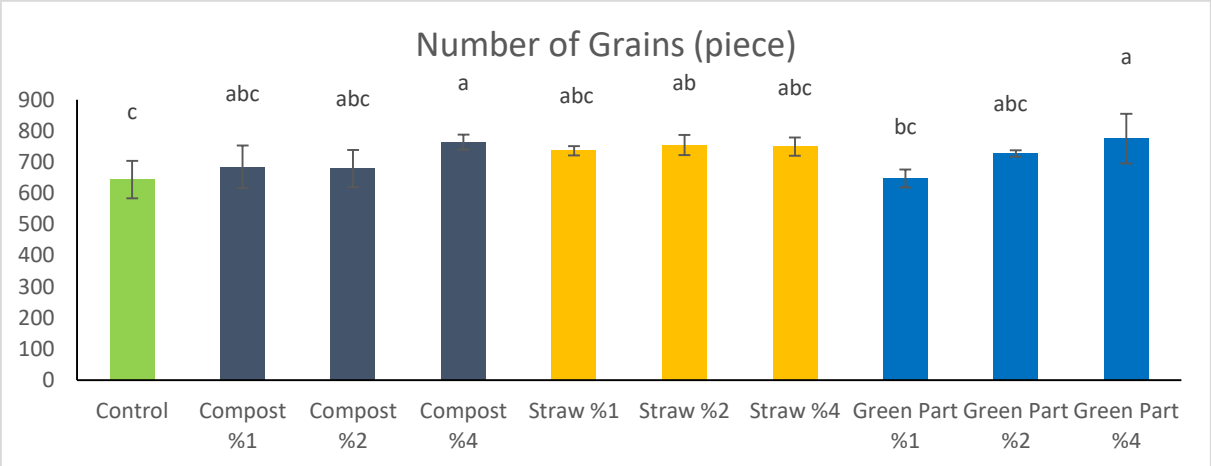
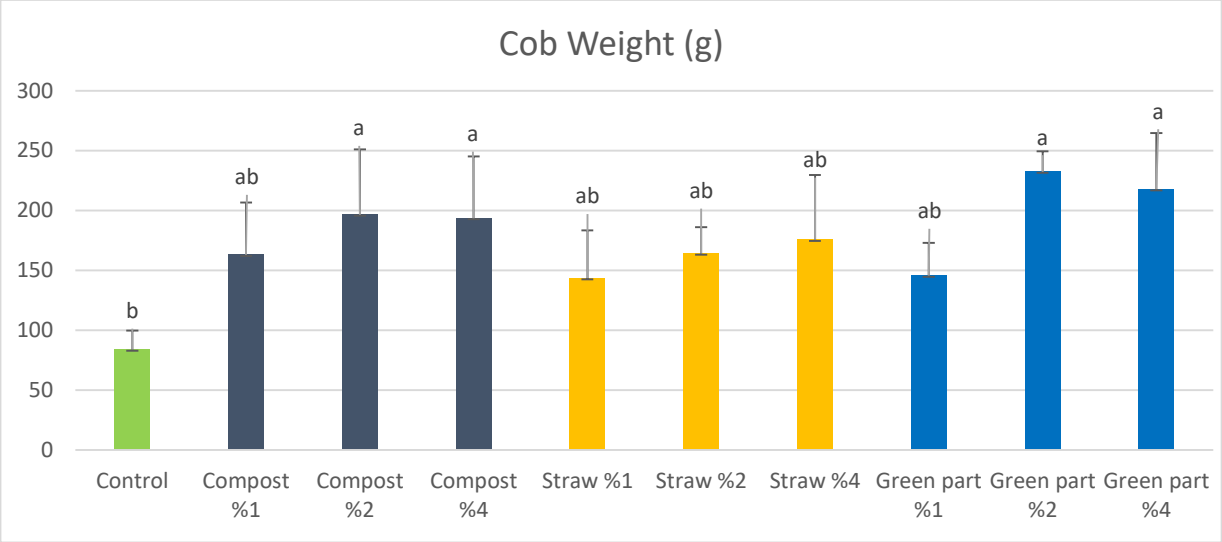


Figure1



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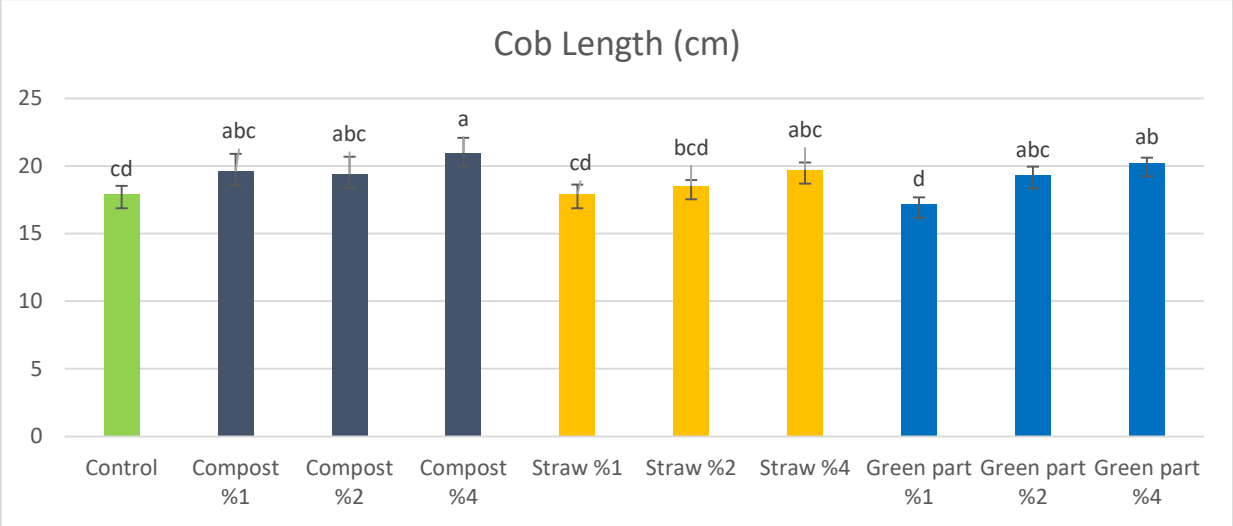

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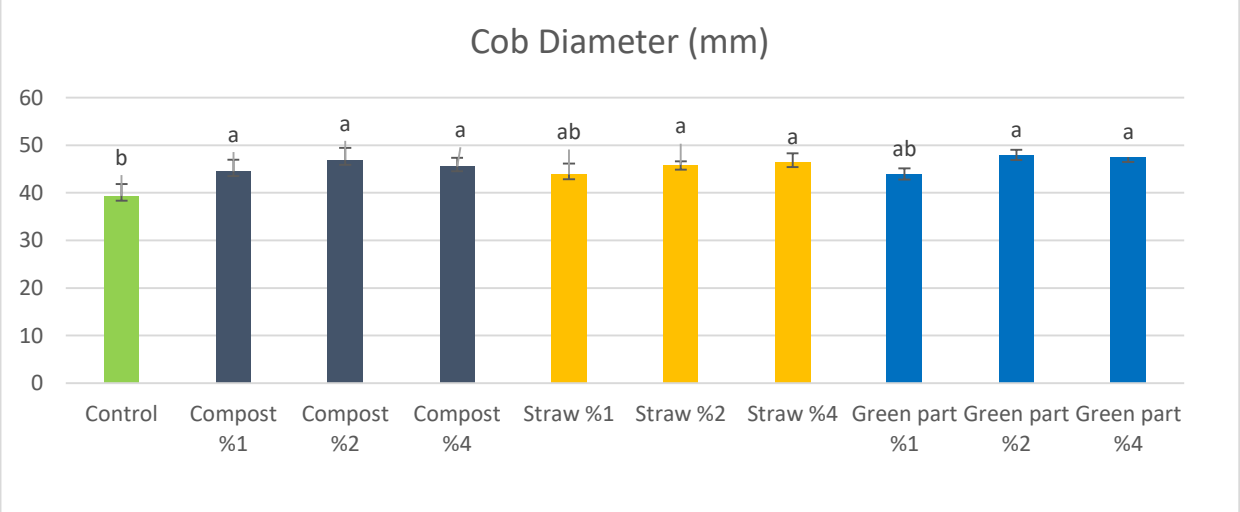

Figure 4

CONCLUSIONS

In this study, the effects of different organic carbon sources on yield parameters in our country were investigated. When the results were examined, it was determined that all organic carbon sources had a significant effect on yield. Corn compost application is recommended, especially in our region with problematic physical properties, as it acts more quickly due to decomposition compared to corn stubble application. It provides both an increase in product quality and an increase in yield. Considering the increasing input costs and degraded soil conditions, compost material will both provide organic matter for the soil and increase microorganism activities, and will be an environmentally friendly and economical method in the evaluation of the straw formed.

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PRESENCE OF MICROPLASTICS IN STOMACH CONTENTS OF BLUE CRAB *CALLINECTES SAPIDUS* (RATHBUN, 1896) IN CANAKKALE STRAIT

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ABSTRACT

The aim of this study is to determine the amount of microplastics in the stomach contents of an individual blue crab. The stomach contents of a total of 45 individuals were evaluated in the study. During the examination of the stomach contents, a total of 46 microplastics were recorded from 15 different stomachs. Microplastics detected were black and blue colored filaments. Microplastics were detected in 10 males and 5 juveniles. No microplastics were detected in the stomachs of females. The %IRI value of microplastic fragments found in all food groups was calculated as 1.40. It was concluded that the microplastics found in the thread group in the study were generally parts of hunting tools. It was determined that microplastics are posing a serious concern and are finding their way into crab diets.

Keywords: *Callinectes sapidus*, microplastic, gut content, IRI, Çanakkale Strait.

INTRODUCTION

In recent 50 years, the world population has increased by 250%. In this context, an increase in the amount of pollutants has also been observed (Browne et al., 2011). Coastal and marine areas are under constant and increasing pressure. Pesticides, organic pollutants, heavy metals, plastics and microplastics generated by human activities are having a greater impact on the marine ecosystem day by day. Today, about 300 million tons of plastic are produced worldwide every year. Pollution in the marine environment by plastics is an issue of increasing concern worldwide, and the transport of plastic residues into the environment depends on the particle size, shape, density and type of polymer. Plastic debris is widely found at the surface of seawater, coastal areas, and on the seabed (Castillo et al., 2016).

Crabs generally accumulate microplastics in their gills, hepatopancreas, stomach, and breast flesh. It is possible that the microplastics in these parts which are also consumed as human food, participate in the food chain and are transferred to humans. They can also accumulate in the tissues and organs of other creatures such as fish, seabirds, and invertebrates that are predators of crabs Crabs take nutrients into their bodies with their millipedes, usually in the form of food through or via sediment. In this way, microplastics, especially on the

sediment surface and in the sediment, enter the crab's stomach through feding (Farrel and Nelson, 2013).

C. sapidus has spread in Turkish waters in recent years and is defined as an invasive species having ecological pressure on native species by establishing dense populations, especially on the coasts of the Mediterranean and Aegean Seas. Records of *C. sapidus* in different locations of Turkish waters were reported by Aydın, 2017; Bilgin, 2019; Ceylan, 2020; Kocataş, 1971; Tuncer & Bilgin, 2008; Yağlıoğlu et al., 2014). Because *C. sapidus* hasa high ecological tolerance (it can live in high temperatures and high salinity), it prefers the sea, brackish water, and freshwaters. *C. sapidus* preys on larger fish and invertebrates. Therefore, microplastic ingestion and entry into the food web is important for exposure. The aim of this study is to determine the presence of microplastics in the diet of the blue crab, which has been widely consumed as human food in recent years.

MATERIAL AND METHODS

A total of 45 specimens of *C. sapidus* were collected between in August 2021 from Çanakkale Strait from the depth between 1.5 and2 m. Microplastics were incidentally observed in the stomachs of crab specimens. Crabs were counted, measured (carapace width, CW and weight), and examined for sex. Stomachs were removed and the contents preserved in ethanol of 70% for dietary analysis and then examined under a binocular microscope (Olympus SZX7). Digital images were measured using ZEN software. Frequency, numerical abundance, volumetric abundance, and relative importance index of food groups were calculated using the data obtained from stomach content analysis [43].

Fi%= number of stomachs with i food x 100 / total number of full stomachs,

Ni%= total number of nutrients i x 100 / total number of nutrients,

Vi%= total volume of nutrient i x 100 / total nutrient volume,

IRI= (N%+V%) x F%

IRI%= IRI value of nutrient i x 100 / total IRI values

RESULTS AND DISCUSSION

A total of 26 males, 4 females, and 15 juveniles were examined in the study (Figure 1). The morphometric characters of the individuals are presented in Table 1. Mean carapace length (CL) was 130.30±2.36 mm, mean carapace width (CW) was 61.22±0.84, and mean weight was 161.34±4.85. The stomach contents of 45 individuals with an average carapace length were examined.

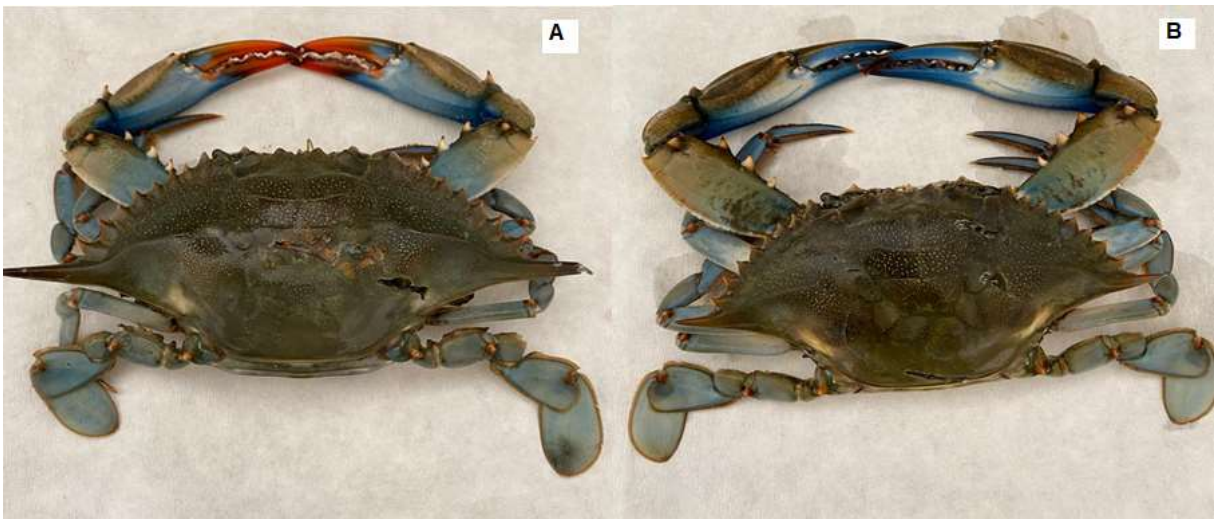

Figure 1. *Callinectes sapidus* individuals sampled from Çanakkale Strait (A: female, B: male) (PhotoFigureed by S. Acar).

Table 1. Morphometric characters (CL, CW and W) of *Callinectes sapidus*

		Carapace length		Carapace width		Weight	
	N	CL (mm)	CL (min-max)	CW (mm)	CW (min - max)	W (g)	W(min-max)
Female	4	133,10±1,85	156,15-177,2	61,85±0,85	64,72-75,02	168,15±3,68	171,37 - 258,34
Male	26	129,32±1,27	102,73-154,49	60,94±0,68	51,16-69,75	159,58±2,24	91,02 - 252,87
Juvenile	15	125,94±1,25	108,77-133,26	59,36±0,66	51,93-62,17	141,11±3,58	94,63 - 151,97
Total	45	130,30±2,36	102,73-177,2	61,22±0,84	51,16-75,02	161,34±4,85	91,02 - 258,34

A total of 46 microplastics were recorded from 15 different stomachs. Microplastics were detected in 10 males and 5 juveniles. No microplastic fragments were found in the stomach of the female individuals. The microplastics found in the stomachs of the crabs are in the form of blue and black colored threads. 26 black and 20 blue microplastics were found in the stomach contents. (Figure 2).

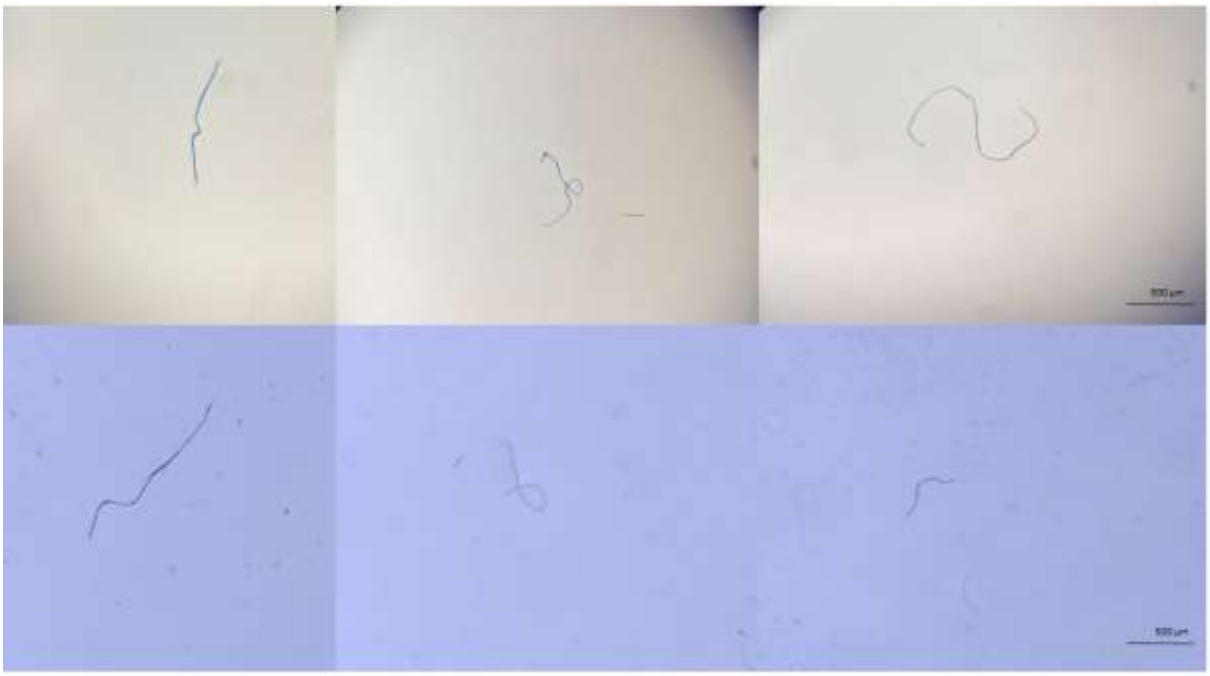

Figure 2. Microplastics (fiber) found in the stomachs of *C. sapidus.*

The N% value of microplastics in the stomach contents of blue crabs was calculated as 1.39, F% as 9.16%, V% as 4.33%, the IRI value as 52.37, and the IRI% value as 1.40. Acar and Ateş (2018), found microplastics, in only 3 male individuals out of 533 stomachs in another portunid crab, *Carcinus aestuarii*. Same authors found that the plastic pieces found consisted of fibers. We also found the microplastic in the stomach contents consisted of fibers. It is generally assumed that the microplastic fragments found originate from fishing gear used in fishing (Acar and Ateş, 2018).

Primary producers (plankton, etc.) and consumers (fish, birds, etc.) are at different levels of the food chain, and consumer organisms (e.g., fish) prey phyto or zooplankton at lower levels. This situation causes a pollutant that enters to the chain at the lowest level to be transported to higher levels. Meanwhile, several active substances such as phthalates, bp-a used in plastic manufacturing can be released again and cause various harmful effects (cancer, etc.) in living organisms (Gündoğdu and Çevik, 2019). Transfer of microplastics to humans is also possible when the soft tissue of the whole organism is consumed (Li et al. 2015) or when the edible parts of organisms overlap contaminated tissue, as in the case of bivalves or soft-shelled crabs (Waddell et al., 2020). The blue crab, whose population has increased in our country's seas in recent years, is also consumed as human food. The place of microplastics, which are thought to accumulate in tissues after consumption, in the food chain and their accumulation in tissues should be investigated in future studies.

CONCLUSION

Aquatic pollution has become an increasing environmental problem in recent years and aquatic ecosystems are constantly polluted with contaminants, especially through inputs from the marine and terrestrial environments. Plastic pollution is increasingly damaging water and

aquatic life. Microplastics, which are pieces of plastic with a size of less than 5 mm, accumulate both in the water column and on the bottom. The present study is on the presence of microplastics in the stomach contents of *Callinectes sapidus* in the Çanakkale Strait. We found that microplastics also enter the crab's body during the crab's food intake.

ACKNOWLEDGEMENT

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SPECTROSCOPIC MONITORING OF THE PHOTOCATALYTIC DEGRADATION OF HUMIC ACID USING BINARY OXIDES

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ABSTRACT

Natural organic matter (NOM) is a key component in an aqueous environment, consisting mainly of humic acids and fulvic acids. The presence of NOM in water leads to notable problems, mainly by causing disinfection by-product formation in drinking water. Therefore, NOM removal is a primary concern for the availability of safe drinking water. The present work was focused on the investigation of the photocatalytic degradation of humic acid (HA) as a representative compound of NOM. Detailed analysis of the degradation process was conducted using ATR-FTIR and Raman Spectroscopy techniques. The previously prepared binary oxides with three different mass ratios of ZnO/TiO_2 (1:1, 1:3, and 3:1) were used as the photocatalyst. The structural changes in the functional groups of HA during the photocatalytic process have been monitored by using ATR-FTIR. Proving the results of the study with the fast and inexpensive ATR-FTIR method showed the feasibility of the photocatalytic study. Raman spectroscopy contributed complementary information to the FTIR results. These two characterization methods showed that the photocatalytic process was quite efficient. In general, slight variations and shifts were observed in the characteristic bands of HA due to the photocatalytic irradiation time and upon binary photocatalyst.

Keywords: Binary oxides, heterogeneous photocatalysis, humic acid, TiO_2, ZnO.

INTRODUCTION

Humic substances, humic acids (HA), and fulvic acids are defined as representatives of natural organic matter (NOM). HA are considered as amorphous macromolecules composed of multifunctional aromatic components with aliphatic moieties such as carboxylic and phenolic functional groups (Chi and Amy, 2004; Conte and Berns, 2008; Uyguner-Demirel et al., 2017; Uyguner-Demirel et al., 2022). Semiconductor photocatalytic treatment has revealed a great potential for photocatalytic degradation by investigating HA removal since it is a lowcost, environment-friendly treatment technology (Birben et al., 2016; Turkten and Bekbolet. 2020; Turkten et al., 2019; Turkten et al., 2018; Uyguner-Demirel et al., 2017). It is well known that

FTIR and Raman spectroscopy are used as binary spectroscopic approaches to characterize the functional groups of HA (Birben et al., 2021; Francioso et al., 1996; Senesi et al., 2003; Uyguner-Demirel et al., 2022). In this study, the spectral profile changes of HA using five different photocatalyst specimens after photocatalytic treatment were monitored by FTIR and Raman spectroscopy. The changes during photocatalysis were explained by the specific band comparison concerning the selectivity of the photocatalyst specimens using these fast and inexpensive spectroscopic techniques.

MATERIALS AND METHOD

All chemical reagents were analytical grade and used without further treatment. HA in the form of Na salt was supplied by Aldrich. The 100 kDa working solution of HA was prepared by using 50 mg/L of HA following filtration through a 0.45 μm membrane filter, and then the ultrafiltration process was performed by using an Amicon 8050 model ultrafiltration stirred cell unit (Kerc. et al., 2004). Photocatalyst specimens were TiO_2 P-25, Evonik (TiO_2), ZnO (Aldrich). The ZnO-TiO_2 binary oxides with three different mass ratios of ZnO/TiO_2 (1:1, 1:3, and 3:1) were prepared by a simple solid-state dispersion method. The detailed information on the preparation and characterization of the photocatalyst specimens was reported in our previous study (Turkten and Bekbolet, 2020). Solar photocatalytic activity testing was performed using a solar simulator, ATLAS Suntest CPS +. The experiments were performed in a 50 mL HA solution, keeping the photocatalyst specimen dose at 0.25 g/L constant. Following each treatment period (t=0-120 min), the photocatalysts were immediately removed through 0.45 μm membrane filters. The filtrates were monitored to follow the alterations during photocatalysis using FTIR-ATR and Raman spectroscopy. FTIR measurements were performed by a Perkin Elmer Spectrum Two model FTIR equipped with a Universal ATR accessory. All spectra were recorded on 64 scans with a scan resolution of 2 cm^{-1}. The Raman spectroscopy measurements were obtained by a Jobin Yvon LabRam confocal microscopy Raman spectrometer with a laser excitation of 632.8 nm.

RESULTS AND DISCUSSION

The photocatalytic degradation of HA using binary oxides was studied and characterized via basic spectroscopic methods. The shift and the change in the intensities of the peaks in the FTIR and Raman spectroscopy showed the state of the degradation. The FTIR spectroscopy measurements were carried out using the ATR technique. The chemical structure of HA is given in Figure 1.

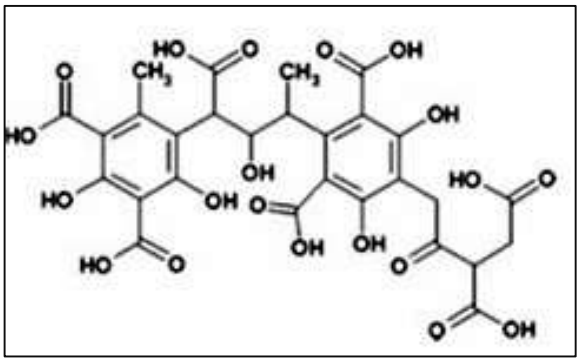

Figure 1. Chemical structure of Steelink's proposed unit structure of HA (Tan, 2003).

ATR-FTIR is a relatively efficient, fast, and inexpensive technique to monitor the structural changes in the functional groups of HA during the photocatalytic process. Three different binary oxides with weight ratios of ZnO/TiO_2 as 1:1(ZT-11), 1:3(ZT-13), and 3:1(ZT31) were used as catalysts, and monitored by using ATR-FTIR spectroscopy.

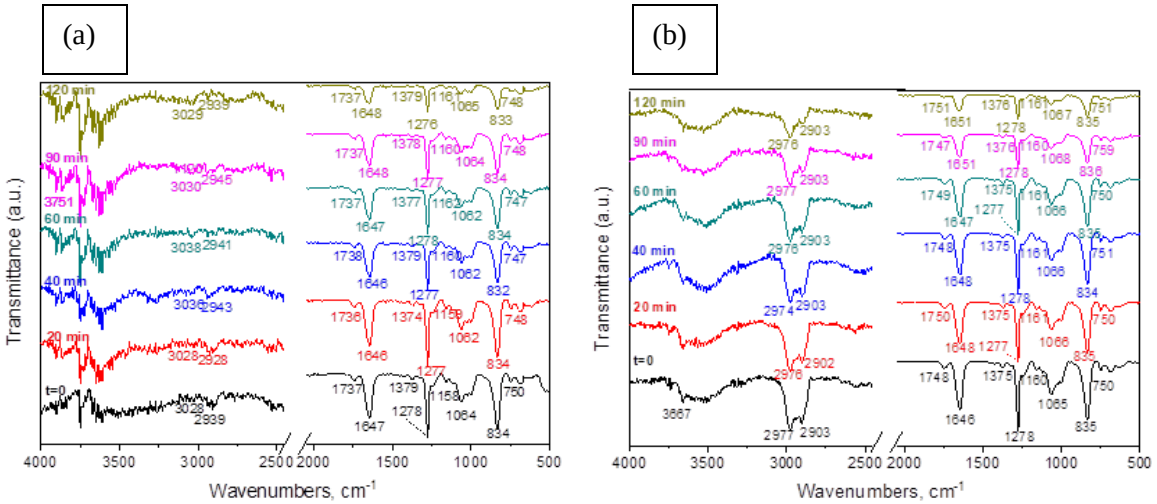

Figure 2. ATR-FTIR spectra of the degradation of humic acid using (a) ZnO and (b) TiO_2 as photocatalyst.

According to, Figure 2, the decrease in the intensity of C=O stretching of amide groups (amide I band), C=O of quinone and/or H-bonded conjugated ketones peak intensity observed in 16301660 cm^{-1} was observed for both catalysts in direct proportion to the exposure time. Likewise, a significant decrease was observed in the intensity of the C–O stretching and OH deformation of COOH, C–O stretching of aryl ethers and phenols (1260-1200 cm^{-1}) peaks, and out-of-plane bending of aromatic C–H, tri- and tetra substituted aromatic rings (975-775 cm^{-1}) peaks for both catalysts depending on the exposure. On the other hand, no change was observed in the intensity of the peak belonging to the C–O stretching of alcohols and aliphatic ethers observed at 1066 cm^{-1} according to exposing time (Francioso et al., 1996). These results indicate that both oxides have photocatalytic effects for the degradation of humic acid separately.

According to these results, the synergetic effects of binary oxides mixed in different ratios were tried as a photocatalyst to reach the optimum result.

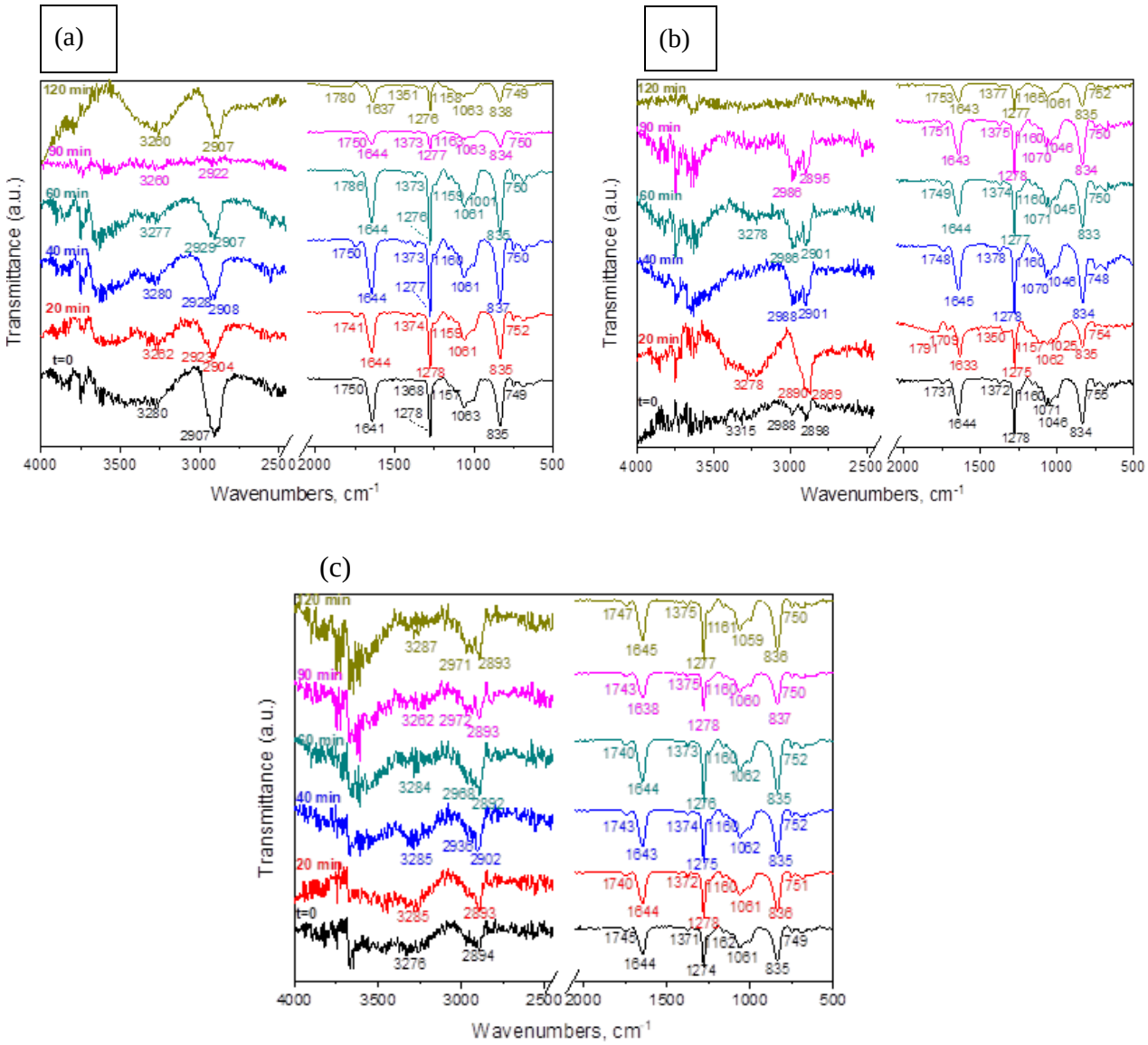

Figure 3.ATR-FTIR spectra of degradation of humic acid by using(a) 1:1(ZT-11), (b) 1:3(ZT13), and (c)3:1(ZT-31) binary oxides as photocatalysts.

Figure 3 indicates that using the mixture of ZnO/TiO_2 at a different ratio as a catalyst gave similar results to binary oxides. Figure 3 shows that using different ratios of ZnO/TiO_2 mixture as catalyst yields similar results with the oxides used separately. Differently, when ZT-31 photocatalyst was used, no significant decrease was observed in the intensity of the main peaks (1644 cm^{-1}, 1275 cm^{-1}, and 835 cm^{-1}) depending on the exposure time compared to other mixtures (Figure 3(c)). This result can be interpreted as meaning that the ZT-31 photocatalyst mixture may have low efficiency in HA degradation. The FTIR Spectroscopy results were supported using Raman Spectroscopy. Figure 4 represents the Raman spectra of the degradation of HA by using all of the photocatalysts (TiO_2, ZnO, ZT11, ZT-13, and ZT-31)

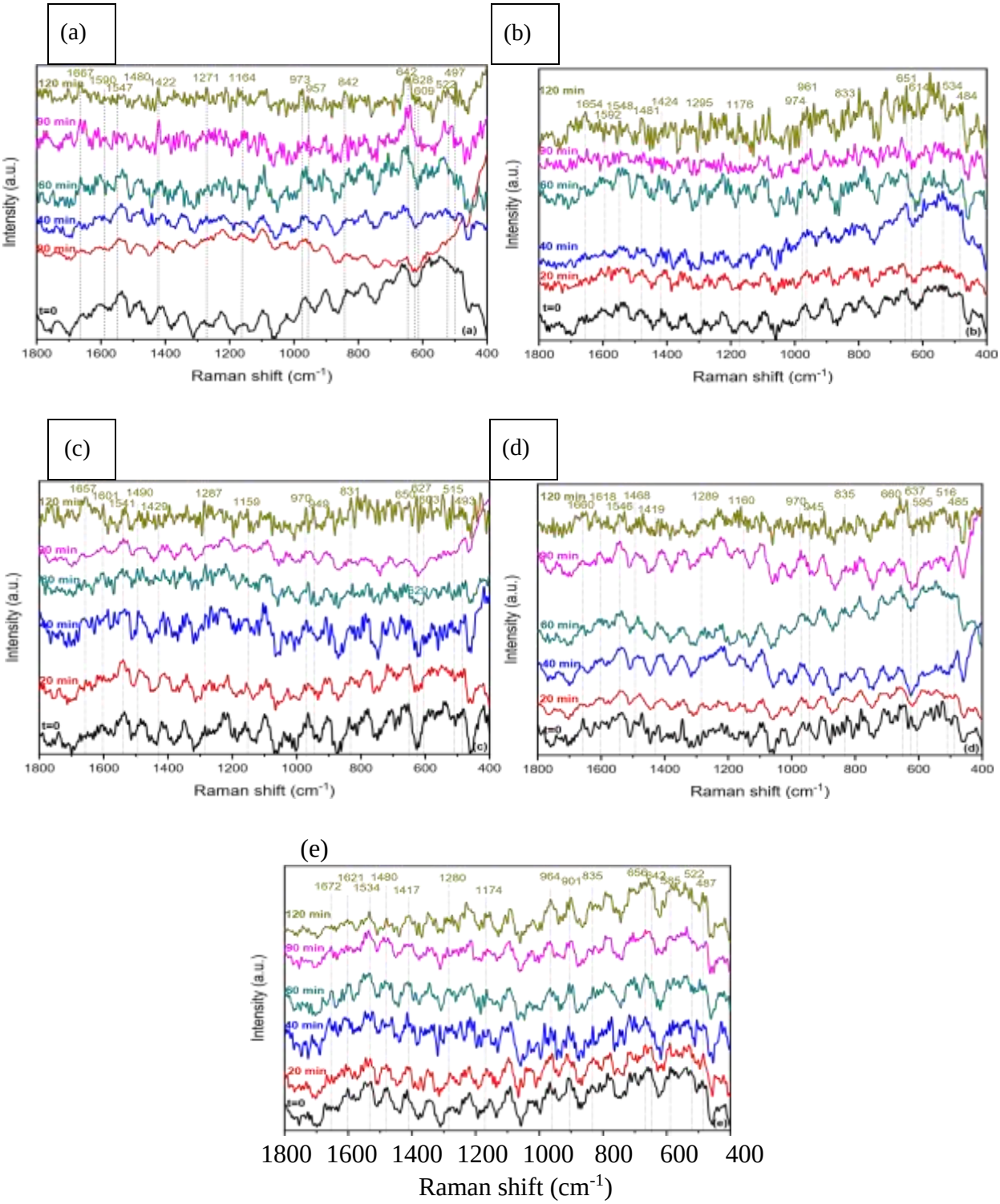

Figure 4. Raman spectra of degradation of humic acid by using (a) TiO_2, (b) ZnO, (c) ZT-11, (d) ZT-13, and (e) ZT-31.

Raman spectroscopy results indicated similar results to the results of FT-IR spectroscopy. Specifically, main Raman shifts(cm^{-1}) at 1680-1635 cm^{-1} (Amide I: C=O, C-N, N-H), 14101400 cm^{-1} (COO- symmetric stretching of carboxylic acids),1275-1210 cm^{-1} (C-O stretching in phenols, CH_2 deformation), and 1130-1030 cm^{-1} (C–C stretching, C–O stretching, C–O–H

deformation) were investigated (Francioso et al., 1996). The results show that all photocatalysts have a similar effect on the degradation of HA, with peak intensities decreasing with exposure time. However, the photocatalyst ZT-31 (Figure 4e) was also observed to be less effective in the degradation of HA than the others because there was not any particular change in the peaks, intensities, and shifts.

CONCLUSIONS

The structural changes in the functional groups of HA during the photocatalytic process have been monitored by using ATR-FTIR. Raman spectroscopy contributed complementary information to the FTIR results. In general, slight variations and shifts were observed in the characteristic bands of HA due to the photocatalytic irradiation time and upon binary photocatalyst.

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THE ROLE OF RURAL WOMEN IN ENSURING FOOD SECURITY: AN EVALUATION FOR TÜRKİYE

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ABSTRACT

Despite the constraints due to as traditions affecting gender discrimination in agriculture, women are one of the essential factors for rural economy and rural development, especially in terms of labor-intensive activities. Rural women have an important role in the agricultural sector for the supply of available, accessible, usable and sustainable food at the global level, as well as in Türkiye. For this purpose, studies are carried out in different scopes in every country so that rural women can maintain and increase their socio-economic conditions. The aim of this study is to evaluate the current situation regarding the role of rural women in the agricultural sector in terms of their contribution to food security in Türkiye. For this purpose, studies on rural women in Türkiye, the activities of organizations related to rural women and the policies followed on rural women were examined. In addition, the contribution of studies aimed at increasing the share of women in agricultural employment and thus empowering rural women economically on food security has been evaluated and recommendations on the subject have been included.

Keywords: Food Security, Rural Woman, Women's Cooperative, Türkiye

INTRODUCTION

Women living in rural areas work from the preparation of the soil to the harvest, and after the harvest, all of the storage, maintenance and sales work. Therefore, rural women can be defined as individuals who produce, evaluate, market in agriculture and produce what they consume due to their role at home. Rural active women make up a large part of the growing world population of about 8 billion, and they produce 60-80% of the food in many developing countries, responsible for half of the food production (Ulker, 2013; OECD, 2022; Worldometer, 2022). The intensive participation of rural women in agricultural production activities in underdeveloped and developing countries shows that rural women constitute the main power of rural economy and development. However, attention is also drawn to the problems experienced on the basis of gender inequality, which poses a threat to the sustainability of the contribution of rural women and their role in agricultural production in these countries. The participation rate of rural women in agricultural production also differs according to the development level of the countries. It is stated that culture, beliefs and socio-economic factors

are effective on the basis of this difference (Ataseven, 2018; Yavuz et al., 2018; Visser and Wangu, 2021).

Although the number of women working in the agricultural sector and the contribution of women to agricultural production are very important in underdeveloped and developing countries, the number of women working in the agricultural sector is decreasing worldwide. While the rate of women employed in the agricultural sector was 44% in 1991, this rate decreased to 27% in 2017. As of 2017, the rate of women employed in the agricultural sector is listed as 2%, 34% and 70% in developed, developing and underdeveloped countries, respectively (WB, 2018). In line with these rates, it can be said that rural women have an important role in the agricultural sector for the supply of available, accessible, usable and sustainable food in many countries and Türkiye (Garcia, 2013). In order for rural women to maintain and increase their socio-economic existence, studies are carried out in different scopes by different institutions and organizations in almost every country. The aim of this study is to evaluate the current situation regarding the role of rural women in the agricultural sector in terms of their contribution to food security in Türkiye.

THE ROLE AND IMPORTANCE OF RURAL WOMEN IN ENSURING FOOD SECURITY

Food security is a comprehensive concept that includes the dimensions of availability, accessibility, utilisation and stability. These dimensions, respectively, mean that people can afford sufficient food, sufficient economic resources to access these foods, the necessary quality and health conditions, and people's access to sufficient food at all times (Keskin and Demirbas, 2012; Tunc and Demirbas, 2022). The importance of this concept, which is defined by FAO as individuals' physical and economic access to sufficient, healthy, safe and nutritious food they need for a quality and healthy life at all times, is increasing with the climate crisis and political crises (FAO, 2006).

Considering the indispensability and economic importance of food products in life, food security as a whole is today a top priority issue in terms of public authority and procedures. While the world is faced with the possibility of a food security crisis with the increasing population, there are two types of nutrition problems. First and foremost is access to adequate food. The other is the intake of healthy and safe food sources that the body needs. It is necessary to ensure that people have the right to buy and consume foods that are sustainable, reliable, affordable, of good quality, and that will develop healthy eating habits (Quisumbing et al, 1996; Dolekoglu, 2002; FAO, 2003).

Rural women, who are actively involved in almost every stage of production, contribute to the provision of production by applying methods such as fallow, alternating planting and spreading, and various techniques in order to protect the soil, increase its productivity and enrich it so that people can reach healthy and sufficient food. It has an important role in ensuring hygiene in animal husbandry and in the production of animal products. At the same time, it produces food at home and plays a decisive role in the purchase of foodstuffs. Therefore, it can be said that women have an important place in the food chain from farm to table (Uzunoz et al.,

2008; STGM, 2010; Brown, 2011). Rural women also contribute to ensuring food safety, environmental protection and soil sustainability.

Although the economic roles and functions of rural women are very important, they work as unpaid family workers in many areas. While women mostly do housework alone, they also contribute to the family budget by doing income-generating (jam, drying for agricultural products, canned food, cheese, yoghurt etc.) works such as handicrafts, turning vegetable and animal raw materials into value-added products (Yilmaz et al., 2019). The economic empowerment of rural women, on the other hand, increases the level of economic access to food and supports the accessibility dimension, which is a very important dimension of food security.

CONTRIBUTIONS OF WOMEN LABOR TO AGRICULTURE SECTOR

The level of women's participation in production in the agricultural sector varies according to the land and livestock owned by the family, to the production structure and income level. Women participate in all stages of plant and animal production, especially seasonal labor. Although the number of women working in the agricultural sector has decreased significantly in Türkiye, as in the world, most women still work in all agricultural enterprises, most of which continue their activities as small family businesses. For this reason, women take part in many stages of agricultural production as unpaid family labor and make a significant contribution to the formation of family income (TURKTOB, 2007; SOFA and Doss, 2011; Yavuz et al., 2018).

The main source of livelihood in rural areas is mostly plant and animal production and seasonal migration. Rural women, on the other hand, participate in production, mostly in post-harvest processing or animal production. If the enterprise has low mechanization level, women are more involved in agricultural activities. Men in vegetable and viticulture in regions where agricultural production is limited; works in garden preparation, collection, irrigation, hoeing. In regions where the main source of livelihood is plant production, female labor is involved in every stage of production except sales. In animal husbandry activities, while men do things like grazing, feeding and shearing, women undertake milking, evaluation of dairy products, barn cleaning and grass collection. In addition, women produce fat, yoghurt and cheese from animal products for the family's self-consumption as well as for sales purposes (Ozcatalbas, 2001; Kızılaslan and Yamanoglu, 2010; Ulker, 2013; Yilmaz et al., 2019).

Despite this active participation, women's labor in the agricultural sector is not seen as an income-generating activity and is not reflected in the statistics, as it is an unregistered and insecure worker in the family and an unpaid family worker in production. Informal employment, which is seen as a problem specific to developing and underdeveloped countries, can also be considered as a problem for Türkiye. According to the statistics of the Social Security Institution, the sector with the highest unregistered employment in Türkiye on a sectoral basis is the agriculture sector. In 2017, informal employment in agriculture was 83.3%. In 2021, 4 million 948 thousand people were employed in the agricultural sector. Informal employment rates in the agricultural sector by gender are remarkable. Accordingly, while

94.2% of women are employed informally in the sector, this rate is 77.7% for men (Candan and Gunal, 2015; Ocal and Senel, 2021; TUIK, 2022).

Informal economy and unregistered employment are explained by the fact that the social and economic policies of the countries with this problem are not carried out in a coordinated manner and the existence of serious structural problems of the economic system. The reasons that push employers to work informally can be listed as the high wage and non-wage social costs, economic crises, tax policies, insufficient inspections, the structure of the workforce and rapid urbanization (Ocal and Senel, 2021).

STUDIES ON RURAL WOMEN IN TÜRKİYE

The National Action Plan for Empowering Women in Rural Areas (2012-2016) was published by the Ministry of Food, Agriculture and Livestock. In the action plan, targets such as improving the position of women in rural areas, making the agricultural sector sensitive to gender, improving Türkiye's international indicators and rankings in statistical data on women, and integrating rural women with national development studies are listed. In this context, for a solution, first of all, workshops were organized on a regional basis in the country and basic problem determinations were made (Ministry of Food, Agriculture and Livestock, 2012).

Another institution, the Ministry of Family and Social Policies, organized a Women Farmer Training Program for the empowerment of women in rural areas within the scope of the Women's Empowerment Strategy Document and Action Plan (2018-2023). In addition, it has been decided to carry out projects in cooperation with institutions and organizations in the creation of models that will increase employment and entrepreneurship, and improve working conditions for rural women to benefit from vocational training and development courses (Ministry of Family and Social Policies, 2018).

In the National Action Plan for Gender Equality (2008-2013), the position of rural women is discussed in terms of education, economy, poverty, authority and decision-making mechanisms, health, media, environment, human rights and violence. Again, in the 61st Government Program, it was aimed to facilitate employment by providing qualifications to the unemployed, especially the young, women and unskilled workforce, by eliminating the rigidity of the labor market within the framework of the flexible flexibility approach and the principle of "protecting people, not the job", in order to increase employment and reduce informality (Prime Ministry, 2008; Official Gazette, 2011).

Turkish Cooperative Strategy and Action Plan (2012-2016) was published in the Official Gazette dated 17.10.2012 and numbered 28444, which is also a working text concerning rural women. (Official Gazette, 2012). The action plan includes measures related to women's cooperatives. Regarding the measures, the Ministry of Customs and Trade, the Ministry of Food, Agriculture and Livestock and the Ministry of Family and Social Policies were included as the responsible institutions. The Ministry of National Education, the Ministry of Development, the Ministry of EU Affairs, KOSGEB, İŞKUR and the Foundation for the Evaluation of Women's Work (KEDV) were counted among the institutions and organizations to cooperate (Ministry of Customs and Trade, 2019).

In the 10th and 11th Five-Year Development Plans, women's participation in decision-making mechanisms, increasing their employment, making producer organizations the leading institutions in social solidarity, cooperation, education and financing and observing positive discrimination, improving occupational health and safety conditions, institutional and development of local capacities, dissemination of women's entrepreneurship, and flexibility with parental leave to reconcile family and work life. In addition, policies to increase awareness with non-formal and formal education starting from early childhood, to develop exemplary practices by raising awareness on gender-sensitive budgeting, and to increase education and skill levels were included. By 2023, it is a priority among the targets to have a female employment rate of 39% and a labor force participation rate of 38.5% (Ministry of Development, 2013; Strategy and Budget Presidency, 2019).

ORGANIZATIONS ABOUT RURAL WOMEN

In all economic systems, it is accepted that women are disadvantaged in terms of working life and participation in the workforce. The most important method that will enable women to be less risky in economic activity and more effective in social life is cooperatives. In Türkiye, on the other hand, in the Turkish Cooperative Strategy and Action Plan, the issue has been approached in the most comprehensive way with the aim of organizing women under the roof of cooperatives in order to increase their entrepreneurial abilities and ensure their participation as an actor in the economy. In order to achieve the goal, measures such as encouraging women who cannot work in another job to organize under the roof of cooperatives, increasing their working, production and marketing opportunities, and carrying out studies to meet their social and cultural needs were included (Emiroglu, 2019; Ministry of Customs and Trade, 2019).

Again, the Cooperatives Support Program (KOOP-DES) aims to support the investment projects that will contribute to the production and employment of the cooperatives and their higher organizations, to ensure efficiency and productivity in their activities, to contribute to the benefit of technology and new production techniques, and to bring individual savings into the economy with appropriate cooperative entrepreneurship models. It is a project announced by in 2020. Only cooperatives and their higher organizations, whose establishment, operation and control were carried out by the Ministry of Commerce, whose members were mostly women, and which aimed to evaluate women's labor, could apply to the project (Ministry of Industry and Technology, 2021). The supports given within the scope of the project are mostly for the purchase of machinery and equipment. In this context, 14 from Tokat, 7 from Adana, 7 from Antalya, 7 from Isparta, 5 from Hatay, 4 from Eskişehir, 3 from Istanbul, 3 from Izmir, 2 from Ankara, and from Nevşehir and one from Bolu, the majority of the members of which are women and which aim to evaluate women's labor, the projects that will contribute to production and employment in accordance with their field of activity have received grant support (Cakir, 2020).

In Türkiye, there are approximately 800 establishments of the "Women's Entrepreneurship Production and Business Cooperative" type registered in the Cooperative Information System. However, detailed and systematic information about these organizations

cannot be reached and a general evaluation cannot be made. However, in the study, it has been tried to shed light on the subject with examples of women's initiatives and production cooperatives. One of these examples, Defne Women's Cooperative, was established in 2010 in Defne district of Hatay. The cooperative produces local products and delicacies such as olives, olive oil, pomegranate syrup, cottage cheese, pickles, dried fruits such as apples, oranges, dates, pepper and tomato pastes, medicinal and aromatic plants collected from nature. The products are processed, packaged and packaged in the facility that has an organic product processing certificate; it is offered for sale under the brand name 'Defne Dalı'. By selling the products they produce to the cooperative, women make an economic contribution to them with the hourly wage they receive. In this way, the cooperative contributes to the economic empowerment of rural women with low incomes by including them in production (Defne Woman, 2022).

Ahatlı Women's Cooperative, located in Kaş district of Antalya province, was established in 2018. The cooperative works in solidarity and cooperation with local women producers. Address-delivered food marketing is carried out and all of the products are produced by women living in the surrounding villages with their own means. Natural and homemade products such as bulgur, tarhana, thyme, dried vegetables, chickpeas, noodles, almonds and carob are marketed (Yildirim, 2020; Ahatlı Women's Cooperative, 2022).

Again, value-added products such as vegetable oils, butter, olive oil, pomegranate syrup, dried fruits and vegetables, tomato paste, spices and pickles are produced and marketed in the Limited Responsibility Women Production and Business Cooperative, established in 2019 by 25 entrepreneur women in the Samandag district of Hatay province. are provided to generate income (Ministry of Commerce, 2022; Samandag Women's Cooperative, 2022).

Mersin Women's Initiative Production and Business Cooperative was established in 2019 by eight founding partners in order to contribute to the family and national economy by increasing women's employment, to increase the value of women in society, to strengthen the unity between women and to brand local products. The cooperative works with a wide network of projects extending to agriculture, tourism, textiles and souvenirs. With the Atalık Yellow Wheat Project, pesticide-free grain production, natural and additive-free jam production made with Silifke strawberries, and the theoretical and practical training given with the Ornamental Plant Cultivation Project, it carries out activities that educate rural women and also support them economically (Senyurt, 2020).

Despite successful examples and high rate of rural women's recognition of producer organizations, it is also known that the level of membership/membership in cooperatives is quite low. As a matter of fact, in a study conducted with women living in the villages of the Thrace Region (Edirne, Kırklareli, Tekirdağ) and based on 323 agricultural enterprises, it was determined that the organizations with the lowest percentage of women were agricultural development cooperatives and the chamber of agriculture. More than half of the women did not want to take an active part in an organization to be established for economic purposes. The most important reasons for these are listed as lack of spouse or family leave, health problems and not needing them (Ozdemir et al., 2019).

EVALUATION OF STUDIES ON RURAL WOMEN IN TERMS OF FOOD SECURITY

Food security is a concept that encompasses all the details the food supply chain from farm to fork. Food should be enough for the ever-increasing population, nutritious and healthy for people, and at the same time, people should always be able to access these foods. Economic empowerment of rural women is seen as the key to ensuring food security (Quisumbing and Meinzen-Dick, 2001). For this reason, the prepared action plans, government programs, development plans and cooperatives-oriented studies are primarily built on the objectives of increasing the quality of life of rural women and then strengthening their role in agricultural production. The formation of a social structure in which rural women can use their right to speak and decide in every ring of the food chain and the implementation of the objectives for this serves the transfer of knowledge and experience to women. A process where women can transfer information, decide and implement at the same time, due to their role in the sanitation and hygiene stages of agricultural production with their environmental protective attitude, is extremely important in terms of realizing the adequacy, usability and stability dimensions of food security.

In addition, it is inevitable to provide economic and social equality in a system where rural women can receive their contribution to agricultural activities based on gender equality. Implementation of the studies planned to reduce the binding housework of rural women, increasing their participation in professional development trainings in line with the agricultural changes of the period, expanding the fields of employment where they can apply their knowledge, developing the awareness of organization in line with the goals of cooperatives will increase the quality of agricultural production and the purchasing power of rural women, thus playing a strong key role in terms of food accessibility (Gokdemir and Ergun, 2012; Ministry of Food, Agriculture and Livestock, 2012). From this point of view, the sensitivity of rural women to the threats of climate change and environmental degradation to food security will also indirectly support food security. Rural women who have an active role in cooking, tillage, heating and waste disposal should be informed about alternative methods. In order to strengthen rural women and increase productivity in agricultural production, it is important to provide productive women with technologies suitable for their production and to train them. Gender-sensitive planning in education and technology development will not only increase production today, but will also contribute to the protection of the environment in the future (STGM, 2010; Ozdemir et al., 2017; Patil and Babus, 2018).

Strengthening employment by raising the awareness of women, increasing their contribution to economic life by improving their quality of life, social and economic conditions will carry women to a stronger position economically, and this will again return as a contribution to food security in terms of access to food (Malinowks and Radon, 2011; Kadim et al., 2014). Developing knowledge and practices through training and course opportunities for women in rural areas, providing support for women to own land and/or animals, and ensuring that young women continue to live in rural areas with high quality through organized activities are extremely important in terms of making access to reliable and sufficient food possible and sustainable (Fidan et al., 2017; Yavuz et al., 2018).

CONCLUSION AND RECOMMENDATIONS

It is known that women working in the agricultural sector experience various deficiencies in many aspects of life (economy, production, education, health, etc.). It should be possible to contribute to economic activities in order to empower women, increase the efficiency obtained from the work they do, and achieve personal empowerment by gaining new skills. In this way, the way for women to have a better quality and humane life will be paved, and it will be ensured that they gain prestige in social relations. However, in this process, the participation of the whole society in the education process and the policies to be followed in the equalization of gender roles should be accelerated. It is especially important to increase the sensitivity of men on this issue (Prime Ministry, 2008; Kaya, 2011; Yavuz et al., 2014).

In this study, the importance of rural women's contribution to agricultural activities was evaluated in terms of food security. Problems such as lack of education for the female workforce, unregistered employment, unequal wages for equal work, guidance through traditions and customs, policies that are not developed and implemented, and insufficient incentives/support for initiatives also limit the contribution of rural women to food security. It is clear that women who are provided with educational opportunities, whose rights are delivered and spaces where they can exercise these rights are created can struggle with negativities more easily. Rural women, who have become more productive and conscious in agricultural production, will contribute to reaching sufficient food both for the present and for the future by observing the protection of the soil during the production phase and contributing to sustainability. At the same time, rural women, who play an active role in the hygiene and sanitation process of food, will understand the importance of this better and will be an important starting point in providing healthy and nutritious food for the society.

Empowering rural women is a basic and indispensable criterion for ensuring food security. All of the studies are aimed at reducing or facilitating the difficulties experienced by rural women. However, the fact that rural women continue to experience the same problems today also reveals the inadequacy of the studies that are applied and thought to be implemented. Improving the quality of women's participation in agricultural production, increasing their productivity in agricultural production and increasing purchasing power will directly positively affect all four dimensions of food security. Therefore, the work done for the economic and social life of rural women should be implemented and monitored effectively, and the planned work should be put into action immediately.

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EVALUATION OF NO-TILLAGE AGRICULTURE WITHIN THE SCOPE OF FOOD AVAILABILITY FOR TÜRKİYE

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ABSTRACT

The issue of the availability of sufficient food to meet the food needs of the growing world population is increasing its importance with the climate crisis and possible famine periods. The importance and diversity of agricultural practices applied to protect and improve the soil destroyed by negative environmental conditions is also increasing. The no-till farming method, which is one of these applications, has come to the fore in recent years in terms of minimizing soil destruction and loss, positive effect on yield and reducing costs.

The aim of this study is to evaluate the economic results of no-till agriculture, which is a technical method in terms of its relationship with the availability dimension of food security by conducting a literature search. In line with the purpose of the study, the literature comparing no-till agriculture and different tillage methods in Türkiye has been researched. The technical results of the obtained literature were evaluated in terms of their direct effects on the farmer income, reducing the production costs and the efficiency effects on the production amount. Suggestions on the subject are also included in the study.

Keywords: Food Security, Availability, Regenerative Agriculture, No-till Agriculture, Yield, Production Cost

INTRODUCTION

Today, the consequences of climate change are one of the most serious problems of the agricultural sectors for all countries of the world. It is not only an environmental and ecological problem affecting all natural systems, but also has important implications for the global development agenda. High temperatures, soil degradation and water scarcity, rainfall variability and extreme weather conditions adversely affect agricultural production and destroy food systems (Tunc and Demirbas, 2022).

From this point of view, countries have been in search of new systems that can protect and sustain existing natural resources in order to avoid food security problems. Unsustainable land use and rapid loss of soil fertility, especially in developing countries, are both a cause and a consequence of world poverty. It is suggested that agricultural systems that cause soil degradation and rely on intensive tillage should be replaced with sustainable production systems in which mulch and plant residues remain as permanent soil cover (Derpsch and Moriya, 2007).

Soil cultivation has been carried out for many years in order to grow crops in the agricultural sector, to prepare suitable seed beds, to control weeds, to mix fertilizers, pesticides, animal manure and other additives into the soil. Initially, replacing the natural ecosystem with tillage helped release nutrients stored in soil organic matter. However, the decrease in organic matter with long-term tillage has deteriorated the soil structure over time and accelerated soil erosion (Papendick and Parr, 1997).

In recent years in Türkiye, the increasing sensitivity about the protection of soil and water resources, the tendency towards economic production and energy saving have brought important changes in soil cultivation to the agenda. Depending on these changes, interest in conservation tillage and especially no-till farming method is increasing as an alternative to traditional tillage (Celik, 2016).

The aim of this study is to evaluate the relationship of no-till agriculture, which is a regenerative agriculture practice, through the availability dimension of food security, in the light of studies on different tillage methods in Türkiye. The scope of the study is limited to the no-till farming method, among the restorative agricultural practices, and other regenerative farming practices are included when appropriate. The study is limited in the framework of no-till agriculture because it improves soil structure, prevents erosion and reduces production costs to a great extent.

MATERIAL AND METHOD

Studies on the effect of no-till agriculture on yield in crop production in Türkiye between 2002-2021 were searched in important databases such as "Google Scholar" and "National Thesis Center". Search terms were “tillage”, “no-till farming”, “zero tillage”, “direct sowing”, “multiple direct sowing”, “different tillage methods”. The literature obtained as a result of the research; method, scope and results are shown in tables on the basis of years.

According to the survey results, studies on the effect of no-till agriculture on production costs in Türkiye cover the period between 2002-2021, and studies on the effect of no-till agriculture on yield between 2006-2021.

REGENERATIVE AGRICULTURE

Soil is a crucial component in the global interconnection of earth, water, air and energy. Beneficial consequences of soil conservation or harmful consequences due to its degradation greatly affect climate change management. At the core of regenerative agriculture is the intention to preserve and restore soil health or restore highly degraded soil so that water quality, vegetation and land productivity can be increased (Rhodes, 2017; Giller et al., 2021). The unifying attitudes in the restorative agriculture system primarily aim to abandon tillage. In a broader context, it aims to protect bare soil against the negative effects of climate change and to repair the damage that has occurred, to encourage plant diversity in planting and to make livestock practices sustainable (LaCanne and Lundgren, 2018; Mitchell et al., 2019).

REGENERATIVE AGRICULTURE APPLICATIONS

Main applications used for the purposes of restorative agriculture systems (Lal, 2020):

No-Tillage Agriculture (NTA): This process, also called direct sowing or zero tillage, is the system in which the soil is continued without using any tillage tools.

Organic Farming: Organic farming is a holistic production system that supports and improves agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. Unlike using synthetic materials to perform any function within the system, production is carried out using agronomic, biological and mechanical methods whenever possible (FAO, 2022).

Drip Fertilization (Fertigation): Since the fertilizers are given to the root zone of the plant together with the water, no fertilizer is given to the places where there is no root development, and the nutrient elements are given to the root zone fully and evenly. With this method, energy and labor gain are provided (Hakgoren, 1993).

Agroforestry: It is defined as the conscious integration of trees and shrubs into crop and livestock systems to create environmental, economic and social benefits. Agroforestry has purposes such as being a diverse, multi-layered food production system and a method of natural resource conservation and/or regenerative land use (Elevitch et al., 2018).

Forage Trees: New agroforestry systems have emerged in the last three decades to feed animals, mostly involving the planting of exotic species and grown in hedges along field boundaries or along contours to limit soil erosion. Forage trees are grown extensively in the East African highlands, including Kenya, Uganda, Tanzania, and Rwanda, primarily by dairy farmers (Wambug et al., 2011; Franzel et al., 2014).

Biochar: Biochar, obtained by heating (pyrolysis) biomass at high temperature (300-10000C) and in an oxygen-free environment (pyrolysis), can improve the physical, chemical and biological properties of the soil due to its properties such as resistant to degradation, high specific surface area and negative surface charge, and can improve plant production. It is used as an additive that can increase its efficiency (Gunal and Erdem, 2018; Alaboz and Oz, 2020).

NON-TILING AGRICULTURE APPLICATION

Ensuring the continuity of soil resources, increasing its productivity and protecting it is of great importance for human life. Agricultural activities are threatened by the misuse and misapplication of land uses, erosion, dispersed and irregular soil and land destruction. It is stated that excessive tillage reduces the organic matter content of the soil, increases the possibility of wind and water erosion due to insufficient surface residues, and causes compaction in the soil due to field traffic (Gozubuyuk et al., 2017; Ministry of Development, 2018).

The traditional tillage method, which leaves the soil surface bare, is one of the main causes of erosion in agricultural areas. With no-till agriculture, the amount of organic matter in

the soil increases as a result of both leaving the stubble in the field and not encouraging the degradation of organic matter by not cultivating the soil. One of the reasons for the emergence of no-till agriculture is the preservation of soil moisture in places where precipitation is insufficient. Although no-till agriculture was initially thought as an effective soil protection method, it later turned into an economical and sustainable production system that improves the physical, chemical and biological properties of the soil, and was seen as a system that also protects the environment by reducing the emission of greenhouse gases (Ergul and Polat, 2009; Bayram et al., 2015).

In no-till agriculture, after the harvest of the previous product, sowing is done directly on the stubble without any tillage before planting. In direct sowing machines, the seeds are placed in the grooves opened by the burial feet that can work in the stubble, they are covered with soil and plant residues and pressed with special pressing elements. Thus, instead of treating the soil with a traditional plow or disc in crop production, a protective and restorative application emerges. Although the success of no-till agriculture depends on the climate and soil conditions, the performance of the seed drill and weed control, weed control is generally done with herbicides (Aykas et al., 2005; Isik et al., 2010).

Less cultivation of the land reduces the wages of labor, fuel, irrigation and machinery. In the no-till system, the yield is increased due to the higher water storage capacity and less soil erosion. Another benefit is that, thanks to its high water content, it provides the opportunity to grow a new product economically instead of leaving it fallow (Dilber and Guler, 2015).

No-till farming technique is difficult and requires more management knowledge, specially produced and expensive seeders are needed in planting mechanization, it is difficult to apply in heavy and poorly drained areas, it can be done mostly in monoculture agricultural areas, the cost is increased due to the use of more chemicals and it will cause environmental pollution. Limiting factors such as concern, low efficiency compared to the traditional system are also stated (Surek, 2004).

RELATIONSHIP BETWEEN NO-TILLAGE AGRICULTURE AND FOOD AVAILABILITY

Global understanding of climate change has shown a shift in meaning from sustainable increase in agricultural and food production to food security over time. There are four dimensions of food security, which has been deepened by adverse developments such as climate change, Covid_19 Pandemic and wars, which the world is experiencing, and which has made it a situation that needs urgent action, and these four dimensions must be realized at the same time in order to ensure food security. These are food availability, food accessibility, food quality and safety, and sustainability of the first three dimensions. The availability dimension relates to the availability of sufficient food. Otherwise, even if all other dimensions are in a position to be provided, the existence of food security cannot be mentioned (Keskin and Demirbas, 2012; Niyaz and İnan, 2016; Demirbas, 2022; Keskin and Demirbas, 2022).

Soil as the bed from which food is produced; It is an increasingly important natural resource in terms of its role in mitigating the effects of climate change and the adaptation

process to climate conditions, as well as its role in supporting many environmental processes such as the water cycle. Sustainability of the soil also supports the availability and sustainability of food. For this reason, the infertility and reduction of the soil that feeds people is one of the biggest risks for ensuring food security (Akin, 2021).

Ensuring the adoption of innovative practices such as the recommended no-till farming method among the practices that greatly help preserve the existence and health of the soil, increase resilience to the challenges of climate change in the agricultural sector, reduce its impacts, and at the same time protect the sustainability of agricultural activity will (Delgado et al., 2011; Demirbas, 2022) help secure the availability of food.

NO-TILL AGRICULTURE WORKS IN TÜRKİYE

Under this title, the technical results of no-till agricultural studies have been evaluated in terms of their effects on the yield and therefore the amount of production in terms of reducing the production costs and directly affecting the incomes.

THE EFFECT OF NO-TILL AGRICULTURE ON PRODUCTION COSTS

The expected benefit from tillage in terms of various factors such as soil structure, product yield and yield elements, cost, work quality, business structure, soil and water conservation varies depending on the selection and correct application of the most suitable tillage method. Among agricultural operations, tillage is the most expensive operation in crop production in terms of cost, as tillage causes the most power consumption, generally more than 50%, even if the soil conditions are in very good condition. The expectation of increasing the crop yield or obtaining the equivalent product at a lower cost by tillage brings to the fore the reduced tillage (RT) and no-till farming techniques (Gozubuyuk et al., 2010; Kasap and Dursun, 2013).

Traditional tillage requires higher inputs in terms of machinery investment, maintenance-repair and workforce compared to no-till agriculture (Aykas et al., 2005). In the no-till farming method, only one transition is made for planting in the field, while this number is at least two or more in the traditional method. Fewer passes mean less machine wear and maintenance costs. It is stated that the no-till farming method provides an annual average of 31.5 liters of fuel savings per hectare compared to the traditional tillage method. The fact that the said savings cover the costs (direct sowing machine and herbicide applications, etc.) required for starting no-till agriculture can make this system more profitable. Therefore, the decrease in expenditures motivates the producers who adapt to agriculture without tillage (Yalcin et al., 2003; Ankara Governorship, 2017).

No-till agriculture can provide 50-75% less total machine requirement, 50-80% less fuel consumption, 60-80% less working time requirement and up to 40% savings in machinery investment costs compared to conventional tillage (Korucu et al., 1998; Korucu, 2002; Aykas et al., 2005; Cikman et al., 2010). Considering that diesel is expensive and producer incomes are low in Türkiye, it is clear that fuel saving is extremely important (Tekin et al., 2017). As a matter of fact, in studies where different tillage and planting methods were measured in terms

of cost and yield, it was revealed that no-till farming method significantly reduced production costs compared to other tillage methods (Tugrul and Dursun, 2002; Cakir et al., 2006; Marakoglu et al., 2010; Çikman et al., 2010; Karaagac et al., 2012; Gozubuyuk et al., 2012; Onen et al., 2012; Ayhan and Cetin, 2014; Ozturk, 2015; Bulut, 2018; Seven, 2019; Aykanat et al., 2019; Kara and Arslan, 2021).

Studies on the effect of no-till agriculture on production costs in crop production in Türkiye between 2002-2021 are shown in Table 1 with their results.

Table 1. No-till agriculture and cost in crop production in Türkiye

Year	Author	Product	Region/ Province/ District	Method	Results	Conclusion
2002	Tugrul and Dursun	Sugar beet	Konya	Traditional Agriculture and No-Tillage Agriculture	The highest cost was realized with traditional agriculture, the lowest cost with NTA.	(+)
2006	Cakir, Yalcin, Aykas, Gulsoylu, Okur, Delibacak and Ongun	Corn	Menemen Region	Traditional Agriculture and No-Tillage Agriculture	While the highest time and fuel consumption was realized with traditional agriculture, the lowest was realized with NTA.	(+)
2010	Cikman, Monis, Saglam and Nacar	Corn and Sesame	GAP Region	Traditional Agriculture and No-Tillage Agriculture	The highest value of fuel, manpower and machinery consumption is in traditional agriculture, and the lowest in NTA method.	(+)
2010	Marakoglu, Ozbek and Carman	Chickpeas	Konya	Traditional Agriculture and No-Tillage Agriculture	The highest value of fuel, manpower and machinery consumption is in traditional agriculture, and the lowest in NTA method.	(+)
2012	Karaagac, Aykanat, Cakir and Korkmaz	Silage Corn	Çukurova Region	Reduced Tillage and No-Tillage Agriculture	While the highest time and fuel consumption was achieved with RT, the lowest time and fuel consumption was achieved with NTA.	(+)
2012	Gozubuyuk, Celik, Ozturk, Demir and Adiguzel	Wheat	Erzurum	Traditional Agriculture and No-Tillage Agriculture	Fuel, manpower and machine consumption are highest in conventional agriculture and lowest in NTA method.	(+)
2012	Cay and Aykas	Tomato	Çanakkale	Traditional Agriculture and No-Tillage Agriculture	The highest fuel consumption was observed in conventional agriculture and the lowest in NTA method.	(+)
2016	Bayhan	Sunflower	Tekirdağ	Traditional Agriculture and No-Tillage Agriculture	Fuel, manpower and machine consumption are highest in conventional agriculture and lowest in NTA method.	(+)
2017	Bektas, Ozyilmaz, Ozer, Altintas, Kaya, Ozgoz and Kocyigit	Wheat and Sunflower	Tokat/ Kazova	Traditional Agriculture and No-Tillage Agriculture	For both products, the highest fuel and manpower consumption is in conventional agriculture and the lowest in NTA method.	(+)

2018	Bulut	Corn	Mardin Plain	Traditional Agriculture and No-Tillage Agriculture	In terms of time and fuel consumption, the highest value is in traditional agriculture, the lowest value is in NTA method.	(+)
2019	Seven	Corn	Şanlıurfa	Traditional Agriculture and No-Tillage Agriculture	The method with the highest cost of fuel, human and machine power is traditional agriculture, and the lowest cost is the NTA method.	(+)
2021	Kara and Arslan	Wheat	Çukurova Region	Ridge Planting and No-Tillage Agriculture	The highest value in fuel, mineral oil, manpower and machine labor consumption is in the back planting method, and the lowest consumption value is in the NTA method.	(+)

THE EFFECT OF NO-TILLAGE AGRICULTURE METHOD ON YIELD

There is generally a negative relationship between tillage density and soil organic matter; Soil organic matter decreases in parallel with increasing tillage practices. Soil tillage practices have a significant impact on the preparation of a good seed bed, as well as on organic matter decomposition, nitrogen mineralization, distribution of carbon and nitrogen, and therefore soil properties and soil fertility, which are directly affected by them (Polat, 2020).

Temperature increases rise the rate of deterioration in the soil. This situation increases the danger of erosion and can cause a decrease in soil fertility. Research on soil fertility in Türkiye reveals that productivity has decreased by 23% in the last 10 years (Ministry of Agriculture and Forestry, 2019). The studies on the effect of no-till agriculture on the yield of crop production in Türkiye between the years 2006-2021 are shown in Table 2.

Table 2. No-till agriculture and yield in crop production in Türkiye

Year	Author(s)	Product	Region/ Province/ District	Method	Results	Conclusion
2006	Cakir, Yalcin, Aykas, Gulsoylu, Okur, Delibacak and Ongun	Corn	Menemen Region	Reduced tillage and No-Tillage Agriculture	While the highest yield was obtained in the RT method, the lowest yield rate was obtained from the NTA method.	(-)
2010	Cikman, Monis, Saglam and Nacar	Corn and Sesame	GAP Region	Traditional Agriculture and No- Tillage Agriculture	The lowest yield in corn was obtained from the traditional farming method. The second lowest yield was obtained from the NTA method. In sesame, the highest yield was obtained from the cotton planting machine and the stubble planting method and the lowest yield was obtained from the NTA method.	(-)
2010	Marakoglu, Ozbek and Carman	Chickpeas	Konya	Traditional Agriculture and No- Tillage Agriculture	The highest yield was obtained from conventional tillage and the lowest yield was obtained from NTA method.	(-)
2012	Karaagac, Aykanat, Cakir and Korkmaz	Silage Corn	Çukurova Region	Reduced tillage and No-Tillage Agriculture	It was determined that the highest yield of silage maize was obtained with RT, and the NTA method contributed 1% to the yield.	(+/-)

2012	Gozubuyuk, Celik, Ozturk, Demir and Adiguzel	Wheat	Erzurum	Traditional Agriculture and No- Tillage Agriculture	Yield (grain + stem) values that make up the total output in irrigated and dry farming conditions were examined on the basis of systems, and the highest yield was obtained in traditional agriculture. The lowest efficiency was obtained from the NTA method.	(-)
2012	Onen, Ozgoz and Ozer	Wheat	Tokat	Reduced tillage and No-Tillage Agriculture	The highest yield values were obtained in the plot where NTA was applied and herbicide applied. The lowest values were obtained from RT agriculture.	(+)
2013	Kasap and Dursun	Chickpeas	Tokat/ Kazova	Traditional Agriculture and No- Tillage Agriculture	In terms of plant and grain yield per square meter, the highest yield was obtained from the traditional agricultural method, and the lowest yield was obtained from the NTA method.	(-)
2014	Gursoy, Ozaslan, Urgun, Kolay and Koc	Lentil	Diyarbakır	Traditional Agriculture and No- Tillage Agriculture	The method that most affected the grain yield of lentils was traditional agriculture. The lowest yield was obtained from the NTA method.	(-)
2015	Ozturk	Soybean	Diyarbakır	Traditional Agriculture and No- Tillage Agriculture	The highest yield was obtained from the traditional tillage method, and the lowest yield from the NTA method.	(-)
2017	Yilmaz and Tuncturk	Saffllower	Muş	Traditional Agriculture and No- Tillage Agriculture	The highest seed yield was obtained with conventional agriculture, and the lowest seed yield was obtained with NTA. Soil tillage increased the yield by about 8%.	(-)
2018	Bulut	Wheat	Mardin Plain	Traditional Agriculture and No- Tillage Agriculture	The highest yield was obtained by conventional tillage, and the lowest yield values were obtained by NTA method.	(-)
2019	Seven	Corn	Şanlıurfa	Traditional Agriculture and No- Tillage Agriculture	Among the tillage methods, the highest yield was obtained from the NTA method, while the lowest yield value was obtained from the traditional tillage method, despite the energy consumption.	(+)
2019	Aykanat, Karaagac, Barut and Sevilmis	Wheat	Çukurova Region	Reduced tillage and No-Tillage Agriculture	For biological yield, straw and grain yield, RT has the highest yield and NTA has the second highest yield.	(+)
2021	Kara and Arslan	Wheat	Çukurova Region	Reduced tillage and No-Tillage Agriculture	The highest grain yield was obtained from RT application, but there was no significant difference between the yield obtained from NTA application and other applications.	(+/-)

EVALUATION AND CONCLUSION

Ensuring food security is related to ensuring product supply security. This necessitates the sustainability of production. As in every business, the decision to continue production in agricultural enterprises is related to economic return, namely profitability. The farmer can ensure the stability and increase of his income by reducing the cost and increasing the efficiency while making a production decision. From this point of view, in this study, the cost and yield effects of no-till agriculture, one of the proposed methods to ensure food security, are examined.

As a result of the literature research, it was determined that the lowest cost among the tillage methods compared for different regions and products was obtained with the no-till farming method. When examined in terms of yield, it is determined that it mostly causes a decrease in yield. Efficiency in no-till agriculture is higher, especially in moisture-constrained regions, and it allows more precipitation to be stored in the soil (Kucukcongar et al., 2014). However, when correct and intensive weed control and disease control are not performed, the yield can be considerably reduced. In addition, yield increases can be achieved in the reduced tillage method. Depending on these data, it can be stated that no-till agriculture has a high potential to contribute to the availability dimension of food security in Türkiye. In order to eliminate the risk of food insecurity brought by climate change, it can be recommended to be combined with other cultural measures according to the product and region.

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EFFECTS OF SALICYLIC ACID APPLICATION ON POSTHARVEST QUALITY OF FRESH CUT APPLE SLICES DURING COLD STORAGE

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ABSTRACT

Salicylic acid occurs spontaneously and creates various metabolic and physiological reactions in plants, affecting growth and development. Salicylic acid is known to be effective on controlling losses and maintaining quality by inhibiting ethylene synthesis in horticultural crops after harvest. In this study, the effects of 4 mM salicylic acid (SA) application on the quality of freshly cut apple slices of Golden Delicious, Granny Smith, Braeburn and Red Chief apple cultivars were investigated during the storage. Fruits harvested at commercial maturity were transported to the Faculty of Horticulture, Faculty of Agriculture, Selcuk University, under suitable conditions. The sliced fruits were immersed in the SA solution for 3 minutes and after the application, the excess water on the apple slices was removed and then placed in polyethylene bags. The bags were stored at 2°C and 90% relative humidity for 28 days. Physical and chemical analyzes were performed on the samples taken 7 days apart during storage. It was concluded that the application of 4 mM salicylic acid could be an effective application in extending the storage life of Golden Delicious, Granny Smith, Braeburn and Red Chief apple slices by maintaining their quality characteristics during 28 days of cold storage.

Keywords: Apple, fresh cut, quality, cold storage, salicylic acid

INTRODUCTION

Apple (*Malus communis* L.) is a climacteric fruit grown in temperate climates and at high altitudes of tropical regions (Seymen and Polat, 2015). Apple is a fruit type rich in phenolic compounds with antioxidant activity such as quercetin, procyanidin B2, epicatechin, phloretin, and chlorogenic acid (Lee et al., 2003).

The International Fresh cut Produce Association defines fresh-cut products as 100% usable fruits and vegetables that are peeled, cut or cut into pieces, packaged, preserved fresh and have high nutritional value. Although different expressions such as less processed, minimally processed, partially processed and prepared have been used for these products, they are generally called fresh cut products. Appearance (size, shape, color, shine and deterioration), texture (firmness, crispness and juiciness), taste (sweetness, sourness, sourness, bitterness) and nutritional value (vitamins, minerals, etc.) are the most important for the consumer in freshly cut fruits and vegetables. In addition, the color changes that occur in the products during processing and shelf life are the most important features that play a role in the quality evaluation of the consumer (Sabir, 2017).

Short shelf life and perishability in fresh cut fruits cause nutrient loss and increase in microbial activity, causing major problems in marketing. Tissue injuries and injuries that occur during slicing cause the enzyme and substrate to be exposed and cause quality losses with the occurrence of enzymatic reactions. However, due to processes such as peeling, cutting and slicing, respiration and ethylene activity increase rapidly in these products and the products can

deteriorate quickly. This causes color changes, softening, microbiological deterioration and changes in taste in the products. As a result of these effects, respiratory rate increases and shelf life decreases (Aguilar et al., 2005).

Salicylic acid (SA), a phenolic compound found in a wide range of plant species, exhibits a high potential in controlling the postharvest losses of horticultural crops. Postharvest SA treatments decrease the ethylene biosynthesis and action, induce the resistance towards disease, prevent oxidative stresses, support the fruit tolerance to chilling injury, decrease respiration rate, delay ripening and senescence, slow down the activity of cell wall degrading enzymes and maintain the crop firmness (Asghari and Aghdam 2010).

In this study, the effects of the use of SA treatment on slice quality and browning of Golden Delicious, Granny Smith, Braeburn and Red Chief apple cultivars were investigated during cold storage.

MATERIAL AND METHOD

Fruits Golden Delicious, Granny Smith, Braeburn and Red Chief apple cultivars harvested at commercial maturity were transported to the Selcuk University Faculty of Agriculture Horticultural Laboratory under appropriate conditions. All fruits were washed with tap water before slicing. Then, in order to prevent any contamination, the fruits were soaked in a solution containing 1% hypochlorite for 5 minutes, then washed with distilled water and dried under room conditions. The fruits are cut into 8 equal slices with the help of a slicer in such a way that they are not damaged. Slices of each variety are divided into two equal groups. While the slices in the first group were immersed in 4 mM salicylic acid (SA) solution for 3 minutes, the slices in the other group were immersed in distilled water and evaluated as a control. After the treatments, the slices were kept at room conditions for a while to remove excess water and then stored in polyethylene bags at 2°C for 28 days. The following physical and chemical analyzes were performed on the samples taken at intervals of 7 days during storage.

In order to determine the weight losses in the slices during storage, the weight losses were calculated by measuring the weight of the samples numbered at the beginning of storage during the storage period, and the results were expressed as %. After squeezing slices with a juicer, total soluble solid content (SSC) in the juice obtained were measured with a hand refractometer and expressed as %. After 5 ml of the juice obtained from the slices was withdrawn and the top was made up to 50 ml with distilled water, it was titrated with 0.1 N NaOH until the pH of the juice obtained was 8.1 and results were expressed as % malic acid.

Slice color was determined by reading L*, a* and b* values on both surfaces of the slices with a colorimeter (Minolta CR 400). Hue angle (h°) value was calculated with the help of hue = arctan (b/a) formula in order to determine the color changes in the slices (McGuire, 1992). In order to determine the darkening of the slices, the whiteness index (WI) value was calculated according to the formula $WI=100-[(100-L^*)^2+(a^*)^2+(b^*)^2]^{½}$ (Bolin and Huxsoll, 1991). The firmness of the slices was measured with penetrometer and the results were given in Newtons (N).

For vitamin C analysis, 5 g of fruit sample was weighed and filtered through filter paper by adding 45 ml of 0.4% oxalic acid. 1 ml of the obtained filtrate was taken, 9 ml of dye solution was added, and a reading was made in the spectrophotometer at a wavelength of 520 nm. A solution to which 9 ml of distilled water was added onto 1 ml of filtrate was used as blank. The results are given as mg/100g (Ozdemir and Dundar, 2006). Total phenol content of slices Singleton et al. (1999) by making some modification in the method. After adding 6.0 ml of

distilled water to 0.1 ml of sample, 0.5 ml of Folin-Ciocalteu was added and shaken and left at room temperature for 3 minutes. At the end of this period, the final volume was completed to 10 ml by adding 20% Na_2CO_3. Measurements were made at 760 nm after 2 hours at 25 °C. The results are given as mg/kg.

The study was established in a randomized plot design with 3 replications for each cultivar and 10 slices per replication. The obtained results were subjected to analysis of variance in the statistical program of JMP 5.1.0 (SAS Institute Inc, Cary, NC, USA). Student's t test ($p \leq 0.05$) was used to compare the means.

RESULTS AND DISCUSSION

The weight loss increased with the progression of storage time in all cultivars and SA treatments on the weight loss of apple slices during storage was found to be statistically significant. Weight loss at the end of 28th day in Braeburn cultivar was 0.19% in control group, 0.17% in SA treated slices, 0.31% in control group in Golden Delicious variety, 0.29% in SA treated slices. Weight loss in Granny Smith cultivar was 0.35% in the control group, 0.24% in SA treated slices. In the Red Chief cultivar, the weight loss was 0.16% in the control group and 0.14% in the SA-treated slices. Maintaining the initial weight of the commodities is one of the prime consideration determining the storage success in horticultural commodities like apples (Butkeviciute et al. 2021). It was determined that the application of salicylic acid on apple slices gave positive results in preventing weight loss in all cultivars compared to the control group (Figure 1).

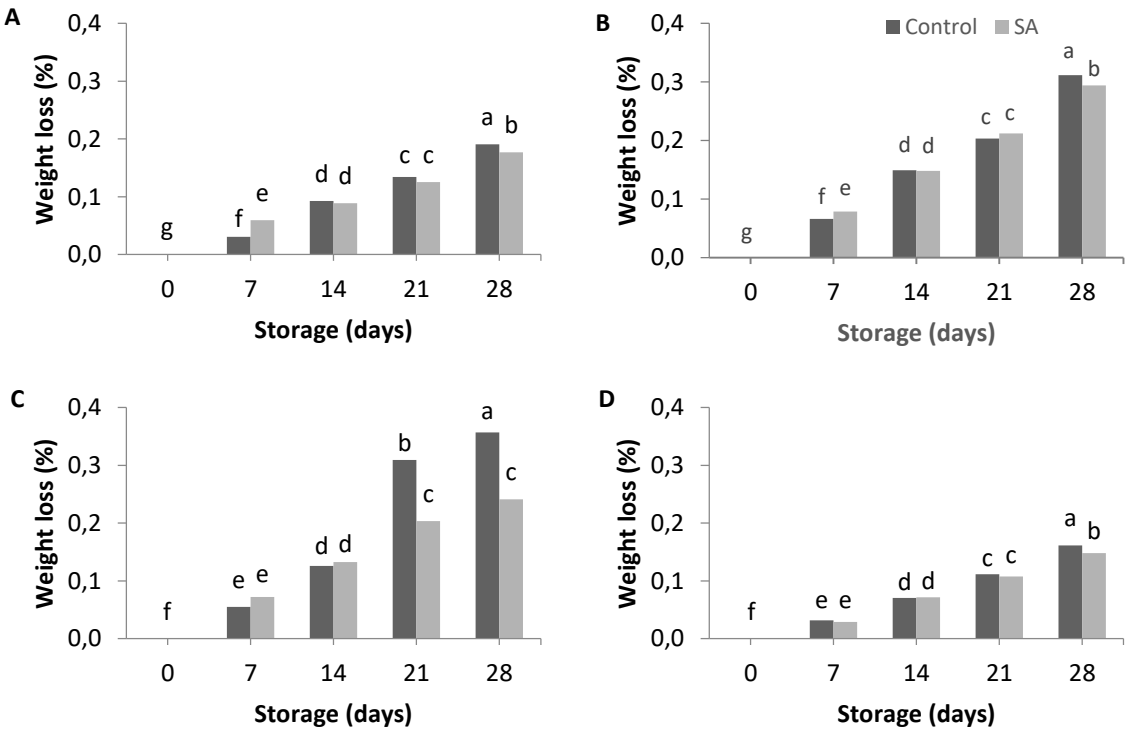


Figure 1. The effects of SA treatment on weight loss of fresh cut apple *cvs*. Braeburn (A), Golden Delicious (B), Granny Smith (C) ve Red Chief (D) slices during cold storage.

SSC value increased significantly in apple slices during the 28 day cold cold storage in all cultivars (Figure 2). The initial SSC value of Braeburn cultivar was 12.7%. At the end of the

28 day storage period, the control group of this cultivar was 13.1% while SA treated slices was 12.8%. The Golden Delicious cultivar had a 13.3% at the begging of the storage. At the end of the 28th day, it was 13.9% in the control group slices and 13.9% in SA treated slices. In Granny Smith, the initial value of SSC was 12.7%. At the end of the 28th day, the SCC value measured in the control group slices was 13.8%, while this value was 13.4% in the SA treated slices. In the Red Chief cultivar, the initial value of SSC was measured as 13%. At the end of the 28 day storage period, the SSC value was 13.8 in the control group slices and 13.7% in the SA treated slices. According to the results, it was determined that salicylic acid was significantly effective in maintaining the total soluble solid content in apple slices.

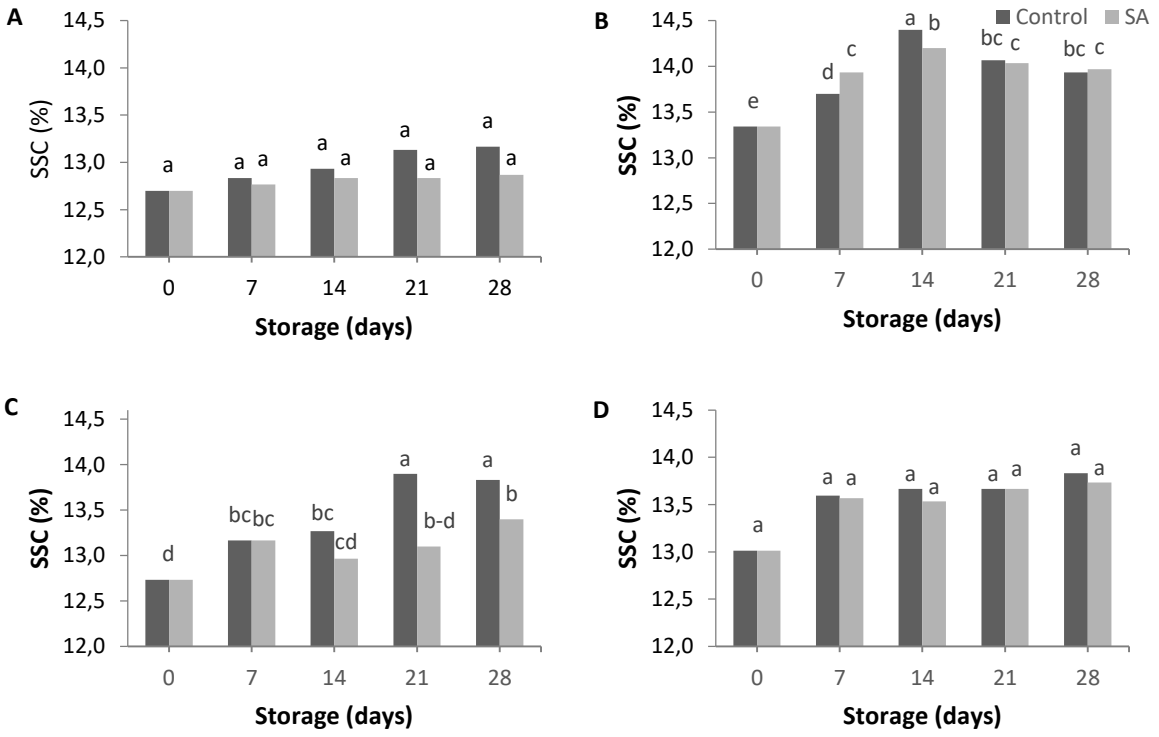


Figure 2. The effects of SA treatment on SSC of fresh cut apple *cvs*. Braeburn (A), Golden Delicious (B), Granny Smith (C) ve Red Chief (D) slices during cold storage.

The effect of SA treatment on TA was statistically significant in all cultivars. TA decreased with the progression of the storage period, and it was determined that SA treatment were effective in delaying such decrease (Figure 3). While the initial TA value of Braeburn cultivar was 0.73%, at the end of the 28th day, TA was 0.61% in the control group slices and 0.68% in the SA treated slices. The initial TA value of Golden Delicious variety was 0.31%. The TA amount of this cultivar was 0.25% in the control group slices and 0.29% in SA treated slices at the end of the storage period. While the initial TA value was 0.66% in Granny Smith cultivar, the TA value was 0.52% in the control group slices and 0.54% in SA treated slices at the end of the 28th day. The initial TA value of Red Chief cultivar was 0.33%, it was 0.22% in the control group slices and 0.24% in the SA treated slices at the end of the storage period. According to the general results, salicylic acid treatment in all four cultivars was more effective in maintaining the amount of titratable acid compared to the control group treatment.

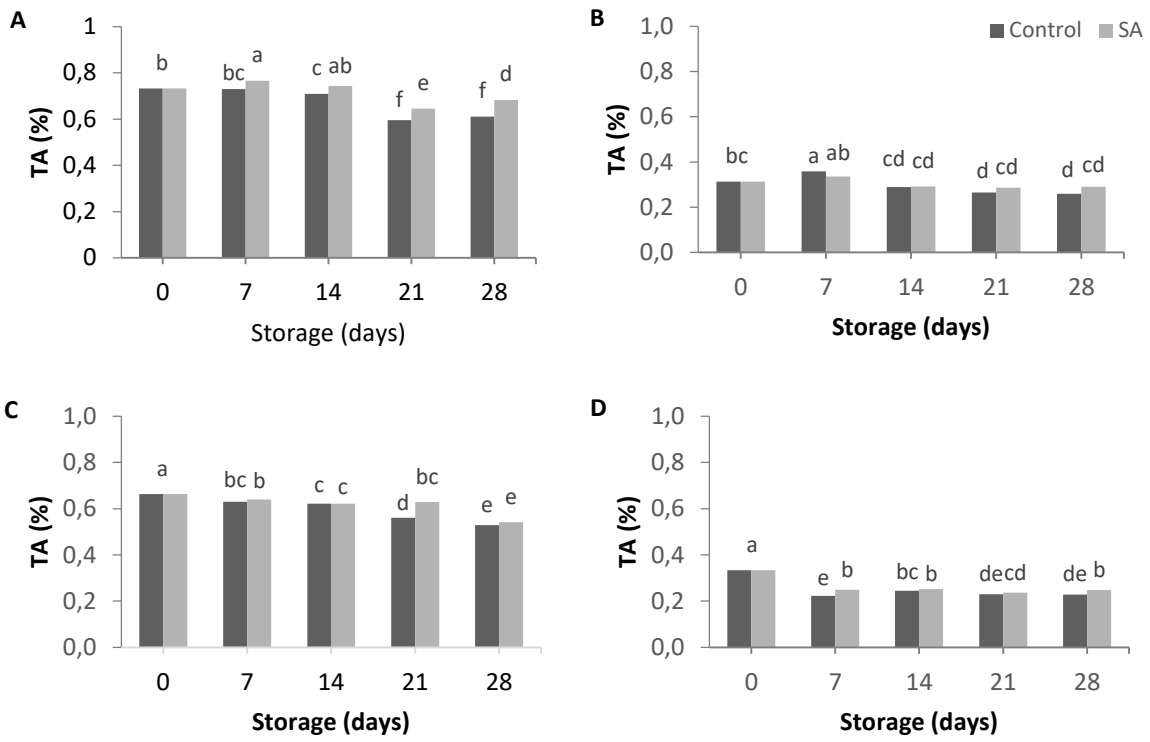
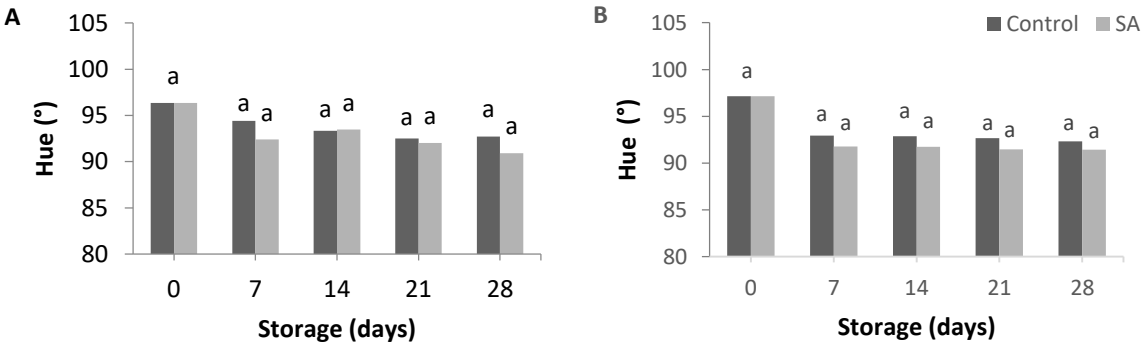


Figure 3. The effects of SA treatment on TA of fresh cut apple *cvs*. Braeburn (A), Golden Delicious (B), Granny Smith (C) ve Red Chief (D) slices during cold storage.

The effects of the SA treatment on hue angle (color) were investigated for 28 days. The initial hue value of Braeburn cultivar was measured as 96.33º. At the end of 28 days, the control group hue value was 92.72º and 90.91º in SA treated slices in Braeburn. The initial hue value of Golden Delicious cultivar was measured 97.13º. The hue value of the control group was 92.34º, while the hue angle value of SA treated slices was 91.45º at the end of the storage period. While the initial hue value of Granny Smith cultivar was 102.68º, at the end of the 28th day, the hue value of the control group was 88.99º and the hue value of the SA treated slices was 92.81º. The initial hue value of the Red Chief variety was 96.29º. The control group hue value was 88.19º, while it was 90.91º in the SA treated slices at the end of the storage period. SA treated cultivars in all cultivars gave significantly positive results in extending the slice color compared to the control group (Figure 4).



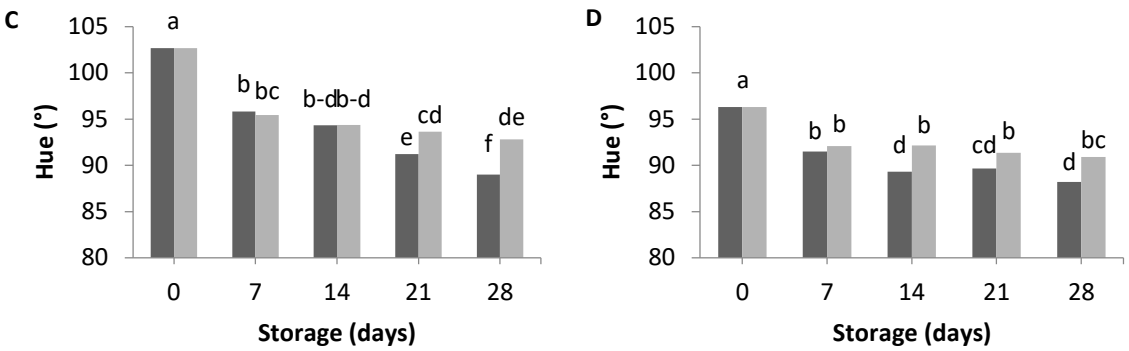


Figure 4. The effects of SA treatment on hue angle of fresh cut apple *cvs*. Braeburn (A), Golden Delicious (B), Granny Smith (C) ve Red Chief (D) slices during cold storage.

The effects of the SA treatment on whiteness index (WI) were investigated for 28 days. The initial value of the WI of the Braeburn cultivar was measured as 62. At the end of the 28 day storage period, the WI of control in Braeburn cultivar was 61 and 59 in SA treated slices, respectively. The initial value of the WI of Golden Delicious cultivar was measured as 63. At the end of the 28 day storage period, the WI value of the control in Golden Delicious was 59.2, and 59.4 in the SA treated slices. The initial value of the WI of Granny Smith cultivar was measured as 68. At the end of the 28-day storage period, the WI value of the control group was 54, and the WI value of the SA treated slices was 61. While the initial WI value of the Red Chief cultivar was measured as 62, the WI value of the control group slices was 53 and the SA treated slices were 57 at the end of the 28-day storage period. According to the results of the analysis, it was determined that salicylic acid treatment delayed fruit flesh browning and was more effective in preserving the slice color compared to the control group treatment (Figure 5).

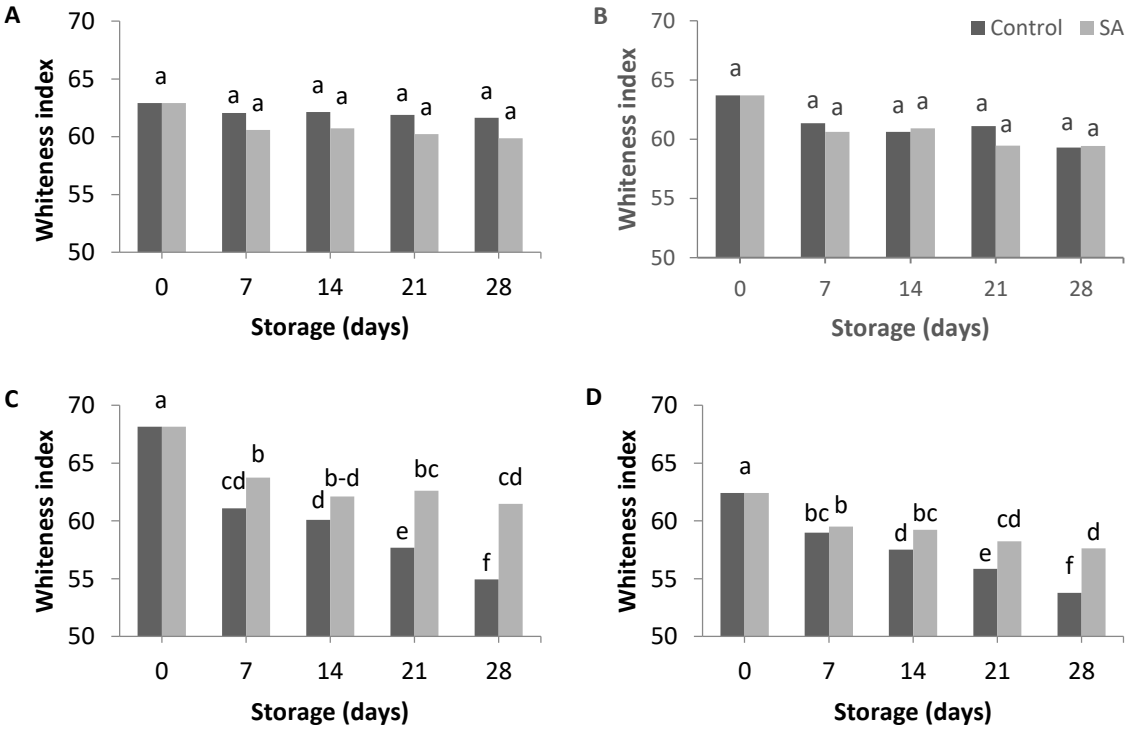


Figure 5. The effects of SA treatment on whiteness index of fresh cut apple *cvs*. Braeburn (A), Golden Delicious (B), Granny Smith (C) ve Red Chief (D) slices during cold storage.

Mechanical strength (firmness and elasticity) is a good indicator for postharvest quality extention for apple slices (Costa et al. 2012). [The initial firmness value of apple slice in Braeburn cultivar was measured as 13.6 N in the. At the end of the 28 day storage period, it was measured as 10.6 N in the control group and 11.9 N in the SA treated slices. The initial firmness value of Golden Delicious was measured as 5.7 N. At the end of the 28 day storage period, Golden Delicious cultivar was measured as 3.5 N in the control group and 5.2 N in the SA treated slices. As for Granny Smith cultivar, tprestorage firmness value was measured as 10.4 N. At the end of the 28 day storage period, the firmness value of the control group in Granny Smith cultivar was measured as 8.8 N, while the hardness value of the SA-treated slices was measured as 9.8 N. In the Red Chief variety, the initial value was measured as 9.8 N. At the end of the 28 day storage period, the firmness was 8.6 N in the control group and 9.3 N in the SA group. According to the results, less firmness loss occurred in the SA treated slices in all four cultivars compared to the control (Figure 6).

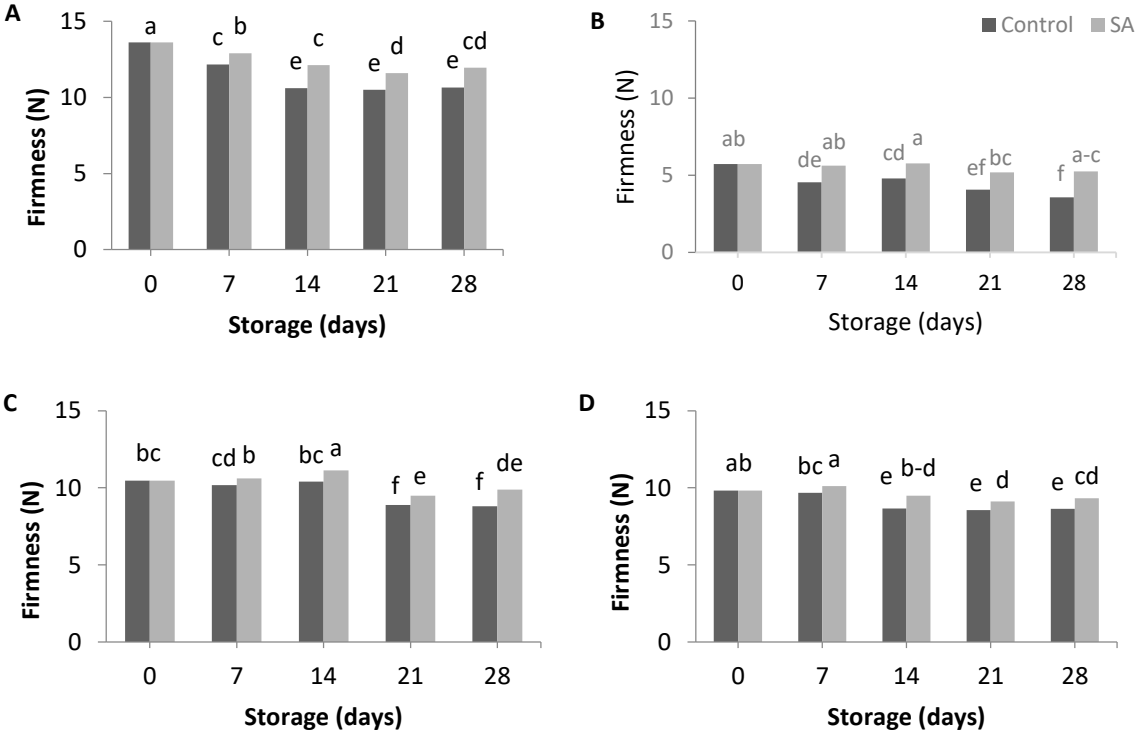


Figure 6. The effects of SA treatment on firmness of fresh cut apple *cvs*. Braeburn (A), Golden Delicious (B), Granny Smith (C) ve Red Chief (D) slices during cold storage.

The effects of the SA treatment on vitamin C content were investigated for 28 days. According to the results, the initial value of vitamin C in Braeburn cultivar was 7.8 mg/100g, at the end of the 28-day storage period, vitamin C of the control group slices was measured 6.9 mg/100g and in slices treated with SA 7.8 mg/100g. While the initial value of vitamin C of Golden Delicious cultivar was measured as 7.7 mg/100g, at the end of the 28th day, the amount of vitamin C in the control group of the same cultivar was measured as 5.9 mg/100g and 7.2 mg/100g in SA treated slices. The initial vitamin C value of Granny Smith variety was 7.3 mg/100g, at the end of the storage period, the amount of vitamin C was measured as 7.5 mg/100g in control slices and 7.7 mg/100g in slices treated with SA. While the initial value of Red Chief cultivar of vitamin C was 7.5 mg/100g, the vitamin C content at the end of the storage period was 7.06 mg/100g for the control group slices and 7.1 mg/100g in SA treated slices. According to the results of the vitamin C analysis, it was determined that the amount of vitamin C was extended for a longer time in the slices treated with salicylic acid compared to the slices

of the control group, and the treatment of salicylic acid was recommended in maintaining the amount of vitamin C (Figure 7).

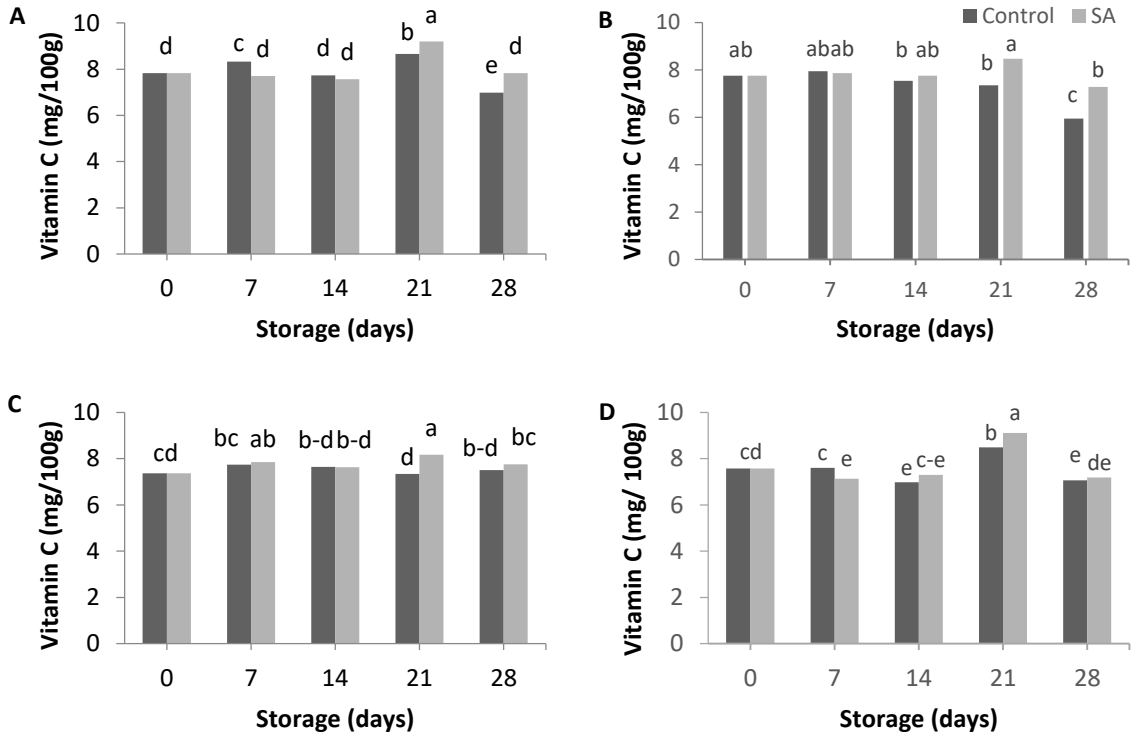


Figure 7. The effects of SA treatment on vitamin C of fresh cut apple *cvs*. Braeburn (A), Golden Delicious (B), Granny Smith (C) ve Red Chief (D) slices during cold storage.

The total phenol of the Braeburn cultivar initially was measured as 370 mg/kg. At the the 21st day storage period, total phenol was measured as 364 mg/kg in the control group slices and 457 mg/kg in the SA treated slices. However, at the end of the storage period, the total phenol values of the control group and SA treated slices in Braeburn were equal to each other (411 mg/kg). The total phenol initial value of Golden delicious cultivar was measured as 438 mg/kg. At the end of the 28 day storage period, total phenol was measured as 459 mg/kg in the control group slices and 525 mg/kg in the SA treated slices. Total phenol initial value of Granny Smith cultivar was measured as 467 mg/kg. At the end of the 28th day, total phenol was measured as 385 mg/kg in the control group slices and 450 mg/kg in the SA treated slices. Total phenol initial value of Red chief cultivar was measured as 468 mg/kg. At the end of the 28 day storage period, it was measured as 633 mg/kg in the control group slices and 677 mg/kg in the SA treated slices. Total phenols in apples display changes during the storage (Hoang et al. 2011). Salicylic acid treatment in all cultivars was more effective in maintaining the total phenol amount in the slices compared to the control group treatment (Figure 8).

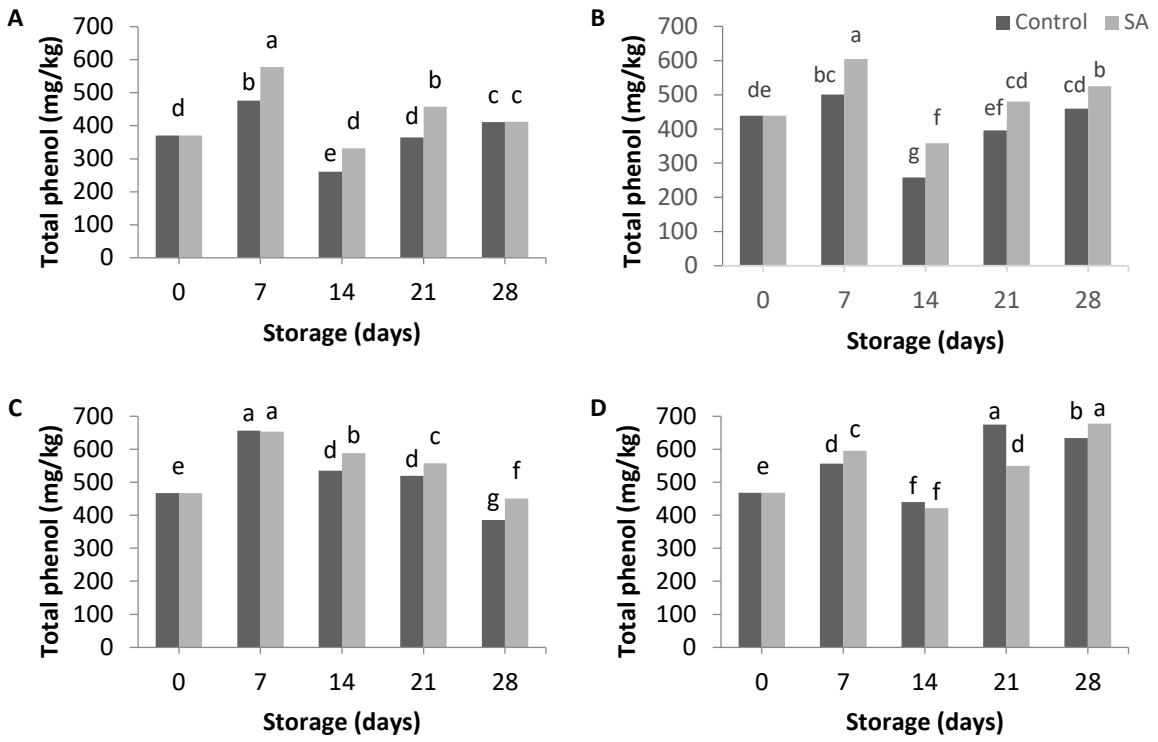


Figure 8. The effects of SA treatment on total phenol of fresh cut apple *cvs*. Braeburn (A), Golden Delicious (B), Granny Smith (C) ve Red Chief (D) slices during cold storage.

CONCLUSIONS

Considering rhe general findings on four different apple cultivars, it was determined that SA was effective in maintaining the firmness and reducing the weight loss in all slices of the cultivars during cold storage. According to the investigations on whiteness index, although the effect of SA on enzymatic browning was not found statistically insignificant for Golden Delicious and Braeburn cultivars, the SA was remarkably effective in preventing enzymatic browning for Granny Smith and Red Chief cultivars. To sum up, SA can be recommended for the maintenance of the postharvest quality in sliced apples during cold storage.

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EFFECTS OF DIFFERENT POSTHARVEST TREATMENTS ON STORAGE DURATION AND QUALITY OF POMEGRANATE *cv.* HICAZNAR

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ABSTRACT

Pomegranate is one of the oldest fruit species with nutritional value and beneficial effects on human health. Pomegranate is grown in large quantities in many subtropical and tropical countries, especially in Mediterranean countries with temperate climates. Pomegranate is rich in folic acid, potassium, phosphorus, iron, antioxidants, vitamin C, polyphenolic substances, alkaloids and flavonoid contents and fiber. Pomegranate, which is perishable after harvest, does not show climacteric properties. Postharvest hot applications, storage at the appropriate temperature, modified atmosphere packaging, storage in controlled atmosphere and surface coating treatments are carried out in order to extend the storage period by preserving the bioactive components and nutritional quality properties of the pomegranates after the harvest. In this study, the effects of different postharvest treatments [control, Modified atmosphere packaging (MAP), salicylic acid (SA) and UV-C light treatment] on the quality characteristics of Hicaznar pomegranates were investigated during storage. Pomegranate fruits harvested at the stage of commercial maturity were brought to the laboratory of the Department of Horticulture, Faculty of Agriculture, Selcuk University under suitable conditions. The pomegranate fruits were stored at 5 °C and 90% relative humidity. The fruit samples were removed from the storage room at 2nd and 4th month of storage period for physical and biochemical investigations (weight loss, fruit skin color, fruit flesh color, soluble solid content, titratable acidity amount, total phenolic substance amount, total antioxidant activity, ascorbic acid (vitamin C) and anthocyanin amount). As a result of the study, it was determined that the prestorage treatments were effective in maintaining the quality characteristics of pomegranates when compared to the control.

Keywords: Pomegranate, Storage, Quality, Salicylic acid, UV-C, MAP

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the oldest known fruit species belonging to the *Punicaceae* subfamily of the *Myrtifloare* (*Myrtales*) order. It is a perennial, shrub-shaped, multistemmed and densely branched plant with a strong root system, which is widely grown in Mediterranean countries (Artes et al., 2000). Pomegranate is rich in vitamins, minerals, organic acids, fibers, polyphenols (tannins, anthocyanins and ellagic acid) polysaccharides and has an important place worldwide due to its beneficial properties for health (Gil et al., 2000). In addition, its antioxidant content is three times higher than green tea, and it helps to increase body resistance by strengthening cell renewal, growth and immune system (Erkan and Doğan, 2018).

Pomegranate is non-climacteric property which is perishable after harvest. It is stated that postharvest hot treatments, storage at the appropriate temperature, storage in a controlled and modified atmosphere and surface coating treatments will give positive results in order to extend

the storage period by preserving the bioactive components and nutritional quality properties of pomegranates after harvest (Mphahlele et al., 2014).

In order to control physiological disorders and pathological diseases that cause product loss after harvest, some physical and chemical treatments are made before and after harvest. In recent years, less harmful or harmless methods have been started to be investigated for products, the environment and human health, since fungicides, which are used especially as reducing agents of decay in chemical treatments leave residues on the product (Klein et al., 1990; Wilson et al., 1994).

Modified atmosphere packaging (MAP) is one of the postharvest techniques used to extend the shelf life of fresh fruits and vegetables. As a principle, MAP delays the aging of fruits and vegetables by decreasing the amount of O_2 and increasing the amount of CO_2 in the package, thus extending their storage and shelf life. It also extend the quality of products by reducing water loss (Sandhya, 2010). Salicylic acid, one of the surface coating methods, is an internally produced plant hormone that regulates the resistance mechanism against physiological changes and diseases, especially in products under biotic and abiotic stress conditions (Davarynejad et al., 2015; Giménez et al., 2017). Treatments performed before storage, affect the storage life of the products. Salicylic acid and its derivatives are effective in maintaining the quality after harvest by preventing ethylene synthesis and movement of the products after harvest. (Asghari and Aghdam, 2010). Another method, ultraviolet-C irradiation (UV-C) gives positive results in the control of postharvest decay. In particular, UV-C treatments at a wavelength of 254 nm create a small amount of stress response in the product, increasing its strength after harvest (Erkan et al., 2009; Turtoi, 2013; Usall et al., 2016).

In this study, the effects of salicylic acid, UV-C light and MAP treatments on the maintenance of fruit quality characteristics of Hicaznar pomegranate cultivar were investigated during cold storage.

MATERIAL AND METHOD

Pomegranate *cv*. Hicaznar was used in the study. Fruits harvested at the stage of commercial maturity were transported to the Horticulture Laboratory of the Faculty of Agriculture of Selcuk University under suitable conditions. Here, the damaged fruits were discarded, experimental fruits was selected in terms of size and color, and they were divided into 4 groups for postharvest treatments.

The first group consisted of the fruits of control group, which were weighed directly without any treatment and placed in the cold storage. The second group of fruits were stored in modified atmosphere packages using passive modified atmosphere. For this purpose, all fruits weighed were placed in Xtend modified atmosphere bags, their mouths were closed and placed in boxes (MAP).

The third group of fruits were exposed to UV-C (1.8 kj/cm^2) light for 5 minutes, then weighed and placed in modified atmosphere packets (UV-C+MAP). The fourth group of fruits were soaked in a solution containing 2.0 mM salicylic acid (SA) for 5 minutes and kept at room conditions for a while in order to remove excess water formed on the surface. After the drying process was completed, the SA treated fruits were weighed and placed in modified atmosphere packets (SA+MAP).

The experimental pomegranates were stored for 4 months in a cold storage at 5 °C and 90% relative humidity. At the beginning of the study (0.month) and at the 2nd and 4th months of

the storage, the fruit samples were taken out of the storage and physical and chemical analyzes were made and the quality changes were determined.

The weight loss was calculated at the beginning of storage and the differences occurred by reweighing the fruits during storage were calculated and expressed as percentage weight loss (%).

Husk color was determined using a colorimeter (Model CR400) after calibration with white tile. Three measurements were made on fruit equatorial axes and results were calculated as hue angle using equations described by McGuire (1992). For the arils color, the measurement process was carried out by placing approximately 100 g samples in petri and taking them from 3 different points and reading the L * a * and b * values. The hue angle (h°) value was calculated to determine the color changes (McGuire, 1992).

Total soluble solid content (SSC) was measured with a hand refractometer in the juices obtained by squeezing the fruits, and the results were given as %. Titratable acidity (TEA) was titrated with 0.1 N NaOH until the pH of the juice obtained was 8.1 and the results were expressed as % citric acid (Cemeroğlu, 2007).

After the juices of the preserved fruits were squeezed, they were homogenized with 25 ml of methanol and kept at 4 °C for 16 hours and then centrifuged. This extraction was used in the analysis of total phenol and total antioxidant (Thaipong et al. 2006).

Total phenolic content (TPC) was determined by spectrophotometric method using Folin-Ciocalteu reagent. 100 µL of the extracted fruit sample was taken, distilled water was added to it, and it was taken into the flask. Folin-Ciocalteu reagent was added to this mixture, shaken and left at room temperature for 3 minutes. At the end of this period, saturated sodium carbonate solution was added and the top was completed with distilled water. The solution, which was incubated for 2 hours at 25 °C, was read at 760 nm wavelength in the spectrophotometer (Singleton et al., 1999).

Ferric Reducing Antioxidant Power (FRAP) method was used to determine the total antioxidant activity (TAA). 2850 µL of FRAP working solution was added to 150 µL of sample extract and kept in the dark for 30 minutes. At the end of this period, readings were made in the spectrophotometer at a wavelength of 593 nm (Benzie and Strain , 1996).

In the total amount of anthocyanin (TMA), the pH differential method was applied. On the fruit extract, 4.5 ml of pH 1.0 and pH 4.5 solutions were placed on it and diluted and readings were made against distilled water at 520 nm and 700 nm. Results were evaluated with respect to Cyanidin-3-rutinoside (Cheng and Breen, 1991).

Ascorbic acid (AsA) amount Pearson et al. (1970), it was determined by spectrophotometric method using dye solution. 45 mL 0.4% oxalic acid was added onto 5 ml of fruit juice and filtered. After taking 1 mL of the obtained filtrate, 9 mL of dye solution ($C_{12}H_6Cl_2NO_2Na$) was added and the reading was made at 520 nm wave length. As a standard, 9 mL of distilled water was added onto 1 ml of filtrate and was used.

The experiment carried out in a completely randomized design with three replications and each replication contained 5 fruits. The data from analyzed parameters was submitted to analysis of variance using JMP statistical software version 5.1 (SAS Institute Inc., Cary, NC, USA). Sources of variation were treatments, storage time and their interaction. Means were compared by Student's t-test at a significance level of 0.05.

RESULTS AND DISCUSSION

Weight loss changes of pomegranate fruit during the storage is shown in Table 1. All postharvest treatments effectively inhibited weight loss compared to the control. Weight loss increased during the storage and reached 12.88%, 1.49%, 1.22% and 0.96% (control, UV-C+MAP, MAP and SA+MAP treatments, respectively) after 4 months at 5 °C. Between the postharvest treatments, the weight loss was less when the fruit were treated with SA+MAP than with MAP or UV-C+MAP at cold storage. It has been well known that SA treatment reduces the respiration in pomegranate (Opara ve ark., 2015). Also, the combined use of SA and MAP could effectively reduce physiological activities in commodities and, by this way, the weight loss in pomegranates would be lower.

Husk color is one of the most important visual attributes for pomegranates. Hue angle value of fruits decreased during storage and the differences between the treatments were statistically significant. The harvest hue angle value of the fruits was 36.68, decreasing to 35.55, 35.01, 33.87 and 31.93 for SA+MAP, MAP, UV-C+MAP and control, respectively after 4 months of storage (Table 1).

Aril color is also an essential feature determining the market value of pomegranates. Hue angle values displayed a gradual decline during the whole storage period compared to the initial values at harvest of pomegranates at 5 °C (Table 1). Hue angle value of arils was determined as 31.88° at the beginning of storage. At the end of 4 month storage, the highest value was determined in SA+MAP treatment (29.03°) while the lowest value was detected in the control group (24.44°) at the end of the storage.

Table 1. Effects of postharvest treatments on weight loss, husk color, aril color, SSC and TEA of pomegranates during cold storage.

Storage (month)	Treatments	Weight loss (%)	Husk color (hue angle)	Aril color (hue angle)	SSC (%)	TEA (%)
Harvest		0.00f	36.68a	31.88a	17.40a	1.50a
2	Control	5.39b	33.64d	25.76de	15.40ef	1.27f
	MAP	0.44def	36.40ab	30.69a	15.47ef	1.37de
	SA+MAP	0.39ef	36.74a	30.31ab	16.46b	1.44bc
	UV-C+MAP	0.55def	35.17bc	28.95b	16.40bc	1.45b
4	Control	12.88a	31.93e	24.44e	15.13f	1.02g
	MAP	1.22cd	35.01bcd	27.12cd	15.67de	1.33e
	SA+MAP	0.96cde	35.55ab	29.03b	16.00cd	1.41cd
	UV-C+MAP	1.49c	33.87cd	28.68bc	16.00cd	1.34e
	$LSD_{0.05}$	0.73	1.50	1.64	0.42	0.04

*Note: For each quality feature, the values significantly different at $p \leq 0.05$ are indicated by different letters according to Student's t-test.

TEA and SSC displayed a gradual decline during the whole storage period compared to the initial values at harvest of pomegranates at 5 °C. The initial TEA was 1.50% and progressively decreased along with the storage time (Table 1). However, postharvest treatments significantly ($p \leq 0.05$) retarded the TEA decline, compared to the control. TEA after 4 months of cold storage were 1.41%, 1.34%, 1.33% and 1.02% for SA+MAP, UV-C+MAP, MAP and

control, respectively. The SSC value, which was measured as 10.53%, showed fluctuations at different rates in all treatments with the progression of the storage time (Table 1). While the highest increase occurred in the control group fruits during the storage period (15.13%), the lowest increase was found in SA+MAP and UV-C+MAP treatments (16.00%).

Changes in AsA of pomegranates during the storage were presented in Table 2. AsA of fruits decreased during storage but the differences between the treatments were statistically insignificant. The initial AsA value of the fruits was 8.37 mg/100 ml, it decreased to 7.19, 6.69, 6.36 and 5.93 for SA+MAP, UV-C+MAP, MAP and control, respectively after 4 months of storage. Selcuk and Erkan (2016) reported a decrease in ascorbic acid content of pomegranates at cold storage and an extended storage period.

The postharvest treatments were significantly effective in maintaining the TPC of the fruits with the progression of the storage period (Table 2). At harvest TPC value was 191.08 mg/100ml. At the end of the storage period, the highest value was determined in the control group (321.08 mg/100ml), followed by fruits stored in MAP and SA+MAP, respectively (285.25mg/100ml and 272.75 mg/100ml). The lowest TPC was determined in the UV-C+MAP treatment (221.91 mg/100ml). Phenolic substances are secondary metabolites responsible for taste and aromatic features of the fruits. The reason for increases in TPC would be most probably due to senescence and stress related factors during storage.

Table 2. Effects of postharvest treatments on AsA (mg/100 ml), TPC (mg/100ml, TAA (µmol/ml) and TMA (mg/l) of pomegranates during storage.

Storage (month)	Treatments	AsA (mg/100 ml)	TPC (mg/100ml)	TAA (µmol/ml)	TMA (mg/l)
Harvest		8.37	191.08de	4.63a	196.74a
2	Control	7.67	246.08bc	4.42c	185.62a
	MAP	8.12	200.25de	4.58ab	195.14a
	SA+MAP	9.62	167.75e	4.56ab	195.94a
	UV-C+MAP	7.52	181.08de	4.51b	184.61a
4	Control	5.93	321.08a	4.13d	120.47d
	MAP	6.36	285.25ab	4.37c	141.02c
	SA+MAP	7.19	272.75b	4.39c	160.16b
	UV-C+MAP	6.69	221.91cd	4.40c	135.28c
	$LSD_{0.05}$	N.S.	42.49	0.08	13.21

*Note: For each quality feature, the values significantly different at $p \leq 0.05$ are indicated by different letters according to Student's t-test. N.S.= Not significant

As shown in Table 2, TAA of the pomegranates gradually decreased during the prolonged cold storage. However, all postharvest treatments significantly maintained the fruit TAA content in comparison to control fruits. Initial TAA content of pomegranates were 4.63 µmol/ml. At the end of the storage period, the highest TAA content was obtained from UV-C+MAP treatment (4.40 µmol/ml), followed by SA+MAP (4.39 µmol/ml) and MAP (4.37 µmol/ml). On the other hand, fruits of control treatment showed the lowest TAA content (4.13 µmol/ml). Previous studies on pomegranate storage also indicated that MAP have positive

effects on preventing antioxidant activity loss (Selcuk and Erkan, 2014; Selcuk and Erkan, 2015).

TMA value increased significantly in pomegranate fruits stored for 4 monhts in cold storage and changed according to the postharvest treatments. (Table 2). At harvest TMA was measured as 196.74 mg/l and it varied between 160.16mg/l (SA+MAP) and 120.47 (control) after 4 months. It has been determined that SA+MAP during the whole storage period was more effective than other treatments in preserving the TMA. The anthocyanins that provides red color to pomegranate arils are the substances showing sensitivities to postharvest operations and storages. Handling temperature, storage conditions, postharvest treatments, structure of anthocyanins, pH, sugars, light exposure and metals could effect the stability of anthocyanin (Maghoumi ve ark., 2013). Postharvest treatments in the present study were found effective on maintaining the anthocyanin contnet of the pomegranate although there was a considerable decrease in the fruits at 4th month of storage. One of the most logical reason for decrease in anthocyanin is color degradation emerging from oxidative reactions along with the fruit senescence. The postharvest tratments performed in the study were found effective on maintaining the anthocyanin content by delaying postharvest senescence and disorders in pomegranates.

CONCLUSIONS

In the present study, the effects of salicylic acid, UV-C light and MAP treatments on the postharvest quality maintenance of Hicaznar pomegranate were investigated. All postharvest treatments effectively inhibited weight loss as well as changes in husk and aril colors compared to the control. Certain biochemical features of pomegranate were also maintained by the treatments by delaying changes in SSC, TEA TPC and TMA during the storage. SA + MAP treatments were more effective in many cases, such as preventing the changes weight loss, TA, husk color and TMA. Therefore, SA + MAP could be recommended for better maintenance of postharvest physiological and biochemical features of pomegranate cv. Hicaznar.

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EFFECT OF MANAGEMENT PRACTICES ON SOIL MICROARTHROPODS IN CONVENTIONAL ORCHARD IN PLOVDIV REGION

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ABSTRACT

Soil biodiversity of apple orchards agrosystems depends on different management (Karov et al., 2006, Kostadinova & Popov, 2015). In conventional orchard usually are used synthetic fertilizers, insecticides and herbicides which influenced negatively on soil fauna. A field study was conducted to examine the influence of agricultural practices on soil microarthropods. The study was carried out on conventional managed apple orchard at Experimental field of Department of Viticulture & Fruit Growing - Agricultural University of Plovdiv for spring 2022. Comprehensive information by monitoring of various agrometeorological factors and agro-technological practices (tillage, mowing, irrigation, pest management and soil analysis) was obtained. Microfauna were observed. Comparing the frequency of species in the different plots, it was found that only two (Collembola and Acari) of all identified taxa were present in the five variants. The most numerous and with the highest frequency of encounter in the five experimental variants are the representatives of Collembola, which were 57-65% of all identified geobionts. Based on the results obtained, it is reported that the highest similarity of soil communities is between sward-drip irrigation and sward- sprinkler irrigation (42.9%).

Keywords: apple orchard, soil biodiversity, conventional farming

INTRODUCTION

Apples are a fruit crop that is grown massively all over the world (FAO). According to the data from the Ministry of Agriculture, Forestry and Food, the South-Central region occupy the leading place in fruit production in Bulgaria https://www.mzh.government.bg/bg/politiki-i-programi/otcheti-i-dokladi/agraren-doklad/,https://www.mzh.government.bg/bg/statistika-i-analizi/izsledvane-rastenievadstvo/danni/. Soil biodiversity of apple orchards agrosystems depends on different management (Karov et al., 2006, Kostadinova & Popov, 2015). In

conventional orchard usually are used synthetic fertilizers, insecticides and herbicides which influenced negatively on soil fauna. Most of the ecosystems services provided by soil biota is achieved from processes in agroecosystems are carried out by specific groups of soil organisms like microarthropods as a part of complex food webs which affect fruit productivity. Soil microarthropods play a key role in the regulation of microbial populations, the decomposition of organic matter, and the cycling of nutrients within soils (Coleman and Crossley, 1996, Doles et al., 2001).

The aim of the current study was to analyze how the agricultural management influenced the soil fauna in conventional apple orchard.

MATERIAL AND METHOD

The study was carried out on conventional managed apple orchard with Florina cultivar – grafted on MM106 rootstock at Experimental field of Department of Viticulture & Fruit Growing - Agricultural University of Plovdiv in Brestnick village.

Comprehensive information by monitoring of various agrometeorological factors and agro-technological practices (tillage, mowing, irrigation, pest management and soil analysis) were obtained. Soil pH was measured using pHotoFlex Set, 2512000, WTW-Germany (ISO 10390), and the soil conductivity was measured using Multiset, F340, WTW- Germany (ISO 11265). Soil texture (pipette method by Wigner), humus content (by Turin) has been analyzed in the Laboratory of the Department of Agroecology & Evironmental Protection and Department of Soil Science & Agrochemistry, Agricultural University of Plovdiv.

Soil microarthropods were extracted via Tullgren funnels. Soil cores were collected once during the spring (in April/May 2022) from soil cores of 50 mm diameter and 400 mm tall of the 25 – 30 cm which was sampled from three plots with 5 variants Sampling was done along transects (of 5 soil cores) in fallow, sward and buffer zone. After extraction, micro-fauna was preserved in 70 % ethanol. The soil samples were observed under steriobinocular microscope sorted and counted and determined to taxonomic level.

In order to establish the faunal similarity between the soil communities in the three subplots, they are compared two by two. The following formula is used:

$$QS = \frac{2C}{A + B}.100$$

Where:
QS - Sorensen similarity index;
A - number of species (taxa) found in habitat A;
B - number of species (taxa) found in habitat B;
C - number of species (taxa) that are common to both habitats.

Simpson's Diversity Index (S) and Simpson's Equality index (E) were established.
The raw data were processed using Statistical Software Statistica 7.0.

RESULTS AND DISCUSSION

Soil properties. The Experimental field of Department of Viticulture & Fruit Growing - Agricultural University of Plovdiv in the village of Brestnik is located 4 km away from Plovdiv, in the South direction. It is located at the beginning of the Rhodope hill "Chernatitsa". This hill is also known as the "Rhodope Collar" of Plovdiv. The land borders the lands of the city of Kuklen and the villages of Brani Pole, Belashtitsa, as well as the Asenovgrad highway connecting Plovdiv and Asenovgrad. This area falls into the Thracian-Strandjan geoFigureical

area of distribution of cinnamon forest soils and more specifically of leached cinnamon soils - Chromic Cambisols according to the international classification of FAO.

The mechanical composition of the studied soils in Brestnik is medium sandy-clay. Carbonates are not observed in either sample. Soil pH is slightly acidic to slightly alkaline. According to the accepted classification for soil organic matter content, the studied soils are characterized by medium content (Table 1).

Table 1. Soil properties in conventional apple orchard in Brestnik village.

Sample/ Depth, cm;	pH (H_2O)	Humus %	Total $CaCO_3$ %	Active $CaCO_3$%	Mechanical composition of the soil ≤ 0,01 mm%
1. Fallow 0-30	7.2	2.1	-	-	43.7
2. Sward 0-30	6.7	2.8	-	-	37.5

The data on the content of agrochemicals is presented in Table 2. The investigated soils are characterized by an average supply of nitrogen, phosphorus and potassium.

Table 2. Agrochemical features in conventional apple orchard in Brestnik village.

Sample/ Depth, cm	NH_4 mg/1000g	NO_3 mg/1000g	$NH_4 + NO_3$ mg/1000g	K_2O mg/100g	P_2O_3 mg/100g
1. Fallow 0-30	12.3	10	22.3	27.65	traces
2. Sward 0-30	18.8	10	28.8	43.92	4.4

Management practices. The following agrotechnological operations were performed in the experimental field. In May and during the five years orchards were treated with the fungicides Tiram 80 VG - 0.3%, Follicur 250 EV - 0.04% (broad spectrum system), Delan 700 VDG - 0.05%, Score 250 EC - 0.02%, Horus 50 EC - 0.03%, Shavit F 72 VDG - 0.2% and Bayfidan 250 EC (systemic) - 0.015%. Against cross moth, lice and mites apple plants were treated a different number of times in different years with Nurele D - 0.04%, Fury 10 EC - 0.125% (contact synthetic pyrethroid of a new generation) and Coragen 20 SC - 16 ml/ha. (Dobrevska et al, 2020).

Soil fauna. Regarding the total number of established invertebrates (Table 3), it can be stated that it is the highest in Plot 2a (Sward-drip irrigation) V_3 and Plot 2 b (Sward-sprinkler irrigation) V^4, followed by Plot 3 (Buffer zone) V_5. Regarding the established number of taxa, it can be stated that it is 2 to 3 times higher in (Buffer zone - V_5). When comparing the frequency of occurrence of species in the five habitats, it is found that only two of all identified taxa are represented in all five sites – Collembola and Acari. The most numerous and with the highest frequency of occurrence in the four experimental variants (V_1-V_4) are the representatives of Collembola, which amount to 57-65% of all established geobionts, while in the control (site V_5) Acari predominate, albeit slightly (28%) (figs.1,2 and 3).

Table 3. Taxons established in different plots of variants at Conventionally managed Apple orchard in Brestnik village.

	2022					
Taxon	Plot 1 a (fallow-drip irrigation)	Plot 1 b (fallow-sprinkler irrigation)	Plot 2a (Sward-drip irrigation)	Plot 2 b (Sward-sprinkler irrigation)	Plot 3 (Buffer zone)	Total
	V_1	V_2	V_3	V_4	V_5	
Collembola	6	12	43	45	14	120
Oribatidae			10	10		20
Protura					2	2
Diptera			1			1
Acari	4	8	16	14	15	57
Diplura		1			10	11
Formicidae					11	11
Coleoptera					2	2
Total number of individuals	10	21	70	69	54	224
Total number taxons	2	3	4	2	6	17

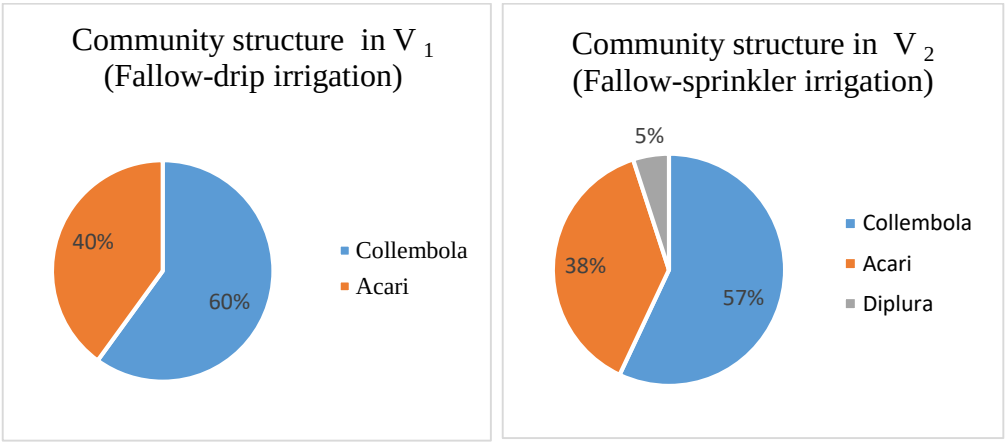

Figure 1. Community structure in fallow variants.

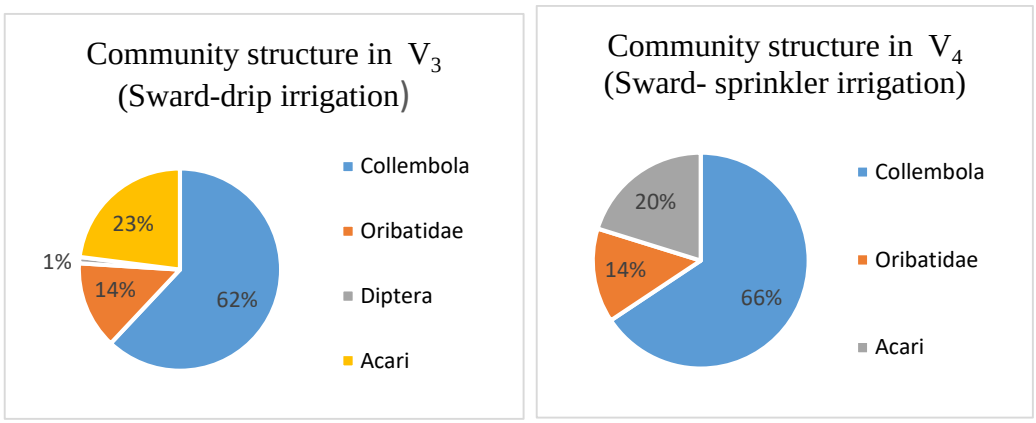

Figure 2. Community structure in sward variants

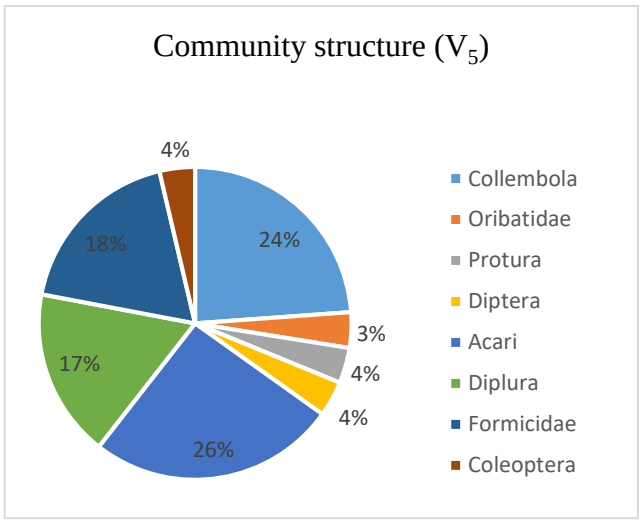

Figure 3. Community structure in Buffer zone.

Based on Simpson's Diversity Index values, the studied plots form the following descending order: V_5 (S=4.318) > V_3 (S=2.273) > V_2 (S=2.174) > V_4 (S=2.083) > Site V_1 (S=1.923). Based on the values of Simpson's Evenness Index, it can be indicated that complete dominance of one taxon over the others is observed in V_1 – Collembola, E=0.195. A similar dominance of one taxon was found in V_3 (Collembola, E=0.568) and V_4 (Collembola, E=0.689), while in V_2 and V_5 two taxa were dominant (Collembola, Acari, E=0.720-0.721).

Sørensen similarity index was used to establish the faunal similarity between the soil communities in the different variants Based on the results, it is reported that the highest similarity is between plot 2 a (V_3) and plot 2 b (V_4) (42.9%), followed by the similarity between V_1 and V_2 (40%) and between V_1 and V_4 (40%). The control (plot 3- V_5) shows the least degree of similarity with the other sites – between 20% and 33%.

Correlation analysis. A significant positive correlation between soil communities is demonstrated in almost all variants except between Site V_5 and Sites V_3 and V_4 (Table 4).

Table 4. Correlation between soil communities in variants.

Correlations (Spreadsheet1) Marked correlations are significant at p < ,05000 N=8

	V_1	V_2	V_3	V_4	V_5
V_1	1,00	1,00	0,93	0,92	0,72
V_2	1,00	1,00	0,93	0,91	0,75
V_3	0,93	0,93	1,00	1,00	0,55
V_4	0,92	0,91	1,00	1,00	0,53
V_5	0,72	0,75	0,55	0,53	1,00

Statistically significant differences in the composition of the soil fauna were demonstrated between V_5-V_1 (t=3.138, p=0.016) and between V_5-V_2 (t=2.74, p=0.0289).

A cluster analysis of the similarity between the five study sites based on the described soil fauna was done. In the cluster analysis, based on the identified soil fauna, the studied sites were divided into two groups, one of which included the four experimental variants, and the control (buffer zone) was in a separate group ($p<0.05$). In turn, the first group is divided into two subgroups, including V_1-V_2 and V_3-V_4, respectively.

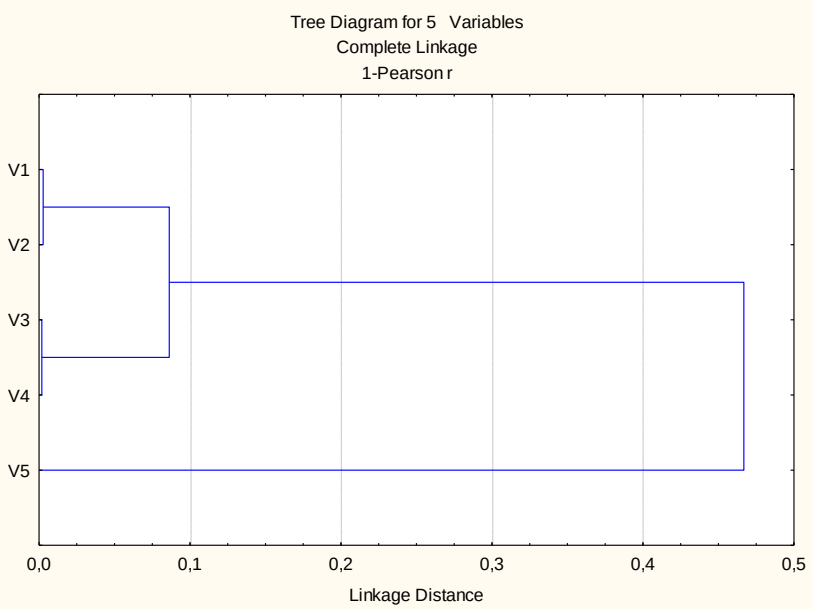

Figure 4. Cluster analysis of five variants of at conventionally managed Apple orchard in Brestnik village.

CONCLUSIONS

One of the most important contributions of soil microarthropods to cropping systems is the impact on decomposition and mineralization process. Microarthropods are negatively affected by pesticides in the conventionally managed orchards. This study gives a snapshot of the state of the soil fauna. More research is needed to more accurately assess the impacts of applied management practices on microfauna which can be a key factor in determining the best soil management strategies in these food agrosystems.

ACKNOWLEDGEMENT

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PHENOLOGICAL AND MORPHOLOGICAL DEVELOPMENT OF DIFFERENT CULTIVARS OF PUMPKINS

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ABSTRACT

The cultivation of pumpkins in Bulgaria is carried out through long-used in practice varieties and local forms and populations. The new varieties are perceived more as "hobby" crops and are of interest for growing small areas or even as landscaping plants. The main reason for conducting this study is to establish and promote up-to-date scientific information on new cultivars of pumpkins to improving the cultivar list of this vegetable crop. The main aim of the study is to determinate the phenological and morphological development of various cultivars pumpkins. Twelve cultivars with different ecological and geoFigureical origin of the following species: Cucurbita pepo (3) Cucurbita maxima (7) and Cucurbita moschata (2) were tested. The experimental design was based on the block method. The phenological phases were determined. The duration of the interphase periods was determined. Morphological characteristic of the fruits was made. The results of phenological observations give us reason to summarize that in the tested varieties from plant emergence to harvest, the period is between 154 and 174 days, not taking into account differences that clearly distinguish between varieties. The tested samples are characterized by vigorous and harmonious vegetative growth, as a result of which they form an active assimilation apparatus, which is a prerequisite for high plant productivity. The complex characteristics of the fruits of the varieties participating in the experiment - shape, color, fruit weight between 1,395 kg and 5,125 kg, and attractive appearance determine them as suitable for sale on the market for fresh consumption.

Keywords: *C. pepo, C. maxima, C. moschata,* morphological characteristic, phenological development

INTRODUCTION

Today, different types of pumpkins are grown all over the world and are an important source of food for a large part of the world's population. Cultivated species are grown in many areas, both in production areas and on private farms. Asia and especially India and China are the largest producers of pumpkins with approximately 58% of the world's total area, followed by Africa with 15%, North America with 11%, Europe with 9%, South America with 3% and Oceania by 0.8%. (FAO, 2009). Apart from the culinary use of the fruits, the pulp of C. maxima cultivars can be dried and then made into flour and mixed with grain flours and used to make breads, pastries, etc. (Cheij, 1984, Facciola, 1990, See et al., 2007).

Vegetative biomass and fruits can also be used as fodder for domestic animals (Mariano and Dirzo, 2002). Pumpkins are also grown for their oil-rich seeds to obtain oil and also as ornamental plants. They can be eaten directly, or made into puree, flour, butter. They are a very good source of fat (50%) and protein (35%) (Robinson and Decker - Walters, 1997). Many people use the flowers of pumpkins for decoration. There are reports in the literature about the use of flowers for culinary purposes. Some authors define them as a separate consumable part of plants (Paris, 2001).

In Bulgaria, from the Cucurbits, new varieties and species are offered more as "hobby" crops and are of interest for growing small areas or even as plants for landscaping. Cultivation of larger areas is carried out by using widely distributed and long-used varieties and local forms and populations.

The main reason for conducting this study is to establish and promote up-to-date scientific information on new cultivars of pumpkins to improving the cultivar list of this vegetable crop.

Cucurbita maxima is one of the most studied and cultivated species of the genus. Its variation includes many local forms and cultivars of original origin, as well as many commercial cultivars, some of which are used for consumption and some of which are used for decorative purposes because of their attractive shapes and coloring. Over 50 cultivars of this species have been described by Tapley et al., 1937. These cultivars differ not only in morphological characteristics but in aspects of agronomic interest such as length of life cycle, productivity and, in some cases, in their ability to adapt to adverse environmental conditions. Castetter (1925), Whitaker and Davis, (1962), Robinson and Decker-Walters (1997), Decker-Walters and Walters, 2000) have studied the diversity of the species.

Cucurbita moschata have also remarkable morphological variations. They are mainly expressed in the appearance and morphological features of the fruits - in color, shape and size and surface of the fruits, as well as in their durability. The presence of numerous varieties developed in different parts of the world, as well as the presence of local forms and populations and local varieties with prominent agronomic characteristics predetermine the great genetic variation of this species (Lira, 1985, 1988). According to Du et al., 2011. Cucurbita moschata has adapted to different agro-ecological conditions, and now shows high variability in terms of many agronomic characteristics, such as fruit shape, flowering biology, leaf morphological features, etc. Cucurbita pepo also shows a very high level of morphological variability. Of the three types of pumpkins, this is the type with the greatest morphological diversity of fruits (Tapley et al., 1937, Mihov et al., 2014). The variety is both in terms of the shape and size of the fruit, and even more so in the color of the skin.

The main aim of the study is to determinate the phenological and morphological development of various cultivars pumpkins.

MATERIAL AND METHOD

The experiments were carried out in the period 2015-2016, in the experimental field of the Department of Horticulture at the Agricultural University - Plovdiv.

The varieties selected in the present study are representatives of the three main types of pumpkins *Cucurbita pepo*; *Cucurbita maxima* and *Cucurbita moschata* with different ecological and geoFigureical origins (Table 1).

Of these, 25% are from France, 33.33% from Poland, 25% from Italy, 16.67% from Bulgaria, as well as 33.33% from the species Cucurbita pepo, 50% from the species Cucurbita maxima and 16.67% from the species Cucurbita moschata. After the observations made in the first experimental year, because of the pronounced decorative effect of the varieties Sweet Dumpling and Ornamentale in miscuglio, they were dropped from the general scheme of the studied varieties. For greater neatness and clarity, in the second experimental year, the original numbering of the varieties was preserved, corresponding to the one presented in Table 1. Under the same numbering from Table 1, the varieties are listed in the tables in the section Results and discussion and are commented on in the text of the article.

Table 1. List of varieties

№	сорт	произход	вид
1	Sweet Dumpling	Vilmorin	*Cucurbita pepo.*
2	Uchiki kuri	Vilmorin	*Cucurbita maxima*
3	Turkish turban	Vilmorin	*Cucurbita pepo.*
4	Bambino	PNOS Ozarow Mazowiecki	*Cucurbita maxima*
5	Ambar	PNOS Ozarow Mazowiecki	*Cucurbita maxima*
6	Danka polka	PNOS Ozarow Mazowiecki	*Cucurbita maxima*
7	Atlantic Giant	Floraland sp. Zo.o. sp.k.	*Cucurbita maxima*
8	Quintale Seme Gialo	Hortus – Buona Luna	*Cucurbita maxima*
9	Buternut Rugosa	Hortus – Buona Luna	*Cucurbita moschata*
10	Ornamentale in miscuglio	Hortus – Buona Luna	*Cucurbita pepo*
11	Plovdivska 48/4	"Genko Enchev" Ltd	*Cucurbita maxima*
12	Muskatna51/17	"Genko Enchev" Ltd	*Cucurbita moschata*

The plants were grown according to the technology for field production of pumpkins adopted for Bulgaria (Cholakov, 2009) with a feeding area for each plant of 2 x 2 m and sowing on 13.05.2015 and 20.05.2016. Each variety was grown in 4 replications. For the optimal development of the plants, the necessary agrotechnical measures were carried out during the growing season, in relation to the applied technology.

Phenological observations were carried out to determine the phases: emergence; formation of the first leaf; flowering of male flowers; flowering of female flowers; formation of first fruit; harvest date (IRGR-Sadovo, 1980, https://ipgrbg.com/).

The following interphase periods (in days) were determined: from sowing to emergence; from germination to flowering of male flowers; from germination to flowering of female flowers; from germination to first fruit; from germination to harvest (IRGR-Sadovo, 1980, https://ipgrbg.com/).

After harvesting the fruits, it was found: fruit weight (g); fruit height (cm); fruit diameter (cm); shape index (I), as a ratio of the height of the fruit to the diameter; the weight of the seeds (g); (IRGR-Sadovo, 1980, https://ipgrbg.com/)

The description of Fruit shape, ribbedness, basic color of skin, secondary color of skin, type of the secondary color, type of skin, thickness of fruit flesh (mm), color of fruit flesh was done according to the methodology of IBPGR (1983) and IBPGR (2008).

RESULTS AND DISCUSSION

The variety is a basic element in the technology of growing vegetable crops. The biological potential of the variety can be revealed only with strict observance of the cultivation technology. On the other hand, the applied technology is adapted to the characteristics of the selected variety. The ecological "passport" of the variety serves for the preparation of the technological "passport" and recommendations regarding the applied technology (Tarakanov, 2002; Goncharov, 2005).

The three species differ in vigor of growth and length of life cycle. The environmental conditions, the type of soil, the availability of nutrients and their assimilation by plants in the different stages of otnotogenesis determine their vegetative behavior and productivity.

The results of the phenological observations (Table 1 and Table 2) show that, in terms of germination, the differences between the individual variants are small and are expressed in a few days difference. The seeds of the varieties originating in Bulgaria (11 and 12) germinate

early - on the fifth day after sowing. Varieties 1, 3 and 7 have the longest germination period. In all the varieties included in the study, the formation of the first real leaf is 7-8 days after germination.

Table 1. Phenological development of plants in 2015

№	sowing	germination	Formation of first leaf	Flowering of ♂ flowers	Flowering of ♀ flowers	Formation of first fruit	Harvesting
1	13.05	21.05	28.05	06.07	28.07	07.08	05.11
2	13.05	20.05	26.05	08.07	27.07	06.08	28.10
3	13.05	21.05	29.05	10.07	24.07	04.08	28.10
4	13.05	20.05	26.05	14.07	30.07	14.08	05.11
5	13.05	19.05	26.05	14.07	30.07	14.08	05.11
6	13.05	20.05	27.05	13.07	27.07	11.08	05.11
7	13.05	21.05	28.05	10.07	24.07	04.08	29.10
8	13.05	20.05	27.05	10.07	24.07	14.08	29.10
9	13.05	19.05	26.05	14.07	30.07	14.08	05.11
10	13.05	19.05	26.05	26.06	20.07	04.08	05.11
11	13.05	18.05	25.05	14.07	30.07	14.08	19.10
12	13.05	18.05	25.05	14.07	30.07	14.08	19.10

Table 2. Phenological development of plants in 2016

№	sowing	germination	Formation of first leaf	Flowering of ♂ flowers	Flowering of ♀ flowers	Formation of first fruit	Harvesting
1	20.05	27.05	03.06	15.07	30.07	16.08	13.11
2	20.05	27.05	04.06	17.07	27.07	14.08	05.11
3	20.05	27.05	06.06	19.07	02.08	24.08	05.11
4	20.05	26.05	03.06	18.07	02.08	24.08	13.11
5	20.05	27.05	04.06	19.07	30.07	20.08	13.11
6	20.05	28.05	06.06	19.07	27.07	14.08	13.11
7	20.05	27.05	06.06	18.07	27.07	24.08	04.11
8	20.05	26.05	03.06	14.07	02.08	24.08	04.11
9	20.05	26.05	03.06	14.07	02.08	24.08	13.11
10	20.05	26.05	03.06	14.07	02.08	24.08	13.11
11	20.05	27.05	03.06	15.07	30.07	16.08	30.10
12	20.05	27.05	04.06	17.07	27.07	14.08	30.10

The flowering of the first male flowers occurs between 44 and 52 days after emergence (Table 3.). Varieties 1 and 10 are distinguished by the earliest flowering of the male flowers. These are representatives of the *Cucurbita pepo* species. Varieties 4, 5, 9, 11 and 12 have the latest flowering of the male flowers. Among them there are varieties of the large-fruited pumpkin - *Cucurbita maxima*, as well as of the *Cucurbita moshctata*. Flowering of the female flowers occurs 20-22 days after the opening of the first male flower. It should be noted that earlier flowering of single female flowers was also observed on single plants, which died very quickly and were not counted as initiation of female flower flowering.

The first visibly well-formed fruits on the plants of the different varieties were reported between 73 and 83 days after germination. Varieties 3, 7 and 10 are distinguished by the earliest fruit formation, followed by 2 and 1. From germination to harvesting, 154 to 174 days pass. For the first experimental year, there were no differences in the interphase periods that would clearly distinguish the representatives of the individual types of pumpkins participating in the experiment. The results are similar in the second experimental year - 2016 (Table 3.).

Table 3. Interphase periods in days in 2015

№	from sowing to emergence		from germination to flowering of ♂ flowers		germination to flowering of ♀ flowers		from germination to first fruit		from germination to harvest	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
1	8	7	44	48	66	67	76	78	174	176
2	7	7	46	50	65	64	75	76	165	169
3	8	7	48	54	62	70	73	88	165	168
4	7	6	52	54	68	70	83	88	174	175
5	6	7	52	53	68	68	83	84	174	175
6	7	8	51	50	65	64	80	76	174	175
7	8	7	48	50	62	64	73	88	166	168
8	7	6	48	54	62	70	83	88	166	168
9	6	6	52	54	68	70	83	88	174	175
10	6	6	44	54	58	70	73	88	174	175
11	5	7	52	48	68	67	83	78	154	156
12	5	7	52	50	68	64	83	76	154	156

From the obtained results with the smallest fruits, the decorative forms of the *Cucurbita pepo* species, varieties 1 and 10, stand out (Table 4). The average weight of one fruit in large-fruited pumpkins changes from 1395g to 5152g, respectively for varieties 2 and 11. The remaining varieties of this species form fruits with an average weight of 1950 to 4810 g. In the representatives of the nutmeg, the average weight is 3085 g and 3732 g, respectively.

Table 4. Morphological characteristics of fruits

№	fruit weight (g)	Fruit height (H) (cm)	Fruit diameter (D) (cm)	Index of fruit I=H/D	Weight of seeds (g)	Net fruit weight (g)
1	262	6.30	7.20	0.88	68.12	193.88
2	1395	14.00	15.50	0.90	205.00	1190.00
3	1950	15.00	29.00	0.52	234.00	1716.00
4	4810	18.00	25.50	0.71	629.00	4181.00
5	3550	13.00	18.20	0.70	637.00	2913.00
6	2800	19.00	22.00	0.86	332.00	2468.00
7	4175	27.50	23.50	1.17	363.00	3812.00
8	1833	18.00	16.00	1.13	407.00	1426.00
9	3085	35.00	16.00	2.19	202.00	2883.00
10	mix-					
11	5152	21.00	23.00	0.91	489.00	4663.00
12	3732	30.00	13.50	2.22	234.00	3498.00

The pumpkin varieties studied also differed from each other in terms of the size of their fruits. With the smallest height of fruits is variety 1 – 6.3 cm. The fruits of the other varieties are higher, and their height varies in the range from 13 to 19 cm. Fruits above 20 cm in height form varieties 7 and 21, and above 30 cm in height are varieties 9 and 12. There are also differences in fruit diameter. It varies from 7.2 cm for variety 1 to 23.5 cm for variety 7. By matching the height to the diameter of the fruit, the fruit index was calculated. The results shows that with globular and oval shape of the fruits are 9 of the studied varieties with elongated shape of the fruits are two of the tested varieties, which are of the species *Cucurbita moschata*. Their fruit index is above 2. The mass of the seeds with the placenta is important because in the culinary use of the fruit, the smaller mass of these fruit organs implies a greater relative proportion of the fleshy part. The relative share varies from 6.27% to 26% of the fruit weight. According to this indicator, the varieties are arranged in ascending order as follows: 12, 9, 7, 11, 6, 3, 4, 2, 5, 8, 1.

Five of the varieties are characterized by a spherical shape (1, 4, 5, 6, 11), two are oval shaped (7 and 8), one is widely-conical (3) and two are elongated pear-shaped (Table5.).

The surface of the fruit in most varieties is superficially ribbed or absent. An exception is variety 1, where the surface is distinctly segmented, with moderate ribbing along the entire length of the fruit. In 50% of the varieties, the main color of the bark is in various shades of green. One third of the varieties have an orange main peel color. One variety has a pale yellow coloration of the bark and one with gray. Eight of the cultivars also have secondary bark color in the form of spots of varying size or stripes. Over 60% of the varieties studied have smooth skin. In the remaining varieties, the skin is shallowly wavy (2 and 7) or with warts (3 and 10). In most varieties, the coloring of the fleshy part is orange. Exceptions are variety 1 with white colored fleshy part and varieties 3 and 6 with yellow colored fleshy part. The thickness of the fleshy part is from 15 mm to 45 mm. In varieties 9 and 12, the thickness of the fleshy part in the region of the neck is 105 mm and 90 mm, respectively, at the base of the fruit – 25 mm and 20 mm.

Table5. Description of fruits

№	Fruit shape	Ribbedness	basic color of skin	secondary color of skin	The type of the secondary color	Type of skin	thickness of fruit flesh (mm)	color of fruit flesh
1	spherical	middle	light yellow	green	on stripes	smooth	15	white
2	pear-shaped	surface	dark green	orange	spotted	rough	30	orange
3	wide-conic	low	dark green	orange	spotted	with warts	22	yellow
4	spherical	surface	grey-green	white	on stripes spotted	smooth	35	orange
5	spherical a	surface	green	grey	spotted	smooth	40	orange
6	spherical	surface	green	yellow	spotted	smooth	40	yellow
7	oval	low	orange	-	-	грапава	45	orange
8	oval	-	orange	-	-	smooth	40	orange
9	pear-shaped	-	green	-	-	smooth	105*/25**	orange
10	ornamental - mix	-	orange, yellow	green	spotted	with warts	-	-
11	spherical	surface	grey	white	spotted	smooth	40	orange
12	pear-shaped	surface	orange	-	-	smooth	90*/20**	orange

CONCLUSIONS

As a result of the conducted researches the following conclusions can be made:

The results of the phenological observations give us reason to summarize that the growth period is between 154 and 174 days, without differences that clearly distinguish the individual varieties.

The tested varieties are characterized by vigorous and harmonious vegetative growth, which is the basis for high plant productivity.

The complex fruit characteristics of the studied varieties - shape, color, fruit weight between 1,395 kg and 5,125 kg, determines them as suitable for realization on the market for fresh consumption.

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MICROPROPAGATION OF *IN VITRO* GERMINATED *PAULOWNIA TOMENTOSA* PLANT

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ABSTRACT

Paulownia (*Paulownia tomentosa*) is a fast-growing tree species belonging to the Paulowniaceae family and is widely cultivated in China and Japan. The wood of the paulownia tree is used in the production of furniture, pulp and coal, as well as the extracts obtained from the leaves, flowers and fruits are used in the pharmaceutical and cosmetic industry. Commercial propagation of paulownia tree is done by seed and cuttings. Due to the low germination performance of paulownia seeds and the limited propagation by cuttings, the production of seedlings by micropropagation method comes to the fore. In this study, micropropagation of paulownia seeds germinated *in vitro* and transfer to outdoor conditions were carried out. During the surface sterilization of the seeds, 70% ethanol and 5% commercial bleach were used. After the seeds were rinsed with sterile distilled water, they were transferred to nutrient medium containing 0 MS and germinated, and at the end of 30 days, approximately 18% germination occurred. Shoots obtained from germinated seeds were used as explants in the regeneration study. In the regeneration study, 6-benzylaminopurine (BAP) was used as a cytokinin in the range of 1 to 4 mg/L. 0.1 mg/L gibberellin (GA) and 0.1 mg/L indole-3-butyric acid (IBA) were added to medium containing BAP. The shoot number and shoot length values were determined 30 days after the culture. The highest shoot growth was observed in the medium containing 4 mg/L BAP + 0.1 mg/L GA + 0.1 mg/L IBA, and the highest shoot length was determined in the medium containing 3 mg/L BAP + 0.1 mg/L GA + 0.1 mg/L IBA. Plants rooted in a medium containing 1 mg/L IBA were subjected to the 21-days acclimatization stage, and then all seedlings were successfully transferred to garden.

Keywords: Paulownia, Chinese poplar, Regeneration, BAP, Seedling

INTRODUCTION

Paulownia (*Paulownia tomentosa*) is a fast-growing tree species native to China and East Asia, belonging to the Paulowniaceae family (Bahri and Bettaieb 2013). Paulownia is grown as an ornamental plant in parks and gardens in Türkiye. In addition, the main reason why it has been the subject of research for more than 20 years is that timber is valuable (Kaymakcı et al., 2011).

The wood, leaves, and fruits of *P. tomentosa* have been used in Chinese traditional medicine for the treatment of tonsillitis, bronchitis, asthma attacks, and bacterial infections such as enteritis or dysentery (Jiang et al., 2004). Today, paulownia wood is used especially in furniture, pulp and coal production. It is known that solutions prepared from fruits and leaves

are used in hair care and as a natural dye for hair (Molcanova et al., 2022). In addition, *P. tomentosa* extracts contain many bioactive compounds such as flavonoids and especially Apigenin (Bahri and Bettaieb 2013).

The production of paulownia seedlings is done with seeds and cuttings. In the seed propagation method, seedlings can be obtained at a low rate and in a long time depending on the germination performance of the seed (Bergmann and Moon 1997). In the multiplication method with cutting, the possibility of high production is limited. Reproduction of tree species has become very practical with the micropropagation method, which is an alternative solution to traditional methods. This method allows the clonal reproduction of genetically uniform, disease and virus-free healthy seedlings in a short time in high quantities (Park et al., 1998).

The aim of this study is to determine the germination performance of *P. tomentosa* seeds germinated *in vitro* and to optimize the micropropagation study in germinating seeds.

MATERIAL AND METHOD

The seeds of *P. tomentosa* (9501 hybrid) used in the study. MS (Murashige and Skoog 1962) was used as the nutrient medium in the study. 3% sucrose was used as a carbon source in the media. 6.5 g/L plant agar was used as a thickener in the prepared media. Plant growth regulators (BAP, GA, NAA and IBA) used in the experiments were dissolved with suitable solvents. Then, stock solutions were prepared in the desired amount and ratio with distilled water and added in appropriate amounts to the needs of the medium. The pH of the nutrient media was adjusted to 5.6-5.8. Then, the nutrient media were sterilized at 121°C, 1.2 kg/cm^2 pressure for 20 min. All cultures were cultured at 24±2°C and under blue-red LED light with 16 hours photoperiod.

Surface sterilization of seeds was first kept in 70% ethyl alcohol solution for 2 min. Then, it was kept in a magnetic stirrer in 5% bleach (5% NaClO) solution for 10 min. Finally, it was passed from autoclaved distilled water 3 times for 5 min. Obtained sterile seeds were transferred to nutrient media containing 0 MS for germination. Germination performance was determined by taking the germination data one month after planting.

Explants taken from plants germinated *in vitro* conditions were transferred to nutrient media containing different plant growth regulators and regenerated. At this stage, different combinations of BAP were added to the solid MS nutrient medium. In addition to different BAP concentrations, GA and NAA were added to the medium at a constant rate (Table 1). The dose of plant growth regulators to be added to the nutrient medium varies between 0.1-4 mg/L. The studies were prepared as five replications and the culture medium providing the best shoot growth was determined (Figure 1).

Table 1. Plant growth regulator combinations used in shoot regeneration media

Treatments	**BAP (mg/L)**	**GA (mg/L)**	**NAA (mg/L)**
1	0	0	0
2	1	0,1	0,1
3	2	0,1	0,1
4	3	0,1	0,1
5	4	0,1	0,1

The resulting shoots were rooted by transferring them to MS nutrient medium containing 1 mg/L IBA. Rooted plants were washed with clean water and purified from their nutrient media. Then it was planted in viols filled with previously sterilized and moistened peat. To prevent sudden moisture loss of the plants, the viol was taken into a suitable container and then completely covered with cling film and taken to the plant growth room. The plants, which were allowed to get used to the external conditions by opening holes in the cling film gradually for three weeks, were transferred to the field conditions at the end of three weeks (Figure 1:6-7).

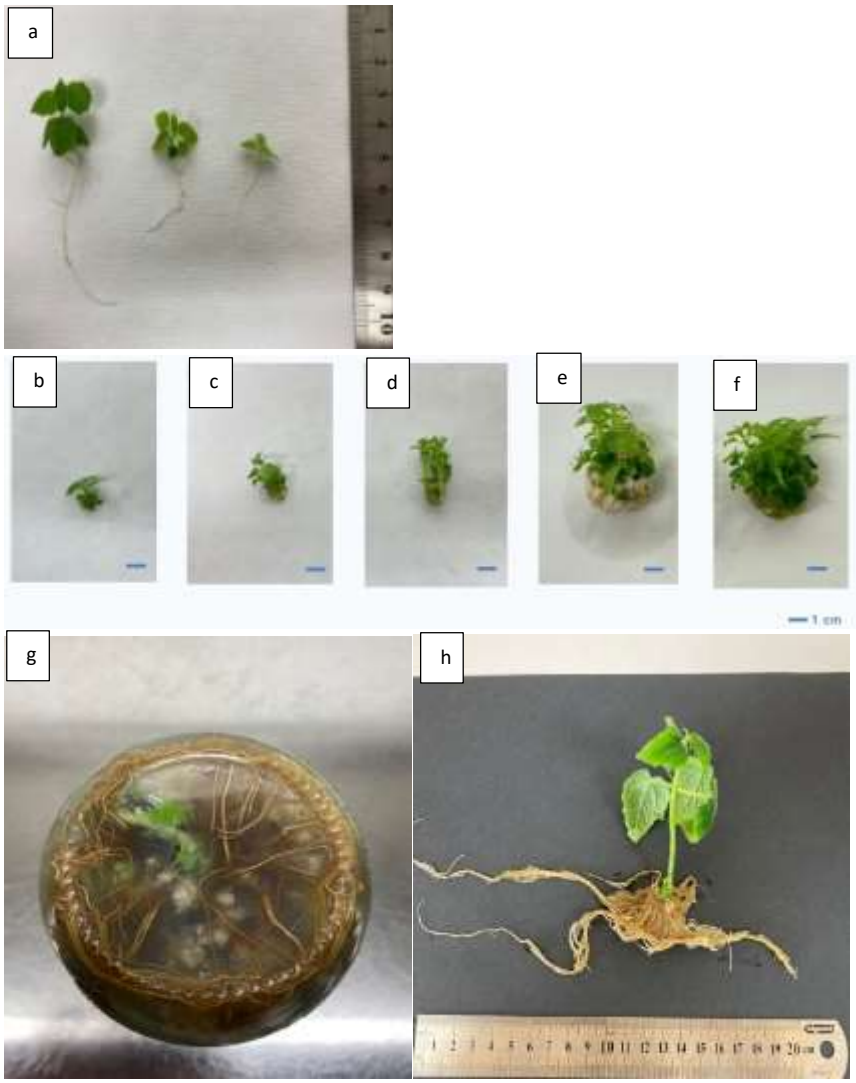

Figure 1. Seed germination, *in vitro* propagation, and rooting stages in *P. tomentosa* (a: Seed germination, b: Growth in media containing 0 MS as a control, c: Growth in media containing 1 mg/L BAP concentration, d: Growth in media containing 2 mg/L BAP concentration, e: Growth in media containing 3 mg/L BAP concentration f: Growth in media containing 4 mg/L BAP concentration g: Rooting phase. h: *P. tomentosa* ready for acclimatization).

Statistical Analysis

All experiments were designed completely randomly. Experiments were made in triplicate. Statistical evaluations of the data obtained from the regeneration studies were made with the Duncan test, one of the One-Way Anova post hoc tests of the SPSS ver 26 statistical program (Snedecor and Cochran, 1967).

RESULT AND DISCUSSION

Because of, the use of paulownia timber in diverse industries and its economic price, its cultivation has increased. In some parts of the world are investing in these plants to diminish

poorness and struggle COVID 19 (Swelum et al., 2020). The reproduction of these plants is faced with numerous problems such as seed-borne microorganisms and pests, poor seed germination and changing development routines. Also, seedling growth is slow compared to rooting cuttings. However, supplementing the plant with auxins speed up rooting and provides superior-quality roots (Zayova et al., 2014). Also, additionally auxins, extra supplement of cytokinins to the media aids to obtain well stems and the formation of adventitious roots (Olatunji et al, 2017; Guan et al, 2019). Consistent with our study, the number of shoots/explants raised notably in direct proportion to cytokinin in studies with *Philodendron selloum* (Hassan and Abdallah, 2015) and *Paulownia hybrid* (Fahmy and Gendy, 2018; Mohamad et al. 2022) plants.

In this study, the viability rates of Pawlonia seeds were also checked. As a result of the viability test, the germination rate of the seeds was found to be 17.77%. After the seeds are sterilized with 70% ethyl alcohol solution for 2 min, 5% hypochloride (5% NaClO) solution for 10 min. Sodium hypochlorite is the most common sterilization tool used for seed and explant sterilization in many plants. Sodium hypochlorite concentration and exposure time may vary from species to species. Sodium hypochlorite-based sterilization protocols are often combined with rinsing with 70% ethanol. Barampuram et al. (2014) used this combined protocol to sterilize cotton seeds. However, none of the protocols he tried yielded stable and reproducible germination ratios, also below tested greenhouse circumstances. Shtereva et al. (2014) determined that the seeds of the genotypes examined by sowing on diverse medium, except Mega and Ganter lines, sprout in 10 days. Seed sprouting rate was found to differ between genotypes. The maximum sprouting ratio for all genotypes was obtained from seeds sown on medium added with 50 mg/L GA3. Sprouting ratios in these variants ranged from 4.2% (*P. tomentosa* hybrid Ganteri × *P. fortunei*) to 40.0% (*P. tomentosa*). Conversely, seeds sprouting in 20 mg/L GA3 supplemented medium showed a decrease in sprouting rate (11.7% - 73%). Victorious sprouting of seeds is straight concerned to plant growing and survival. *P. tomentosa* seeds were found to have the maximum sprouting talent compared to another genotypes. The results of this study were similar to our study.

Cultures supplemented with MS medium prepared with 0.1 mg/L IBA and 3 mg/L BA recorded the maximum shoot inductions (100%), average shoot size and average number of nodes each material in *Cryptolepiss anguinolenta* (Monney et al., 2016). Similar results were obtained from *P.tomentosa* (Al-Tinawi et al., 2010; Bahri and Bettaieb, 2013; Krishna Vrundha et al. al., 2021) and *Ficus anastasia* (Elmeer and Al Malki, 2010) plants. In our study, the maximum shoot number at 4 mg/L BAP + 0.1 mg/L GA + 0.1 mg/L NAA and shoot length were obtained from the medium containing 3 mg/L BAP + 0.1 mg/L GA + 0.1 mg/L NAA (Table 2). In another study, MS medium supplemented with BAP or IBA was used with shoot bud initiation using lateral buds of mature *P. tomentosa*. This result observed 99% success of sprouting meristem tissue of *P. taiwania* in MS medium (Yang and Chang 1989). It was stated that the best medium for shoot induction from nodal explants of *P. tomentosa* was MS medium containing 1 mg/L BAP.

Table 2. Shoots number and shoot length as a result of plant regeneration mediums

Mediums	Shoot number	Shoot length
0 MS	1,33±0,21e	0,86±0,10d
1 mg/L BAP + 0,1 mg/L GA+ 0,1 mg/L NAA	6,00±0,85d	1,26±0,10d
2 mg/L BAP + 0,1 mg/L GA+ 0,1 mg/L NAA	11,00±1,00c	1,86±0,07c
3 mg/L BAP + 0,1 mg/L GA+ 0,1 mg/L NAA	17,33±0,91b	3,26±0,12a
4 mg/L BAP + 0,1 mg/L GA+ 0,1 mg/L NAA	23,33±1,78a	2,48±0,11b
p=0,01		

Lobna et al. (2008) addition of 1 mg/L BA to *P. kawakamii* growth medium gave the best results in terms of shoot formation, shoot number per explant (2.6) and maximum shoot length (35.37 mm). In addition, Rahman et al. (2004) showed that *in vitro* propagation by nodal segments in *Ficus benghalensis* L. tree responds better than shoot tip explants. However, 90% of the node explant cultures developed shoots in modified MS medium with 0.5 mg/L BA.

In this study, we determined the optimum starting environment as 0 ms. For shoot development, the longest shoot is 3 mg/L BAP + 0.1 mg/L GA + 0.1 mg/L IBA and the maximum shoot number is 4 mg/L BAP + 0.1 mg/L GA + 0, 1 mg/L was obtained from the medium. According to Marcotrigiano and Stimart (1983), media containing BA (1 mg/L) and IBA (0.25 mg L^{-1}) were the most effective media for shoot propagation. In another study similar to ours, Rajbahak and Sah (2010) determined that a medium containing 1 BAP mg/L and 0.1 mg/L NAA gave the best results in micropropagation of *P. tomentosa.* Ozaslan et al. (2005) stated that the highest number of shoots from the stem material of *P. tomentosa* were obtained from MS medium containing 3 mg/L BAP+0.1 mg/L IAA. These results were consistent with our study. Ahmed et al. (2022) investigated the average survival rates, shoot number, and shoot length of *P. tomentosa* at different explant types and different BAP concentrations. Using WPM with shoot tips and stem segments, it was determined that the main effect of BAP survived 80% and 87% in explants, with a very high level of significance. In addition, in the same mediums, it was found that the number of shoots was successful with a high value in 3.06 for shoot tips and 3.2 for stem segment. In addition, shoot tips and stem segments, 0.5 BAP mg/L and the highest mean shoot length in MS environment, were determined to be 3.06 and 3.1 cm. In our study, the longest shoot was 3.26 (p=0.01 significance level) and the longest shoot was 23.33. Our study has quite a lot of shoots when compared to the studies done.

Bahri and Bettaieb (2013) transferred the seedlings with well-developed roots to plastic containers and kept them under controlled conditions for two weeks, then transferred to the soil under greenhouse conditions. It was determined that the transplanted seedlings survived 100%. Zayova et al. (2014), it was determined that it had the highest rooting percentage and the highest number of roots in 0.5 mg/L IBA medium with 1⁄2 MS. In this study, the best rooting and the longest root number were obtained in MS medium with 1 mg/L IBA. After acclimatization, all plants taken into the greenhouse developed well and survived (Figure 2).

Figure 2. The acclimatization of *P. tomentosa* ready (a: The seedlings in vitro medium, b: in greenhouse and, c: at outdoor land).

CONCLUSION

Ultimately, we developed an *in vitro* propagation protocol using nodal stem segments of *P. tomentosa*. The shoots obtained by germinating in nutrient media containing 0 MS were used as explants in the regeneration study. MS medium containing 4 BAP mg/L + 0.1 mg/L GA + 0.1 mg/L NAA in shoot growth was the medium with the highest shoot growth. On the other hand, MS medium containing 3 BAP mg/L + 0.1 mg/L GA + 0.1 mg/L NAA was determined to be the medium containing the highest shoot length. Statistical analyses were determined by shoot number and shoot length values 30 days after culture. The shoots were rooted in a medium containing 1 mg/L IBA and the plants with 100% rooting were subjected to the 21-days acclimatization stage, and then the rooted seedlings were successfully taken into the garden. This study includes successful *in vitro* micropropagation and rooting protocol of *P. tomentosa*. This protocol can be used for rapid and healthy reproduction of *P. tomentosa* and other Paulownia species in the future.

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DETERMINATION OF YIELD AND SOME QUALITY PROPERTIES OF CHICORY AND SOME FORAGE CROPS MIXTURES AT DIFFERENT HARVEST STAGES IN THE ESTABLISHMENT YEAR

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ABSTRACT

This study was carried out to determine the yield and some quality characteristics of chicory and some forage crops mixtures at different harvest periods. It was established in the field of Research and Application Centre of Ondokuz Mayıs University Faculty of Agriculture according to the randomized complete blocks design in 2017. Forage mixtures comprised combinations of alfalfa (*Medicago sativa*), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), birdsfood trefoil (*Lotus corniculatus*), perennial ryegrass (*Lolium perenne* L.) and orchard grass (*Dactylis glomerata* L.) along with chicory as pure stand, binary mixtures (chicory+legume/gramineae) and triple mixtures (chicory+legume+gramineae). Plants were harvested at the grazing maturity and budding stage of chicory. During the grazing maturity and budding stage, the highest total green grass and hay yield was determined in the red clover+perennial grass+chicory mixture between 5859.7 - 1148.1 kg/da and 5284.3 – 1318.5 kg/da, respectively. In the grazing maturity stage, the average crude protein, ADF, NDF and NYD contents were determined as 20.1%, 26.6%, 39.2% and 166.5 while, in budding stage, the same values were 17.0%, 31.4%, 41.8%, and 147.0, respectively. According to the results obtained from the study, it can be said that the yield and quality values of triple mixtures were higher than binary mixtures and pure, moreover, the yield and quality values of grazing maturity stage was higher than budding stage. In this ecological conditions, mixtures of red clover + perennial ryegrass + chicory or red clover + orchard grass + chicory look like more beneficial. But, to make any recommendation the study need to be continued for many years.

Keywords: chicory, sowing pasture, mixture, legumes, grasses.

INTRODUCTION

The application of breeding schedules in natural grassland is difficult because it is not economical and lasts a long time. Therefore, there is a necessity to establish forage plant species and cultivars suitable for the climatic conditions of the region and sowing meadow and pasture facilities to meet the need for high-quality roughage. Moreover, more yield and quality were obtained from perennial legume-grass forage plant mixtures compared to pure sowing. (Koç et al., 2004; Seydoşoğlu and Bengisu, 2019).

It is necessary to increase the number of species and varieties cultivated to raise the production and quality of roughage obtained from sowing pastures in our country. Alternative forage plant species and cultivars have been developed from wild forms in many countries taking care of this issue. Although a drought occurred in New Zealand in 1978, the high yield and nutritional

value of chicory plants attracted great attention. The distribution and frequency of the chicory plant in our country's natural flora clearly reveal the plant's adaptation to our country's ecology and its potential diversity (Başaran et al, 2018). For this purpose, chicory will contribute to our agriculture and animal production, significantly, as an alternative plant.
This study was carried out to determine the yield and quality properties of some legumes and grass forage plants mixtures in the grazing maturity and budding stages of chicory (*Cichorium intybus* L.), in the year of establishment.

MATERIALS AND METHODS

This study was carried out in the field of Agricultural Application and Research Centre of Ondokuz Mayıs University Agricultural Faculty in 2017 for one year. According to the results of the soil analysis of the area where the study was carried out; The soil is clayey in terms of texture (45%), slightly acid character (6.34), salt-free (0.48 mmhos/cm), organic matter content is high (3.37%) and less calcareous (2.18%), nitrogen content is sufficient (0.16%), phosphorus and potassium contents is very high (65.40 and 340 ppm, respectively). The average temperature and total precipitation values for 2017 were 15.2 ºC and 657.3 mm, respectively (Table 1).

Table 1. Some climatic data of Samsun province for long years and trial years.

	Average temperature			Total precipition			Proportional humidity (%)		
Months	Long years	2017	2018	Long years	2017	2018	Long years	2017	2018
January	7.2	6.2	8.9	8.9	78.8	153.6	66.4	60.2	65.9
February	7.2	7.4	7.4	10.1	40.1	37.0	68.9	58.8	71.2
March	8.2	9.4	9.4	11.5	65.1	118.9	74.5	71.3	72.7
April	11.3	10.2	10.2	12.7	78.3	6.8	78.0	73.7	69.5
May	15.5	15.3	18.2	18.2	70.9	18.9	79.0	75.5	76.4
June	20.1	20.9	22.9	22.9	45.1	29.7	74.5	71.7	66.5
July	23.1	24.2	25.3	25.3	0.4	39.7	72.3	64.8	64.6
August	23.5	25.5	25.7	41.5	13.7	65.3	72.1	64.8	60.8
September	20.2	22.3	21.9	51.5	29.8	90.2	73.6	63.5	65.5
October	16.3	16.4	18.5	82.5	34.6	88.4	74.6	61.6	69.9
November	12.6	13.3	13.6	83.1	59.4	67.9	68.8	61.5	67.8
December	9.3	12.2	9.8	81.5	141.1	76.3	65.8	54.5	63.9
Average	**14.5**	**15.2**	**16.6**				**72.3**	**65.1**	**67.9**
Total				**712.5**	**657.3**	**792.7**			

In the study, Grassland Puna II cultivar of chicory (C) (*Cichorium intybus* L.), Dimitra cultivar of alfalfa (AA)(*Medicago sativa* L.), Liflex cultivar of white clover (WC) (*Trifolium repens* L.) cultivar), Suez cultivar of red clover (RC) (*Trifolium pratense* L.), Lotus cultivar of birdsfood trefoil (BT)(*Lotus corniculatus* L.), Perennial ryegrass of genotype (*Lolium perenne* L.), Lidacta cultivar of orchard grass (OG) (*Dactylis glomerata* L.)) were used. The study was established according to randomized complete block design with 3 replications. Cultivars used in the research were sowed as pure, binary (chicory + leguminous/grass) and triple (chicory +

leguminous + grass) mixtures and consisted of total 21 treatments (mixture). The row spacing was 20 cm, the plot length was 3.5 m. In addition, the number of rows were determined as 4 rows for pure sowing, 6 rows for binary mixtures sowings and 9 rows for triple mixtures sowings. The experiment was established on 28.02.2017 and plants used in the study were sowed by hand. 6 kg/da P205 and 5 kg/da N fertilizer were applied with the sowing. Irrigation was not done in the experimental area, the plants groved in the rain fed conditions. Chicory plants were harvested when they reached 25-35 cm plant height for grazing maturity stage. The second harvest period was made when chicory plants were budding period. Two mowing (June 13 and July 10) were made for grazing maturity period and one mowing (July 1) was made for budding period. After the plants cut from mixed plots were separated according to their types, the fresh grass yield per decare was calculated by weighting. 500 gr samples from each plant species were taken from the harvested plots, dried at 60 ^{o}C until they reached a constant weight, and hay yield was calculated. The dried samples were weighed and the hay yield per decare was calculated by using the hay rates obtained. Dried samples were grinded in a mill that sieve diameter is 1 mm. The crude protein, ADF and NDF proportion of the samples were determined using the IC-0904FE calibration program on the Foss NIRSystems Model 6500 Win ISI II v1.5 instrument (Starks et al, 2004). The relative feed value (RFV) was calculated using ADF and NDF values (Horrocks, 1999). The results obtained from the study were analysed according to the 'Randomized Complete Blocks Trial Design' using the SPSS 17.0 statistical package program, separately for each harvest (mowing).

RESULTS AND DISCUSSION

The pure and mixed plots of chicory and other forage crops were harvested in grazing maturity and budding periods, and fresh yield and hay yields were calculated. The calculated yield values are given in Table 2. Statistically significant differences were determined between the mixtures in terms of both fresh yield and hay yield values. At the grazing maturity period, in both 2 harvest (first and second mowing) highest fresh and hay yields were obtained from triple mixture (RC+LP+C). Moreover, RC+LP+C and RC+OG+C mixtures statistically are found in the same group (Table 2). At the grazing maturity period, in first mowing, the lowest hay yield was determined in pure birdsfood trefoil plot (68.0 kg/da) whereas in second mowing, the lowest dry grass yield was determined in pure orchard grass plot (88.1 kg/da). At the budding stage, the highest fresh yield was obtained from the mixture of RC+LP+C as 5284.3 kg/da, similar to the grazing maturity period. The lowest fresh yield was obtained from the pure birdsfood trefoil plot (794.7 kg/da) while the highest hay yield was obtained from mixture of RC+LP+C (1318.5 kg/da). In addition, it was determined that RC+LP+C mixture (1318.5 kg/da) and RC+C mixture (1224.1 kg/da) were in the same statistical group (Table 2). In the establishment year, development of birdsfood trefoil that is perennial legume plant were weak compared to the other legume plants. Effects of red clover on fresh and hay yields in the mixtures more than the other legumes and grasses. More fresh and hay yields were obtained from binary and triple mixtures with red clover.

Table 2. Fresh and hay yields obtained from mixtures of chicory and some forage crops harvested during the grazing maturity and budding stages (kg/da)

Mixture	1. mowing grazing maturity period (kg/da)		2. mowing grazing maturity period (kg/da)		Budding period (kg/da)	
	Green grass yield	dry grass yield	Green grass yield	dry grass yield	Green grass yield	dry grass yield
AA	981,9 eh	222,2 dg	350,0 f	115,1 gh	1013,1 ef	335,0 eg
WC	1579,1 df	218,4 dg	434,4 ef	153,8 fh	1112,2 ef	321,2 fg
RC	2885,0 bc	434,5 bc	805,3 de	250,6 cf	2507,8 cd	808,6 bc
BT	**389,5 h**	**68,0 g**	427,5 ef	132,1 gh	794,7 f	219,4 g
LP	812,5 fh	171,8 fg	263,1 f	92,9 gh	1172,2 ef	379,6 dg
OG	436,6 gh	94,4 g	**248,3 f**	**88,1 h**	**728,9 f**	**212,6 g**
C	1473,6 df	181,1 eg	878,9 ce	194,8 dg	2295,8 ce	590,6 cf
AA+C	1497,6 df	222,5 dg	816,3 de	191,7 eh	2795,3 cd	607,4 cf
WC+C	2020,3 ce	292,2 cf	1165,7 bd	311,7 c	3117,3 bd	593,6 cf
RC+C	3673,3 ad	549,3 ab	1553,3 ab	425,2 ab	4408,0 ab	1224,1 a
BT+C	1429,6 dg	215,6 dg	1095,3 bd	246,1 cf	3198,0 bd	568,2 cf
LP+C	2090,0 cd	405,3 bc	1123,7 bd	254,0 cf	3132,7 bd	721,0 bc
OG+C	2497,3 cd	355,9 cd	1310,7 bc	246,5 cf	3595,0 bc	940,5 b
AA+LP+C	2360,0 cd	417,7 bc	1312,3 bc	349,7 bc	3544,0 bd	765,9 bc
WC+LP+C	2471,3 cd	376,9 cd	1337,1 bc	338,9 bc	2974,5 cd	637,2 cd
RC+LP+C	**4008,1 a**	**643,0 a**	**1851,6 a**	**505,0 a**	**5284,3 a**	**1318,5 a**
BT+LP+C	2013,8 ce	314,3 cf	1144,3 bd	287,1 ce	2761,3 cd	677,8 bc
AA+OG+C	1901,2 ce	319,0 cf	978,3 cd	259,0 cf	2717,6 cd	627,5 ce
WC+OG+C	2280,8 cd	340,1 ce	941,2 cd	254,6 cf	2162,6 de	546,6 cf
RC+OG+C	3838,3 ab	619,7 a	1565,7 ab	449,9 a	3680,5 bc	934,4 b
BT+OG+C	1928,6 ce	292,0 cf	1134,0 bd	300,8 cd	3464,8 bd	694,2 bc

* There is no difference at the 0.01 level between the averages shown with the same letter in the same column. AA: Alfalfa, WC:White clover, RC:Red clover, BT:Birdsfood trefoil, LP:Perennial ryegrass, OG:Orchard grass and C: Chicory.

It was determined that the difference between the treatments in terms of average ADF ratio was statistically very significant (Table 3). At the grazing maturity stage, the highest ADF proportion was obtained in the pure orchard grass plot with 31.5% while the lowest ADF proportion was obtained in the pure birdsfood trefoil plot with 16.3%. In the second mowing, the highest ADF ratio was obtained from the pure orchard grass plot with 36.5% whereas the lowest ADF ratio was obtained from the pure white clover plot with 21.5%. At the budding stage, the highest ADF ratio was obtained from the pure orchard grass plot with 37.1% while the lowest ADF ratio was obtained from the pure birdsfood trefoil plot with 26.2%. When the ADF ratios of all plots are examined; In the grazing maturity stage, all mixtures were placed statically in the quality group (ADF< 31), except pure orchard grass. At the budding period, all plots were placed statically in quality group except white clover, birdsfood trefoil and chicory in pure plots, WC+C plot and BT+C plot in binary mixtures, RC+LP+C and RC+OG+C plots

in triple mixtures. It was detected that there is significance statically between plots in terms of NDF value (Table 3). At the grazing maturity period, the highest NDF ratio was obtained from pure orchard grass plot with 51.8% while the lowest NDF ratio was obtained from pure birdsfood trefoil plot with 27.6%. In the second mowing, the highest NDF ratio was obtained from pure orchard grass plot with 62.9% whereas the lowest NDF ratio was obtained the pure white clover plot with 30.1%. In the budding stage, the highest NDF was obtained from pure orchard grass (61.3%), and the lowest was obtained from BT+C binary mixture plot (35.2%). When NDF ratios of all plots are examined; pure legumes and mixtures being with chicory point out compared to the other plots. Different plant species and different harvest stages affect the average NDF value.

Table 3. ADF and NDF values of mixtures of chicory and some forage crops harvested during grazing maturity and budding period*

Mixture	**1. mowing grazing maturity period (%)**		**2. mowing grazing maturity period (%)**		**Budding period (%)**	
	ADF	**NDF**	**ADF**	**NDF**	**ADF**	**NDF**
AA	24,8 eg	33,4 hı	26,5 cf	35,3 f	33,2 be	41,7 df
WC	21,7 h	31,6 ıj	**21,5 h**	**30,1 g**	29,0 gh	35,8 gh
RC	28,3 bd	42,0 be	30,1 bc	42,1 cd	35,7 ab	46,2 c
BT	**16,3 ı**	**27,6 j**	22,3 gh	34,8 f	**26,2 ı**	38,3 fh
LP	26,6 cf	44,0 bc	30,4 b	49,2 b	34,6 bc	52,9 b
OG	**31,5 a**	**51,8 a**	**36,5 a**	**62,9 a**	**37,1 a**	**61,3 a**
C	26,0 cg	36,9 fh	25,9 dg	36,1 ef	30,4 fg	36,4 gh
AA+C	24,2 fh	33,8 hı	25,4 eg	34,5 f	31,3 dg	39,4 eh
WC+C	24,2 fh	35,0 gı	24,8 fh	34,7 f	29,1 gh	35,9 gh
RC+C	26,9 bf	40,7 cf	30,0 bc	41,2 cd	33,6 bd	43,7 ce
BT+C	23,4 gh	34,5 hı	24,4 fh	34,7 f	27,3 hı	**35,2 h**
LP+C	29,7 ab	46,3 b	30,4 b	44,2 c	31,8 df	44,0 cd
OG+C	27,7 be	40,9 cf	29,4 bd	41,8 cd	32,4 cf	40,4 dg
AA+LP+C	28,6 bc	41,4 cf	26,9 bf	37,9 df	30,8 eg	41,7 df
WC+LP+C	24,0 fh	35,8 gı	25,8 dg	37,5 df	30,1 fg	39,9 dg
RC+LP+C	28,3 bd	42,2 be	27,3 bf	38,9 df	33,7 bd	44,1 cd
BT+LP+C	25,2 eg	38,0 dh	25,4 eg	37,6 df	30,3 fg	41,5 df
AA+OG+C	25,5 dg	37,7 eh	28,8 be	40,1 ce	31,0 eg	39,9 dg
WC+OG+C	26,2 cg	39,8 cg	27,5 bf	38,4 df	28,7 gh	37,3 fh
RC+OG+C	28,5 bc	42,8 bd	29,8 bc	42,0 cd	33,8 bd	44,0 ce
BT+OG+C	24,9 eg	37,7 eh	26,3 cf	38,1 cd	28,8 gh	37,5 fh

* There is no difference at the 0.01 level between the averages shown with the same letter in the same column. AA: Alfalfa, WC:White clover, RC:Red clover, BT:Birdsfood trefoil, LP:Perennial ryegrass, OG:Orchard grass and C: Chicory.

In terms of average crude protein content of hay, there are statistically significant differences both in the grazing maturity period and in the budding period amongst the treatments. Crude protein ratio of hay were varied between 14-8 and 25.7% in grazing maturity stage and between 13.0 and 19.1% in budding stage. In both stages, the highest crude protein ratio was obtained from the pure birdsfood trefoil plot. RC+C and RC+LP+C plots were prominent at the grazing maturity stage. At the budding stage, WC+C plot in binary mixtures and AA+OG+C plot in triple mixtures were point out compared to other plots in terms of the yield and quality values. There are differences between plant species in terms of crude protein ratio. Legumes have a higher crude protein ratio than grasses. The crude protein ratios of chicory determined in the study were found to be higher than the values reported by some researchers (Barry, 1998). This situation may be due to soil and climatic conditions, plant species, mixture ratios, and harvest time differences.

Table 4 Crude protein and relative feed values of chicory and some forage crop mixtures harvested at grazing maturity and budding stages*

Mixture	**1. mowing grazing maturity stage (%)**		**2. mowing grazing maturity stage (%)**		**Budding stage (%)**	
	Crude protein	**RFV**	**Crude protein**	**RFV**	**Crude protein**	**RFV**
AA	24,3 ac	194,5 bc	21,1 ab	184,7 b	19,0 ab	141,7 eg
WC	25,1 ab	213,8 b	20,9 ac	**224,0 a**	17,9 ac	**173,1 a**
RC	23,7 ad	148,5 eh	20,0 ac	144,6 df	16,2 ce	123,3 gh
BT	**25,7 a**	**257,6 a**	20,6 ac	191,8 b	**19,1 a**	168,3 ac
LP	16,7 ıj	143,9 gh	15,6 ef	123,5 f	**13,0 f**	109,6 h
OG	**16,3 j**	**115,6 ı**	**14,8 f**	**89,4 g**	14,2 ef	**91,2 ı**
C	18,7 gj	173,2 ce	19,6 ac	178,1 bc	17,0 ad	166,7 ac
AA+C	22,0 ce	193,0 bc	**21,4 a**	186,3 b	17,6 ac	152,7 be
WC+C	21,9 de	186,3 cd	20,0 ac	187,0 b	18,1 ac	171,8 ab
RC+C	22,8 bd	155,3 eh	20,5 ac	148,1 df	17,3 ad	133,8 eg
BT+C	21,6 de	190,8 bc	20,7 ac	187,7 b	17,8 ac	179,2 a
LP+C	17,6 hj	134,0 hı	17,2 de	137,4 ef	15,0 df	136,1 eg
OG+C	18,3 gj	153,8 eh	19,2 ac	147,4 df	16,6 bd	147,3 df
AA+LP+C	19,9 eh	150,1 eh	19,9 ac	166,8 bd	16,7 ad	145,0 ef
WC+LP+C	20,2 eg	182,8 cd	19,2 bc	170,9 bd	16,6 bd	152,7 be
RC+LP+C	21,5 de	147,1 eh	19,3 ac	163,2 be	16,9 ad	132,3 fg
BT+LP+C	18,9 fı	170,1 cg	19,2 ac	171,2 bd	17,2 ad	146,8 df
AA+OG+C	19,5 eh	170,2 cg	18,8 cd	154,2 ce	18,8 ab	151,2 cf
WC+OG+C	19,9 eh	160,1 dh	19,4 ac	165,0 be	17,5 ac	165,8 ad
RC+OG+C	21,4 df	144,8 fh	19,3 ac	145,7 df	16,3 ce	132,6 fg
BT+OG+C	20,1 eh	172,0 cf	19,7 ac	168,0 bd	18,2 ac	165,6 ad

* There is no difference at the 0.01 level between the averages shown with the same letter in the same column. AA: Alfalfa, WC:White clover, RC:Red clover, BT:Birdsfood trefoil, LP:Perennial ryegrass, OG:Orchard grass and C: Chicory.

When the relative feed values were examined, RFV values varied between 89.4 and 257.6 at the grazing maturity stage and between 91.2 and 173.1 at the budding stage. At the grazing maturity stage, the highest RFV value was determined in the pure birdsfood trefoil stands. At the budding stage, many mixtures took place in 1st and 2nd quality group. In both stages, the hay of pure orchard grass had low quality. In general, the RFV value of grasses is lower than legumes. The RFV content of hay obtained from the mixtures varies according to the climatic factors, the plant species and their ratios, and the maturity period at harvest.

CONCLUSION

In this study, which was carried out to determine the yield and some quality characteristics of mixtures of chicory and some forage plants in the establishment year, at the grazing maturity and budding stages, the highest fresh and hay yields were obtained from the triple mixture of red clover + perennial ryegrass + chicory. Considering the crude protein rate, yield and quality characteristics, chicory can be evaluated with legumes and grasses in binary or triple mixtures. Regarding the diversity of ecological conditions in our region and country, it was necessary long term and more studies.

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MOLECULAR MARKERS FOR GENETIC DIVERSITY AND CHARACTERIZATION STUDIES IN *Orobanche* spp.

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ABSTRACT

The broomrapes (*Orobanche* spp.) are holoparasitic plants, devoid of roots and chlorophyll, completely dependent on their hosts. *Orobanche* is a large genus of parasitic, herbaceous plants that are mainly found in the Northern Hemisphere. They are mainly located in the Mediterranean-basin, North Africa, Southwestern Asia, and some other regions with Mediterranean type climate. Five of *Orobanche species are considered important agricultural pests (O. crenata, O. cernua, O. cumana, O. ramosa, O. aegyptiaca). They particularly choose Asteraceae,* Solanaceae, Fabaceae, Umbelliferae, and Cucurbitaceae. Some molecular markers, such as random amplified polymorphic DNA, amplified fragment length polymorphism, cpDNA diagnostic markers, inter simple sequence repeats, and microsatellites have been successfully used for genetic diversity and characterization studies in *Orobanche* species. This study focuses on the use of various molecular markers that reveals the genetic diversity of broomrapes in different regions. Based on the studies in the literature, it is necessary to deepen the studies by using more populations and effective molecular markers belonging to the distribution area of the species in order to understand and reveal events such as global genetic characterization and diversity, distribution and evolutionary mechanism of race formation.

Keywords: Broomrape, Genetic Diversity, Molecular Markers, Races

INTRODUCTION

Orobanche spp. which is an angiosperm parasite is one of the major threatening factors for important agro-economic crop production particularly in Europe and Asia. *Orobanche* genus is the largest genus among the holoparasitic members of Orobanchaceae family (Zare and Donmez, 2013). *O. crenata, O. cernua, O. cumana, O. ramosa, and O. aegyptiaca are f*ive of *Orobanche species considered as important agricultural pests in the world. These parasitic plants particularly choose Asteraceae,* Solanaceae, Fabaceae, Umbelliferae, and Cucurbitaceae family as their hosts. Molecular marker studies about genetic diversity, population structure, race determination, gene flow, and virulence genetic mechanisms for broomrape populations are scarce (Velasco et al., 2016); but recently molecular marker studies with highly informative markers is increasing.

Molecular markers are selected predominantly because of their abundance in number, unaltered by environmental factors or the developmental stage of plants. Various classes of DNA mutations such as insertions, deletions, substitution (point mutations), or errors in replication of tandem repeated DNA provided development of different types of molecular markers (Gangapur et al., 2018). **M**olecular markers based on single or multi-locus analysis, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), cpDNA diagnostic markers, inter simple sequence repeats (ISSR), PCR-Restriction Fragment Length Polymorphism (PCR-RFLP or CAPS), and microsatellites (SSRs) have been successfully used for genetic diversity and characterization studies in various

Orobanche species. In this study, the use of various molecular markers that reveals the genetic diversity of broomrapes in different regions of dispersal area was evaluated.

GENETIC DIVERSITY STUDIES IN *Orobanche* spp. BY MOLECULAR MARKERS

Randomly amplified polymorphic DNA (RAPD)

RAPD analysis is known as rapid, cost-efficient, and simple molecular marker technique whereas its optimization can be time-consuming. Its reliability is low because of using random decamer primers. In RAPD analysis there is no need for prior information about DNA sequence of studied organism. The first molecular marker studies about genetic diversity and characterization of the genus *Orobanche* were done with RAPD analysis. In RAPD studies, various *Orobanche* populations from Israel, Spain, Egypt, Bulgaria, Turkey, Romania, France, Russia, **Ukraine**, Hungary, Tunisia, and Serbia were investigated from 1996 to 2021 (Table 1). The RAPD analysis has revealed that low to moderate level of genetic diversity. Paran et al. (1997) reported that all the detected genetic diversity within the populations not among the population for studied *Orobanche* species. In Gagne et al. (1998), 47% of the total genetic differentiation was within population for *O. cumana* from 4 different countries. Roman et al. (2001) also detected that high level of genetic diversity within populations (77%) *O. crenata from Spain.* Molinero-Ruiz et al. (2014) studied *O. cumana* and reported that 60% of the genetic diversity was among populations from three different countries (Spain, Turkey, and Hungary) but 40% of the variability was due to among populations within the same country. Ivanovic et al. (2021) studied 11 Serbian *O. cumana* populations and reported great genetic heterogeneity in studied populations.

Amplified fragment length polymorphism (AFLP)

AFLP analysis is the combination of RFLP and PCR techniques and useful for determination of polymorphism especially between closely related genotypes. Although AFLP analysis is fast, highly variable, trustworthy, and relatively cheap, being dominant marker is restricted the determination of heterozygous genotypes. Gagne et al. (2000) and Vaz Patto et al. (2008) were used AFLP analysis for genetic diversity determination of *O. cumana* and *O. foetida*, respectively (Table 1). Gagne et al. (2000) studied two broomrape populations from Spain and Bulgaria and reported AFLP has a higher discriminating power of closely related germplasms than RAPD analysis. In Vaz Patto et al. (2008), 86.3% of the genetic diversity was due to differences among individuals within populations of *O. foetida* from Morocco.

Inter simple sequence repeats (ISSRs)

ISSR technique is based on amplification of the region between two microsatellites with the use of primers having repeat motifs of two, three, four etc. nucleotides. ISSR is fast, cheap in cost, polymorphic, and have good reproducibility. In addition of these advantages, ISSR analysis requires no prior information about DNA sequences of the studied genome. Benharrat et al. (2002), Abedi et al. (2014), Abdalla et al. (2016), and Abdalla et al. (2020) were used ISSR analysis for genetic diversity detection of *O. hederae / O. amethystea, O. aegyptiaca, and O. crenata, respectively* (Table 1). Benharrat et al. (2002) studied *O. hederae and O. amethystea* populations from west and center of France with five ISSR primers and stated that ISSR is useful for detecting intraspecific variation in *O. hederae, also ISSR h*as high reliability to detect new strains of *O. cumana.* Abdalla et al. (2020) studied 30 plants of *O. crenata from Egypt and the similarity matrix was ranged from 83.7% to 65.5% means moderate to high similarity*

between genotypes. Abedi et al. (2014) reported that 1% of the total genetic diversity was among the populations and remaining (99%) was within the populations for studied 44 *O. aegyptiaca* individuals from Northwest Iran.

Table 1. Molecular marker studies of *Orobanche* spp. since 1996

Orobanche spp.	**Country**	**Molecular Marker Type** / No. of Primers Used	**References**
O. aegyptiaca, *O. ramosa*, *O. cernua*, *O. cumana*, *O. crenata*	**Israel, Spain**	**RAPD / 31**	Katzir et al. 1996
O. aegyptiaca, *O. mutelii*, *O. cernua*, *O. cumana*, *O. crenata*	**Israel**	**RAPD / 22**	Paran et al. 1997
O. aegyptiaca, *O. ramosa*, *O. crenata*, *O. oxyloba*	**Egypt**	**RAPD / 20**	Zeid et al. 1997
O. cumana	**Bulgaria, Turkey, Romania, Spain**	**RAPD /23**	Gagne et al. 1998
O. crenata	**Spain**	**RAPD / 23**	Roman et al. 2001
O. alba, *O. amethystea*, *O. arenaria*, *O. ballotae*, *O. cernua*, *O. clausonis*, *O. cumana*, *O. crenata*, *O. densiflora*, *O. foetida*, *O. foetida var. broteri*, *O. gracilis*, *O. haenseleri*, *O. hederae*, *O. latisquama*, *O. mutelii*, *O. nana*, *O. ramosa*, *O. rapumgenistae*, *O. santolinae*	**Spain**	**RAPD / 5**	Roman et al. 2003
O. ramosa	**France**	**RAPD / 2**	Brault et al. 2007
O. cumana, *O. cernua*, *O. ramosa*	**Bulgaria, Turkey, Romania, Spain, Russia,** Ukraine, Israel	**RAPD / 6**	Atanasova et al. 2014
O. cumana	Hungary, **Romania, Spain, Turkey**	**RAPD / 18**	Molinero-Ruiz et al. 2014
O. foetida	Tunisia	**RAPD / 10**	Bouhadida et al. 2015
O. cumana	**Serbia**	**RAPD / 5**	Ivanovic et al. 2021
O. cumana	**Bulgaria, Spain**	**RAPD, AFLP / 23, 3**	Gagne et al. 2000
O. foetida	**Morocco**	**AFLP / 3**	Vaz Patto et al. 2008

O. crenata, O. ramosa, O. cumana	**Spain**	**cpDNA diagnostic marker**	Roman et al. 2007
O. crenata	**Spain, Israel**	**RAPD, ISSR / 1, 5**	Roman et al. 2002
O. hederae, O. amethystea O. cernua, O. cumana	**France**	**ISSR / 5**	Benharrat et al. 2002
O. aegyptiaca	**Iran**	**ISSR / 20**	Abedi et al. 2014
O. crenata	**Egypt**	**ISSR / 5**	Abdalla et al. 2016
O. crenata	**Egypt**	**ISSR / 5**	Abdalla et al. 2020
O. cumana	**Spain**	**SSR / 15**	Pineda-Martos et al. 2013
O. cumana	**Russia, Romania, Kazakhstan**	**SSR / 9**	Guchetl et al. 2014a, 2014b
O. cumana	**Bulgaria, Spain, Turkey**	**SSR / 79**	Pineda-Martos et al. 2014a
O. cumana	**Bulgaria, Spain**	**SSR / 15**	Pineda-Martos et al. 2014b
O. cumana	**Spain, Romania, Greece, Turkey, Bulgaria, Russia,** Ukraine	**SSR / 20**	Martin-Sanz et al. 2016
O. cumana	**Spain**	**SSR / 20**	Jebri et al. 2017a
O. cumana	Tunisia**, Spain, Serbia, Romania, Israel, Turkey, Bulgaria**	**SSR, SNP / 27, 164**	Jebri et al. 2017b
O. cumana	Moldova	**SSR / 12**	Duca et al. 2017
O. cumana	**Turkey**	**SSR / 24**	Ziadi et al. 2018
O. cumana	**Turkey**	**SSR / 8**	Bilgen et al. 2019
O. crenata	Ethiopia	**SSR / 11**	Belay et al. 2020
O. cumana	Moldova, Romania, Bulgaria, Serbia, Turkey, China	**SSR / 15**	Duca et al. 2021
O. cumana	**Turkey**	**SSR / 8**	Arı 2022

Simple sequence repeats (SSRs)

Microsatellites (simple sequence repeats) are region of DNA with 1-6 base pairs of repeat motifs. SSRs are codominant, polymorphic, fast, highly effective/reproducible, and relatively expensive markers. Recently SSR analysis is preferred for determination of genetic diversity, genetic characterization and molecular phylogenetic analysis of various plant species. Pineda-Martos et al. (2014a) designed 298 SSR primer pairs and 79 of them were polymorphic among *O. cumana* populations from Bulgaria, Spain, and Turkey. After their development of SSRs, SSR marker usage increased for molecular marker studies on *O. cumana* (Table 1). The comparison of some genetic diversity parameters from studies of *Orobanche* spp. by using SSRs was given in Table 2. The results obtained from different studies were found to be compatible with each other (Table 2). Small variation of calculated genetic diversity parameters might be due to the number of studied populations, geoFigureic origin of populations, the number of individuals per populations, the number of SSR marker etc.

Table 2. Genetic diversity parameters of *Orobanche* spp. by using SSRs (Na=Mean number of alleles per locus, PIC=Polymorphic information index, I=Shannon's information index, Ho=Observed heterozygosity, He=Expected heterozygosity)

References	**Na**	**PIC**	**I**	Ho	He
Guchetl et al. 2014b (Pop1-Russia-Kazakhstan/Pop2-Romania)	**2.33/1.11**	**-**	**0.63/0.05**	0.44/0.04	0.41/0.03
Pineda-Martos et al. 2014b	**1.733**	**-**	**0.299**	0.008	0.186
Martin-Sanz et al. 2016	**1.00<Na<1.88**	**-**	**0.00<I<0.51**	**0.00<Ho<0.19**	**0.00<He<0.35**
Jebri et al. 2017b	**-**	**-**	**0.12<I<0.32**	**0.00**	**0.06<He<0.23**
Bilgen et al. 2019	**2.271**	**0.289**	**0.547**	0.207	0.340
Belay et al. 2020	**7.25**	**0.801**	**0.45**	0.37	0.78
Duca et al. 2021	**7.857**	0.745	**-**	0.782	-
Arı 2022	2.016	0.199	0.386	0.199	0.241

In Guchetl et al. (2014b), 22% of the total genetic variance was due to differences between the studied populations and 78% due to differences between the individuals within the each gene pool. In Pineda-Martos et al. (2014b), the 11 Bulgarian broomrape populations have within population variation level of 59.54% and among population variation level of 40.46%. Jebri et al. (2017b) studied four populations from Tunisia and reported that 25.35% of the total genetic diversity was among the populations and remaining (74.65%) was within the populations. Bilgen et al. (2019) reported that high proportion of the genetic variation (66%) was due to within population variation and 34% of the diversity was due to among population variations for studied *O. cumana* populations from Thrace region of Turkey. Arı (2022) calculated the within population variation as 53% and among population variation as 47% for *O. cumana* populations from Thrace region and Adana city of Turkey. From the investigated studies we

can conclude that SSR markers are suitable markers for genetic diversity and characterization of *Orobanche* spp. and have high discriminating power between genotypes/populations.

CONCLUSION

The review suggests that the analysis of *Orobanche* spp. by using various molecular markers have led to determination of within and among genetic variation levels of studied populations, and generally high level of within population variation was observed. According to the review of literature, the data obtained from molecular markers studies is very significant for future studies to detect the genetic structure, diversity, and new race determination of *Orobanche* spp. as well as breeding of crops for parasite resistance. In conclusion, it is necessary to deepen the studies by using more populations and effective molecular markers belonging to the distribution area of the species in order to understand and reveal events such as global genetic characterization and diversity, distribution and evolutionary mechanism of race formation.

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MACROMORPHOLOGICAL AND MICROMORPHOLOGICAL STUDY OF ACHENES (CYPSELA) IN *Lactuca serriola* L. AND *Lactuca saligna* L. (*ASTERACEAE*)

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ABSTRACT

Turkey; It has an extremely rich flora due to reasons such as its geoFigureical location, physical structure and historical development. The flora of Turkey has over 8,500 species of flowering plants, and this figure exceeds 10,000 with the addition of subspecies and varieties. One of Turkey's richest family is Asteraceae. And the genus Lactuca L. is one of the larger genera of the Asteraceae family. This genus is represented by 17 taxa in the Flora of Europe. Morphological studies in the field of botany are one of the most important stages used in the identification and taxonomy of plants. Clarifying the boundaries of morphology in taxa, detecting possible variations in morphology (because of phenotype-environment interaction) will reveal the traces of evolutionary transformation in a region/area, solving taxonomic problems arising from these variants, boundaries of biodiversity. For this purpose, in our study, the macromorphological and micromorphological properties of Cypsela species of *Lactuca serriola* L. and *Lactuca saligna* L. belonging to the subfamily Cichorioideae subfamily Lactuceae of the Asteraceae family were examined under stereomicroscopy and scanning electron microscopy (SEM). In the samples collected following the flowering season, macromorphological features of cypselas (Cypsela), cypsela length measurements, color, shape, surface texture, abscission trace features and surface micromorphological features were investigated. In *L. saligna*, cypsela is dark brown, elliptical, 2.5-3 mm in body length, ribs 9-11 striped on the face, beak filamentous and 6.5 mm, pappus white and 5-6 mm. Cypsela faces often rugulose, having many small wrinkles carpopodium U shaped. In *L. serriola* is light brown, obovate shaped, 3.5-5 mm in size, 5-7 ribs, 2-2.5 mm in body length, 3-5 mm in beak length, thin, white 4-5 mm, straight, whitish, pappus uniseriate. Cypsela surface was found to be scabrate, carpopodium round shaped. Micromorphological features of cypselas were similar in both species. However, the other remaining features were proved to be primarily diagnostic at the species level.

Keywords: *Lactuca serriola, Lactuca saligna,* Asteraceae, Cypsela morphology, SEM, Surface microsculpture.

INTRODUCTION

The *Asteraceae* is one of the largest families of flowering plants and contains major globally naturalized flora. The genus *Lactuca* L. belongs to tribe Lactuceae, subfamily Cichorioideae, family Asteraceae (Bano and Qaıser, 2011; Davis, 1988). It is represented worldwide by more than 97 wild species. Several of them have been cultivated for their economic and medicinal importance since ancient times (Lebeda et al., 2001; Doležalová et al., 2002). *Lactuca* includes wild species of annual, biennial or perennial herbs with erect or ascending habit, which are predominantly self-pollinating (Thompson et al., 1958; Sida O, Tan

K 2000). It is distributed throughout the temperate and warm regions with 17 species in Europe, 12 species in America, 43 species in Africa and 51 species in Asia (Ferakova 1976, Lebeda et al., 2001; Doležalová et al., 2002). Some of these species are naturalized in Australia (Doležalová et al., 2002). There are 12 species of this genus in the Balkans 1 of which are endemic (Polunin 1987). 8 species of *Lactuca* in Flora of Turkey are recorded (Jeffrey, 1975). Recently, because of the transfer of certain species of the *Cicerbita* genus to *Lactuca* (Kilian et al.,2009), the number of species of *Lactuca* has increased in Flora of Turkey. According to recent records, *Lactuca* is represented in Turkey with 36 taxa (Ekim, 2012 and Güzel et al., 2018). Only 2 *Lactuca* species (*L. saligna* L. and *L. serriola* L.) are present in European Turkey (Jeffrey, 1975).

In this study, macro and micromorphological characteristics of cypsela (frequently termed an cypsela) of the species have been reported in detail.

MATERIAL AND METHODS

Material

The specimen upon which this study is based were collected from Edirne province in European Turkey. During the master thesis in 2012-2014 years, titled "The morphological, Anatomical and Palynological Investigation on Lactuca L. (Asteraceae) in Edirne" (Köse, 2014) in Trakya University. *Lactuca serriola and Lactuca saligna* specimens collected from European Turkey. These specimens were determined according to the Flora Europaea (Ferakova 1976). Description of the species and descriptive illustrations are given based on the collected specimens. In morphological studies, biometric measurements of the cypsela have been studied. The collected specimens are deposited in the Herbarium of Trakya University (EDTU).

Cypsela (frequently termed an cypsela) morphology

Macro and micromorphological characteristics of cypsela in *Lactuca serriola* L. and *Lactuca saligna* L. were examined under stereomicroscopy and scanning electron microscopy (SEM). The cypsela length data are based on measurements of 10 fruits randomly chosen using a stereomicroscope equipped with an ocular micrometer. The cypsela macro and micromorphological features including; cypsela length measurements, colour, shape, surface texture and the number of ribs when present as well as the abscission scar characteristics and details of the surface microsculpture peculiarities were investigated. For scanning electron microscopy (SEM) samples were mounted on metal stubs**,** coated with a thin layer of gold in ion sputtering device. Micromorphological properties of cypsela were exemined with a Zeiss Evols10 scanning electron microscope and microFigures were taken using the same microscope at the Technology Research and Development Application and Research Central Laboratory of Trakya University (TUTAGEM). The terminology for surface sculpturing patterns is based on Zhu et al. (2006), Abid and Qaiser (2015) and Husseın and Eldemerdashh (2016).

RESULTS

Cypsela (frequently termed an cypsela) morphology

Subgen. *Lactuca L.*

L. saligna L., Sp. Pl., ed. 1: 796 (1756). (Table 1, Figure 1)

Specimens examined in European Turkey:

A1E: Edirne: Centre, at the road side, stony place, 41°39'28"N, 26°31'25"E,156 m alt, 05.07.2013, coll. F. Dane (EDTU 13406).

Uzunköprü; Centre, roadsides, 41°16'16."N, 26°41'58."E, 08.08.2013, coll. B. Köse (EDTU 13424). In *L. saligna*, cypsela is dark brown, elliptical, 2.5-3.5 mm in body length, beak filamentous and 6.5 mm, pappus white, 5-6 mm; ribs 9-11 on each face, faces often transversely rugulose, having many small wrinkles; usually glabrous; carpopodium U shaped.

L. serriola* L., Cent. Pl. 2: 29, no. 189 (1756). *(L. scariola L.). (Table 1, Figure 2)

Specimens examined in European Turkey:

A1E: Edirne: Centre, at the road side, stony place, 41°39'28"N, 26°31'25"E,156 m alt, 05.07.2013, coll. F. Dane (EDTU 13410).

A1E: Edirne: Centre, the Balkan Campus, at the roadside, 41°39'28"N, 26°31'25"E, 18.07.2013, coll. F. Dane (EDTU 13379).

Uzunköprü; Centre, roadsides, 41°16'16."N, 26°41'58."E, 08.08.2013, coll. B. Köse (EDTU 13381). In *L. serriola* is light brown, oblanceolate-obovate shaped, 3.5 - 5 mm in size, 5-7 ribs, 2-2.5 mm in body length, 3-5 mm in beak length, thin, straight, whitish, white pappus uniseriat and 4-5 mm, cypsela surface was found to be scabrate-pylos, carpopodium round shaped. Macro and micromophological features of cypsela of examined specimens of *L. saligna* and *L. serriola* in Table 1.

Table 1: Macro and micromophological features of cypsela of examined specimens of *L. saligna* and *L. Serriola*

Cypsela	*Lactuca saligna*	*Lactuca serriola*
Length (mm)	3 + 6.5 mm	2 +3 mm
Colour	Dark Brown	Light Brown
Cypsela shape	Oblong	Obovate
Beak	Long(5-6 mm)	Long(3-5 mm)
Direction	-+ curved	-+ curved
Cypsela surface	Winged	Winged
Abscission scar;Position	Basal	Basal
Carpopodium	+	+
Ribs	9-11	5-7

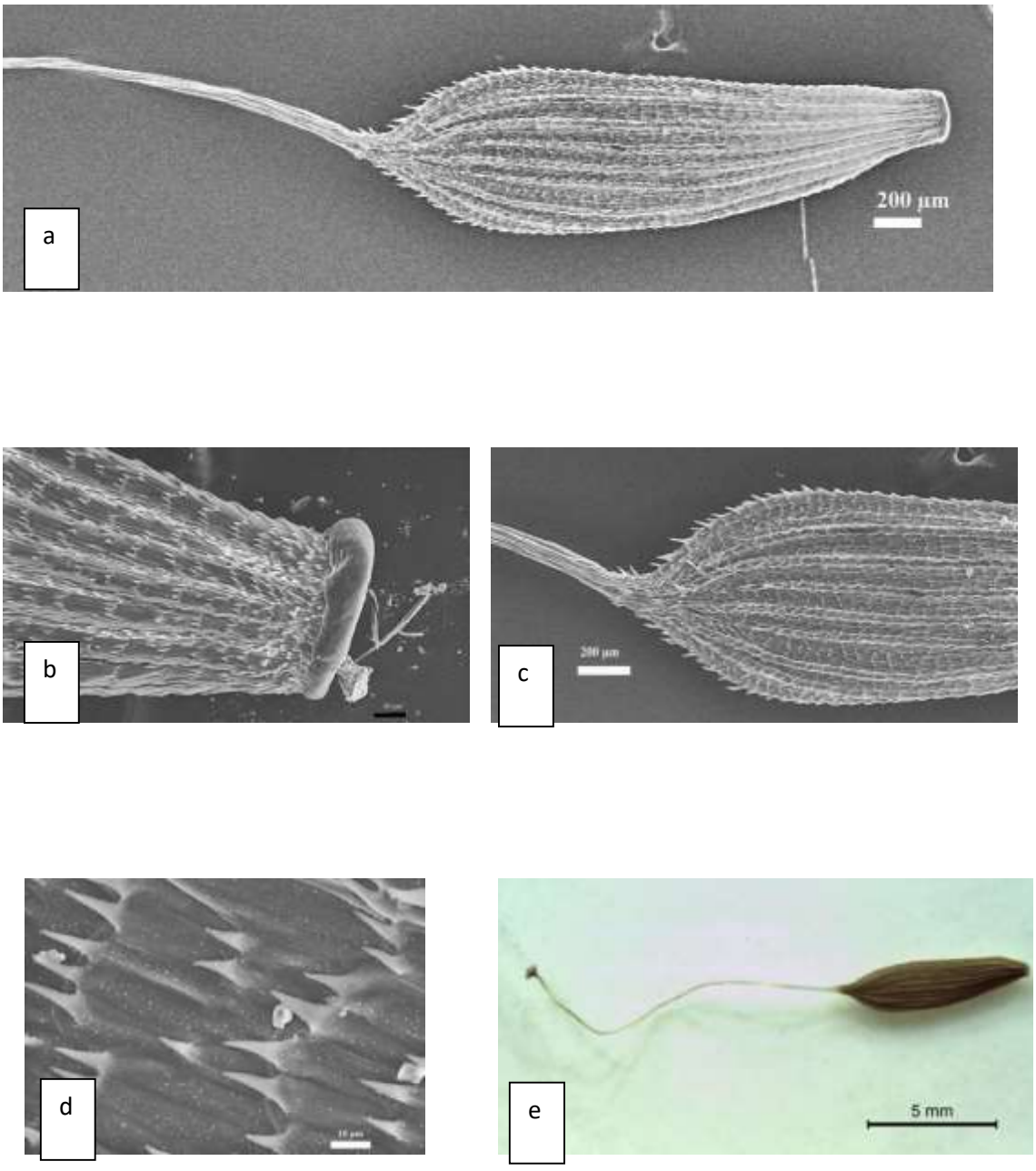

Figure 1 . SEM ans LM microFigures cypsela of *Lactuca saligna*

a. Cypsela with hairy and 9-11 ribs

b. Carpopodium

c. Beak

d. Surface detail. Ovary, (SEM),

e.LM microFigures cypsela of *Lactuca saligna*

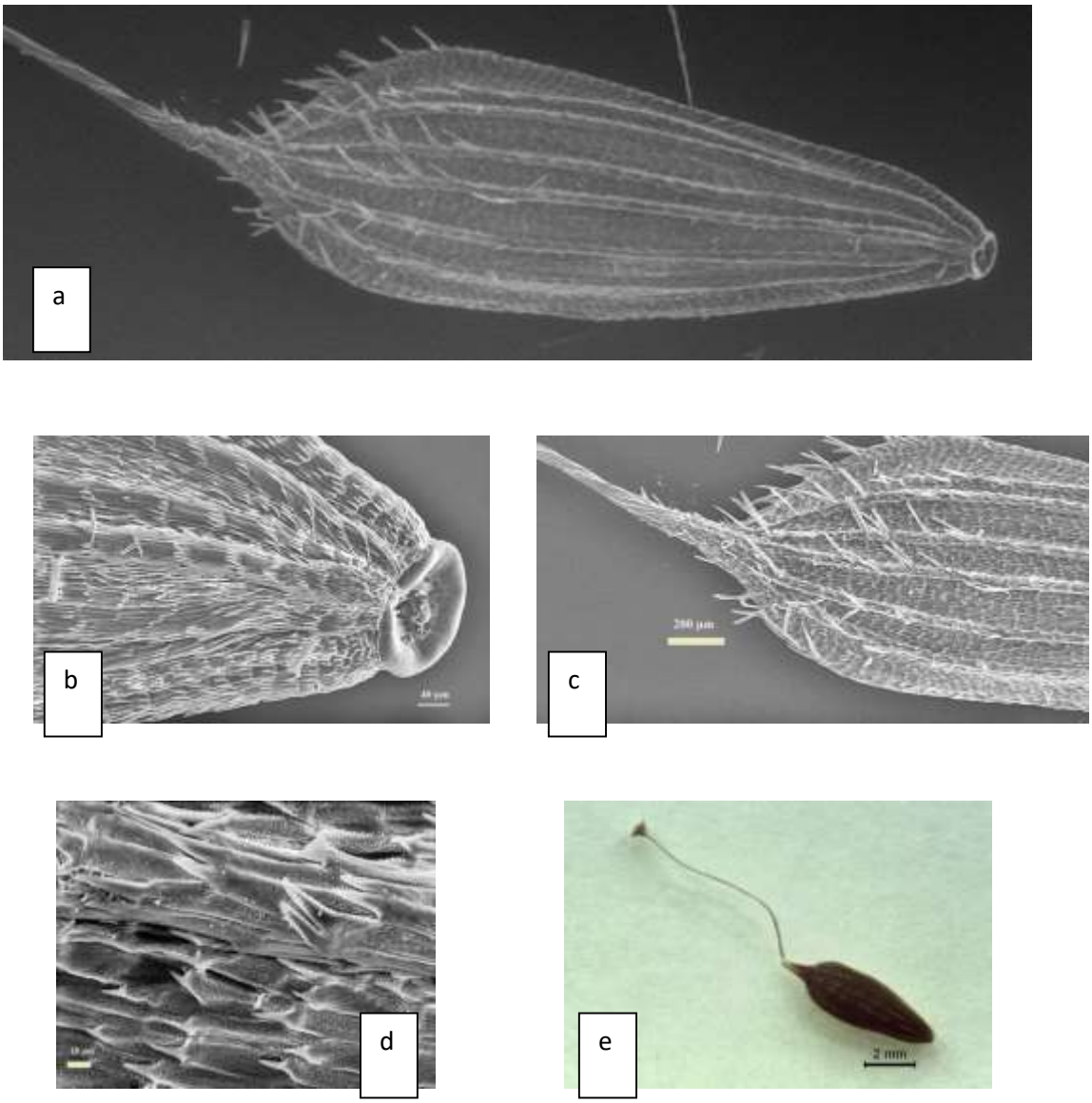

Figure 2: SEM and LM microFigures cypsela of *Lactuca serriola*

a. Cypsela with hairy and 5-7 ribs

b. Carpopodium

c. Beak

d. Surface detail. Ovary, (SEM)

e.LM microFigures cypsela of *Lactuca serriola*

DISCUSSION

The morphology of the collected specimens of this species is identical with that given by Ferakova (1976). According to Feráková (1977), the European *Lactuca* species are divided in four sections (*Phoenixopus*, *Mulgedium*, *Lactucopsis* and *Lactuca*). The species most closely

related to cultivated lettuce (*Lactuca sativa L.*) belong to the section *Lactuca* (subsection *Lactuca*) which contains the best understood wild species: *L. serriola L.*, *L. saligna L.*, *L. virosa* L., and *L. altaica Fisch. & Mey*. These species are ruderal and prefer disturbed soils (Feráková 1977). *L. serriola* and *L. saligna* widely spread in Anatolia and European Turkey especially in the Mediterranean area in fields, ditches, roadsides, waste places, ruderal habitats and woodland communities. We found *L. saligna* and *L.serriola* on road side and stony places, mainly along roads which was recorded in Turkey. Cypsela blackish (dark Brown), flower head pale yellow, about 1 cm cross, in a long pyramidal cluster (Polunin 1987) while stem spinulose, the leaves are deeply lobed, cypsela brownish in *L. serriola* as in the present study Köse, 2014 (Jeffrey 1975, Ferakova, 1976).

Recently, cypsela morphology of some *Lactuca* species has also been studied by various authors (El-Naga 1997, Zhu et al. 2006, Abid and Qaiser 2015, Hussein and Eldemerdash (2016). Güzel et al. (2018), Sadeq and Aliwy (2019). El-Naga (1997) investigated the carpopodium of 40 species belonging to the tribe Lactuceae (Asteraceae) to assess their taxonomic significance. Zhu et al. (2006) studied the cypsela wall anatomy and surface sculpturing of 14 species of the genus *Lactuca* L. and its related genera. Abid and Qaiser (2015) studied the cypsela surface sculpturing of 13 species of the genus *Lactuca* from Pakıstan. Hussein and Eldemerdash (2016) studied the cypsela surface sculpturing of 3 species of the genus *Lactuca* and its related genera from Egypt. Güzel et al. (2018) studied the cypsela surface sculpturing of 4 species and 4 subspecies of the genus *Lactuca* from Turkey (Anatolia). Sadeq and Aliwy (2019) studied the cypsela surface sculpturing of *L. serriola* from Iraq. There are some differences between the cypsela morphology of investigated species. They are different morphologically from each other in terms of cypsela length, color, shape, surface texture and the number of ribs, the abscission scar characteristics and details of the surface microsculpture peculiarities. *L. serriola* have hair on cypsela body and hair is only distributed at the top of the ribbed faces in different sizes and begin to fade into fine spines in the base of the cypsela as in *L. saligna*. Both of them are with carpopodium. But is different some features of cypsela. Cypsela of *L. serriola* based on Iraq material; cypsela colour brown, shape oblanceolate-obovate, beak 4-5 mm, carpodium U shaped, surface scabrid-pilose, number of ribs 7-9 (Abid and Qaiser 2015, Sadeq and Aliwy 2019).

Our results provided the first cypsela morphology data on this species based on Turkish material. Such characteristics can be useful for designation primarily at the species level and sometimes at the genus level.

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METHODOLOGICAL APPROACH FOR ASSEMBLE DATA FROM VEGETABLE CROPS FOR USE IN REMOTE SENSING

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ABSTRACT

Remote sensing is increasingly used in contemporary methods for observe the biological status of agricultural crops. Early identification of yield problems can significantly help reduce losses and achieve target results and profit. The main aim of this article is to outline the framework of the methodological scientific approach for the range of analytical indicators suitable for assessing the ecobiological status of plants compatible with remote sensing. The present approach includes data on land characteristics, analysis of physicochemical parameters of soil and morphometric characteristics of plants, indicators of biological status of vegetable varieties in the different stages of their development. Based on the results of the studies, the practical significance of remote sensing methods and vegetation indices will be determined. The collected scientific information and establishing the dependencies of the analyzed data and spectral analyzes on remote sensing will be the basis for making of expert decisions for the agronomic management of the vegetable production.

Key Words: Vegetables, remote sensing, ecobiological status, vegetation indices

INTRODUCTION

Vegetables crop production is an integrate part of Bulgarian agriculture. Its development in Bulgaria is determined by a number of preconditions - appropriate soil and weather conditions, various species composition and structure of adequate cultivars, different production ways.

Planning and management of arable land in vegetable production are fundamental to sustainable land use (Godschalk, 2004). These activities are based on constant field research and analysis. Safalary et al. (2022) point out that obtaining modern, up-to-date and accurate information on arable land and crop development can be accomplished by using remote sensing and GIS. Because of the influence of climatic factors during the cultivation of vegetables, it is not always possible to obtain objective information necessary to establish the current situation, its assessment and forecasting (Francis et al., 2013). According to Antamoshkin et al. (2022) it is important to know the status of crop plants and the assessment of the environment for making management decisions to guide the productivity of agricultural crops and protect soil fertility of intensively used areas. The authors point out that this monitoring and evaluation can be done by analyzing the spectral characteristics of the remote sensing and satellite images of the earth in the different phases of the vegetation period of the plants. Parallel field studies are also conducted to validate the results.

The importance of the use of remote sensing in agriculture has been highlighted by numerous studies in recent years - Castaño et al. (2010), Rao et al. (2014), Widiatmaka (2016), Segara et al. (2020), Hara et al. (2021), Valero et al. (2021), Radocaj et al. (2022), Safdary et al. (2022). Diagnostic methods based on remote sensing are increasingly entering in Bulgarian agriculture, but scientific information is limited. Concrete, practically applicable recommendations are lacking. These facts, as well as the topicality of the problem, are the main motive for this article.

The main aim of this article is to outline the framework of the methodological scientific approach for the range of analytical indicators suitable for assessing the eco-biological status of plants compatible with remote sensing.

MATERIAL AND METHOD

Pepper has been determined as the main crop for Bulgarian vegetable production through the analysis of statistical data from the Agrostatistics Department of the Ministry of Agriculture (https://www.mzh.government.bg/bg/statistika-i-analizi/), conversations with producers and experts and own studies (Haytova et al., 2014). Pepper is planted on the field in May and the area is cleared in autumn after the first frost. This cultivation scheme is repeated every year (Cholakov, 2009).

Based on the literature reviewed (Gary et al, 1998; Francis et al., 2003; Sönmez and Sari, 2006; Castaño et al. 2010; Xu et al. 2019; Purnamasari et al., 2022; Radocaj et al., 2022 ; Safdary et al., 2022), generally accepted methods for field research - in situ, analysis of plant samples (Dimova and Marinkov, 1999), pepper growing technology (Cholakov, 2009), botanical and growth characteristics of plants (Mihov et al., 2014) the methodology for assemble data from vegetable crops for use in remote sensing was developed.

The following stages for work are defined:

1. Identification of suitable areas for pepper cultivation by assessing their suitability;
2. Outline the field research framework by defining the main indicators;
3. Determining the compatibility of in situ data with those from remote sensing and preparing recommendations for practical use;

RESULTS AND DISCUSSION

For the implementation of Stage 1 of the work algorithm, a conceptual scheme for assessing the suitability of the land was drawn up (Fig. 1). For the needs of vegetable production, it is necessary to analyze the main climate indicators, the agrobiological requirements of vegetable crops and to evaluate the infrastructural factors. This evaluation is done through GIS. According to Mendas and Delali, (2012) GIS is very useful in land evaluation due to its capability in acquisition, storage and extraction of georeferenced data. Malczewski (2004) and Malczewski (2006) claim that land suitability evaluation and mapping even become one of the most useful applications of GIS in planning (Malczewski, 2004, 2006). The method that is currently widely used in land use planning is integrating the land evaluation method with the geoFigureic information system (GIS) and remote sensing. Remote sensing gives its contribution in a broad view, both spatial and temporal (Ambarwulan et al., 2010). The integrated use of land evaluation, remote sensing and GIS can be done in a decision-making system in land evaluation methodology, which is known as multi-criteria decision-making. In this manner, a lot of criteria that affect land suitability can be considered (Widiatmaka, 2016).

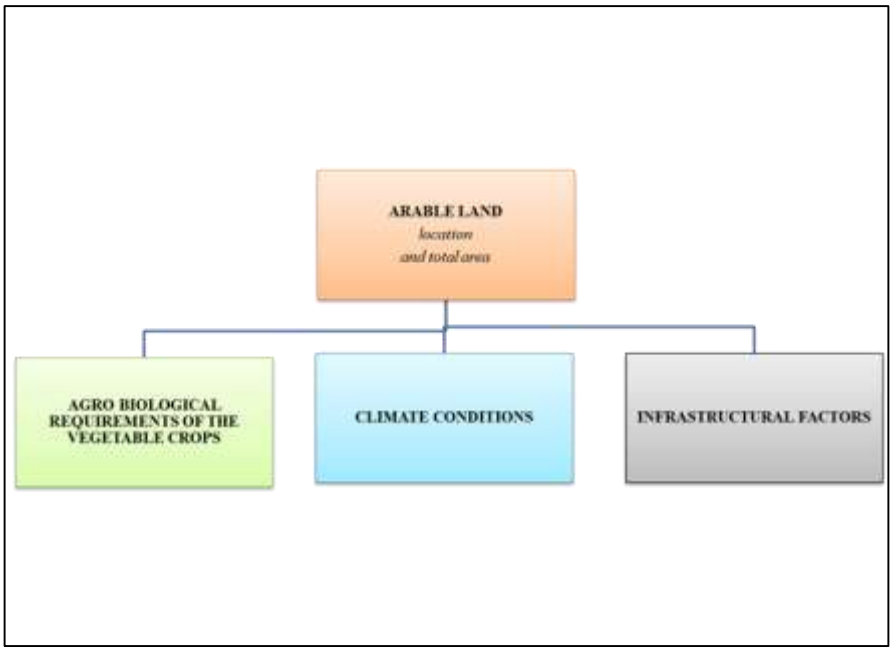

Figure 1. Conceptual scheme for land suitability assessment

This analysis is very necessary according to Gary et al. (1998). The analysis would be included as part of the database for horticultural land use planning (Rao et al., 2014).
Similar research is conducted by Purnamasari et al. (2022), who identified suitable areas to evaluate the sustainability of land use for cassava production using a multi-criteria model integrating with GIS, remote sensing and AHP.

The present approach in Stage 2 includes data on land characteristics, analysis of physicochemical parameters of soil and morphometric characteristics of plants, indicators of biological status of vegetable varieties in the different stages of their development (Fig.2).

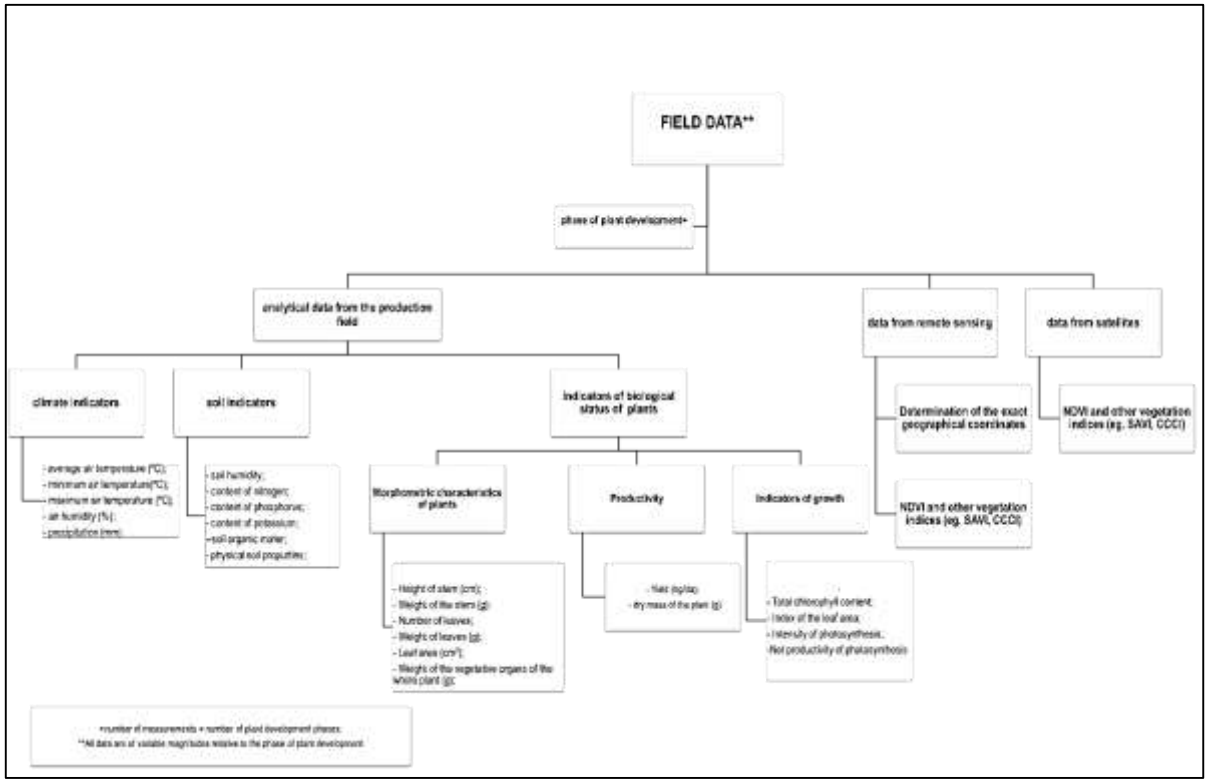

Figure 2. Framework of the methodological scientific approach

For field research and the construction of the geo-database for characterizing the phenological development of agricultural crops and soil differences, the following classifications are used for: registering phenological phases - the BBCH scale (Meier, 2001, 2018); soil map - (IUSS Working Group

The basic climate indicators are determined by a meteorological station measuring the indicators in dynamics.

The basic physico-chemical parameters of the soil are determined in two depths (30 cm, 60 cm): pH of the soil; Humidity; Content of organic matter and humus; content of nitrogen, phosphorus and potassium. Soil pH: determined potentiometrically in the field using Soil pH meter, PCE-PH20S (PCE Instruments, United Kingdom) and in laboratory conditions (H2O, KCl) using pHotoFlex Set, 2512000 (WTW, Germany). Soil moisture: determined indirectly (in the field) by Soil Humidity Meter TR 46908 (Turoni, Italy) and in laboratory conditions by the weight method. Mechanical composition of the soil - determined according to ISO 11277; Content of organic matter in the soil - determined according to Tyurin's methodology; Humus content: determined according to ISO 14235:02; Nitrogen, phosphorus and potassium content (mobile forms): determined according to ISO/TS 14256-1:2003 and GOST 26209:1991.

Plant and soil samples are taken in a randomized method in the test fields (Fig.3). The sites have an area of 50 m^2 with 400 plants each (Fig 3.).

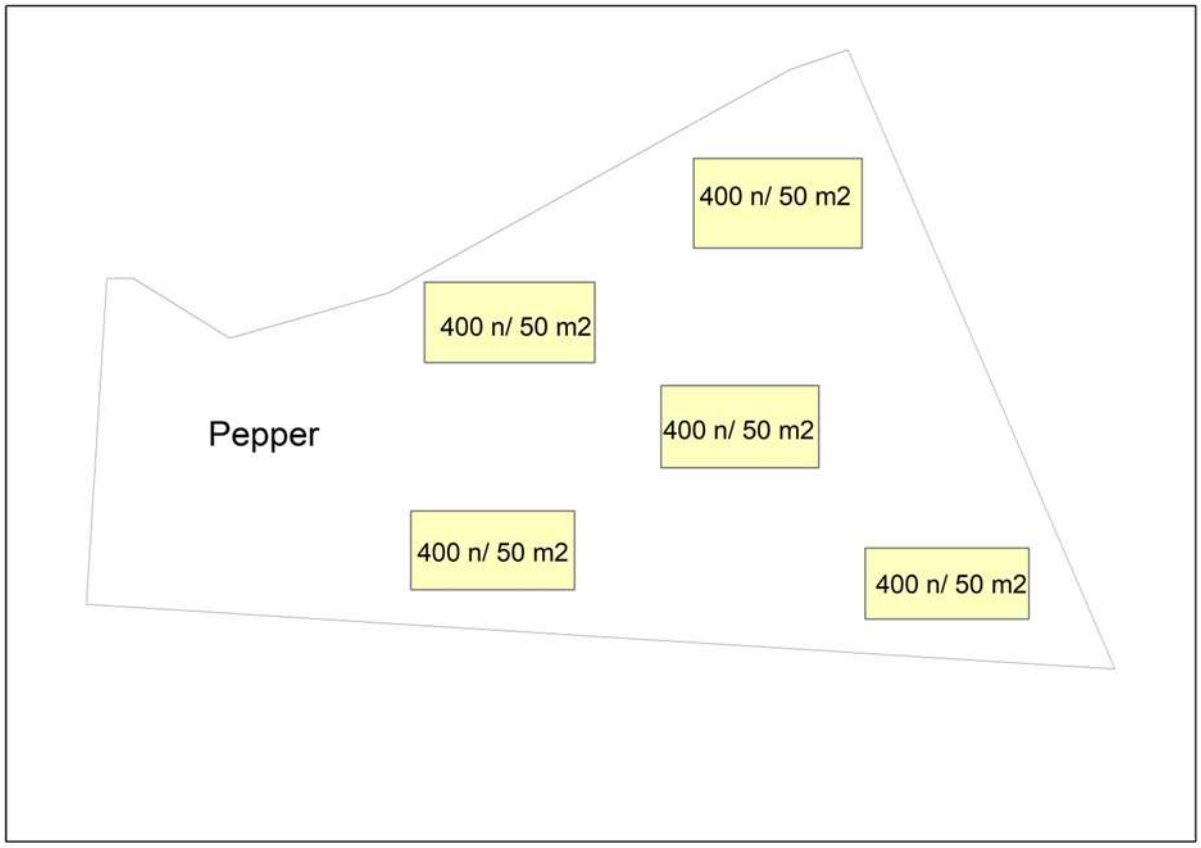

Figure 3. Scheme of the basic sites for soil and plant samples

The assessment of the condition of the crop is carried out in the mass occurrence of the phenophases. It is visual for the development and health status of the plants. The morphometric indicators are determined through randomized samples of plants. In the phenophases of fruiting, the productivity of the plants is also determined by taking into account the yield and the dry mass of the whole plant (Georgiev et al., 1980). Calculation of net productivity of photosynthesis according to Nichiporovich's method (Lawlor and Cornic, 2002); Calculation of the photosynthetic potential - the sum of the leaf surface of the crop for each day of the growing season (Lawlor and Cornic, 2002; Transpiration (E, μmol m-2 s-1) was measured on three fully developed, undamaged leaves of the same physiological age, taking at least 50 values for each individual leaf. It is expressed as the amount of water transpired (in grams or milliliters) per unit mass or leaf surface per unit time Q-box CO650 Portable Photosynthetic System, Quibit Systems Inc., Canada; Camspec M108 spectrophotometer, UK. Intensity of photosynthesis (A, μmol m-2 s-1) is measured on three fully developed, undamaged leaves of the same physiological age, taking at least 50 values for each individual leaf. It is expressed as the amount of CO_2 absorbed per unit mass or leaf area per unit time (Q-box CO650 portable photosynthetic system, Quibit Systems Inc., Canada; Camspec M108 spectrophotometer, UK).

A similar work approach was used by Hong et al (2012), Lee et al (2016), Jeong (2018) Kim et al. (2018), Na et al. (2018).

Stage 3 is to establish a relationship of "in situ" results with vegetation indices, such as with NDVI – normalized vegetation index, EVI – enhanced vegetation index, CCCI – index of chlorophyll content in plant cover, SAVI – index for adaptation of plants to the soil. After building the model and validating the data, direct monitoring of cultivated land can be carried out. It is compared with observations on plant development.

It is determining the parameters of the optimal growing plants through observations on their architectonics. It is determining the time to achieve the optimal index of leaf area and the period during the vegetation for which it is preserved.

The presented methodology for Stage 3 is in line with those of Na et al. (2017a) and Na et al. (2017b).

CONCLUSIONS

Based on the results of the studies, the practical significance of remote sensing methods and vegetation indices will be determined. The collected scientific information and establishing the dependencies of the analyzed data and spectral analyzes on remote sensing will be the basis for making of expert decisions for the agronomic management of the vegetable production.

The guidelines for future research work are: development and testing of regression models based on the developed algorithms for quantifying certain parameters of the investigated vegetable crops; development of a methodology for monitoring vegetable crops through remote sensing methods.

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LAND SUITABILITY ASSESSMENT FOR PEPPER CULTIVATION AROUND KATUNITSA VILLAGE, BULGARIA

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ABSTRACT

Horticulture is one of the most important brunches in Bulgaria. Plovdiv region is a special area of vegetable production. The purpose of this study is to give assessment of the natural local factors - climate, terrain, and soil characteristics for sustainable pepper cultivation in Katunitsa village, Bulgaria by GIS. In this research were investigated physical land characteristics, especially analysis of physic-mechanical parameters of the soil and terrain characteristics which is very important for crop cultivation. All information is prepared for using of GIS application by converting it into spatial database. The analysis of the created database will be bases for using the main vegetation indices of remote monitoring for the pepper cultivation in the observed region.

Key words: vegetables, land evaluation, remote sensing, vegetation indices

INTRODUCTION

Vegetable crop cultivation provides valuable and cheap food for the population. In the last few years, fewer and more expensive vegetables have been produced in Bulgaria. Large quantities are imported from Greece and Turkey, both fresh and canned. The horticulture is the one of the main branches of agriculture and is still territorially fragmented. In Plovdiv region the vegetable production is specialized of the field mid-early and late cultivation.

Pepper is the main crop of horticulture. It is cultivated in South Central Region of Bulgaria, due to the peculiarities of the climate, more than 80% of the yields in the country are obtained. On average, about 3,000 hectares of pepper are planted in the country per year, with yields varying over the years according to climatic conditions.

Over the past three years, pepper production has gradually shrunk, with 3,500 ha planted in 2016, and 2,800 ha open in pepper farms two years later. https://www.dfz.bg/

Among the main objectives of the strategic plan for the development of agriculture and development of rural areas for the period 2023-2027 of the Ministry of Environment and Water are:

- Promoting sustainable development and the effective management of natural resources such as water, soil and air.
- Modernizing the sector by stimulating and sharing knowledge, innovation and digitalization in agriculture and rural areas and encouraging their greater use.https://www.moew.government.bg/

Careful planning of the land use resources is based on land evaluation, which is the process of assessing the suitability of land for alternative land uses. (Fresco et al, 1994).

Agricultural land cultivation is act of making use of environmental resources to get production for livelihood. For profitable land use and getting more quality yields of vegetable productions is important to know based factors about crops development. They are complex of the land, climate and soil characteristics depending on biologically requirement of vegetables. (Banaya, 2008)

Information on land resources is a key to their careful and effective evaluation. GeoFigureic information system (GIS) provides powerful spatial data management and analysis function has been widely used for managing spatial resources. (Addharu E et al., 2021)

The purpose of this study is to the purpose of this study is to give assessment of the natural local factors - climate, terrain, and soil characteristics for sustainable pepper cultivation in Katunitsa region, Sadovo municipality and to suggest a framework for input the data in a GIS for land assessment, according to FAO classification.

MATERIAL AND METHOD

Study area

Katunitsa village is in the South-central part of Bulgaria in Plovdiv region and Sadovo municipality. Total area is 2273ha.

The land around Katunitsa village is a plane terrain with altitude varies from 140m to 190m. Study area is located near the Maritsa River and have high groundwater levels, which can use for crop irrigation.

The arable fields are with area around 2-5 ha. The use of fields for vegetable cultivation ranging from 0.5-2 ha. Figure 1.

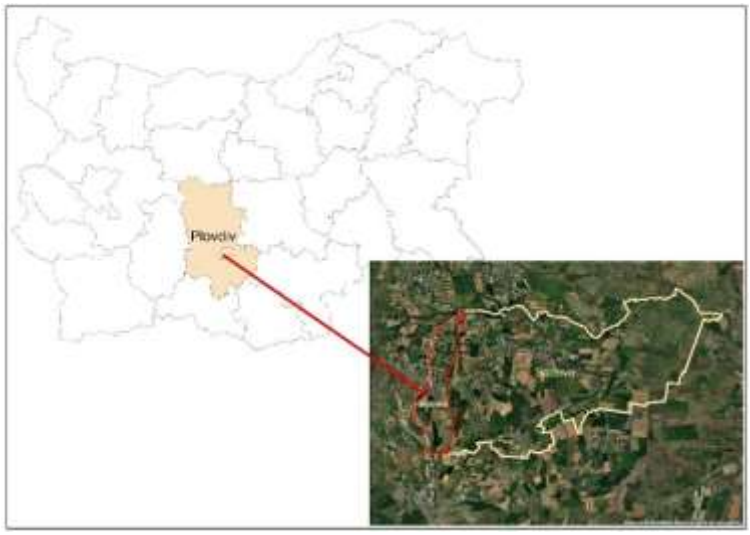


Figure 1. Study area

GeoFigureic information

To assessment of terrain and soil types were used:

Digital Elevation Model

Digital soil map large scale 1:400 000 Information - source Institute of Soil Science, Agrotechnology and Plant Protection, Sofia, Bulgaria (http://www.issapp-pushkarov.org/en/resources).

Attribute information

Climate

To characterize the climate in the region, the multi-year values (for a 40-year period) of the main agrometeorological indicators for the region of Plovdiv and Sadovo were used Climatic reference books of Bulgaria (1979, 1983, 1990). The following indicators were monitored: Average monthly air temperature, °C; Minimum monthly air temperature, °C; Maximum monthly air temperature, °C; Precipitation, mm;

Soil characteristics

To evaluation of the soil characteristics were used: soil type, soil texture, organic matter content (%), organic matter depth (cm), soil reaction (pH в H_2O). (Yanchev and Popova, 2000; Popova and Sevov, 2010), Institute of Soil Science, Agrotechnology and Plant Protection, Sofia, Bulgaria (http://www.issapp-pushkarov.org/en/resources).

Assessment of abiotic factors

The influence of abiotic factors and requirements of the pepper for climate, soil and terrain characteristics are determined according to Panayotov 2006, Cholakov (2009), Stefanova 2017

The land suitability evaluation was made according to FAO classification in three classes: Class S1 Highly Suitable, Class S2 Moderately Suitable, Class S3 Marginally Suitable.

The methodology is based on matching soil/land characteristics against agronomic requirements of crop and then the suitability classification will be assessed. The physical land suitability evaluation used limiting factors method assigning the suitability classes, in which the lowest suitability class will limit for the rest of factor (FAO classification 1976, 1983,1993, 2000, 2004)

Table 1. Land Suitability evaluation by FAO classification

Suitability classes	Description
Highly Suitable -S1	Suitable capacity of locations is high and satisfies all criteria set up
Moderately Suitable-S2	Suitable capacity of locations is medium and satisfies most of the criteria set up, but some criteria are not satisfied
Marginally Suitable -S3	Suitable capacity of locations is low and satisfies some of the criteria set up, but most of the criteria are not satisfied

RESULTS AND DISCUSSIONS

Analysis of the natural conditions

Climate

Region of the Katunitsa village is in the central part of the Thracian lowland and it located in a transitional-continental climate area, which borders the Mediterranean climate area in the south. Compared to Northern Bulgaria, the continental climate is significantly milder. In the

north, mountain Stara planina appears as a natural barrier for cold air masses, and along the valley of the Maritsa River, a weak Mediterranean influence reach.

By using data from the Climatic reference books of Bulgaria (1979, 1983, 1990), the average multi-year values for a forty-year period of the main agrometeorological indicators, accepted as a climatic norm characterizing the climate of the region, were determined.

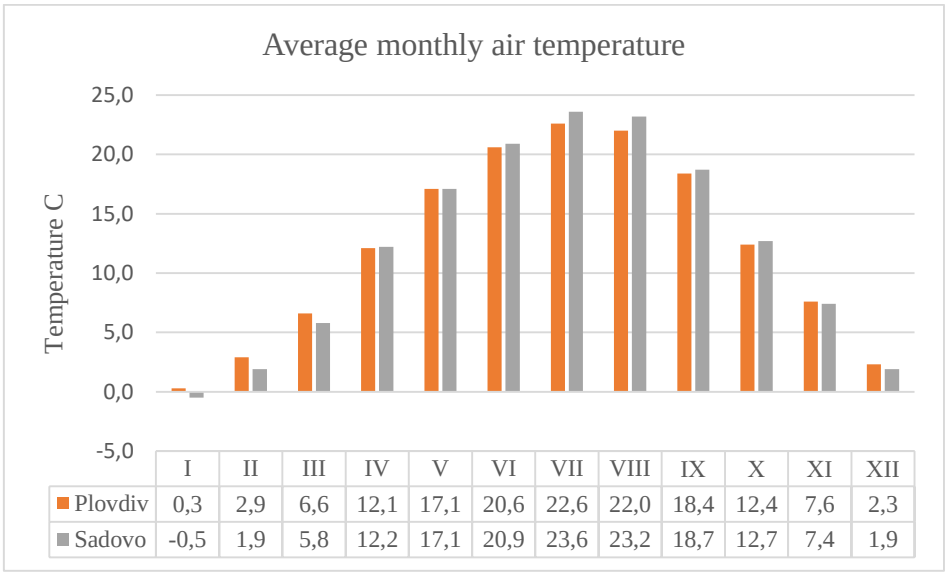
Winter is relatively mild, with the average temperature for the winter months being 1.3°C. Spring comes early. The permanent increase in temperatures above 5°C occurs in the first ten days of March. The average date of the last frost is April 13, and the last possible date is May 20. Summer is hot and autumn is warm with sharp and rapid transitions to winter.

The average minimum temperatures for the region are within the limits of -5°C, which makes the region suitable for growing cold-resistant vegetable crops with pre-winter sowing.

During the summer months, the temperature values range from 29 - 30°C, which approach the upper limit of the biological requirements of vegetable crops.

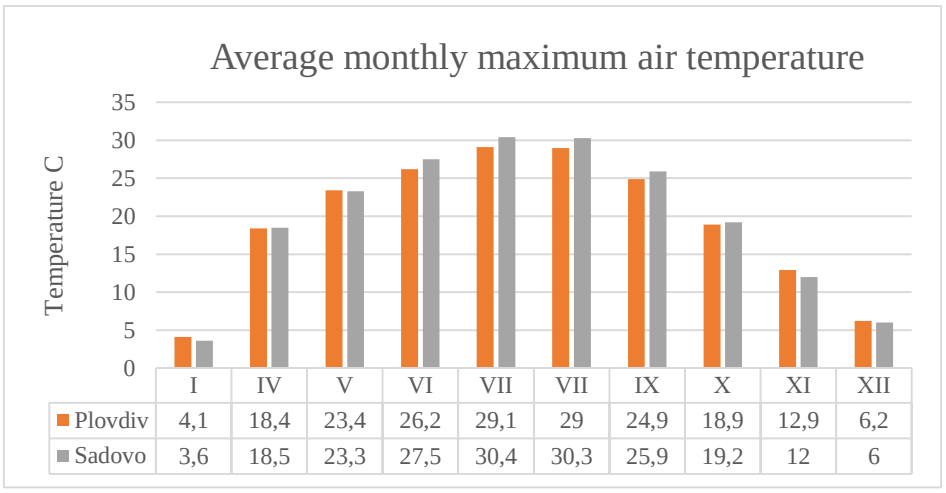
Smaller annual temperature amplitudes are characteristic of the climate in the area. The annual temperature amplitude varies between 19.3ºC - 23.2ºC.

Precipitation has a continental character and is unevenly distributed throughout the year. Their largest amount is in June – 61-68 mm, and droughts are most frequent in August (25-31 mm). The average annual amount of precipitation is 551.6 mm and is below the national average. The natural provision of water from the precipitations, requires the organization of the production of vegetables in the area to be carried out with ensured regular irrigation. The direction of the winds is mostly westerly. (Figure 2 (a, b, c, d)).



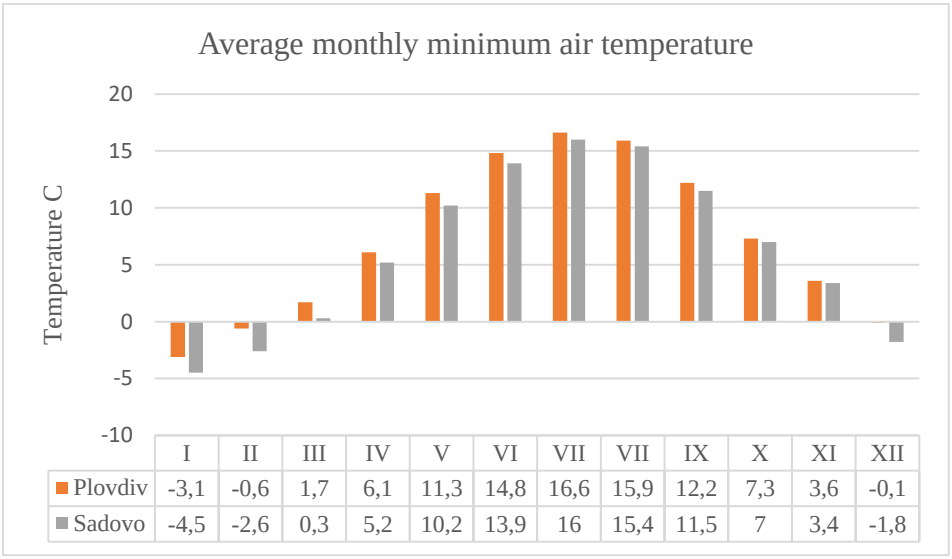
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Plovdiv	0,3	2,9	6,6	12,1	17,1	20,6	22,6	22,0	18,4	12,4	7,6	2,3
Sadovo	-0,5	1,9	5,8	12,2	17,1	20,9	23,6	23,2	18,7	12,7	7,4	1,9

(a)



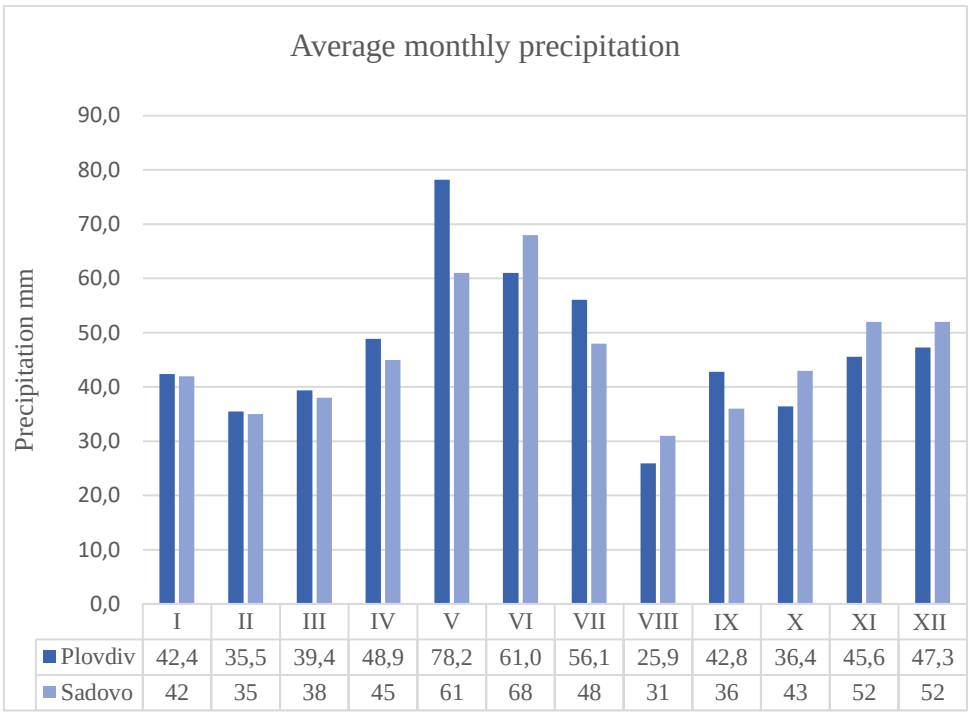
	I	IV	V	VI	VII	VII	IX	X	XI	XII
Plovdiv	4,1	18,4	23,4	26,2	29,1	29	24,9	18,9	12,9	6,2
Sadovo	3,6	18,5	23,3	27,5	30,4	30,3	25,9	19,2	12	6

(b)



	I	II	III	IV	V	VI	VII	VII	IX	X	XI	XII
Plovdiv	-3,1	-0,6	1,7	6,1	11,3	14,8	16,6	15,9	12,2	7,3	3,6	-0,1
Sadovo	-4,5	-2,6	0,3	5,2	10,2	13,9	16	15,4	11,5	7	3,4	-1,8

(c)



	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Plovdiv	42,4	35,5	39,4	48,9	78,2	61,0	56,1	25,9	42,8	36,4	45,6	47,3
Sadovo	42	35	38	45	61	68	48	31	36	43	52	52

(d)

Figure 2. *(a, b, c, d)* Average air temperatures and precipitation

Soil characteristics

The largest part, according to the small-scale soil map, in the region around Katunitsa is soil type by FAO classification District Fluvisols. Other soil types in the region are District Planosols, Calgari-glay eutric vertisols and Stagni-glayic Chernozems. They occupy a smaller part of the territory and have no agricultural importance for vegetable growing (http://www.issapp-pushkarov.org/en/resources). (Figure 3)

In terms of geoFigureical distribution, they belong to the first sub-district of the Thracia-Strandzha region. They occupy the valley of the Maritsa River from Pazardzhik to Dimitrovgrad, the valley of the Tundzha River between Sliven and Yambol. The soils here are developed on sandy-clay and sandy-gravel Quaternary sediments. They refer to the second terrace of the Maritsa River and are formed by sandy and gravelly Quaternary alluvial deposits, interspersed in places with clayey sands and clays with a thickness of 0.5 to 10-12 m. The width of this terrace varies from 300 m to 4-5 km. It is of great agricultural importance - there are fields, vegetable, orchards etc. located on it. The groundwater level is usually 1-3 m. They were formed on alluvial sediments, have a well-formed organic matter accumulative horizon, which gradually passes into the C horizon, and deep (below 100 cm) in the soil-forming materials, siltation is observed. The horizon of organic matter in the soil is most often 20-40 cm thick, but it can be more powerful. Along the Maritsa River, the color of the humus horizon is mostly gray-brown.

Usually, this horizon also has a well-defined fine-grained structure. The mechanical composition is sandy and sandy clayey. Despite the light mechanical composition in the lower part of the profile, in the upper horizons the content of physical clay reaches 50%.

The content of organic matter is usually not high - no more than 2-2.5%. Most of the alluvial-meadow soils are carbonate with a slightly alkaline reaction (pH 7.3 - 7.7), and the non-carbonate soils are neutral to slightly acidic. The cation sorption capacity varies around 20-30 mequ/100g soil.

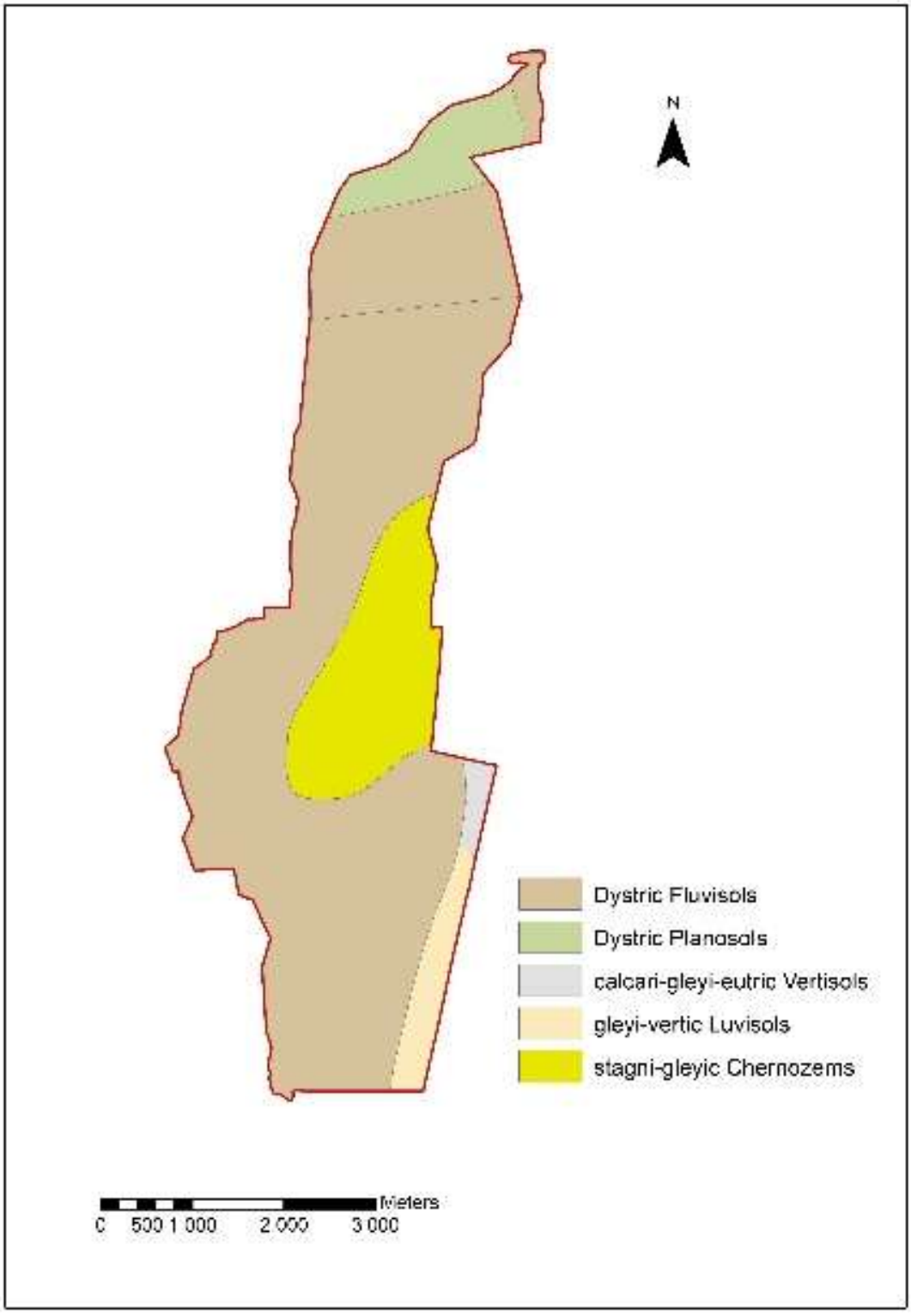


Figure 3 Map of soil type, Katunitsa

The carbonate content is 7.2 - 6.7% and decreases from top to bottom. The significant amount of calcium carbonate affects the improvement of the physic-chemical and water properties of the soil. (Yanchev and Popova, 2000; Popova and Sevov, 2010)

Pepper requirements and land suitability assessment

Climate

A suitable temperature for pepper growth and development is between 18 and 25°C. Low temperatures below 12°C led to physiological disturbances in seedlings. Pepper can be grown for industrial purposes under field conditions in areas where there is at least 3,5 months frost-free period and the average daily temperature is around 20°C. Pepper is a heat-loving vegetable crop. At a temperature of 20-25ºC and 75-80% of soil moister capacity (SMC) the seeds germinate in 7-10 days. The optimal temperature for normal development of pepper plants is 18-25ºC. Below this temperature, growth slows down, below 12ºC - it stops and at -0.3˚ -0.5ºC the plants die. Above 25ºC, growth accelerates, which is associated with elongation of the internodes, and above 35ºC, generative organs do not set, and previously formed flowers and cords fall off. (Panayotov et al. 2006, Cholakov, 2009)

Soils and soil characteristics

High yields and quality production are obtained only on structural soils well stocked with nutrients and organic matter. Clay-sandy and sandy-clay soils are also suitable for cultivation. Wet and cold soils, as well as soils with an acidic reaction, are not suitable for growing pepper, which develops well in a neutral or slightly alkaline reaction. Clay-sandy and alluvial soils with a lighter mechanical composition, water-permeable and quickly warming are more suitable for early field production, and heavier and moisture-retaining soils for mid-early and late field production. Pepper grows best on soils with a neutral to slightly alkaline reaction. On cold and acidic soils, it does not give good results. Very light sandy soils, as well as acidic and strongly alkaline soils are unsuitable for it (Panayotov et al., 2006). The reaction of the soil should be neutral to slightly alkaline - pH - 6.5-7.5 (Cholakov, 2009).

Land suitability assessment

In land suitability analysis, the evaluation the criteria with classes (S1, S2, S3) indicate the degree of suitability with importance of the criteria. These classes have to be rated, how important is the class S1 with respect to a particular criterion to contribute for the final goal (suitability). In this particular land suitability analysis, the criteria are mainly related to climate factors, terrain and soil characteristics, infrastructure and environmental information.

Based on the requirements for pepper cultivation, climatic, soil and relief characteristics can be considered as: dominant and non-dominant (those that can be changed, by alternative methods). If most dominant factors meet the agrobiological requirements of the plants, then the studied territory is favorable. As a final result of the study of the soil and relief characteristics, the suitability of the land from S1 to S3 is indicated.

As a result of the studies done on the requirements of pepper for climate, soils and terrain, we can summarize the data in Table 3. the data are presented with the FAO land suitability ratings. Our observations are in line with what was found by Stefanova (2017).

Hierarchical data model allows analysis of many factors involved in the valuation of agricultural land. By applying AHP analyses, the influence of different levels of the factor space is established by comparing quantitative and qualitative indicators. (Burrough P. A., 1986, Carver, S. J., 1991, Saaty T. L, 1977, 2008, Stefanova et al., 2014, Kazemi H. 2016)

GeoFigureic Information System (GIS) is widely used tool to solve complex tasks. Based on such type of analysis, combining AHP by Saaty and overlay analysis, the influence of land evaluation factors for pepper cultivation can provide to a scientific basis for sustainable land evaluation and selection the field.

Table 2. Requirements for the pepper cultivation to climate, soil and relief

Parameters	Potential ratings by FAO classification		
	Highly Suitable -S1	Moderately Suitable-S2	Marginally Suitable -S3
Air temperature (°C)	18 -26	15 -18, 28 -35	< 15 & > 35
Soil texture (<0.01mm/%)	30-45	20-30; 45-60	<20 & >60
Organic matter (%)	> 2	1.5-2	< 1.5
Soil reaction (pH в H_2O)	7 – 7.5	6.5-7	< 6.5 & > 7.5
Organic matter depth (cm)	30-50	20-30	10-20
Slope (%)	Flat to 5	5-7	>7

To achieve the aim of the research, it is necessary to develop a data model that will be involved in the assessment and suitability of agricultural land for growing vegetable crops.

The guidelines for intelligent crop production are using of remote sensing for identifying land use and crop diagnostics through vegetation indices.

Land suitability evaluation is part and first step of the process of land-use planning and the results should be useful for intelligent land use management.

The selection of the main criteria and sub-criteria for each soil and topoFigureic characteristics are first step to suitability evaluation process.

For a comprehensive assessment of abiotic factors - soil and topoFigurey in relation to the suitability of the territory for growing vegetable crops, it is necessary to evaluate their characteristics by degree of importance. The framework is presented on the Figure 4.

Terrain assessment refers to the following characteristics: average elevation of the terrain, slope in percent, and exposure relative to the directions of the world. For this purpose, an irregular spatial terrain model (TIN) is created.

In the evaluation of the soil and soil characteristics, the following are included: soil type, organic matter content in %, soil reaction – pH (H_2O), soil texture (content of physical clay with particles < 0.01 mm), organic matter depth in cm.

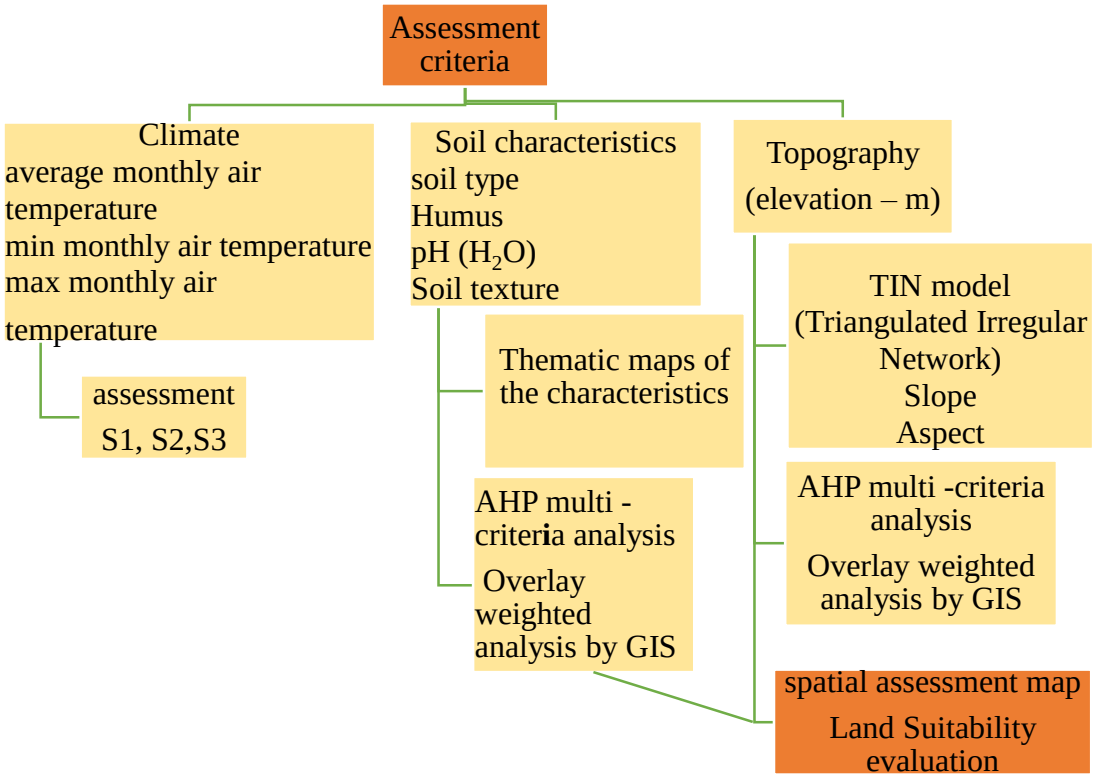


Figure 4. Framework for multicriteria assessment

CONCLUSIONS

The climate conditions around Katunitsa and Sadovo regions are suitable for vegetable cultivation. The temperature and precipitation values are within the limits of their biological requirements or deviate insignificantly from them. The most of cultivated area in Katunitsa region are suitable for pepper cultivation. More of the physical land characteristics fitted on crop requirements with class S1. Obtaining spatial assessment maps of using weighted analysis with GIS overlay by combining AHP analysis needs large-scale soil and cadastral maps.

The guidelines for intelligent crop production are using of remote sensing for identifying land use and crop diagnostics through vegetation indices. Land suitability evaluation is part and first step of the process of land-use planning and the results should be useful for intelligent land use management. The land use assessment is a tool to help land users, to plan arable area in a way, that current land use problems are reduced and specified.

ACKNOWLEDGEMENT

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DETERMINATION OF THE BIOCHEMICAL CONTENTS OF WHITE AND RED FRUIT PITAYA (*HYLOCEREUS SP*.) FRUIT SPECIES

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ABSTRACT

Pitaya is one of the tropical fruit species that arouses curiosity in terms of appearance and taste and is increasing in popularity day by day in Turkey. In this study, the biochemical contents of red-fleshed (*Hylocereus polyrhizus*) and white-fleshed (*Hylocereus undatus*) pitaya species grown in Antalya province were investigated. The amount of water-soluble dry matter, pH, titratable acidity, phenolic compounds and organic acids were determined. The most abundant phenolic compound in both fruit types was protocatechuic acid (14.140 mg kg^{-1}: white, 9.498 mg kg^{-1}: red). It has been determined that the red flesh fruit has higher content of gallic acid, rutin, quercetin, ferulic acid and p-coumaric acid. Organic acid content of white-fleshed pitaya fruit was found to be higher than that of pink. The white-fleshed fruit type had 774.322 mg $100g^{-1}$ and the red-fleshed fruit type had 511.146 mg $100g^{-1}$ malic acid content. A positive correlation was found between the amount of vitamin C and organic acids, protocatchuic acid, vanillic acid and syringic acid. A negative correlation was determined between citric acid and gallic, p-coumaric and ferulic acid, rutin and quercetin contents. Rutin content was found to be positively correlated with gallic acid, quercetin, ferulic acid and p-coumaric acid content. Consumption of red-fleshed fruit should be increased in terms of phenolic compounds, and white-fleshed fruit should be increased in terms of vitamin C content.

Key Words: Pitaya, white, red, phenolic compound, vitamin C

INTRODUCTION

Pitaya fruit (*Hylocereus sp.)*, also called dragon fruit, is a tropical fruit that belongs to the cactus group. As well as its homeland is South and Central America, its cultivation under greenhouse conditions has become widespread in many countries in recent years (Jiang and Yang, 2015). Although the genus *Hylocereus* has 14 different species, especially *Hylocereus undatus*, which has white flesh, is known as the species cultivated throughout the world. *H. polyrhizus* and *H. costaricensis* species, which have flesh colors ranging from red to purple, are also among the other commonly cultivated species (Mizrahi and Nerd, 1999).

In recent years, Pitaya has been popular in European countries, as well as markets such as Costa Rica, Mexico, Vietnam, and its popularity is slowly increasing in countries such as Thailand, Australia, Malaysia, India, Türkiye and Israel (İbrahim et al., 2018; Attar et al., 2022). Vietnam is the country with the highest share of world production with 640000 tons of production in thirty thousand hectares of land. Pitaya is grown in greenhouse in Türkiye, especially in Antalya, Mersin, Adana, Aydın, İzmir and Hatay provinces (Anonymous, 2022).

Pitaya's consumption is increasing by people due to their nutrient-rich content and beautiful appearance although pitaya fruit species have different characteristics. This fruit, which is widely consumed fresh, contains vitamins A, C, B and E, and is among the fruit types rich in potassium, magnesium and phosphorus (Thokchom et al., 2019). It gains value in terms of health due to phenolic compounds (Tenore et al., 2012), betalains (Thu and Quang, 2019) and karatonoids (Joshi and Prabhakar, 2020) found in its fruit. Due to the phenolic compounds it contains, it scavenges free radicals and has antioxidant activity (Parmar et al., 2019). Researchers have found that pitaya fruit prevents tissue damage (Harahap and Amelia, 2019), reduces total cholesterol (Hossain et al., 2021), prevents prostate and breast cancer (Joshi and Prabhakar, 2020) and also has antimicrobial and antifungal effects (Ibrahim et al., 2018) reports. It is also known that white pitaya seeds are rich in linoleic acid, which cannot be synthesized by the body but is important for nutrition (Pomeranz, 2003).

In this study, the chemical contents of two different types of white and red fleshed pitaya fruit species were investigated.

MATERIAL AND METHOD

White fleshed (*Hylocereus undatus*) and red fleshed (*Hylocereus polyrhizus*) pitaya species harvested in Antalya province were used to conduct the research. The harvested fruits were analyzed in Bolu Abant İzzet Baysal University Faculty of Agriculture. Some of the samples were stored at -20 °C for organic acid and phenolic compound analysis.

The amount of water-soluble dry matter was determined with a refractometer, the pH value of the fruit juice was determined with a table-type pH meter and titratable acidity was determined in terms of malic acid by titration method. Fruit flesh color was made with a Konica Minolta brand colorimeter device. In color readings, L represents the brightness of the color, +a the red value of the color, -a the green value of the color, +b the yellow value of the color, -b the blue value of the color, and the Chroma value the intensity of the color.

Organic acids

The method described by Bevilacqua and Califano (1989) has been modified. 5 g of pitaya fruits were taken, homogenized with sulfuric acid and centrifuged. Extraction was passed through a 0.45 µm membrane filter and SEP-PAK C18 cartridge. The acids were analyzed in high performance liquid chromatoFigurey (HPLC). Aminex HPX - 87 H, 300 mm x 7.8 mm was used in the HPLC system and the device was controlled by a computer with Agilent package program. The DAD detector in the system is tuned to 214 and 280 nm wavelengths.

Phenolic Compounds

Rodriguez-Delgado et al. (2001) was used for the determination of phenolic compounds. 5 g of fruit sample was homogenized. The extract was filtered with a 0.45µm millipore filter and transf1erred to HPLC. ChromatoFigureic separation was performed on Agilent 1100 HPLC system, using a DAD detector and a 250*4.6 mm, 4µm ODS column.

Vitamin C

The method specified by Cemeroğlu (2004) was used. 5 g of fruit sample was completed with 2.5% M-phosphoric acid solution and centrifuged. The extract was passed through a 0.45 µm Teflon filter and transferred to the HPLC device. In HPLC analysis, vitamin C was carried out on a C18 column.

Statistical Analysis

SPSS (ver. 26) package program was used to evaluate the data. The t test was used to compare binary data. Pearson correlation analysis was performed in SPSS 26.0 package program in order to determine the relationship between the data.

RESULTS AND DISCUSSION

The biochemical contents of white and red pitaya species were examined and it was observed that there were differences between the two species in general.

Flesh colors of different pitaya species were examined and statistical difference was determined (Table 1). It was observed that white fleshed fruits had higher L and hue (31.48) values. The red pitaya had higher a (4.34) and chroma (4.68) values. It was determined that the white pitaya had a brighter white color, and the red pitaya had a more intense red color. In the study conducted in the red-fleshed *H. polyrhizus* species, they found 32.64 L, 2.41 a, 0.60 b, 27.42 chroma and 1.82 hue values (Wu et al., 2019). In another study of the same species, L values between 5-15, chroma values between 20-25 and hue values between 25-30 were determined (Jamaludin et al., 2010). Similar to the findings obtained, it was stated that Garcia-Cruz et al. (2013) 19.4 L and 19.8 hue value in red pitaya, Jamilah et al. (2011) 23.89 a and 4.61 b values has found. In general, although L, a, b and hue values are in harmony with the literature, it was observed that the chroma value was more matte compared to the results obtained.

Table 1. The amount of color value and some biochemical of white and red pitaya

	White fleshed	Red fleshed		White fleshed	Red fleshed
L	28.61 ± 1.23 a*	16.40 ± 0.05 b	SSC (%)	9.00 ± 0.23 b	12.87 ± 0.35 a
a	0.55 ± 0.05 b	4.34 ± 0.20 a	pH	5.32 ± 0.04 b	5.87 ± 0.03 a
b	0.14 ± 0.26 a	0.73 ± 0.05 a	TA (%)	0.13 ± 0.01 a	0.34 ± 0.23 a
Chroma	0.69 ± 0.06 b	4.68 ± 0.16 a			
Hue	31.48 ± 1.71 a	8.79 ± 0.18 b			

* The difference between the means shown with the same letter in the same line is not significant at the $p<0.05$ level.

The pH, titratable acidity and pH values of pitaya species are given in Table 1. While the amount of water-soluble dry matter and pH amount were determined higher in red-fleshed fruit species, there was no difference between species in terms of titratable acidity. The red-fleshed species had a 12.87% SSC rate and 0.34% TA rate. In the study, the amount of SSC between 13.5-14.9% and 0.16% TA was determined in red pitaya (Jiang et al., 2020). According to Zahid et al. (2013), while obtaining similar SSC results with the obtained results, the TA value (0.62%) was very high. Likewise, Arivalagan et al. (2021) reported between 9.83-10.1% in white pitaya and 9.15-10.9% in red pitaya depending on the cultivars and Magalhaes et al. (2019) reported 8% SSC value in white pitaya. The findings show that higher SSC was obtained in red pitaya, although in agreement with white pitaya. The amount of TA (0.4%) was obtained, similar to the findings obtained in the study on red pitaya (Wu et al., 2020). The amount of pH was higher in red fleshed pitaya than in white fleshed pitaya, in line with the literature (Arivalagan et al., 2021; Senadheera and Abeysinghe, 2015). Researchers have reported pH of 5.4 in red fleshed and 4.8 in white fleshed (Arivalagan et al., 2021). It has been observed that red pitaya is at the forefront in terms of SSC, pH and TA amount, as in the literature.

The amount of organic acid and vitamin C of pitaya fruits were examined and given in Table 2 and Figure 1. The highest organic acid type in white and red fleshed fruits was determined as malic (774.32 and 511.15 mg 100 g^{-1}), succinic and citric acid, respectively. Oxalic acid (14.81 mg 100 g^{-1}) was the least abundant organic acid in the white pitaya, while tartaric acid (10.35 mg 100 g^{-1}) was found in the red pitaya. All acids were statistically higher in white fleshed fruit. The amount of fumaric acid was determined more than 3 times in white pitaya compared to red

pitaya. While the amount of citric acid was 14.89 mg 100 g^{-1} in red-fleshed fruit species, it was more than doubled in white-fleshed fruit species at 41.03 mg 100 g^{-1}. The amount of vitamin C (Figure 1), like organic acids, was determined to be 18% higher in white fleshed pitaya (31.64 mg 100 g^{-1}). In a previous study, it is stated that malic acid (13.3 mg g^{-1}) in red fleshed pitaya fruit constituted two-thirds of all acids (Wu et al., 2019). Again, the percentages of organic acids contained in red pitaya were examined and contrary to this study, it was reported that oxalic acid was the dominant acid and 20% less malic acid content (Jamilah et al., 2011). Morais et al. (2019) obtained 0.24 g l^{-1} citric acid, 2.47 g l^{-1} malic acid and 0.53 g l^{-1} succinic acid in their study on red pitaya. In parallel with this findings, in the study where the two species were compared, it was determined that white pitaya (31.11 mg 100 g^{-1}) contained 50% more vitamin C compared to red pitaya (20.0 mg 100 g^{-1}). Again, Zitha et al. (2022) obtained 18.92 mg 100g^{-1} vitamin C in red pitaya (*H. polyrhizus*), while Nurul and Asmah (2014) obtained a smaller amount (24.66-30.21 µg 100 g^{-1}) vitamin C. In this study, it was seen that all organic acids and vitamin C were higher in white fleshed pitaya in accordance with the literature.

Table 2. Malic, succinic, fumaric, oxalic and tartaric acid amounts of white and red pitaya

	White fleshed (mg 100 g^{-1})	Red fleshed (mg 100 g^{-1})
Malic	774.32 ± 0.03 a	511.15 ± 0.03 b
Succinic	60.08 ± 0.03 a	38.84 ± 0.04 b
Fumaric	39.10 ± 0.02 a	12.26 ± 0.03 b
Oxalic	14.81 ± 0.01 a	13.51 ± 0.01 b
Tartaric	17.50 ± 0.02 a	10.35 ± 0.03 b
Citric	41.03 ± 0.06 a	14.89 ± 0.04 b

* The difference between the means shown with the same letter in the same line is not significant at the $p<0.05$ level.

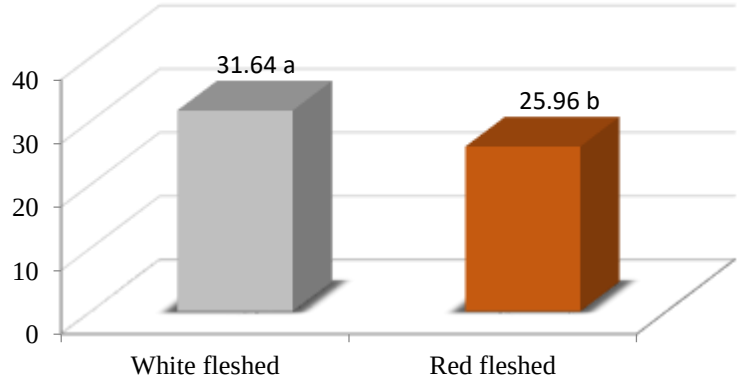

Figure 1. The vitamin C contents of white and red pitaya (mg 100 g^{-1})

In terms of specific phenolic compounds, white and red fleshed pitaya were examined (Figure 2 and 3). The highest amount of phenolic compounds were protocatechuic acid, quercetin and ferulic acid, respectively. Protocatechuic acid, which was determined as 14.140 mg kg^{-1} in white fleshed fruit, was determined as 9.498 mg kg^{-1} in red fleshed fruit. Rutin (1.282 mg kg^{-1}) was the least phenolic compound in white pitaya, and syringic acid (1.226 mg kg^{-1}) was the least in red pitaya. The white fleshed pitaya had the highest protocatechuic, vanillic and syringic acid content, while the red fleshed species had the highest p-coumaric, ferulic and gallic acids, rutin and quercetin. Rutin was determined as 1.282 mg kg^{-1} in white fleshed pitaya

and 2.993 mg kg^{-1} in red fleshed pitaya, more than twice that. It was determined that the white pitaya contained 47% less quercetin and 29% less ferulic acid compared to red. The amount of vanillic acid was determined as 2.325 mg kg^{-1} in white pitaya and 1.924 mg kg^{-1} in red species. In a study investigating the total amount of phenolic compounds in dried fruits, the total phenolic content was determined between 8.75-10.80 mg g^{-1} in red pitaya and 2.43 mg g^{-1} in white pitaya (Al-Mekhlafi et al., 2021). In a study examining total flavonoid amounts, 9.56 mg g^{-1} flavonoid amount was determined in red pitaya, while 3.52 mg g^{-1} in white pitaya (Kim et al., 2011). Likewise, Hua et al. (2018) reported that the amount of phenolic compounds in red fleshed pitaya is higher than in white fleshed pitaya. Similar to the findings, in a study conducted with *H. polyrhizus* species in Brazil, 0.11 mg 100 g^{-1} vanillic acid and 0.003 mg 100 g^{-1} gallic acid were found in fruit flesh (Zitha et al., 2022). In the same genus of study, 144.05 mg 100 ml^{-1} gallic acid, 65.10 mg 100 ml^{-1} syringic acid and 26.57 mg 100 ml^{-1} rutin amount were obtained (Morais et al., 2019). In different studies, which were higher than the results obtained in the research, 14.37 mg 100 g^{-1} ferulic acid (Chen et al., 2021), 10.32 mg 100 g^{-1} p-coumaric acid (Benta-Silva et al., 2018), 1.31 mg g^{-1} quercetin (Saenjum et al., 2021) and 26.37 µg 100 g^{-1} rutin (Fernandes et al., 2016) were measured. In general, it was observed that red pitaya had more phenolic compounds, which was consistent with the literature. The difference between the types and amounts of phenolic compounds is thought to be due to the variability of the growing place or the cultivar.

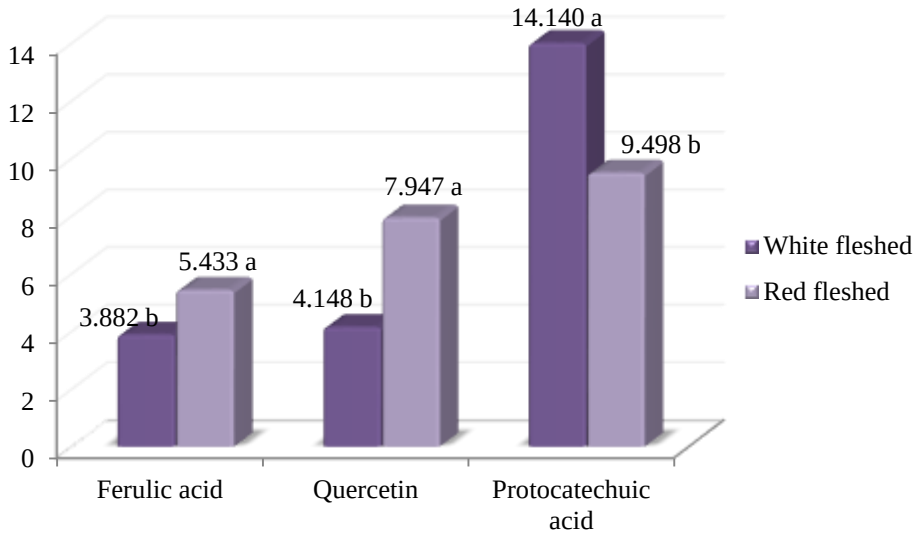

Figure 2. The ferulic acid, protocatechuic acid and quercetin contents of white and red fleshed pitaya (mg kg^{-1})

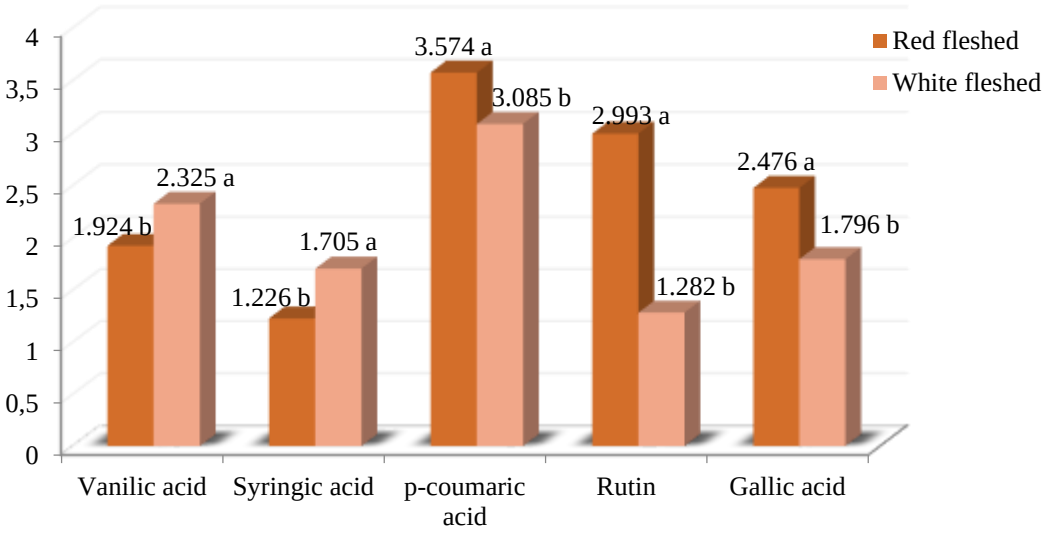

Figure 3. The vanillic acid, syringic acid, p-coumaric acid, rutin and gallic acid contents of white and red fleshed pitaya (mg kg^{-1})

The relationships between phenolic compounds, organic acids, SSC, pH and TA in white pitaya fruit are given in Table 3. A positive correlation was found between gallic acid and all organic acids, and an excellent (100%) correlation was obtained especially in oxalic acid. Very strong correlations were found between vanillic acid and syringic acid (99%), between ferulic acid and TA (100%), between rutin and pH (100%), between fumaric acid and vitamin C (99%) and it was observed that they increased together. There was a weak positive correlation (6%) between vanillic acid and rutin, and a moderate (23%) positive correlation between quercetin and ferulic acid. There was a weak negative correlation between pH and organic acids. Tartaric acid was found to provide 100% positive correlation with malic, succinic and vitamin C. It has been determined that p-coumaric acid has a strong but opposite correlation with all organic acids. Again, the only component in which SSC (12%) and TA (36%) showed a negative relationship was determined as p-coumaric acid.

The relationships between SSC, pH, TA, phenolic compounds and organic acids in red pitaya fruit are given in Table 4. A negative relationship was observed between vitamin C and other components except rutin and oxalic acid. It has been concluded that when these components increase, vitamin C decreases. Very strong correlations were found between ferulic acid and syringic acid (99%), between quercetin and citric acid (100%). An excellent level of positive correlation was obtained between gallic acid and ferulic (99%), tartaric (100%), malic acid (100%) and pH (100%). Similarly, a significant and positive relationship was determined between protocatechuic acid and vanillic and succinic acid. It was found that p-coumaric acid showed a positive relationship with all components except vitamin C. A moderate positive correlation was found between quercetin and p-coumaric acid (20%) and SSC (32%). While it was determined that fumaric acid had a negative relationship with rutin, oxalic acid and vitamin C, it was determined to have a strong and positive correlation with other components.

Table 3. Correlation relationships between phenolic compounds, organic acids, SSC, pH and TA in white pitaya fruit

White fleshed pitaya	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18
Gallic (V1)	1	.988	.923	.903	-.808	.958	-.142	.208	1.000**	.998*	.998*	.999*	.998*	.995	.999*	.833	-.115	.957
Protocatechuic (V2)		1	.971	.958	-.708	.990	.011	.355	.988	.996	.977	.979	.977	.968	.983	.908	.038	.990
Vanillic (V3)			1	.999*	-.518	.995	.251	.569	.921	.946	.897	.902	.897	.879	.910	.982	.277	.995
Syringic (V4)				1	-.476	.988	.297	.609	.901	.930	.875	.880	.874	.855	.888	.990	.324	.989
p-coumaric (V5)					1	-.604	.699	.408	-.810	-.766	-.842	-.836	-.843	-.863	-.826	-.347	.678	-.602
Ferulic (V6)						1	.148	.481	.957	.975	.938	.942	.938	.924	.948	.957	.176	1.000**
Rutin (V7)							1	.938	-.145	-.076	-.203	-.191	-.204	-.242	-.174	.429	1.000*	.150
Quercetin (V8)								1	.205	.273	.148	.159	.146	.108	.176	.715	.948	.483
Oxalic (V9)									1	.998*	.998*	.999*	.998*	.995	1.000*	.831	-.118	.956
Citric (V10)										1	.992	.993	.992	.986	.995	.868	-.048	.974
Tartaric (V11)											1	1.000**	1.000**	.999*	1.000*	.798	-.175	.938
Malik (V12)												1	1.000**	.999*	1.000*	.804	-.164	.942
Succinic (V13)													1	.999*	1.000*	.797	-.177	.937
Fumaric (V14)														1	.998*	.773	-.215	.923
Vitamin C (V15)															1	.815	-.147	.947
SSC (V16)																1	.454	.958
pH (V17)																	1	.178
TA (V18)																		1

** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level V:Value

Table 4. Correlation relationships between phenolic compounds, organic acids, SSC, pH and TA in red pitaya fruit

Red fleshed pitaya	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18
Gallic (V1)	1	.943	.949	.992	.805	.998*	-.552	.891	-.446	.878	1.000**	1.000*	.937	.996	-.660	.879	1.000*	.974
Protocatechuic (V2)		1	1.000*	.978	.562	.963	-.798	.991	-.718	.987	.939	.936	1.000*	.910	-.873	.670	.949	.994
Vanillic (V3)			1	.981	.576	.968	-.787	.989	-.706	.984	.945	.942	.999*	.917	-.864	.683	.955	.996
Syringic (V4)				1	.723	.998*	-.653	.941	-.555	.932	.990	.989	.974	.977	-.750	.812	.994	.995
p-coumaric (V5)					1	.763	.051	.447	.173	.423	.812	.817	.548	.854	-.086	.990	.793	.648
Ferulic (V6)						1	-.607	.920	-.505	.909	.997	.996	.959	.988	-.710	.845	.999*	.987
Rutin (V7)							1	-.870	.992	-.883	-.542	-.535	-.808	-.475	.991	-.087	-.568	-.728
Quercetin (V8)								1	-.804	1.000*	.885	.881	.993	.847	-.929	.567	.900	.971
Oxalic (V9)									1	-.819	-.435	-.427	-.729	-.364	.966	.035	-.463	-.638
Citric (V10)										1	.873	.868	.990	.832	-.939	.544	.887	.964
Tartaric (V11)											1	1.000**	.933	.997*	-.651	.885	1.000*	.971
Malik (V12)												1	.930	.998*	-.645	.889	.999*	.969
Succinic (V13)													1	.903	-.881	.658	.944	.992
Fumaric (V14)														1	-.591	.918	.994	.949
Vitamin C (V15)															1	-.222	-.675	-.814
SSC (V16)																1	.870	.747
pH (V17)																	1	.978
TA (V18)																		1

** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level V:Value

CONCLUSION

In this study, in which the fruits of *H. undatus* and *H. polyrhizus* pitaya species were examined, different results were obtained in terms of biochemical contents. It has been determined that white pitaya has a brighter and more intense color, while red has a duller and darker color. While white pitaya gained value in terms of organic acids and vitamin C, red pitaya came to the fore more in terms of phenolic compounds and SSC. When phenolic compounds were evaluated in themselves, p-coumaric acid in white pitaya and those other than rutin in red pitaya showed positive correlation. When organic acids were evaluated within themselves, it was determined that while all of them had a positive relationship in white pitaya, oxalic acid in red pitaya had a negative relationship. As a result, it was concluded that red pitaya has superior properties in terms of healthy nutrition, appearance and taste.

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DETERMINATION OF THE GENETIC RELATIONSHIP BETWEEN DIPLOID AND TRIPLOID BANANA GENOTYPES BY FLOW CYTOMETRY AND SIMPLE SEQUENCE REPEAT (SSR) ANALYSIS

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ABSTRACT

Banana is an important crop in tropical and subtropical regions, where it has many wild species. In this study, ploidy levels of commercial Grand Nain and Dwarf Cavendish, local Azman, Erdemli Yerli, and Küllü Erdemli Yerli, wild F4 (*Ensete glaucum* the Yunnan banana), F5 (*Ensete ventricosum* the Abyssinian Banana), and F3 (*Ensete glaucum* the Snow Banana) genotypes were determined through flow cytometry. Also, genetic relationships among genotypes were assessed by ten Simple Sequence Repeats (SSR) markers. Of the F3, F4, and F5 genotypes obtained from seeds, F4 and F5 were determined as diploids, while F3 was determined as spontaneous triploid. Others were triploid. Genetic similarities of banana genotypes were between 0.3750 and 0.8814 based on SSR molecular markers. According to the dendrogram formed by the Unweighted Pair Group Method With Arithmetic Averages (UPGMA) analysis, genotypes were collected in three main groups and 'Dwarf Cavendish' was the most distinct from the others. In conclusion, there is a genetic variation among banana genotypes grown in Turkey. Determining the degree of genetic relationship of diploids with the other genotypes will be beneficial, especially in terms of their use in future breeding studies.

Keywords: banana, ploidy, molcular markers, NTSYS

INTRODUCTION

Banana, is an important crop in tropical and sub-tropical regions, believed to be originated from Indochina and South-East Asia, where it has many wild species (*Musa acuminata* AA and *Musa balbisiana* BB) now (Simmonds, 1959). The cultivated genotypes can present different genomic combinations: AA, AB, AAA, AAB, ABB, AAAA, AAAB, AABB and ABBB, diploids, triploids and tetraploids, depending on the basic number of chromosomes, two, three or four, respectively, being eleven, the basic number of chromosomes of the species (Carreel et al., 2002). Some banana clones can be cultivated in subtropical regions between 20° and 30° North and South of the Equator (Gubbuk et al., 2004). In Turkey, the cultivation is only carried out in the Mediterranean climate strip and most of banana growers produce 'Azman', 'Dwarf Cavendish' and 'Grand Nain' cultivars as cultivation material and this cultivars have A genom (*M. acuminata*). 'Azman' is thought to be one of the clones of 'Dwarf Cavendish' or 'Grand Nain' mutated over time. But, there is not clear information about Azman. But it is commercial a variety for country. In addition to these genotypes, there are also 'Erdemli Yerli' and 'Erdemli Yerli Küllü' genotypes that have no commercial importance but are offered for sale in local markets. Many phylogenetic studies on the genus *Musa* (Gawel and Jarret, 1991; Gawel et al.,

1992; Ude et al., 2002; Jarret et al., 2002, 2003; Jarret et al., 1992; Jarret and Gawel, 1995; Shepherd, 1999; Nwakanma et al., 2003; Bartos et al., 2005; Heslop-Harrison and Schwarzacher 2007; OECD, 2009; Li et al., 2010; Liu et al., 2010; Nayar, 2010; Christelova et al., 2011; Hribova et al., 2011; Das et al., 2011) demonstrated that none of the five sections of *Musa* previously defined based on morphology was recovered as monophyletic. Recently, to identfy *Musa* genomes and determine the level of genetic variability between genotypes, a number of techniques have been used via molecular markers, including Random Amplified Polymorphic DNA (RAPD) (Das et al., 2009), Restriction Fragment Length Polymorphism (RFLP) (Hippolyte et al., 2010), Amplified Fragment Length Polymorphism (AFLP) (Opara et al., 2010), Inter Simple Sequence Repeats (ISSR) (Lu et al., 2011), Sequence Related Amplified Polymorphism (SRAP) (Phothipan et al., 2005) and Simple Sequence Repeats (SSR) markers (Miller et al., 2010). In the genus *Musa*, flow cytometry has frequently been used in ploidy analysis (Awoleye et al., 1994; Dolezel et al., 1997; Johnson et al., 1998; Egesi et al., 2002; Emshwiller, 2002; Stacy et al., 2002; Beatson et al., 2003; Walker et al., 2005). It has an advantage over the traditional chromosome counting technique in that it can be used to screen many plants in a short time and can be practically applied to any plant tissue (Roux et al, 2003; Dolezel and Batros, 2005). In this study, nuclear DNA content of commercial (Grand Nain and Dwarf Cavendish), local (Azman, Erdemli Yerli and Erdemli Yerli Küllü), and wild (F3, F4, and F5) genotypes were determined through flow cytometry and also genetic relationships among genotypes were assessed by Simple Sequence Repeats (SSR) markers.

MATERIAL AND METHOD

Plant material

International ‘Dwarf Cavendish’ and ‘Grand Nain’; local ‘Azman’, ‘Erdemli Yerli’ and ‘Erdemli Yerli Küllü’; wild F3, F4, and F5 banana genotypes were used as the plant material of the study. All plants were grown in the greenhouse in Mersin city (34°E 36°N, sea level, average annual temperature: 23.3/14.7˚C, mean RH: 70%, mean annual precipitation:138 mm), Erdemli, Southern Turkey (one of the most important area of banana cultivation in the country). F3, F4, and F5 banana genotypes were obtained from the seeds. The images of the individuals used in the study are given in Figure 1.

Figure 1. Original images of banana individuals. a. AZ, b. DC, c. GN, d. Küllü Erdemli Yerli, e. Erdemli Yerli, f. F4, g. F5, h. F3

Estimation of ploidy level

Ploidy level determination was performed using the Cystain UV Precise P kit from Sysmex. Leaf samples taken from young leaves were placed in petri dishes containing 0.4 mL buffer and finely chopped with a razor blade. The samples were filtered through 167 µm nylon filters, into a 3.5 mL tube, and 1.6 mL of the solution containing DAPI fluorescent dye was added. The samples were kept at room temperature for 15 minutes and then analyzed in a Partec brand Flow cytometer. The triploid variety was used as a control during the analysis. For ploidy screening, the instrument was calibrated using "Dwarf Cavendish" as a reference (standard) triploid (3x) with its peak set at channel 150. The peaks of the unknown samples were determined by examining the position of their peaks relative to the reference cultivar. All the samples with peaks at channel 100±5 were considered diploids, while those at channel 150±5 were triploids.

DNA isolation

Total genomic DNA was extracted from fresh leaf tissues for molecular analysis by using the CTAB procedure as described by Doyle and Doyle (1990). DNA concentration was measured with a microplate spectrophotometer (BioTek Instruments, Inc., Vinooski, USA), and 10 ng/mL DNA templates were made using TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) buffer.

SSR (Simple Sequence Repeat) Analysis

PCR cycle and reaction conditions of SSR analysis; 13.5 µl amplification reaction 1 mM $MgCl_2$, 0.1 % Tween 20, 0.25 mM dATP, 0.25 mM dTTP, 0.25 mM dGTP, 0.25 mM dCTP, 0.4 mM primer, 1.0 units Taq DNA polymerase and 20-25 ng DNA will include. PCR conditions; Initial denaturation 94°C for 5 min 1 cycle, denaturation 94°C for 1 min, annealing 55°C for 40 sec, 72 °C for 30 sec 38 cycles, Final extension at 72°C for 7 min and incubation at 4°C. The products obtained from the PCR reaction are separated by electrophoresis in 3% ultra pure agarose gel. After imaging, amplification fragments are present (1) or absent (0) has been scored. The list of forward and reserve primers used in the study is given in Table 1.

Table 1. SSR primers and sequences used in the study

	Forward Primers (5'-3')		Reserve Primers	
1	Ma3	GGA ACA GGT GAT CAA AGT GTG A	Ma3	TTG ATC ATG TGC CGC TAC TG
2	Ma1	TGA ATC CCA AGT TTG GTC AAG	Ma27	CAA ACA CAT GTC CCC ATC TC
3	Ma1	TTT GCC TGG TTG GGC TGA	Ma18	CCC CCC TTT CCT CTT TTG
4	Ma3	TGG CTG ACA ATT ACA TGA CA	Ma60	GCG CAC TGT GGT GTG T
5	Ma1	GAG CCC ATT AAG CGT AAC A	Ma24	CCG ACA GTC AAC ATA CAA TAC A
6	Ma1	TTT GCC TGG TTG GGC TGA	Ma16	CCC CCC TTT CCT CTT TTG C
7	Ma1	ATT GGG CAG GCA TCA AGT AC	Ma19	GCA ATG GTG CTA CCC ACC
8	Ma3	CAA CAG CTC TCG CACA TTC	Ma64	AAC CTT TAA TGT ATC TGC
9	Ma3	GCA CGA AGA GGC ATC AC	Ma90	GGC CAA ATT TGA TGG ACT
10	Ma1	AGA TGG CGG AGG GAA GAG CCG	Ma5	GAT CCA AGC TTA TCG A

Data analysis

Molecular analysis was carried out as follows: each band was scored as present (1) or absent (0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software package (Rohlf, 2000). The genetic similarity matrix was calculated using the coefficients of Nei and Li (1979). Cluster analysis was conducted based on the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) using NTSYS-pc version 2.0 software (Rohlf, 2000).

RESULTS AND DISCUSSION

A number of ploidy levels exist in *Musa* spp. (Tenkouano et al., 2011). Knowledge of ploidy level in Musa accessions is vital for breeding, conservation and tissue culture as they are affected by ploidy (Suman et al., 2012). Ploidy level influences fertility of banana. For instance, most triploids are sterile while diploids and tetraploids are fertile (Tenkouano et al., 2011). In the study, we determined ploidy level of Grand Nain, Azman, Dwarf Cavendish, F3, F4, F5, Erdemli Yerli, and Küllü Erdemli Yerli. As Grand Nain and Dwarf Cavendish are international genotypes, ploidy levels were determined as triploid as expected. However, since Azman, Erdemli Yerli and Erdemli Yerli Küllü genotypes are local genotypes unique to Turkey, their ploidy levels were not known before. As a result of the study, it was understood that three genotypes were triploid. Seeds of wild F4 (*Ensete ventricosum* the Abyssinian Banana), F5 (*Ensete glaucum* the Snow Banana), and F3 (*Ensete glaucum* the Yunnan banana) genotypes were brought from abroad for breeding works. F4 and F5 were determined as diploids, while

F3 was determined as spontaneous triploid. The peak images of diploid and triploid genotypes are shown in Figure 2.

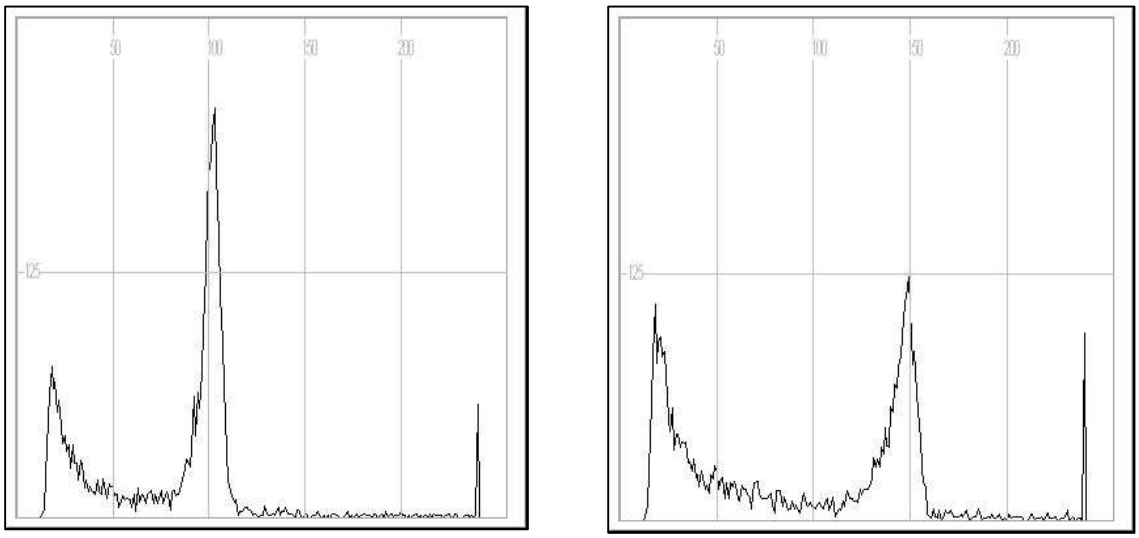

Figure 2. Histogram showing F4, a diploid with its peak set channel at 100 and Dwarf Cavendish as a triploid (control genotype) with its peak at channel 150.

Polyploids such as bananas are often apparent by their distinct and robust morphology (Briggs and Walters, 1984). However, diploids are delicate in nature, lean in size and even perish in harsh weather conditions. Following this system, plants with erect leaves are considered diploids while those with drooping leaves as tetraploids, and those with an intermediate leaf orientation as triploids. This method of ploidy determination is subjective and not always reliable. It becomes even more unreliable when evaluating hybrids from a breeding program that consist of a mixture of ploidy levels including aneuploids. Morphological markers do not often reflect genetic relationships because of interaction with the environment, epistasis and the largely unknown genetic control of traits (Smith and Smith, 1989). In contrast, DNA markers are found in abundance and are not influenced by the environment or developmental stage of a plant, making them ideal for genetic relationship studies in plant species (Reddy et al., 2002). SSR markers combine several features of an ideal genetic marker, owing to their abundance and widespread dispersal in genomes, hypervariability, co-dominant nature, accessibility, reliability, and ease of interpretation (Gaudeul et al., 2004). Therefore, assessment of *Musa* genetic variability solely by using SSR markers continues to be widely used (Amorim et al., 2008; Jesus et al., 2009). The banana individuals were assessed concerning genetic diversity by SSR analysis. The total number of amplified bands was 356, and 48 of them were polymorphic. The total and polymorphic bands per primer ranged from 2 to 8. In terms of the number of polymorphic bands, the primer Ma3 x Ma64 produced the lowest number of bands while Ma3 x Ma90 was gave the highest number of bands. The mean polymorphism rate of the SSR primers was 14.09% (Table 2.). When the dendrogram formed by UPGMA analysis using SSR data using the Nei and Li (1979) similarity coefficients were examined, it was seen that the individuals were divided into three groups (Figure 3). Dwarf Cavendish variety was completely separated from all of them and formed a branch on its own. The large main group was again divided into four groups within itself. Küllü Erdemli Yerli and F3 were alone in the small group and the similarity matrix was determined as 0.6462. On the other hand, Erdemli Yerli was separated from the others. F4 and F5 were gathered in a single branch that were seen as the most closely related clones (0.9020) (Figure 4). Azman formed a single branch and separated from Grand Nain and Erdemli Yerli. The correlation matrix (r) of the study was

determined as 0.90. Brown et al. (2009) reported that 'Grande Nain' (AAA), 'Williams' (AAA), and 'Dwarf Cavendish' (AAA) were in the same cluster after a molecular characaterization by RAPD markers. Pinar et al. (2020) aimed to determine the genetic variability within banana cultivars using SRAP marker. They determined GN, AZ and DC as close relatives. In an another study, As a result of the SRAP assessment, DC and GN were the closest related clones. Contrary to the SRAP results, GN and AZ were identified as the most genetically related clones in morphological and RAPD assessments (Baysal and Ercisli, 2022). In this study, although GN and AZ were in the same group, DC was in a completely different group.

Table 2. Number of bands and polymorphism ratios of banana individuals obtained with SSR primers

Primers	Total number of bands	Number of polymorphic bands	Polymorphism ratio (%)
Ma3 x Ma3	40	7	17.5
Ma1 x Ma27	32	6	18.8
Ma1 x Ma18	40	7	17.5
Ma3 x Ma60	15	2	13.3
Ma1 x Ma24	8	2	25.0
Ma1 x Ma16	34	5	14.7
Ma1 x Ma19	61	6	9.8
Ma3 x Ma64	8	0	0.0
Ma3 x Ma90	46	8	17.4
Ma1 x Ma5	72	5	6.9
Mean	356	48	14.09

Jingyi et al. (2009) investigated 56 banana accessions by SSR marker. Due to the complex SSR parterns in banana, it was difficult to score each band with number 1 and 0. They scored each SSR pattern with an alphanumeric code instead of with pairs of 1 and 0. Ravishankar et al. (2012) used 26 SSR markers for identify 15 genotypes of banana belonging to different groups. The markers amplified a total of 88 alleles, with two-to-six alleles per locus (mean = 3.38). Similar results were observed by Oriero et al. (2006), who tested 44 SSR markers to assess polymorphism in five accessions of *Musa*. In the latter work, only nine SSR markers yielded amplification products which were then used to screen 40 *Musa* accessions. Creste et al. (2003) used 33 SSR primer pairs in diploid and triploid accessions of *Musa* from Brazil. Only 15 gave amplification products, as the anchor sequences flanking the microsatellite loci probably differed sufficiently to prevent amplification.

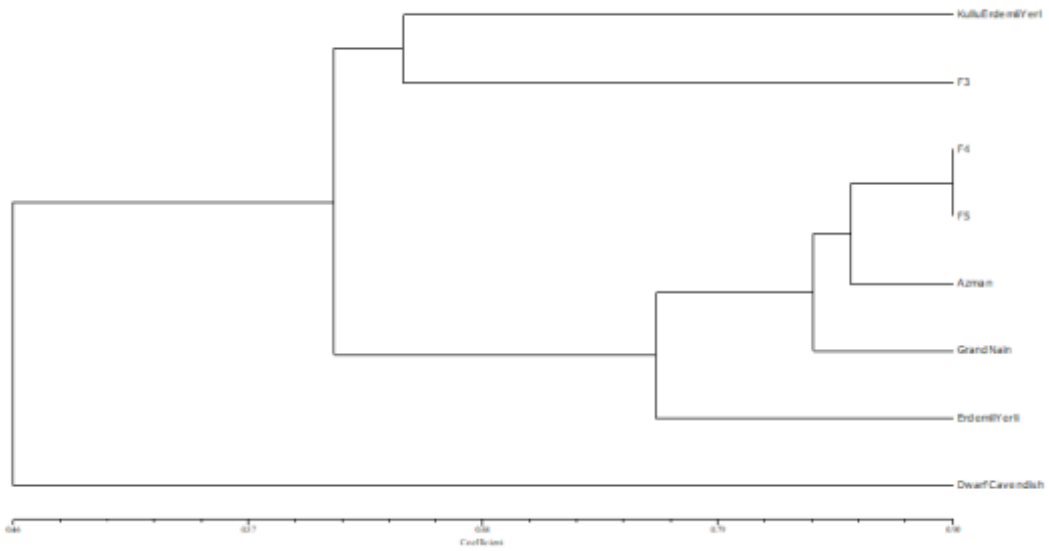

Figure 3. Dendrogram of eight banana individuals using UPGMA method obtained from SSR markers

Although many *Musa* SSRs have been developed thus far by using a number of different strategies (Jarret et al., 1994; Crouch et al., 1997; Lagoda et al., 1998; Creste et al., 2003, 2006; Wang et al., 2008; Miller et al., 2010), the number of SSR loci available for genetic analysis is still limited, with many more validated and polymorphic SSRs required (Creste et al., 2004; Wang et al., 2009).

	Küllü E. Yerli	F4	F5	Grand Nain	D.Cavendish	Erdemli Yerli	F3	Azman
Küllü E. Yerli	1.0000							
F4	0.6415	1.0000						
F5	0.5769	0.9020	1.0000					
Grand Nain	0.6000	0.8571	0.8333	1.0000				
D.Cavendish	0.6250	0.3404	0.3913	0.4545	1.0000			
Erdemli Yerli	0.4815	0.7925	0.7692	0.7600	0.3750	1.0000		
F3	0.6462	0.6250	0.5714	0.5246	0.5285	0.6154	1.0000	
Azman	0.7667	0.8814	0.8276	0.8214	0.5556	0.7333	0.7324	1.0000

Figure 4. Similarity index of eight banana individuals obtained by UPGMA analysis using SSR molecular marker data

CONCLUSION

Knowledge of the ploidy of bananas is valuable for banana breeding schemes as it involves interploidy crosses leading to several possible ploidy levels in the progeny. Ploidy

detection devices are very fast and thus a spontaneous triploid individual emerging from seed was detected in our study. In genetic evaluation using SSR marker, the rate of polymorphism was determined as 14.09%. While the number of monomorphic bands obtained was high, the number of polymorphic bands was found to be low. As a result of the study, it was understood that the study would be more effective with more primer combinations.

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THE BENEFITS OF APPLYING BIOFILTERS TO IMPROVE THE WATER QUALITY IN AQUARIUMS

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ABSTRACT

The objective of the study was to determine whether biofilters can improve the water quality in fish-rearing aquariums and to analyze how ventilation and stocking density affect water quality. Experiments were conducted in four aquaria using Nile tilapia at average body weights 19.3±1.67 – 21.4±1.83g. Treatment "1" included 12 fishes reared without ventilation and biofilter. Treatment "2" included 12 fishes reared with ventilation and without biofilter. Treatment "3" included 12 fishes with ventilation and biofilter. Treatment "4" included 24 fishes reared with ventilation and biofilter. Fishes in all treatments were fed on the same diet which contained 35% protein. Each week, pH levels, ammonia, nitrite, and nitrate concentrations were measured and recorded. The temperature of the water was observed daily and noted. All fishes in each aquarium were weighed at the beginning and end of experiments as well as weekly. The study revealed that, biofilters removed ammonia which converted into nontoxic nitrate, it also showed that ventilation system can aid to some extent reduce the ammonia concentration. This study also indicates that, fishes growth were adversely affected in the first and fourth treatments that characterized by higher concentrations of ammonia and/or nitrite in most measurements as compared to the second and third treatments. Rate of mortality was almost higher in the first and fourth treatments. In the fourth treatment, low growth and high mortality rates showed the negative effects of high stocking density on water quality. In order to maintain acceptable water quality by eliminating high concentrations of ammonia and converting it into nitrate, which is safe to fishes, the current study suggests the significance of equipping fishes in aquaria with biofilters.

Key words: Biofilter, water quality, Nile tilapia, stocking density, growth performance

INTRODUCTION

The last two decades have seen a rise in recognition of the importance of the fisheries and aquaculture sectors to global food security and nutrition (FAO, 2022). Aquaculture is one of the most economically important applied strategies all over the world and fish is a food commodity that is distributed worldwide and regarded as an important source of animal protein (Osuigwe and Obiekezie, 2007) as well as other essential elements that are necessary for the maintenance of healthy body to the human especially in the developing countries (Afolabi et al., 2020). Factors affecting the aquatic environment change frequently and put organisms under

stress. These environmental changes have a variety of physiological effects on species. They may be deadly, change the impact of other factors, be directive or control something.

Biomonitoring has been recommended as the most effective management technique for evaluating the condition of the environment. The biofiltration process is a process that utilizes microorganism attached on a solid matrix surface and forms a biological layer (known as biofilm). This process can filter out pollutants physically, and also degrade pollutants through biological activity (Chaudhary et al., 2003). The biofiltration process has been reported as a cost effective method for the elimination of a variety of organic and inorganic pollutants, even for substances classified as relatively harmful, toxic, and hardly biodegradable compounds (Suprihatin, et. al., 2017). The biofilter is populated by two distinct physiological groups of nitrifying bacteria: those that oxidize ammonia to nitrite and others that oxidize nitrite to nitrate (Bock and Wagner, 2006).

Ammonia is the major end product of nitrogen metabolism, excreted by aquatic animals and high levels of ammonia can have negative effects on fish growth and survival (Guan et al 2010). Ammonia can be controlled by using the nitrification process, plants uptake and immobilization by bacteria (Hargreaves, 2006). While still there is limited information and the efficiencies of these filtration techniques. This study aimed to evaluate the effect of bio-filtration and the fish density on water quality and fish growth performance.

MATERIALS AND METHODS

Fish sample and experimental procedures:

The experiment for this study was carried out in the fish laboratory of Al-Neelain University's Faculty of Agricultural Technology and Fish Sciences. The biofilter's ability to remove ammonia from the water, the impact of ventilation on that ability, and the impact of fish density were all tested during the experiment's four treatments. The experiment took place in four glass aquariums with measurements of (90 cm x 40 cm x 40 cm) and holding approximately 100 liters of water for a period of 7 weeks, from 9/3 to 27/4/2018. The Nile tilapia *(Oreochromis niloticus*), which was procured from the college's fish farm, was utilized in the experiment at average body weights ranging from 19.3±1.67 – 21.4±1.83g. Following that, the fish were weighed and divided into the four aquariums used for the experiment, with 12 fish each. As a control, the aquarium was left without ventilation and a biofilter in the first treatment. In the second treatment, the aquarium received ventilation but not a biofilter, and in the third aquarium, a biofilter and ventilation were provided (treatment 3). The fourth aquarium had ventilation and a biofilter added (treatment 4).

Feeding and growth performance analysis:

Fish were fed 2% of their body weight per day in all experimental treatments. The fish received their daily amount of food in two doses, the first at roughly 9:00 am and the second at about 4:00 pm. The diet for the fish in each of the four treatments was designed to have the same 35% protein content. At the start of the experiment and then once a week, all fish were weighed to monitor their growth. All fish were weighed at the end of the experiment, and the

percentage increases in fish weight, daily growth rate, and survival rate in each treatment were calculated.

Measurements of water quality variables:

Weekly water samples from each aquarium were taken for chemical analysis at the Federal Ministry of Health's National Laboratory (Astak). Using an Atomic Absorption Spectrophotometer (AAS), total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), and nitrate nitrogen (NO_3-N) were measured and calculated in (mg/L) accordance with the (AOAC, 1980) technique. Temperature and pH were measured in aquarium by using a mercury bulb thermometer and a portable pH scan respectively.

Statistical Analysis:

SPSS version 20.0 for Windows was used to conduct all statistical analyses. The distribution of the data was checked for normality, and the variance was examined for homogeneity. The one-way analysis of variance (ANOVA) was used to test if the treatments differed in terms of certain parameters. The significance level was determined to be at the 5% level of probability.

RESULTS

Treatment 1 (Control):

The results obtained in the 7-week study indicated that the total ammonia concentration in the first treatment (control) that contained 12 fish without biofilter and ventilation began gradually to increase from zero at the beginning of the experiment before introducing the fish to 20 mg/L at the end of the first week, and then increased again at the end of the seventh week (end of the experiment) to 9.2 mg/L (Fig 1). Nitrite (NO_2-N) concentration was zero mg/L at the beginning of the experiment and at the end of the first week. It then gradually increased, reaching at 0.23 mg/L at the end of the second week and 0.23 mg/L at the end of the seventh week (Fig.2). Nitrate (NO_3-N) concentration was zero at the beginning of the experiment, as well as at the end of the first and second weeks. It then raised to 4.3 mg/L at the end of the third week, and then gradually decreased to 2.3 mg/L even by the end of the seventh week (Fig. 3). The pH in the first treatment varied throughout the trial period, with the lowest value being 7.0 at the end of the third week and the highest reading being 7.9 at the end of the second week (Fig.4).

Treatment 2:

In the second treatment with ventilation and without biofilter, the ammonia concentration started to rise from zero mg/L at the beginning of the experiment before introducing the fish to 15 mg/L at the end of the first week, then decreased to 0.9 mg/L at the end of the second week and then increased to 6.5 mg/L by the end of the seventh week (Fig1). Nitrite concentration

started to rise from 0 mg/L before fish were introduced to 1 mg/L at the end of the first week, and then increased to 1.32 mg/L by the end of the seventh week (Fig2). The nitrate concentration increased from zero mg/l at the beginning of the experiment to 4 mg/L at the end of the first week and to 60.9 mg/L at the end of the second week, then decreased by the end of the experiment to become 50.9 mg/L (Fig. 3). The pH in the second treatment ranged during the trial period between 7.02 at the end of the third week as the lowest reading recorded and 7.9 at the end of the second week as the highest reading (Fig 4).

Treatment 3:

In the third treatment with ventilation and biofilter, the ammonia concentration appeared to gradually increase slightly from zero at the beginning of the experiment and the end of the first and second weeks to 0.9 mg/L and then decreased by the end of the seventh week to 0.15 mg/L (Fig1).The nitrite concentration increased from zero at the beginning of the experiment to 15.0 mg/L by the end of the first week, and then decreased to 0.2 mg/L by the end of the seventh week (Fig 2). The concentration of nitrate increased from zero before introducing the fish to 84.0 mg/L at the end of the first week, and then decreased to 44.6 mg/L at the end of the seventh week (Fig 3). The pH in the third treatment ranged during the experiment period between 6.80 at the end of the fifth week as the lowest reading recorded and 7.76 at the end of the second week as the highest reading (Fig 4).

Treatment 4:

In the fourth treatment, which included 24 fish with ventilation and biofilter, the ammonia concentration increased from zero at the beginning of the experiment to 18.0 mg/L at the end of the first week, and then to 7.8 mg/L at the end of the seventh week (Fig1). The nitrite concentration increased from zero before introducing the fish to 0.4 mg/L at the end of the first week and then to 16.5 mg/L at the end of the seventh week (Fig2). The nitrate concentration increased from zero to 5.0 mg/L at the end of the first week, and then to 62.9 mg/L at the end of the seventh week. The pH in the fourth treatment ranged during the trial period between 6.80 at the end of the fifth week as the lowest reading recorded and 7.80 at the end of the second week as the highest reading.

Growth and survival rates of Fish:

The results of growth performance of fingerlings Nile tilapia fed on diets containing 35% protein are presented in (Table 1). There were no significant differences ($p>0.05$) in the growth rate, and the survival rate in the various treatments. The increase in average fish weight was 2.1± 0.98 gm. in the first treatment, 7.2±0.78 gm. in the second treatment, 6.0±0.78 gm. in the third treatment, and 5.2±0.65 g in the fourth treatment (Table 1). Accordingly, the percentage increase was 10.3±1.54% in the first treatment, 33.6±2.12% in the second treatment, 28.8±1.93% in the third treatment, and 26.9±1.67% in the fourth treatment (Figure 5). The daily growth rate of fish in the first treatment was 0.04±0.71 g/day, in the second treatment it was 0.15±0.65 g/day, in the third treatment it was 0.12±0.32 g/day, and in the fourth treatment it

was 0.11±0.23 g/day (Fig 6). The survival rate of fish in the first treatment was 66.7±2.01%, the second treatment 75.0±2.22%, the third treatment 83.3±2.31%, and the fourth treatment 54.2±2.05% (Table 1).

Temperature:

The average water temperature during the experimental period was 26.2°C in the first and second treatments, and 26.3°C in the third and fourth treatments. The highest temperature was 32°C and the lowest was 21°C (Fig 7).

Table 1. Fish Performance in 7-weeks

Parameter	Treatment 1 (control) without biofilter and ventilation	Treatment 2 with ventilation and without biofilter	Treatment 3 with biofilter and ventilation	Treatment 4 with biofilter and ventilation (24 fish)
Average of Initial weight (g)	20.3±1.23	21.4±1.30	20.8±1.23	19.3±1.08
Average of final weight (g)	22.4±1.22	28.6±1.67	26.8±1.78	24.5±1.54
Weight gain (g)	2.1±0.98	7.2±0.78	6.0±0.78	5.2±0.65
Weight gain %	10.3±1.54	33.6± 2.12	28.8±1.93	26.9±1.67
Daily gain (g)	0.04±0.71	0.15±0.65	0.12±0.32	0.11±0.32
Survival %	66.7±2.01	75.0±2.22	83.3±2.31	54.2±2.05

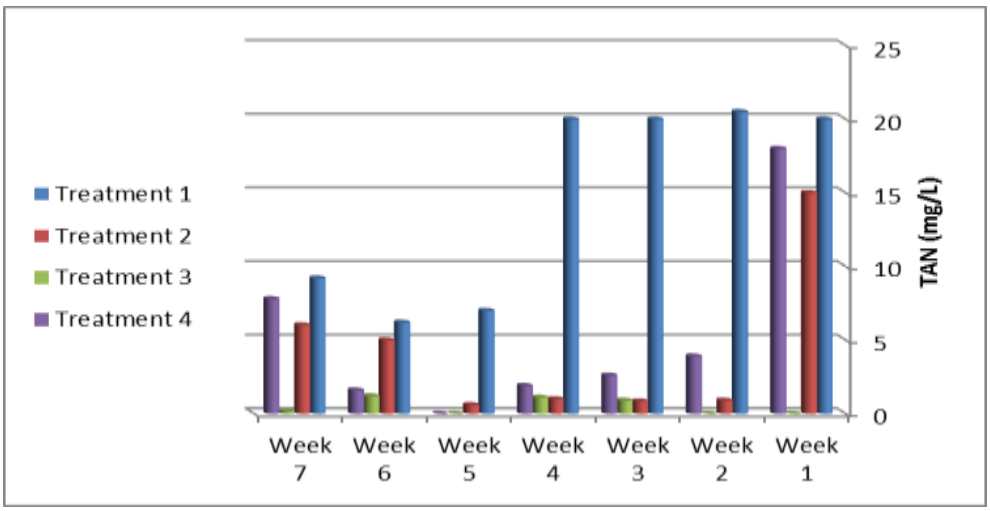

Figure 1. Weekly mean concentrations of total ammonia nitrogen in 4 treatments

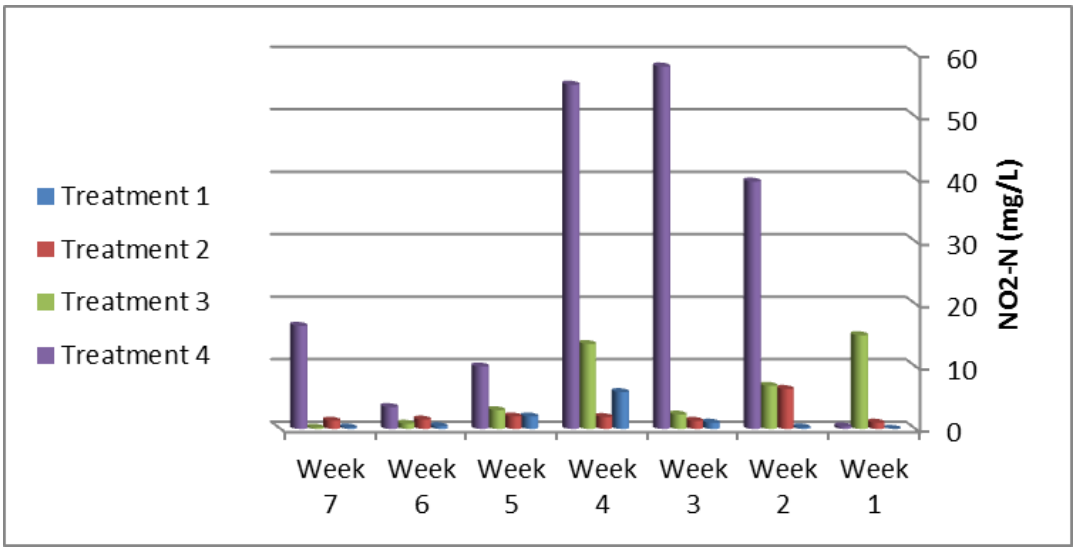

Figure 2. Weekly mean concentrations of nitrite-nitrogen in 4 treatments

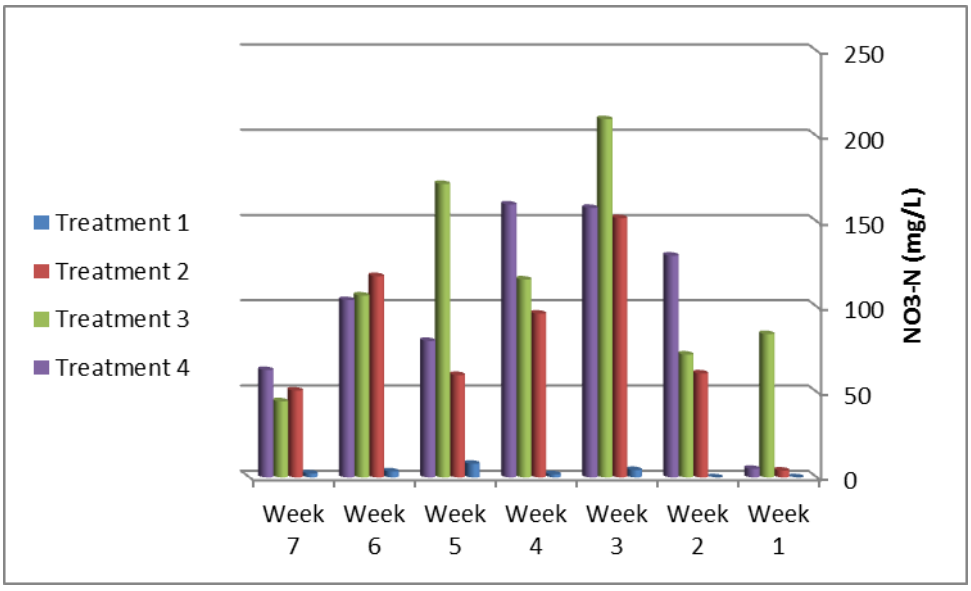

Figure 3. Weekly mean concentrations of nitrate-nitrogen in 4 treatments

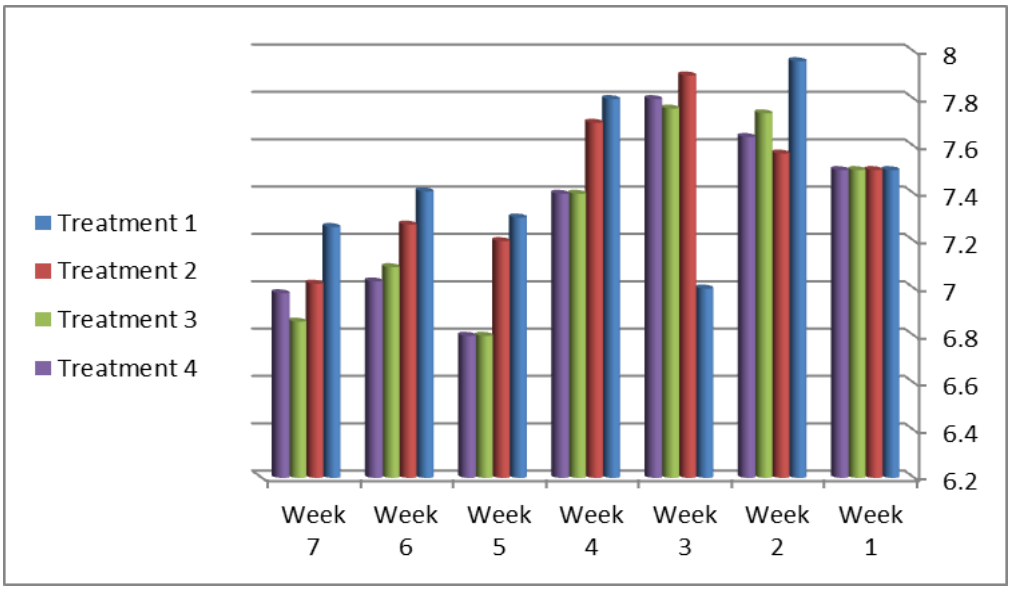

Figure 4. Weekly pH in 4 treatments

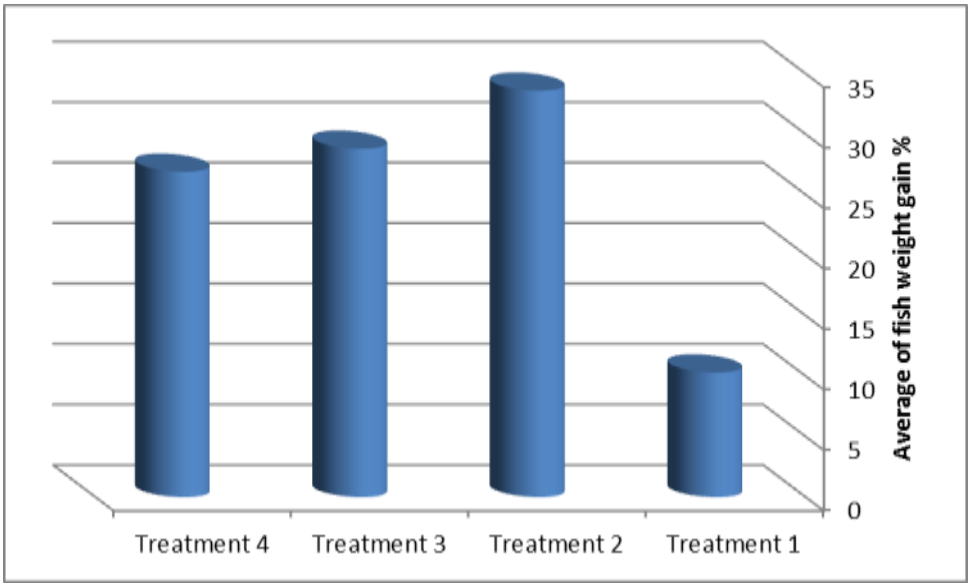

Figure 5. Growth rate (%) of fish at four treatments

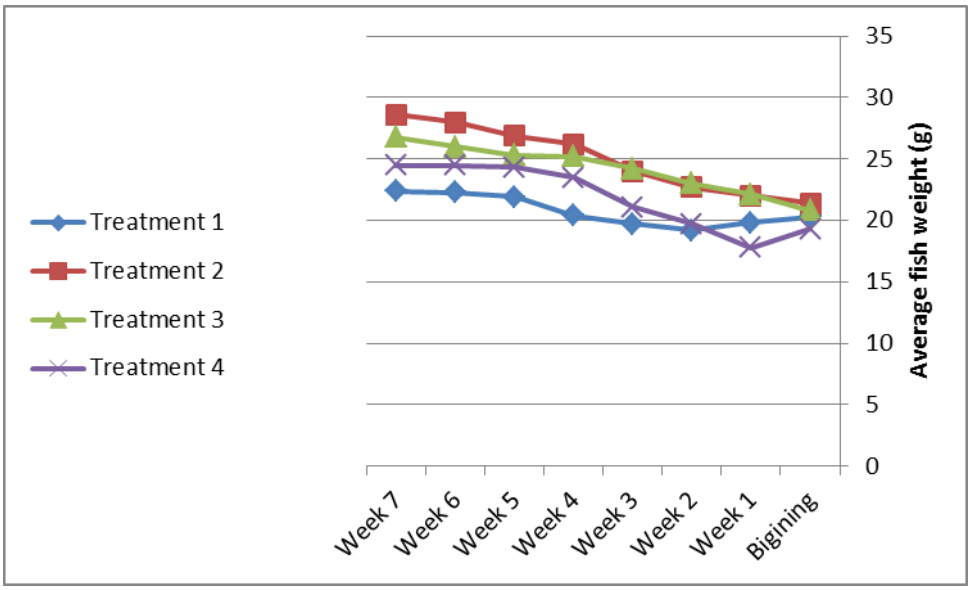

Figure 6. Changes in the weight gain (g) of fish at four treatments

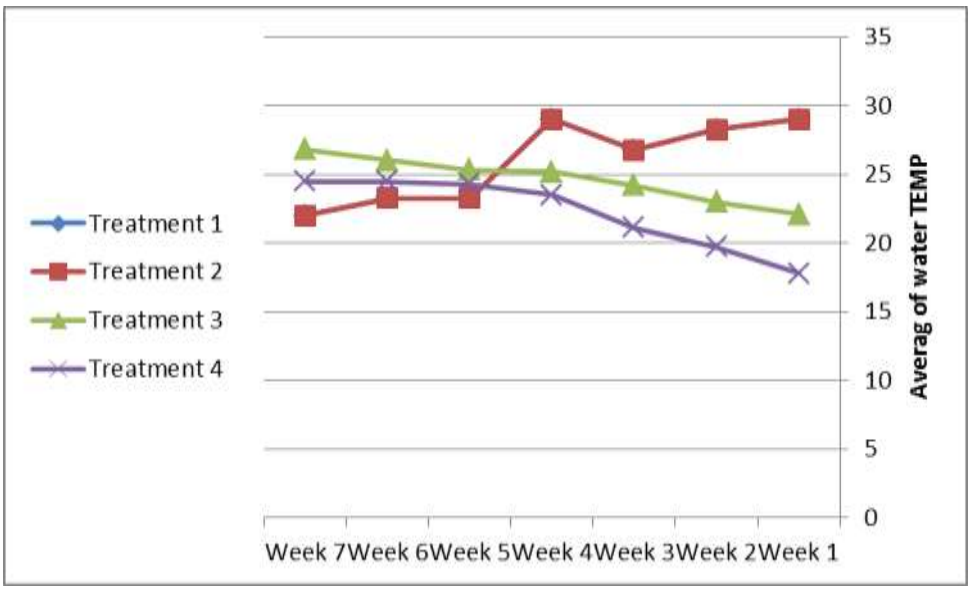

Fig 7. The average water temperature during the experimental period (°C)

DISCUSSION

Various types of pollutants can be eliminated by using the biofiltration system. In addition to the filtration effects of the quartz sand matrix, bacteria in the biofilter (biofilm) also oxidized the organic materials into simpler compounds, such as water and carbon dioxide (Neori, 2004). The effect of the bio-filter was evident since the first week, as the ammonia concentration was high in the first aquarium (without filter and without ventilation) and the second one (without filter with ventilation) Figure 1, as the concentration reached by the end of the first week 20 and 15 mg/L, respectively. Noted that the concentration of ammonia in the second aquarium (15 mg/L) is less than in the first aquarium (20 mg/L). The effect of ventilation in reducing the

ammonia concentration, was observation of the occurrence of the nitrification process because the nitrifying bacteria are aerobic bacteria and indicated by the presence of a small concentration of nitrite (1 mg/liter) Figure 2 and nitrate (4 mg / liter) Figure 3. Nitrite is one of the toxic substances for fish and high concentrations of it may lead to the death of fish as it affects the efficiency of oxygen transfer (Boyed, 1979). The values of ammonia concentration was within the limit of (EIFAC, 1973).

The third aquarium (filter with ventilation), there was no ammonia by the end of the first week, which indicates that the bio-filter had removed it, and this is confirmed by a high concentration of nitrite (15 mg /L) and a higher accumulated concentration of nitrate (84 mg/l). No ammonia was recorded (zero mg/l) at the end of the second week, then it appeared with a small concentration of 0.15 mg/L at the end of the seventh week. The biofilter kept removing ammonia with high efficiency, confirms that, throughout the weeks of the experiment. This aquarium recorded the lowest ammonia concentration reading among all the treatments, and recorded the best survival rate of fish, which amounted to 83.3±2.31%. This results agrees with (Suprihatin et. al., 2017) who found that an application of biofilter can significantly improve the quality of raw water, as shown by the decreased values of organic matter, and ammonia. .

The fourth aquarium had a high concentration of ammonia (18 mg/l) at the end of the first week, despite the fact that it contained a bio-filter. This is due to the high density of fish (24 fish/aquarium). Our results indicated that an inverse relationship between the fish density and the total ammonia excreted into the fish aquarium. This negative relationship was also found with different fish species such as koi carp (Irhayyim & Fotedar, 2019) and rainbow trout (Bucking, 2017), haddock (Lankin et al 2008) .This relationship has been partly explained in terms of the physiological changes and also be related to the variations in the surface of respiratory organs (Post & Lee 1996). The survival rate in this aquarium (54.2±2.05%) was lower than in other aquariums, and the reason for the mortality may be due to the exposure of fish throughout the experiment period to high concentrations of nitrites (up to 39.6, 58 and 55.1 mg/L,).This agree with Konikoff (1975) who stated that the nitrite concentration led to the death of half of the channel catfish within an 96 hour was 24.8 mg/L. The high ammonia concentrations that were sometimes recorded in some of the experimental aquariums did not lead to a high mass death rate in those aquarium, perhaps because of the low pH of the water recorded throughout the experiment period. The highest reading recorded during the experiment is pH 7.96. Swingle (1961) reported that the appropriate range of pH for freshwater fish 6.5-9.5, and fish production decreased at pH levels above 9.5, and below 6.5

Although there was no significant difference ($p < 0.05$) between the four treatments in the mean weight of the fish at the end of the experiment, the performance of the fish seemed to be affected by the ammonia concentration Table 1. Growth stunted during the first two weeks coincided with the high ammonia concentration, especially in the first and fourth aquarium, which both of them, the fish lost part of their weight, especially during the first week. While the second and third aquarium witnessed weak growth during the first two weeks. Then, as the experiment progressed, the growth improved in all aquariums with a decrease in the level of ammonia, including the first and fourth ponds (Figure 5&6).

From this study was clear that the biofilter removed ammonia from the water and converted it into non-toxic nitrate, and ventilation helped to reduce the concentration of

ammonia in the absence of the biofilter by providing a better oxygen environment for the aerobic nitrifying bacteria that are already present in the water. As for the aquarium that was not provided with a biofilter or ventilation, the ammonia concentration increased, the mortality rate increased slightly (survival rate of 66.7±2.01%) compared to the other two aquariums that contained the same density of fish, but one was provided with ventilation (survival rate 75±2.22%) and the other was provided with ventilation and filter Vital (survival rate of 83.3±%) (Table1). This result agreed with (Colt & Tchobanoglous, 1976) who found that 50% of the fish died when they were exposed for 96 an hour to ammonia values that ranged from 0.5-3.8 ppm.

CONCLUSION

This study demonstrated the importance of providing aquariums with a bio-filter to maintain water quality by removing ammonia and converting it into non-toxic nitrates for fish. It also indicated that good ventilation helps somewhat in reducing ammonia concentration. The absence of a biofilter to provide a large surface area for nitrifying bacteria, it may affect the performance of fish. Overcrowding of fish in the aquarium that exceeds the ability of the biofilter to remove ammonia affects water quality and fish performance and leads to the death of some of them. It can be said that the suitable density for a aquarium with a volume of water of about 100 liters and equipped with a bio-filter and good ventilation is 250-300 g of Tilapia fish.

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USE OF IMAGEJ IMAGE PROCESSING SOFTWARE IN FUNGAL GROWTH TRACKING

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ABSTRACT

Fungi are eukaryotic microorganisms that have important functions in nature. They can be categorized as obligate parasites, facultative parasites and saprophytes. Most of the facultative parasites and saprophytes can be grown in artificial media, under laboratory conditions. Determining the growth dynamics of fungi in artificial media is important for mycological studies. Measuring colony development at certain time points manually using ruler is a common practice. Using this technique in fungal cultures that do not form smooth, circular colonies causes problems in during calculations. ImageJ is an open-source, Java-based image processing program that can calculate area and pixel value statistics for user-defined selections. The program has a user-friendly interface and it can easily distinguish and select the object of interest from its background on any photoFigureic image. After photoFigureing the fungi in the medium, using ImageJ program, the fungal growth data can be obtained much faster and more precisely. With this method, the effects of irregularities in fungal growth on data acquisition can be minimized. In this study, the use of ImageJ software in the case of the wheat leaf pathogen Z*ymoseptoria tritici* is described. Steps such as visualization of fungal growth, selection of the area to be calculated from the images, distance calibration, processing of the obtained images are explained with visuals.

Keywords: Fungal growth, ImageJ, Image processing

INTRODUCTION

Growth measurements in petri dishes

For growth, measurements the fungus of interest is first spread on the solid medium and let grow at optimal temperature. After fine growth is established in all directions, a specific (generally 5-mm) diameter disk is removed with help of a cork borer. The removed disk is placed reversed in a new solid medium so that the fungus can colonize the new environment. Growth parameters are recorded at specific time points by measuring the diameter of fungal growth in the arbitrarily chosen x direction and the y direction, which is perpendicular to the x. (Fig. 1) Two measurements are then averaged and recorded. These two measurements are made in order to reduce the margin of error.

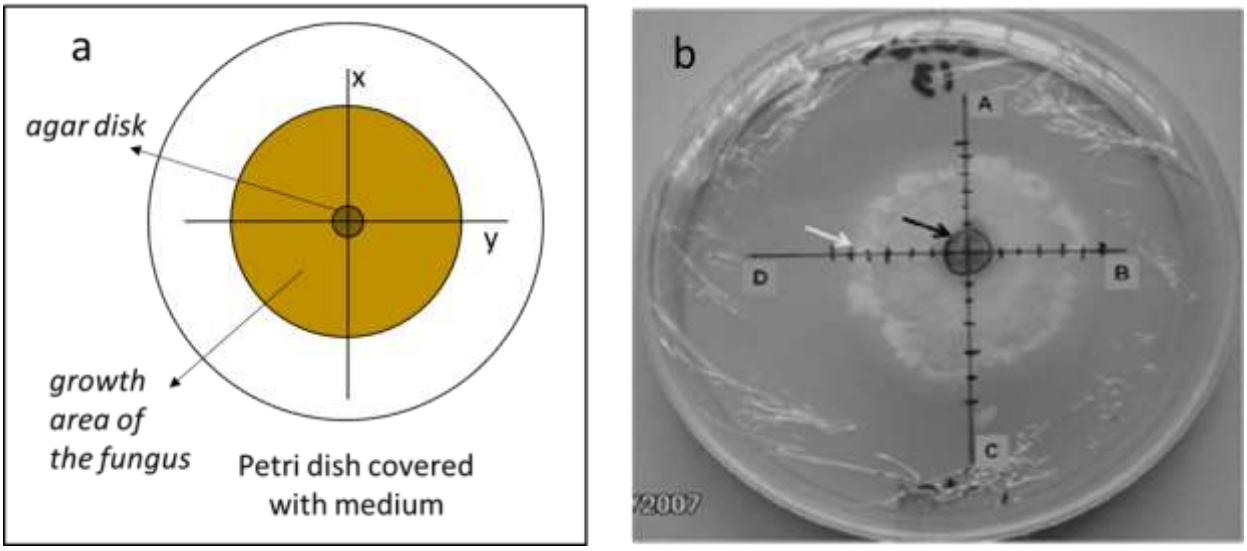

Figure 1. Fungal growth on petri dish. a) Representation of measurement procedure, b) Measurement of fungal growth (Miyashira 2010)

However, since these measurements are made with a ruler and the data are taken by looking, there is a chance of error occurring during the measurements. Since the measurements to be taken are millimetric, the margin of error increases in manual measurements. Moreover, there is a small distance between the ruler and the planes where the fungus grows, different data can be obtained regarding the angle of view during the measurements made with the eye. There may be also a slippage in the ruler at the time of measurement. Other difficulties in measurements can be due to fungal growth shape. Some of these problems are the growth that is not perfectly round, asymmetrical and uneven growth from the origin and irregular growth (Fig. 2). These abnormalities can not be easily addressed by classic measurement method.

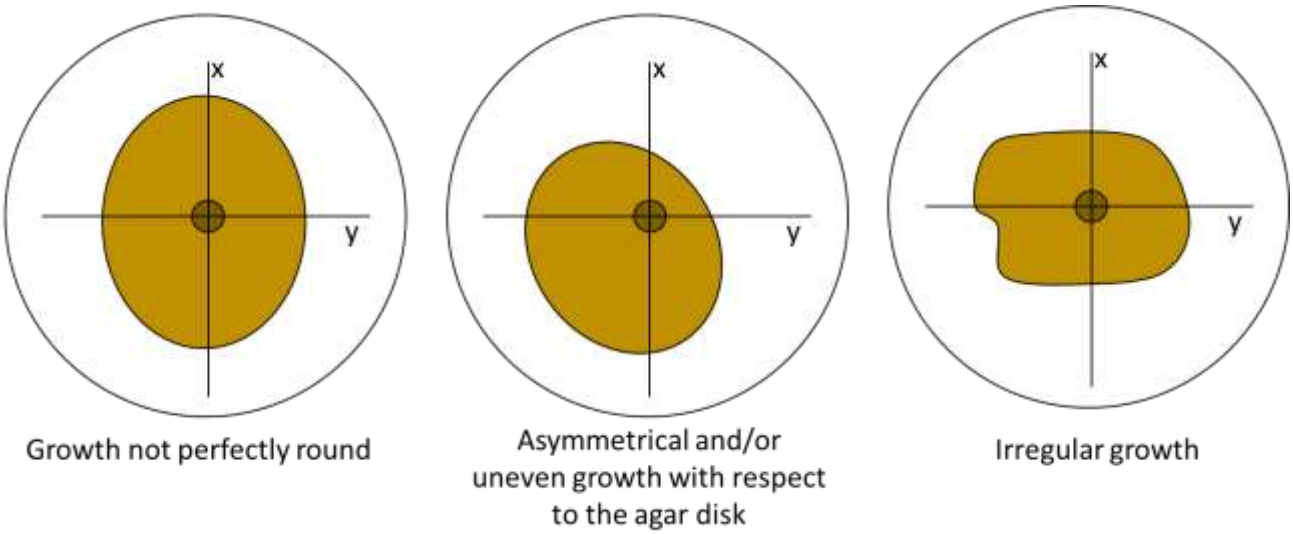

Figure 2. Some of the most common growth abnormalities

ImageJ: an image processing software

ImageJ is an open source Java image processing program (Fig. 3) that can calculate area and pixel value statistics of user-defined selections. It can display, edit, analyze, process, save and print 8-bit, 16-bit and 32-bit images in different formats such as TIFF, GIF, JPEG, BMP, DICOM, FITS and RAW. It can calculate distance, angle and area and pixel value statistics of user-defined selections. It supports standard image processing functions such as contrast manipulation, sharpening, smoothing, edge detection and median filtering.

Figure 3. ImageJ 1.53k user interface

To open any image with ImageJ from the File drop menu Open can be selected, or ctrl+o shortcut can be utilized (Fig. 4). From the window the file in your PC should be found. It also supports drag and drop of the files.

Figure 4. The opened picture is displayed in a new window

An important aspect of using ImageJ is the area selection. It should overlap the growth area as precisely as possible. If the growth area is rounded you may select it by using "Oval" box and adjust it around the growth (Fig. 5 a). To adjust selection area polygon selections or freehand selection can be used as well (Fig. 5 b and c). Since polygon selections and freehand selection have larger margin of error their use should be avoided using unless necessary

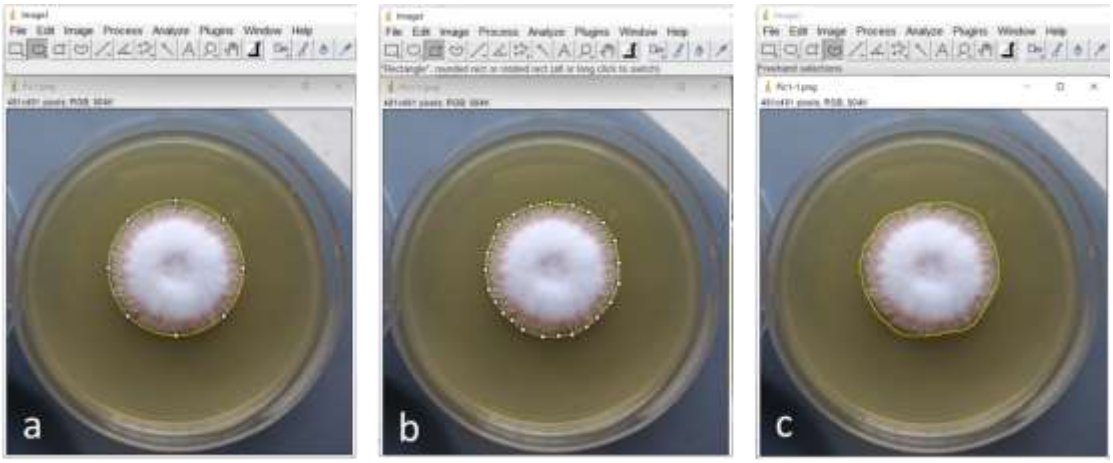

Figure 5. Different selection options. a) Oval selection, b) Polygon selections, c) Freehand selections

Another versatile selection tool is Wand (tracing) tool which make selection automatically and will produce decent results in good light conditions (Fig 6a). By double-clicking the Wand tool box an option window will appear where tolerance can be adjusted to

match the intended selection (Fig 6b). It is possible to use other selection tools to compensate for the unselected regions by holding the shift key (Fig 6c).

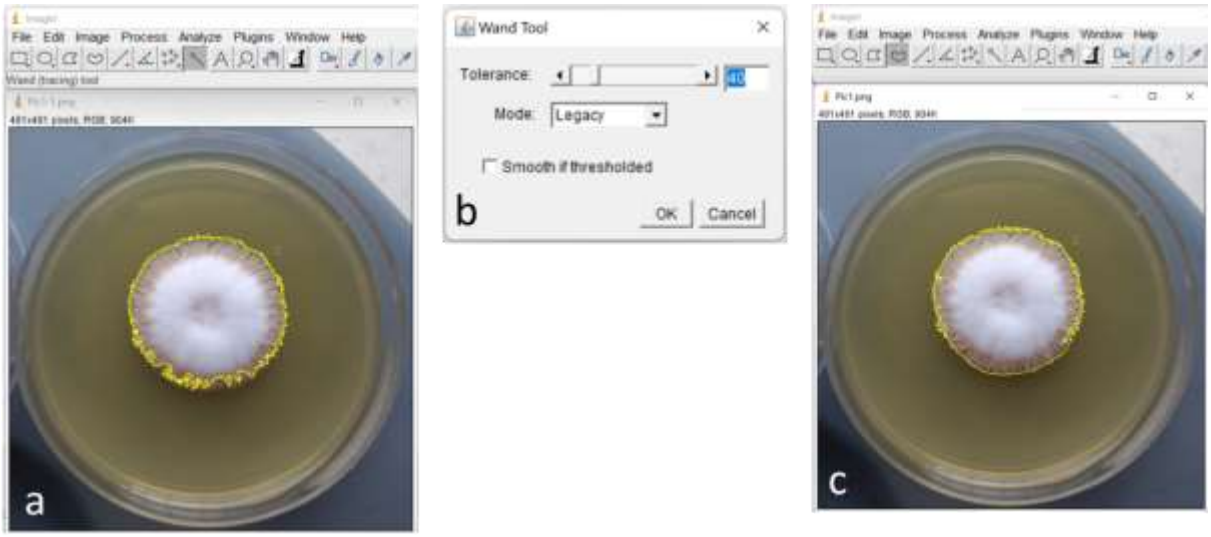

Figure 6. Selection using Wand tool. a) Result of wand tool selection, b) Options window, c) addition of missing parts with free hand selection tool

There is also a possibility to select all pixels based on their color hue, saturation and brightness thresholds by selecting Image → Adjust → Color Threshold. There are other color selection criteria in drop menu of Color Space such as RGB, Lab and YUV (Fig. 7)

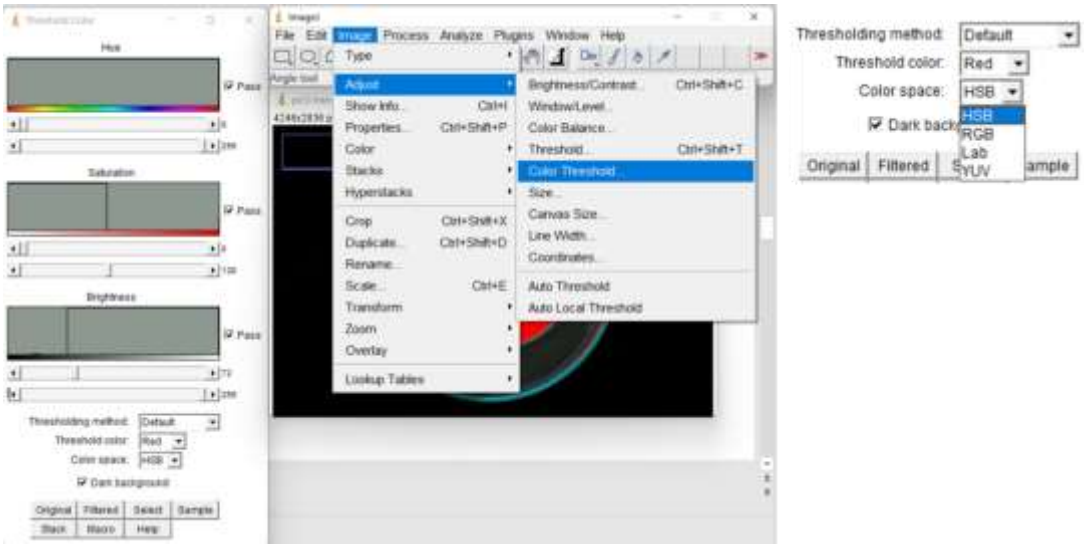

Figure 7. Selection using color threshold

However, the selection of the correct area is highly depended on the color and light conditions. Even in high contrast images the selection may not work properly (Fig. 8).

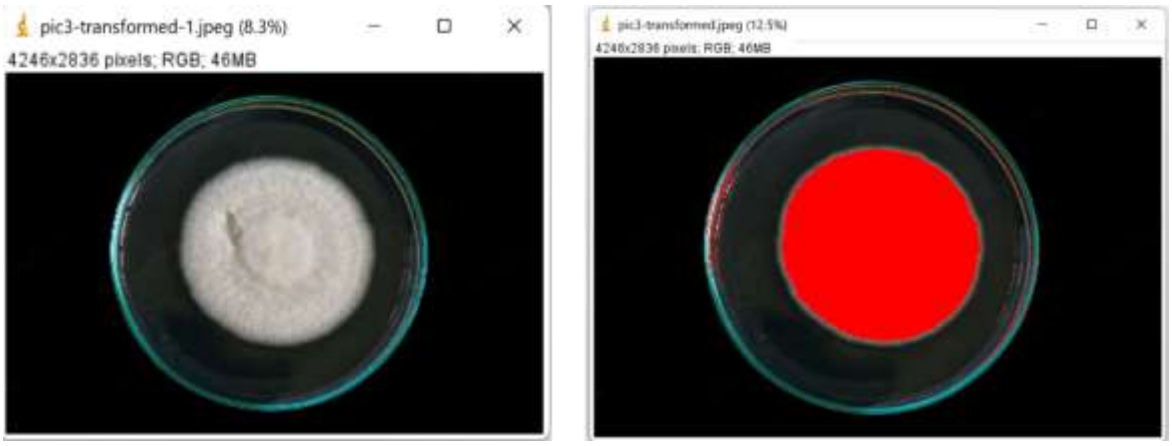

Figure 8. Selection using color threshold. Original image (left) and the selection (right). The selection in the picture is not perfect as lines on the edge are also selected

To solve this problem edges of petri dish can be cropped. For cropping after selecting a greater than the growth area by oval selection tool using Edit → Clear Outside function will remove the edges. Than by adjusting color parameters it is possible to select the exact area of growth (Fig. 9).

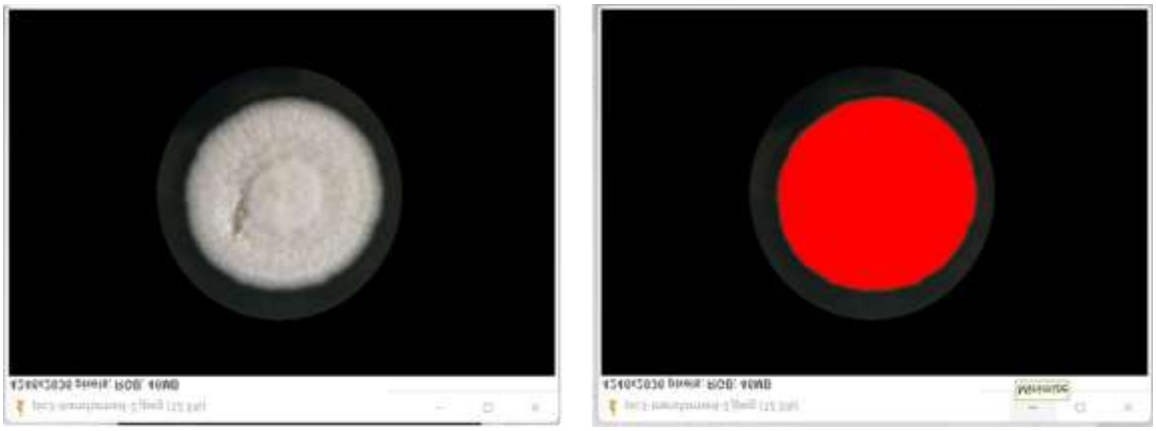

Figure 9. Cropped original image (left) and the selection (right). The seleciton is correct.

To make measurements in ImageJ it need to be calibrated to a known-length object. It can be a ruler or even a petri diameter itself, if the petri size is known. First a staight line is drawn through diameter of petri dish (Fig. 10).

Figure 10. Calibration with 60 mm petri dish

From the Analyze drop menu of the program Set Scale should be chosen (Fig. 11). In the Known distance box, the length of the known object over which the line was drawn should be entered. In the unit length a specific unit by which the calibrator length is measured should be entered. Program will specify the number of pixels per the measured length as the scale ratio. The later measurements will be performed by using to this ratio.

Figure 11. Set Scale options. Since the distance of of the drawn line is known to be 60 mm it is adjusted accordingly.

To cacluate area in the program Analyze → Measure (ctrl+m) should be used (Fig. 12). Since the area is calculated, the measurement can be converted to the diameter by following formula:

- R=(A/3.14)^0.5
- D=2xR

where, R-radius, D-diameter, A-area

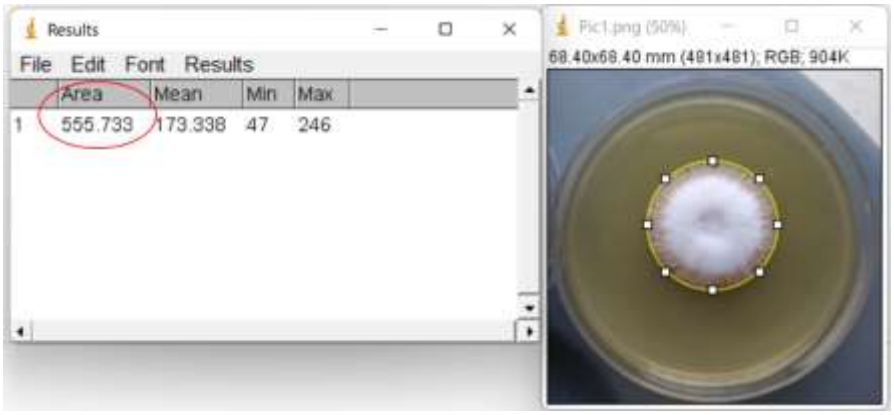

Figure 12. Area calculation after calibration. Area of the selection was measured to be 555.733 mm^2. Diameter is calculated to be 26.6 mm

To shoot the images for ImageJ an appropriate setup would be very helpful. A card box can be used with one face cut for light. Interior should be a dark color. Above a small hole for camera can be craved (Fig. 13). To minimize the margin of error, the distance from which the photo is taken should be the same and the same camera should be used. On these conditions single calibration will suffice. If the distance and/or camera are changed while taking a data for the fungus, it is necessary to recalibrate the program. Photos can also be taken with a smartphone camera that can capture millimeter intervals on the ruler scale.

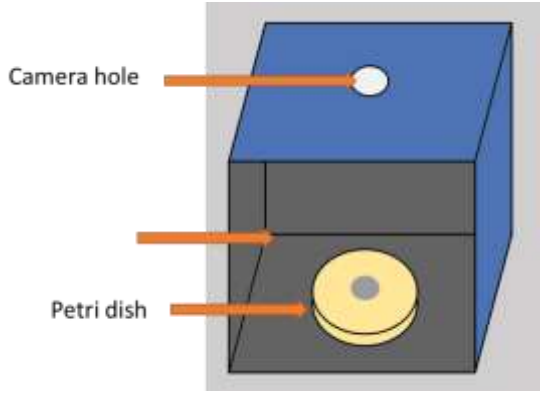

Figure 13. Setup for taking pictures

Advanteges of using ImageJ

- After the setup is prepared, picture taking and data acquisition are faster compared to manual measurements
- Since the diameter is found from the area, the most appropriate average data can be calculated
- Irregularities in growth are automatically added to the selected area with the ImageJ program. Thus, the average of all possible diameter planes is taken
- Since the photoFigures of the measurements are stored, when in doubt that there is any irregularity in the data, the measurement can be confirmed by searching the photoFigure of the measurement taken.

Disadvantages of usning ImageJ

- The researcher is required to have an intermediate level of computer skills
- Before starting the measurements, the camera setup needs to be prepared
- In order to determine the fungus' growth area, the selected area in the pictures must match the growth of fungus. For the best results, the most appropriate parameters with regard to the growth type and color of the fungus, the background and the lighting conditions should be determined.
- Once the parameters are adjusted, they can be used as long as the pictures are taken in same conditions. However, if the lighting conditions are changed the parameters should be re-adjusted.

Application of ImageJ for determination of growth parameters of fungus was adapted and conducted in the Ph. D. Thesis: "Functional Analysis of Fungal Effector Candidate SSP (Small Secreted Protein) Genes of Septoria Leaf Blotch Pathogen (*Zymoseptoria tritici*) In Wheat" (2019). Growth curve of *Z. tritici* was calculated using this method.

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EFFECT OF DIFFERENT FERTILIZATION IN CARROT SEED PRODUCTION ON THE MATHEMATICAL APPROACH OF SEEDLING MORPHOLOGY AND WEIGHT OF 1000 SEEDS

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ABSTRACT

The main goal of the present study was to establish the effect of different fertilization regimes, applied during carrot seed production, on the sowing parameters and seedling morphology behaviors and weight of 1000 seeds. A mathematical approach was used for their group formation – a cluster, correlation and factor analysis, as well as for evaluating the similarity and remoteness of the influence of the studied regimes and their grouping based on main morphological indicators of carrot seedlings. The experiments were carried out in the period 2017 - 2019 at the experimental field of the Agricultural University – Plovdiv, Bulgaria. The increasing levels of nitrogen, phosphorus and potassium fertilizers in two periods of application – once and twice for Tushon variety were studied. Taking into account the average sample, the germination energy, germination, length of embryo root, length of hypostyle, fresh weight of one seedling and dry weight of seedlings were examined. Higher influence on the sowing parameter was established with relation to the higher doses of mineral fertilizers, which provoked the better development of carrot seedlings. Cluster analysis results corresponded to the conclusions about the effect of fertilizer regimes on the sowing qualities. The positive correlation coefficients were registered between the fresh weight of the seedlings and length of embryo root and hypocotyls: r=+0.40 and r=+0.42. The greatest effect of fertilization regimes on the clusters was due to the germination capacity and fresh weight of the seedlings.

Keywords: carrot, correlation, cluster analysis, factor analysis, seedlings, seed

INTRODUCTION

According to Copeland and McDonald (2001) and Panayotov (2015), the yield of vegetable seeds is extremely dependent on the applied nutritional regime. Ilyas et al. (2013) in their study conducted with two applications for planting carrot stecklings for seed production and 30 days later applying 100 kg ha^{-1} nitrogen found a significant improvement in seed quality and seed viability. According to Stepuro (2008), the maintenance of optimal balance of nutritional macronutrients, with a view to obtain high yield and quality of carrot seeds, it could be achieved through fertilization with $N_{45}P_{55}K_{75}$ kg ha^{-1}. Hooda et al. (2014) tested three levels of fertilization in carrot seed production and highlighted that the highest fertilizer rate of 100:50:50 (N:P:K kg.ha^{-1}) led to the greatest increase in seed production, as they also had the highest vital indicators. According to them, this is due to the better development of the plants, especially the central umbel, as well as the formation of more second-order umbels. The highest

seed yield of Nantes carrot varieties, Rao and Maury (1998) was obtained when 80 kg.ha^{-1} nitrogen and 60 kg.ha^{-1} phosphorus were applied. They also pointed out that the influence of nitrogen on seed formation, as well as on their quality features, respectively, were stronger than that of phosphorus. Szafirowska et al. (1996) expressed the opposite opinion that increasing the amount of applied nitrogen had no particular effect on the germination process of carrot seeds. Anouar et al. (2001) pointed out that there was a strong correlation between carrot seed size and germination. The application of different fertilizer rates of nitrogen and potassium significantly affects the germination of carrot seeds, the weight of 1000 seeds and their whole sowing qualities (Amjad et al., 2005).

According to Panayotov et al. (2021), in order to establish the relevant dependencies and trends in various experiments, it is essential that through the application of various mathematical analyzes it is possible to establish the similarity and remoteness of individual signs and indicators and their grouping into clusters, determination of their correlation relationships, as well as by means of factor analysis to unite the factors that are correlated with each other. In this direction, in agronomic science, these possibilities are widely used for grouping and evaluation of individual varieties or different soils properties (Doneva et al., 2008; Krasteva et al., 2010; Panayotov et al., 2010; Milev, et al, 2015).

The main goal of the present study is, by applying different mathematical methods, to establish the relationships, dependencies and trends between individual morphological features of carrot seed seedlings, as well as the weight of 1000 seeds with relation to the use of different fertilizing regimes in seed production.

MATERIAL AND METHODS

The experiments were carried out in the experimental field of the Department of Horticulture and in the Scientific Laboratory of the Department of Mathematics and Informatics at the Agricultural University-Plovdiv, Bulgaria with Tushon carrot variety in the period 2017-2019. The adopted technology in Bulgaria for carrot seed production with previous stecklings described by Minkov (1984) was used. The fertilization was studied in two regimes: once fertilization regime - the whole amount of phosphorus and potassium fertilizers was applied before deep plowing in the autumn, while nitrogen - during steckling planting; twice fertilization regime - before the autumn deep plowing the half of the phosphorus and potassium fertilizers were applied, the other half - in the spring before planting, but nitrogen fertilizer - half during planting, and the other part at the phase beginning of flowering. The experiments were carried out in four replicates with plot size of 6 m^2 in order to establish the seed production for each variant (Barov, 1982). Different levels of fertilization in kg.ha^{-1} were tested:

Once fertilization: 1.$N_0P_0K_0$– control; 2.$N_{70}P_{140}K_{150}$ (recommended by Madzharova, 1968; Kolev, 1977); 3.$N_{50}P_{90}K_{100}$; 4 .$N_{50}P_{90}K_{200}$; 5.$N_{50}P_{190}K_{100}$; 6.$N_{50}P_{190}K_{200}$; 7.$N_{90}P_{90}K_{100;}$ 8.$N_{90}P_{90}K_{200;}$ 9.$N_{90}P_{190}K_{100;}$ 10.$N_{90}P_{190}K_{200;}$

and twice fertilization: 11.$N_{50}P_{90}K_{100}$; 12.$N_{50}P_{90}K_{200}$; 13.$N_{50}P_{190}K_{100}$; 14.$N_{50}P_{190}K_{200}$; 15.$N_{90}P_{90}K_{100;}$ 16.$N_{90}P_{90}K_{200}$; 17.$N_{90}P_{190}K_{100}$; 18.$N_{90}P_{190}K_{200}$.

Seeds were harvested in stage of 60 % of full botanical maturity and the rest - in waxy maturity with subsequent post-harvest ripening for 10 days. Seeds from each umbel were extracted. Analyses were performed on an average sample for each four replicate. Weight of 1000 seeds was determined in four replicates. The energy of germination (first count) and germination (final count), according to ISTA recommendation (ISTA, 2018) were established.

In the moment of germination recording the seedling morphology as length of embryo root and length of hypocotyls on 10 seedlings of four replicates were measured. The fresh weight of one seedling was assessed by measurement of each developed seedling from the four replicates on scale accurate to the third digit and divided into the number of these seedlings. The dry weight of seedlings was established in oven through the method application described by Black et al. (2006). Due to similarity in the trend of the obtained results, the presented data were the average values from the three-year studies. Statistical data processing and the correlation analysis were conducted via methods described by Fowel and Cohen (1992).

The assessment of the effect of different fertilization types was established by comparing the following sowing parameters and seedling morphological indicators: X_1 – energy of germination, X_2 - germination, X_3 – length of embryo root, X_4 – length of hypocotyls, X_5 – fresh weight of one seedling, X_6 – dry weight of seedlings, X_7 – weight of 1000 seeds.

The grouping of the 18 tested variants of fertilization in the above mentioned two regimes was established by a hierarchical cluster analysis. The intergroup connection method (Ward, 1963; Dyuran and Odelly, 1977) was used. The Euclidean intergroup distance was used as a measure of similarity:

$$D(x, y) = \sqrt{\sum_{i=1}^{n} (x_i - y_i)^2}$$

The formed clusters are presented Figureically through the dendrogram, where the rescaled distances at which the clusters are formed by the dotted horizontal lines. The statistically significant correlations between the studied indicators were established through the performed correlation analysis. In order to reduce the number of the initially included indicators for weight the factor analysis technique were done by the by the principal components method (PCA) (Kline, 1994). By the number of eigenvalues of the correlation matrix being greater than 1 (Kaiser's criterion), the number of principal components was determined. The explanation of the total variance in the variables was achieved by the contribution of the eigen-factor that was shown by the eigenvalues. Data processing were performed via the statistical program SPSS.

RESULTS AND DISCUSSION

The bigger amounts of tested mineral fertilizers improved the viability of carrot seeds (Table 1). The highest values of germination energy and germination were found in a once fertilization with $N_{90}P_{90}K_{200}$ -78.88% and 80.88%, respectively, followed by the option with $N_{90}P_{190}K_{100}$. The same trend in the twice fertilization was observed after the application of $N_{50}P_{190}K_{100}$. Krarup et al. (1976) and Gray et al. (1983) also concluded that higher fertilizer rates increased germination energy and germination of carrot seeds. Similar results were reported by Kong LingJuan et al. (2004), Kushawala (2009) and Hooda et al. (2014). Differences for germination in all variants compared to the control were statistically significant, most of them at the p=1.0% level.

For a more detailed assessment of seed sowing qualities, the morphological characteristics of the sprouts are extremely important (Panayotov, 2015). The largest embryo root of 2.45 cm was developed by the seeds from plants fertilized with $N_{50}P_{90}K_{200}$, both in the once and twice regimes. Next were those applied with $N_{50}P_{190}K_{200}$ (twice regime) and of $N_{50}P_{190}K_{100}$ and $N_{90}P_{90}K_{200}$ (once regime). What these variants have in common is that most of them have the highest potassium level of all tested. The hypocotyl is characterized with a longer length. Its values ranged from 4.44 cm for zero fertilization to 5.20 cm and 5.18 cm,

respectively, for varieties with once application of $N_{90}P_{90}K_{200}$ and $N_{50}P_{90}K_{100}$. Taking into account the twice fertilization regime, the greatest hypocotyls growth was observed in application of $N_{90}P_{190}K_{100}$ and $N_{90}P_{190}K_{200,}$ respectively. Statistical significance of differences with the control was established for these variants.

Table 1. Sowing parameters of carrot seeds after application of different fertilization regimes

Variants	Viability indexes		Morphology of seedlings				Weight of 1000 seeds (g)
	Energy of germination (%)	Germination (%)	Length of (cm)		Weight		
			embryo root	hypocotyls	fresh (mg)	dry (%)	
Single fertilization							
$N_0P_0K_0$	59.22	60.88	2.20	4.48	11.23	2.66	0.921
$N_{70}P_{140}K_{150}$	64.55	67.88	2.04	5.05	10.99	4.21	1.028
$N_{50}P_{90}K_{100}$	62.66	69.55	2.09	5.18	11.77	4.11	1.148
$N_{50}P_{90}K_{200}$	69.22	73.10	2.45	5.00	11.65	3.88	1.095
$N_{50}P_{190}K_{100}$	68.44	70.44	2.22	4.83	10.27	4.10	0.923
$N_{50}P_{190}K_{200}$	66.88	70.44	2.20	5.11	10.19	3.33	0.897
$N_{90}P_{90}K_{100}$	61.77	72.21	1.95	5.04	9.88	3.55	0.944
$N_{90}P_{90}K_{200}$	78.88	80.88	2.21	5.20	11.24	3.66	1.083
$N_{90}P_{190}K_{100}$	73.99	76.66	2.06	4.97	9.82	3.21	0.938
$N_{90}P_{190}K_{200}$	63.55	68.22	2.24	4.64	8.73	3.44	0.923
Twofold fertilization							
$N_{50}P_{90}K_{100}$	69.32	74.55	2.02	4.44	9.15	3.88	0.889
$N_{50}P_{90}K_{200}$	69.44	76.22	2.45	4.74	10.46	3.22	1.064
$N_{50}P_{190}K_{100}$	77.10	79.77	1.98	4.70	9.44	3.66	0.931
$N_{50}P_{190}K_{200}$	65.10	69.10	2.30	4.91	9.40	3.11	0.895
$N_{90}P_{90}K_{100}$	66.44	70.44	2.21	4.90	9.06	3.88	0.887
$N_{90}P_{90}K_{200}$	68.88	75.66	2.19	4.79	10.07	4.11	1.166
$N_{90}P_{190}K_{100}$	66.11	71.10	1.94	5.01	9.64	4.10	0.835
$N_{90}P_{190}K_{200}$	62.11	65.44	2.09	4.94	10.28	3.44	0.847
p=5.0%	7.24	7.34	0.41	0.52	1.90	0.88	0.090
GDp=1.0%	9.70	9.84	0.55	0.70	2.55	1.18	0.130
p=0.1%	12.81	12.98	0.73	0.92	3.36	1.55	0.170
	r*=+0.40	r**=+0.42					

Correlation dependence between fresh weight and: r* length of embryo root; r**length of hypocotyls

Several authors pointed out that the fresh weight of seedlings was directly related to the vigour of the seeds (Copeland and McDonald, 2001; Black et al., 2006). In this regard, applied fertilization levels have a significant impact on this trait. The seeds obtained after a once fertilization with $N_{50}P_{90}K_{100}$ and with $N_{50}P_{90}K_{200}$ developed the highest fresh weight per seedling - 11.77 mg and 11.65 mg, respectively. A certain reduction in this fertilization regime was found for the variants with application of $N_{70}P_{140}K_{150}$, $N_{90}P_{90}K_{100}$, $N_{90}P_{19}0K_{100}$ and $N_{90}P_{190}K_{200}$. Taking into account the twice application of mineral fertilizers, better results were found for combinations with $N_{50}P_{90}K_{200}$ and $N_{90}P_{190}K_{200}$. It can be pointed out that even

regarding the fresh weight, as was emphasized for the embryo root, these varieties are characterized with higher amounts of potassium. A positive correlation was found between the fresh weight of seedlings and the length of the embryo root and also hypocotyls length, with correlation coefficients of r=+0.40 and r=+0.42, respectively.

A more detailed idea for the vital status of seeds, besides the fresh weight of seedlings, can also be obtained from their dry weight. After a once application of $N_{70}P_{140}K_{150}$ and $N_{50}P_{90}K_{100}$, the dry weight reached values of 4.21% and 4.11% against 2.66% for the control. A similar increase was obtained with a twice application of $N_{50}P_{90}K_{200}$ and $N_{90}P_{190}K_{200}$. Statistical significance of the difference was found, except for variants with $N_{50}P_{190}K_{200}$ and $N_{90}P_{190}K_{100}$ (once) and for $N_{50}P_{90}K_{200}$, $N_{50}P_{190}K_{200}$ and $N_{90}P_{190}K_{200}$ (twice).

The weight of 1000 seeds is the highest after the twice fertilization with $N_{90}P_{90}K_{200}$ – 1.166 g. It was followed by the $N_{50}P_{90}K_{100}$ variant with the once application - 1,148 g. Relatively high results were observed for both $N_{90}P_{90}K_{200}$ (once) and $N_{50}P_{90}K_{200}$ (twice), where the increase compared to the control was 17.58% and 15.52%. The differences with the control of these variants were mathematically proven. Amjad et al. (2005) also pointed out that nitrogen and potassium fertilization increased the weight of 1000 carrot seeds, especially when applying 75 kg.ha^{-1} N and 90 kg.ha^{-1} K_2O.

The performed cluster analysis indicated that the effect of the fertilization regime on the morphological parameters of carrot seed seedlings was grouped into three main clusters. The results are presented both tabularly, with the steps of combining the clusters and intergroup distances (Table 2), and Figureically through a dendrogram (Figure 1).

The first cluster was dived into three sub-clusters. The first sub-cluster consisted of variants with once fertilization 5 ($N_{50}P_{190}K_{100}$) and 10 ($N_{90}P_{190}K_{200}$), and with twice fertilization - 15 ($N_{90}P_{90}K_{100}$). The second sub-cluster included variants 6 ($N_{50}P_{190}K_{200}$ once) and variants with twice application of mineral fertilizers 14 ($N_{50}P_{190}K_{200}$ and 18 ($N_{90}P_{190}K_{200}$). The third sub-cluster consisted of variants with once fertilization 7 ($N_{90}P_{90}K_{100}$) and with twice fertilization 17 ($N_{90}P_{190}K_{100}$), all are similar in terms of indicators: seed germination, length of embryo root, length of hypocotyls, dry weight of sprouts and weight of 1000 seeds.

The second main cluster included variants 9 ($N_{90}P_{190}K_{100}$), 13 ($N_{50}P_{190}K_{100}$) and 11 ($N_{50}P_{90}K_{100}$) of once application, which were most similar in terms of: length of embryo root, length of hypocotyl, fresh weight per seedling, seedling dry weight, weight of 1000 seed.

Variants 2 ($N_{70}P_{140}K_{150}$), 3 ($N_{50}P_{90}K_{100}$), 4 ($N_{50}P_{90}K_{200}$) and 8 ($N_{90}P_{90}K_{200}$) of the once regime and also 12 ($N_{50}P_{90}K_{200}$) and 16 ($N_{90}P_{90}K_{200}$) of the twice regime form the third cluster were identical in terms of the following parameters: length of embryo root, length of hypocotyls, fresh weight per seedling and 1000 seed weight.

The most distant were variants 1 ($N_0P_0K_0$) and 2 ($N_{70}P_{140}K_{150}$) of the once fertilization regime, with intergroup distance having a coefficient of 25.590 shown in Table 2.

During the performance of the factor analysis and the application of the method of principal components, it turned out that two of the factors had values of eigenvectors greater than 1, which determined the selection of two principal components (Figure 2).

The influence of the individual features on the grouping is according to the scree test criterion. Three components were formed. Table 3 gives the factor weights and shows how the variance is distributed among the principal components. The data from the table show that the first component is most strongly correlated with germination energy, germination, hypocotyls length and weight of 1000 seed, i.e. have high factor weights. It could be defined that this factor

that have the greatest relative weight in the grouping of the morphological indicators of the carrot seedlings and their weight of 1000 seeds. The second component is in the highest correlation mainly with germination energy, germination and fresh weight of a seedling, the third component – with the length of the embryo root and absolute dry weight of the seedlings.

Table 2. Combining clusters and inter-cluster distances

Stage	Cluster Combined		Coefficients
	Cluster 1	Cluster 2	
1	5	15	2,446
2	6	14	2,494
3	2	3	2,883
4	7	17	3,599
5	9	13	3,820
6	6	18	3,946
7	5	10	5,189
8	5	6	5,533
9	4	12	5,993
10	5	7	7,201
11	9	11	7,714
12	4	16	8,264
13	5	9	11,252
14	4	8	11,455
15	2	4	14,144
16	2	5	16,390
17	1	2	25,590

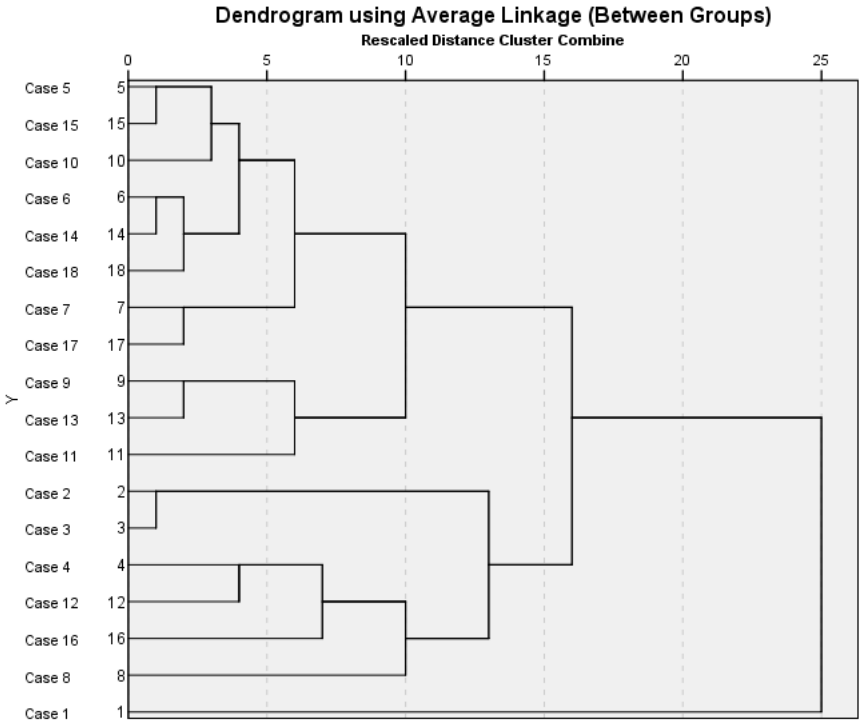

Figure 1. Dendrogram based on mean intergroup distances

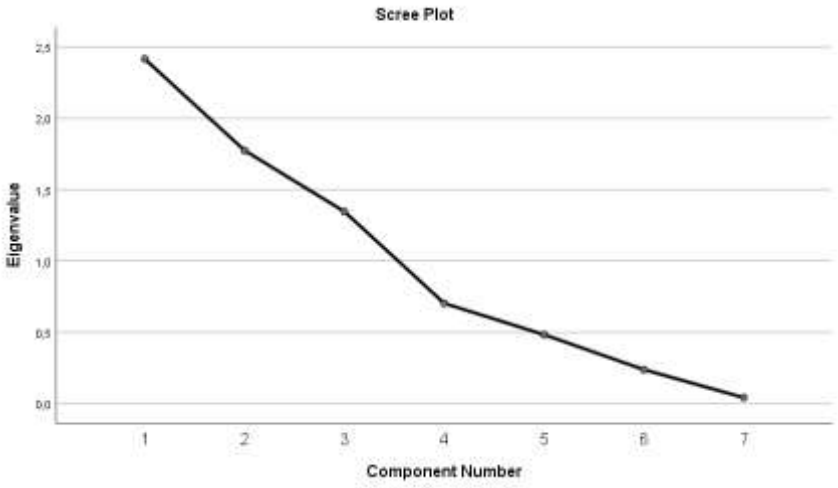

Figure 2. Values of the eigenvectors

They explain 79.08% of the total variance of the sample. The first principal component (the first factor) explains 34.51% of the variance, the second - 25.34% of it, and the third factor - 19.22%. The results obtained through the applied factor analysis are synchronous with the results of the cluster analysis when they are distributed into clusters according to their proximity

of the base on the same indicators. The correlations between the variable values of the studied features and the main components are expressed Figureically (Figure 3)

Table 3. Factor matrix obtained by the method of principal components

N	Indicators	Main components		
		1	2	3
1.	Energy of germination	0,680	-0,603	0,301
2.	Germination	0,741	-0,609	0,208
3.	Length of embryo root	0,178	0,451	0,742
4.	Length of hypocotyls	0,539	0,266	-0,484
5.	Fresh weight of one seedling	0,488	0,757	-0,040
6.	Dry weight of seedlings	0,492	-0,119	-0,638
7.	Weight of 1000 seeds	0,776	0,422	0,133
Percentage of the total variation ,%		34,51	25,34	19,22
Cumulative percentage of the total variation ,%		34,51	59,86	79,08

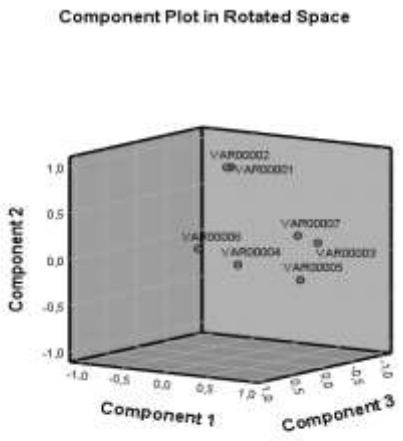

Figure 3. Projection of the considered indicators in the PC – plane

The proposed mathematical approach allows the increase of objective evaluation of the complex effect of the applied fertilization on the increase of 1000 seed weight, fresh weight of a seedling and embryo root length.

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CONCLUSIONS

The higher fertilizer rates improve significantly the germination energy and germination of carrot seeds. Embryo root length is the highest at $N_{50}P_{90}K_{200}$ application, despite the application regime, and the hypocotyl length is the largest after once fertilization with $N_{90}P_{90}K_{200}$ и с $N_{50}P_{90}K_{100}$. The fresh weight of seedlings is improved most significantly after once fertilization with the following combinations: $N_{50}P_{90}K_{100}$ and $N_{50}P_{90}K_{200}$. As a result of the mineral fertilization, their dry weight also increases significantly. The high values of the weight of 1000 seeds are observed after the once application of $N_{50}P_{90}K_{100}$ and $N_{90}P_{90}K_{200}$ at both fertilization regimes. The conducted cluster analysis shows that the effect of fertilization regime on the morphological indicators of carrot seedlings is grouped into three main clusters. The main indicators having the biggest influence on the division of variants are as follows: germination energy, germination, hypocotyl length, weight of 1000 seed. It means that thy have high factor weights combined into the first factor explaining 79,08 % of the total dispersion of variables. The performed classification of the included variants leads to higher objectivity at the evaluation of the effect of the examined indicators. The factor analysis results, where the examined indicators are grouped into three new factors, allow more effective planning of the further stages of the study.

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FUNGAL DISEASES IN LAVENDER (*Lavandula spp*.)

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ABSTRACT

Lavender (*Lavandula spp*.) is a plant that draws attention worldwide as an aromatic and ornamental plant especially, and is used for many different purposes. It is preferred because of its ability for adaptation to different climatic and soil conditions, and for resistance to drought and diseases. However, the studies from different countries showed that fungal diseases caused by *Phytophthora spp., Fusarium spp., Septoria lavandulae, Rhizoctonia solani, Botrytis cinerea, Sclerotinia sclerotiorum, Phoma lavanduae* and *Phomopsis lavandulae* caused significant losses in lavender. *L. angustifolia, L. stoechas, L. pubescens, L × intermedia, L. × allardii, L. dentata* and *L. hybrid* are among the lavender species which these diseases appeared. In this review, the symptoms of fungal diseases reported in different lavender species and some morphological characteristics of the fungal species are mentioned in the light of the current literature.

Key Words: Lavanta, *Lavandula spp.*, fungal diseases

INTRODUCTION

Water resources needed for agricultural production are decreasing under the influence of climate change. This situation is one of the main factors limiting agricultural development between 2000 and 2025, especially in the Mediterranean region (Chartzoulakis et al. 2002; Rey et al., 2017). Global surface temperatures are expected to increase by 1.1°C to 6.4°C by the end of the current century according to the 4th report of the International Panel on Climate Change (IPCC) (Fischlin et al. 2007; Xu et al., 2010). Drought as a result of the increase in average temperatures has a negative impact in Turkey as outher countries of the world due to its geoFigureical location (Partigöç and Soğancı, 2019). It is predicted that the Balkans, North Africa and the Iberian Peninsula, especially Turkey, will experience higher temperature values of 40-50% than the global average (Doblas-Reyes et al 2021; IPCC, 2021). The decreasing water resources should be used efficiently to counteract climate change and ensure the sustainability of agricultural production. For this reason, it is necessary for the producer to grow economic plants that can be grown with limited irrigation, that are resistant to diseases and pests, and to take care of these plants.

Lavender (*Lavandula spp*.) is a plant that tends towards resistance to drought, diseases and pests. It takes its name from the Latin word “lavare” which means to wash. It was found in the texts of the Greek botanist Theophrastus, who lived between 370-285 before Christ. It has been used by the Romans for a long time in baths and therapy (Lis-Balchin, 2002). It is still

used in Tibetan Buddhist medicine to treat psychological diseases (Basch et al, 2004) while it was used in mummification by the Egyptians in ancient times,

Lavandula spp. are a perennial plant belonging to the Lamiaceae family and 603 species of this family have been identified in Turkey (Renaud et al., 2001; Celep and Dirmenci, 2017). Three important types produced are lavender (*Lavandula angustifolia* Mill.), lavandin *(Lavandula intermedia*) and spike lavender (*Lavandula spica* L. = *L. latifolia* Medik.) (Kara and Baydar, 2013; Tucker 1985). Lavender was planted on 22,188 decares and production of 3.499 tons was made in Turkey (TUIK 2021). Thus, it has taken its place as the most produced plant after oil rose among aromatic plants.

The lavender plant has a 15-20 cm long flower spike-panicle axis at the tip of the flower stalk. Its flowers are purple-lilac colored and fragrant. Essential oils are obtained from the flower and flower stalks of the plant (Karık et al., 2017). The roots of the lavender plant, which form many lateral branches, go deep to the soil until approximately 80 to 100 cm. The color of the fruits changes from dark brown to black (Sönmez et al., 2018).

Lavender species have many different uses such as perfume, cosmetics, aromatherapy, pharmacy, and are also grown as an ornamental plant. In the beekeeping industry, lavender honey is obtained from the pollen of *L. latifolia*, which is known as Spike lavender (Castro-Vázquez et al., 2014). It has curative effect for insomnia, stress and anxiety when dried plant parts are consumed by brewing (Üstü and Uğurlu, 2019). The oils obtained from lavender are also effective on bacteria and fungi. *L. angustifolia* prevent the conidial germination and growth of the germ tube of *Botrytis cinerea* (Cavanagh and Wilkinson, 2002). Turgut et al. (2017) reported that the essential oil of *Lavandula angustifolia* completely inhibited the contamination of cosmetic producs by microorganisms.

In this review, fungal diseases observed during the cultivation of *Lavandula spp.* were examined in the light of current literature. The review was supported with images of the symptoms of some diseases. In order to understand the biology and infection mechanisms of the pathogens, some characteristics were also given.

Phytophthora ROOT AND CROWN ROT

The disease is caused by different *Phytophthora* species, occurs generally in field conditions, rarely in greenhouses and has worldwide economic importance. The pathogen fungus absolutely requires water to complete its life cycle and therefore prefers extremely moist soils. It can remain dormant in the soil for long periods, however, it becomes active and reinfects the plants under favorable conditions (Gonzalez, 2016). Sexual reproduction occurs through oospores. They survive with chlamydospores in the soil or in plant debris under unfavourable environmental conditions. The disease spreads easily during mild weather and high humidity conditions (Giesbrecht et al., 2014).

P. parasitica, which causes symptoms such as wilting, browning of root and stem on lavender plant, was observed in Taiwan, and it was found that the pathogen showed good hyphal development at 28℃ (Tsay, 2002). *Phytophthora palmivora* and *P. nicotiana* have been reported to cause wilt on *L. angustifolia* in Italy (Davino et al., 2002). These two *Phytophthora* species were isolated in subsequent years in the United States from hybrid lavenders (*Lavandula x intermedia*) that exhibited symptoms of root rot, and were reported to be pathogenic (Dlugos and Jeffers, 2019). *P. nicotiniana* was also found on lavender leaves with gray coloration and wilted plants in Spain (Alvarez et al. 2007).

Derviş et al. (2011) detected root rot symptoms in 2-year-old seedlings of *Lavandula angustifolia* grown in Hatay, Turkey. These authors identified the fungus as *Phytophythora palmivora* based on classical (chlamydospore size, sporangium structure, etc.) and molecular tests. In addition, they determined it as pathogenic based on pot tests.

L. angustifolia grown as rootstock was observed to have crown rot and blackening of roots, browning and wilting of shoots (Figure 1) in Lithuania. Yellowing, brown discoloration under the bark, dark gray spots later turning brown on the shoot and death of the plants in severe infections were other observed symptoms. *Phytophthora cinnamomi* was isolated from the infected parts. The pathogen caused lesions of different sizes depending on the cultivar when it was inoculated on stems and leaves of different lavender cultivars (Orilowski and Valjuskaite, 2007).

Figure 1. Wilting of lavender cuttings caused by *Phythophythora* (Orliovski & Valjuskaite, 2007).

Phytophthora x pelgrandis, a hybrid of *P. nicotiana* and *P. cactorum*, caused root and crown rot, yellow spots on leaves, and killed the plant in the late growth stage of *L. stoechas* in Italy. As a result of isolation in 2 different media, whitish colonies composed of non-aerial mycelium developed on the PDA (Potato Dextrose Agar) medium and a colony with patterned and aerial mycelium developed in the medium of V8A (Faedda et al. 2013).

Chen et al. (2017) observed in the Taiwan region of China that the roots of *L. angustifolia* turned brown, the plant became pale and the tips of the plant turned gray. In the study, a pathogenicity test was conducted using isolates from diseased plants, *P. cactorum* was found to be pathogenic and this pathogen was reported for the first time in China.

Fusarium WILT

The disease is caused by different *Fusarium* species. These species have a fairly wide host range and are soil-borne. They prefer hot and humid weather conditions. *Fusarium* species generally survive for a long time in the soil as chlamydospore form. Contaminated seeds with these species are transported with tools, equipment and water. *Fusarium* spp. pose a threat to

cultivated lavender varieties. To date, three species have been identified on lavander. Among them *F. solani* caused wilting of the entire plant resulting in yield losses. The disease, which appeared in *Lavandula angustifolia* and which exhibited symptoms of necrosis and chlorosis, was detected in China. Colony development with wooly-cotton appearance, thick-walled and curved macroconidia were observed on PDA (Ren et al. 2008). *F. sphorotrichioides*, caused stunting, chlorosis, and dieback in addition to wilting symptoms in late growth stages of *L.* × *intermedia* species. On PDA medium, the fungus initially formed white colonies, which then turned red in color (Cosic et al., 2012). In *L. pubcens* grown in a greenhouse in Saudi Arabia, wilting, browning of the vascular tissues and dark coloration on many parts of the plant were observed, and the pathogen was identified as *F. oxysporum* (Perveen and Bokhari, 2010). The pathogen was also detected in the hybrid *L.* × *allardii* in northern Italy in 2018, it had macroconidia with an average size of 7.4 × 3.3 μm, with 3 septa within the pale orange sporodocium in the CLA (Carnation Leaf Agar) medium (Ortu et al., 2018). In 2021, it was detected in *L. angustifolia* in Turkey, and the pathogen was reported to produce macroconidia with an average size of 27.5 × 4.8 μm on PDA medium. It was also found that the agent was a pathogen (Ozer et al., 2021).

Sclerotinia STEM ROT

The pathogen, *Sclerotinia sclerotiorum* has a large number of hosts. The agent generally causes water-soaked spots on the leaves and stems of plants, and dark lesions on the stem, the typical fluffy white mycelium of the pathogen develops on the lesions (Bolton et al., 2005).

The pathogen was isolated from *L. hybrida* grown in a greenhouse in Argentina and caused necrosis of the stem, abnormal coloration of the leaves, wilting and death in the following days. In the pathogenicity tests performed with *L. hybrida* and *L. dentata*, the formation of sclerotia on the stems of the plants was observed at 10 days after inoculation (Cicerchia and Wright, 2010).

S. sclerotiorum was first observed on *Lavandula stoechas* in northern Italy. Symptoms such as necrosis on roots, darkening and wilting of leaves of infected plants were observed, and plants were covered with a white mycelial layer when relative humidity was high (Garibaldi et al., 2015).

Rhizoctonia ROT

Rhizoctonia spp. are soil-borne fungal pathogens that have a destructive effect especially on young plants (Ajayi and Bradley, 2017). It is known that they can maintain their vitality for a long time in plant residues in soil with resistant fungal tissues they produce (Baysal-Gürel et al., 2019).

Based on the hyphal fusion of isolates used to determine the anastomosis groups formed by *Rhizoctonia solani* (Carling, 1996), the presence of the anastomosis group AG -1-1B was found in *L. angustifolia* in northern Italy (Garibaldi et al. 2013) and was also reported in *L. stoechas* in Italy (Bertetti et al., 2015). Recently, it was reported in a lavender cultivated areas in Gangwon Province, Korea (Aktaruzzaman et al. 2020). The authors reported that the disease called web blight and appeared as blight on the leaves and the development of white mycelium on the stem was caused by *R. solani*. The pathogen also caused round and wet spots on the leaves of the plants. It was observed that it initially showed white mycelia at 20°C in the nutrient

medium and dark brown sclerotia formed on the mycelium 10 days after inoculation, and it was reported that the isolate belonged to the AG -1-1B anastomosis group.

GREY MOLD

The disease agent *Botrytis cinerea* is a common pathogen in many areas, has a wide host range and develops very rapidly. It is found in the host as a saprophyte. Its conidia are ovoid, unicellular and non-septate, and appear gray due to the dark color of its conidiophores (Oliveira Michelle, 2014).

Botrytis cinerea was first observed on *Lavandula stoaches* in Italy, causing spots on the leaves and stems of the plant, which initially yellow and later became necrotic, after which the typical gray-colored mycelia of the pathogen developed. The sclerotia formed by the pathogen on the PDA medium were small, dark colored and irregular. The presence of *B. cinerea* was confirmed by molecular analyzes and pathogenicity tests (Garibaldi et al. 2010). This study reported that this pathogen was also observed in *L. angustifolia* in different countries.

***Septoria* LEAF SPOT**

Septoria lavandulae, which causes the disease, is known for the pycnidia it formed on the spots (Crous et al., 2009). The disease poses a serious threat in lavender production as it causes deterioration of the essential oil quality of the plant, loss of yield, and poor rooting (Vasileva, 2015). The pycnids of the pathogen, which was determined in *L. dentata*, are between 40 and 49 µm in diameter, and their conidia (22-36x1.5-1.7 µm) with 3 to 4 septate are in the form of filiform (Buonaurio et al., 1996). This species was detected in the samples of diseased *L. angustifolia* in Bulgaria, and the severity of the disease increased with the presence of *Phoma lavandulae* (Nakova, 2011).

Vrandecic et al. (2014) determined the disease in *Lavandula* × *intermedia* in Bulgaria. They detected oval necrotic spots (Figure 2 and Figure 3) on the branches and leaves of the plant, and pycnidia in the middle of these spots. The first symptoms were usually seen on the lower leaves, with the progression of the disease the leaves turned yellow and remained small. The authors reported that the pathogen has long, straight or 3-4-septate conidia with curved ends and they determined that it was pathogenic in pathogenicity tests.

Figure 2. Necrotic spots on the upper part of the lavender leaf (Steenwinkel, 2018).

Figure 3. Necrotic spots on the lower part of the lavender leaf (Steenwinkel, 2018)

Phoma and *Phomopsis* ROT

Phoma spp. are fungi commonly found in soil and usually attack the roots of plants. The species are distinguished on the basis of their structures such as conidia, chlamydospores and pycnidia. The colonies is variably colored, powdery, and has a velvety texture (Bennett et al. 2018; Rai et al. 2013).

Phoma lavandulae, which occurs on lavender, causes wilting and loss of color on infected plants in the period when air temperatures begin to rise, and then it causes death as in Figure 4 (Metcalfe, 1931). While yellow spots form on the leaves and branches, these spots gradually dry on the stem and turn into grayish necrotic spots. *Phomopsis lavandulae*, which forms dark pycnidia in lavender, causes small yellow spots on leaves and long necrotic spots on the branches and forms cracks (Vasileva and Nakova, 2015). Pycnidia are round in roots and conidia are two-celled and pointed (Nakova, 2011).

Figure 4. Dieback of the plant caused by *Phoma lavandulae*. (Cattlin, 2019).

OTHER IDENTIFIED FUNGAL DISEASES

In addition to these known fungal pathogens, *Alternaria alternata* has been observed in *L. spica*, *L. stoaches* (Carta et al. 1983), and *L. officinalis*. *Colletothricum* species have also been detected in this lavender species (Perello and Dal Bello, 1995). Vasileva (2015) determined *Armillaria mellea* on *L. officinalis* and noted that it caused darkening along with structures called rhizomorphs in the roots, and informed that *Rosellinia necatrix* with a white mycelium has also been reported in Bulgaria.

CONCLUSION

Lavender, cultivated as an ornamental, medicinal and aromatic plant, is important worldwide. The quality and productivity of its oil, obtaining healthy plants are the commercial characteristics as desired for other plants. This review has shown that numerous fungal diseases have been detected over the years in different countries where different species of lavender were cultivated although lavender was reported as resistant to the diseases. However, there is no study on the control methods of these diseases in lavender. The detection of pathogens that can cause economic losses in this plant, whose production is increasing, will help to determine control methods against these diseases. Based on the information on isolation and morphological characteristics of pathogens on lavender species that cause diseases, studies on biological and chemical control can be conducted. In addition, there is a need for research on how fungal diseases affect the yield of this plant and, the quality, quantity and amount of oil obtained from the plant.

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EFFECTS OF MYCORRHIZA EFFECTIVENESS AND DIFFERENT ADHESIVE AGENTS USED IN BACTERIA INOCULATION PROCESS TO CHICKPEA FOR NODULATION AND SOME SEEDLING GROWTH

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ABSTRACT

In this study, the effects of different adhesives agents used in bacteria inoculation and mycorrhiza application on nodulation and some seedling properties of chickpea were investigated. The research was carried out in as pot trial at Isparta University of Applied Sciences, Faculty of Agriculture in 2020. Yaşa-05 chickpea cultivar was used in the study. Seeds were inoculated with *Rhizobium ciceri* using different adhesive agents (sugar, molasses, guar, whey, seaweed, gum arabic). In the study, 83 g / da Glomus spp. ($1x10^4$ w/w) mycorrhiza species were used for each a pot. In the study, 48 pots of 2.8 lt size and 2 kg soil-peat (1:1) mixture mortar were used in each pot. The experiment was set up in a randomized plot design with 3 replications. In the experiment, seedling and root length, number of nodules, seedling fresh and dry weight, root fresh and dry root weight properties were investigated. As a result, according to the data obtained from the study, mycorrhiza application with *Rhizobium ciceri* the use of gum arabic as bacteria adhesive agents had positive effects on the nodule number, seedling and root development.

Keywords: Chickpea, *Rhizobium ciceri*, mycorrhiza, bacterial adhesive agents, nodulation and seedling properties

INTRODUCTION

In our country, chickpea plant has been grown for a long time and used in human and animal nutrition. In addition, chickpeas are also grown to rehabilitate degraded soils. An important source of nitrogen fixed to the soil in agricultural areas is Rhizobium-legume mutualist lives. As a result of this symbiosis, 6.4-21.6 kg of nitrogen per decare is attached to the soil annually thanks to edible legumes (Şehirali, 1988). Although chickpea farming is so widespread, it is still not widespread to plant it by inoculating it with bacteria that positively affect its production and yield. The absence of natural *Rhizobium ciceri* bacteria specific to chickpea in many growing regions necessitates nitrogen fertilization, which is needed for plant growth in these regions. Making the necessary nitrogen fertilization causes both extra cost, labor and environmental pollution. With nitrogen fixation, chickpea can meet 60% to 70% of the total nitrogen it needs. Considering both the cost and environmental damage of fertilization, bacterial inoculation will be more beneficial (Meral et al., 1998). As a result of inoculation of legumes with bacteria, there is an increase in their yield, which has been determined by many researchers (Kaya, 2002; Söğüt, 2005; Kağan, 2012). Saleh et al. (2013) in their study

examining the effects of different bacterial adhesives (sucrose, molasses, peptone and glycerol) on the yield of black gram, reported that the sticker they used as peptone had the highest yield in black gram. Many different substances are used in bacterial adhesion. Adhesives used in the experiments are substances such as sugar, gum arabic, peptone, guar, methyl cellulose, polyvinyl pyrollidone, caseinate salts etc.

"Myco" means fungus, "Rhiza" means root. It is the mutual and beneficial relationship between the mycorrhizal fungi in the roots and the plant. Mycorrhizal fungi colonize plant roots and as a true extension of the root system, they enter the places where the roots cannot enter and reach, ensuring that the plant nutrients and water can be taken by the plant. Mycorrhizal infection can reduce the negative effects of polluted soils on the plant body. Mycorrhiza protects the plant against plant diseases and damages by feeding it well, and it becomes effective by fighting with other organisms directly in the rhizosphere (Anonymous, 2022). Various researchers have determined that there are different growth increases in different plants at the end of their studies on mycorrhizae (Palta et al., 2010; Erzurumlu and Kara, 2014; Küçükyumuk et al., 2014).

In this study, the effects of nodulation and mycorrhizae activity of different adhesives used in bacterial inoculation and some agricultural properties in drought-resistant chickpeas were investigated.

MATERIAL AND METHOD

The research was carried out in Isparta University of Applied Sciences, Faculty of Agriculture, Cereals and Edible Legumes Laboratory in 2018. In the study, the seeds of the Yaşa-05 chickpea cultivar were divided into two groups, both bacteria (*Rhizobium ciceri*) and mycorrhiza were applied to one group, and only bacteria were applied to the other. Inoculation was made using different adhesives (sugar, molasses, whey, guar, seaweed, gum arabic), which is another factor in the study. In the study, 48 pots of 2.8 lt size and 2 kg soil-peat (1-1) mixture in each pot were used. 3 g of *Rhizobium ciceri* bacteria were applied to each pot. 47 mg of mycorrhiza was applied to the pots in which mycorrhiza was applied.

The study was set up in a randomized plot design with 3 replications. Properties such as seedling length, number of nodules, fresh weight, dry weight, root length, fresh root weight and dry root weight were investigated on 5 randomly selected plants. The results obtained from the experiment were subjected to analysis of variance (TOTEMSTAT). LSD multiple comparison test was used to determine the differences between the means of the applications ($P < 0.05$).

RESULTS and DISCUSSION

In the study in which the effects of different bacterial inoculation adhesives and mycorrhizal activity on chickpea were investigated, the effect on root length was statistically significant at the 0.01 level. In terms of seedling length, the interaction of bacterial inoculation adhesives and bacterial inoculation adhesives x mycorrhiza application was found to be significant, while the mycorrhizal application was found to be statistically insignificant. In the study, the highest root length was determined in gum arabic (25.8 cm) applications, and the lowest in pure bacteria (12.7 cm) applications. Mycorrhiza application had a positive effect on root length. When the interaction was examined, the highest root length was obtained from the application of gum arabic whether mycorrhiza was applied or not. The seedling length characteristic varied between 13.6 and 24.1 cm according to the bacterial adhesives. While the

highest root length was obtained in the application of gum arabic, it was obtained from the application of pure bacteria. Mycorrhiza applications were found to be statistically insignificant. When the interaction was examined, the highest seedling length was obtained in the arabic gum application in both applications. The lowest seedling length was determined in seaweed in mycorrhiza applied sticker applications, and the pure bacteria application and seaweed applications were statistically in the same group. In the sticker applications without mycorrhiza, the lowest seedling length was determined in guar glue, and there was no statistical difference between the guar glue and pure bacteria application (Table 1). Gum arabic is a complex carbohydrate extracted from Acacia and generally used in legume inoculation (Deaker et al, 2004). Vincent et al. (1962) found that gum arabic not only protected cells against desiccation on beads but resulted in better survival on seeds than maltose suggesting some protection against toxic seedcoat factors. In our study, arabic gum both root length and seedling length have the highest values. Therefore, we think that arabic gum is more effective than other applications.

Table 1. Seedling and root length averages as a result of different adhesive and mycorrhiza applications

	Root length (cm)			**Seedling length (cm)**		
	M0	**M1**	**Mean**	**M0**	**M1**	**Mean**
Control	12,80 c	21,02 bc	***16,91 B***	17,33 cd	18,30 b-d	***17,81 BC***
Bakteria	9,27 d	16,07 e	***12,67 C***	13,05 e	14,07 e	***13,56 D***
Sugar	13,03 c	18,80 cd	***15,92 B***	17,62 cd	16,40 c-e	***17,01 BC***
Molasses	12,60 c	22,57 b	***17,58 B***	16,78 d	19,63 ab	***18,20 B***
Guar	9,23 d	21,68 b	***15,46 B***	11,94 e	18,87 bc	***15,41 CD***
Whey	14,57 c	18,13 de	***16,35 B***	19,75 c	15,82 de	***17,79 BC***
Seaweed	20,26 b	15,86 e	***18,06 B***	23,34 b	13,88 e	***18,61 B***
Arabic gum	26,07 a	25,46 a	***25,77 A***	26,07 a	22,03 a	***24,05 A***
Mean	**14,73 B**	**19,95 A**		**18,23**	**17,38**	

M0: Without mycorrhiza applications; M1: mycorrhiza applications

In the study in which mycorrhiza and different adhesives were applied to chickpeas, the interaction of mycorrhiza application x adhesive and adhesive substance on seedling and root dry weight was found to be significant ($P<0.01$). The average seedling dry weight varied between 3.64 g and 6.13 g according to the adhesive application. While the highest average seedling dry weight was determined in the seaweed application, the all of adhesives except the bacteria application (3.64 g) were in the same statistical group with the seaweed application. According to the adhesive application, the average root dry weight varied between 1.44 and 3.47 g, the highest root dry weight was determined in the gum arabic application, and the lowest in the bacteria, sugar and guar applications. Examined mycorrhiza application x adhesive interaction, the highest root dry weight was determined arabic gum, the lowest was pure bacteria in both mycorrhiza applications. In root dry weight pure bacteria with molasses were in the same statistical group without mycorrhiza applications (Table 2). In the study, it was determined that the application of adhesive on fresh seedling and root weight and the interaction

of mycorrhiza*adhesive material were significant (P<0.01), application of mycorrhiza on fresh weight was significant, and application of mycorrhiza on dry weight was insignificant. While application of mycorrhiza on fresh seedling weight was significant, fresh root weight was not. According to the adhesive application, the highest average fresh root and seedling weight was determined in the gum arabic application (33.1 g and 30.3 g, respectively). The lowest fresh seedling weight was obtained from the bacteria application (18.1 g), and the lowest fresh root weight was obtained from the sugar application (15.78 g). Although mycorrhiza application had a positive effect on fresh weight, this difference did not occur on dry weight. While Hoben et al. (1991) determined that the seedling fresh weight was 0.7 g in the control group, It was determined that in the application of bacteria inoculation in peanuts, it was 1.8 g with the application of peanut oil as a sticker; 1.0 g with mineral oil application and 1.5 g with arabic gum application. As a result of the examined study, sticker applications increased the seedling weight compared to the control. As a result of our study, we determined that the sticker applications increased the seedling weight characteristics compared to the control, which shows that it is similar to the literature results.

Mycorrhiza, adhesive application and interaction of mycorrhiza*adhesive were significant (P<0.01) on the number of nodules. According to the adhesive application, the average number of nodules was obtained from the bacteria application (9.45) the least and the gum arabic (20.43 units) application the highest. Mycorrhiza application (15.37) significantly increased the number of nodules. Waggoner et al. (1979), have applied arabic gum glue for bacterial inoculation adhesive on white clover and determined a very effective increase in the number of nodules (increase %280 to control) as a result of the study. Literature source supports the obtained results of the study.

Table 2. Dry seedling and root weight averages as a result of different adhesive and mycorrhiza applications

	Dry Seedling Weight (g)			**Dry root Weight (g)**		
	M0	**M1**	**Mean**	**M0**	**M1**	**Mean**
Control	5,05 bc	4,91 ab	***4,98 AB***	1,68 cd	2,26 b	***1,97 BC***
Bakteria	3,52 cd	3,75 b	***3,64 B***	1,48 d	1,40 d	***1,44 C***
Sugar	5,22 bc	4,32 ab	***4,77 AB***	1,76 cd	1,58 cd	***1,67 C***
Molasses	4,63 bc	5,35 ab	***4,99 AB***	1,52 d	2,33 b	***1,93 BC***
Guar	2,76 d	5,96 a	***4,36 AB***	1,59 cd	2,12 bc	***1,85 C***
Whey	5,32 bc	5,56 ab	***5,44 AB***	2,15 c	1,84 b-d	***2,00 BC***
Seaweed	7,88 a	4,39 ab	***6,13 A***	3,01 b	1,94 b-d	***2,47 B***
Arabic gum	5,79 b	5,99 a	***5,89 A***	3,61 a	3,33 a	***3,47 A***
Mean	**5,02**	**5,03**		**2,10**	**2,10**	

M0: Without mycorrhiza applications; M1: mycorrhiza applications

Table 3. Fresh seedling and root weight averages as a result of different adhesive and mycorrhiza applications

	Fresh Root Weight (g)			**Fresh Seedling Weight (g)**		
	M0	**M1**	**Mean**	**M0**	**M1**	**Mean**
Control	19,46 bc	26,30 b	***22,88 BC***	20,50 bc	24,01 c-e	***22,3 CD***
Bakteria	22,71 b	18,05 c	***20,38 C***	15,55 c	20,57 de	***18,1 D***
Sugar	16,22 cd	15,34 c	***15,78 D***	24,34 b	29,92 a	***27,1 AB***
Molasses	17,06 cd	24,25 b	***20,66 C***	23,00 b	29,45 ab	***26,2 A-C***
Guar	13,27 d	23,55 b	***18,41 CD***	16,61 c	28,01 a-c	***22,3 CD***
Whey	21,74 b	17,88 c	***19,81 CD***	23,90 b	24,89 b-d	***24,4 BC***
Seaweed	35,27 a	18,25 c	***26,76 B***	17,63 c	19,21 e	***18,4 D***
Arabic gum	33,22 a	33,04 a	***33,13 A***	30,92 a	29,62 ab	***30,3 A***
Mean	**22,37**	**22,08**		**24,12 B**	**25,71 A**	

M0: Without mycorrhiza applications; M1: mycorrhiza applications

Table 4. Nodule number of plant averages as a result of different adhesive and mycorrhiza applications

	Nodule number of plant		
	Mo	**M1**	**Mean**
Control	11,47 c	16,13 bc	***13,80 BC***
Bakteria	6,57 d	12,33 d	***9,45 D***
Sugar	12,39 c	14,40 c	***13,39 BC***
Molasses	11,71 c	17,26 b	***14,49 B***
Guar	8,29 d	16,70 b	***12,49 C***
Whey	13,14 c	14,02 cd	***13,58 BC***
Seaweed	16,05 b	12,25 d	***14,15 BC***
Arabic gum	20,95 a	19,91 a	***20,43 A***
Mean	**12,57 B**	**15,37 A**	

M0: Without mycorrhiza applications; M1: Mycorrhiza applications

CONCLUSION

In addition to the adhesives actively used in the study, the study was planned whey and seaweed applications by adding industrial waste. As a result of the study, gum arabic was the most effective substance on nodulation as a bacterial adhesive in seedling growth. The lowest seedling growth was detected in plain bacteria application. Mycorrhiza application which is effective in root development in arid areas, had a positive effect on the characteristics of root length, fresh seedling weight and number of nodule in the study.

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VARIETIES AND CHARACTERISTICS OF LETTUCE/SALAD (*Lactuca Sativa* L.) RECORDED IN TURKIYE

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ABSTRACT

The most significant crop among the leafy vegetables is Lactuca sativa L., most commonly known as lettuce. Lettuce, which is grown as a one-year cool climate vegetable belonging to the Compositae family, has been registered in our country as ıceberg, cos, lollo, and butterhead lettuce types. But different types of lettuces registered in recent years, in addition to the number of leaf divisions, thickness and size. The shift in lettuce types that have been authorized for registration through 2022 has been thoroughly investigated with the help of this study. The registered lettuces were also examined in the study in terms of some characteristics, their dendrograms were taken out, and an attempt was made to determine their relatedness to one another. As a result, these descriptors are effective analytical tools for researching the intricate morphological variability of this species and interactions between the varieties.

Keywords: Lettuce, Distinctness, Uniformity, Stability, Registration, Diseases

INTRODUCTION

The most significant crop in the Asteraceae family of leafy vegetables is lettuce (*Lactuca sativa* L.), which is estimated to be the largest plant family and has between 23.000 and 30.000 species (Bayer and Starr, 1998). It came from the coast of the Mediterranean (Lebeda et. al. 2007), but it has just been mentioned by Pitrat (2012) and (Balkaya and Özgen 2019) that Europe and Anatolia, Caucasia, Iran and Turkistan are the primary regions of origin for lettuce. Within *Lactuca sativa* L., there are numerous cultivars of lettuce. They are divided into several different varieties, including butterhead lettuce (var. *capitata* L. *nidus tenerrima* Helm), crisphead lettuce (var. *capitata* L. *nidus jagger* Helm), cos lettuce (var. *longifolia* Lam., var. *romana* Hort. in Bailey), cutting lettuce (var. *acephala* Alef.), stalk (Asparagus) lettuce (var. *angustana* Irish ex. Bremer), Latin lettuce (without a scientific name) (Lebeda et. al., 2007; Mou, 2008). Each group has a different growth pattern, leaf form, color, and texture.

Lettuce is extensively consumed globally and used in several items, from salad to medicines, adding a lot of nutritional value to diets (Kenny and Beirne, 2009). A nicotine-free cigarette is made from lettuce leaves. Edible oil and dried latex are found in the seeds and stems (Hassan et al., 2021). Fiber, iron, vitamins C, A, K, beta-carotene, and folate are all present in lettuce. It is a vegetable that is low in calories, fat, and sodium. In addition, lettuce is a rich

source of additional health-promoting bioactive substances with anti-inflammatory, cholesterol-lowering, and anti-diabetic properties (Mou, 2009; Materska et al., 2019).

It is grown commercially in a mild climate and subtropical areas in the world and planted annually in fields and greenhouses in a range of environmental conditions, lettuce also has significant economic advantages due to vertical farming and hydroponic methods. Lettuce is grown as a garden crop in the majority of locations and is commercially produced in many nations across the world. The Food and Agriculture Organization of the United Nations (FAOSTAT, 2020) study states that the global output of lettuce and chicory climbed from 11.5 million tons in 1990 to more than 27.6 million tons in 2020, with China producing 38.7% of that total, followed by the USA (15.1%) and Spain (3.4%). Our country ranks 7th in the world with a total lettuce production of 540.569 as of 2021 (Turksat, 2021). In the past years, it was generally grown as a winter crop in the southern regions of Turkey where the climatic conditions are suitable. However, in recent years, as a result of the resistance to biotic and abiotic stresses with the number of varieties, lettuce cultivation is carried out in every period. Especially, the demand for freshly cut lettuce, minutely prepared, or "ready to eat" green vegetables has been rising steadily in recent years. In lettuce, consumer acceptance is associated with leafy vegetables visual characteristics such as shape, color, size, freshness, texture and flavor (Nicola and Fontana, 2014).

In addition to disease resistance, the primary lettuce diseases are *Bremia lactucae* (BI) races, *Fusarium oxysoprum* f.sp. Lactucae and *Lettuce leaf aphids* are accepted by UPOV. Lettuce root aphid, *Lettuce mosaic potyvirus,* and *Tomato bushy stunt virus* that are not found in the UPOV test guidelines are not available but this resistance is in the booklet of companies. This resistance is also very important for lettuce cultivation around to worldwide and can cause severe damage to crops (Obermeier et al., 2001; Miller et al., 2003; German-Retana et al., 2007). It must be remarked, however, that registered varieties of Turkish lettuce varieties have not been comprehensively detected to be disease-resistant characterized until today. Moreover, most of this information is not found in VRSCC records.

When choosing the optimal genotypes to increase output, genetic diversity in vegetable crops is crucial. Effective strategies for differentiating grown lettuce from variety to variety include certain morphological features. Varieties of *Lactuca* from Turkey are maintained in various seed companies. Variation in lettuce is recently found in leaf length, shape, color, texture, size and heading type. This study aim to a detailed study will be given about the status of lettuce varieties from 1964 to today and to evaluate the agronomical and morphologic properties of lettuce varieties. The principal component analysis also emphasized the relationship between the various parameters under investigation. The breeders and researchers studying Turkish lettuce varieties can rely on these findings for guidance and information.

MATERIALS AND METHODS

In this study, quarterly reports were published and protocols were prepared by VRSCC (Variety Registration and Seed Certification Center) and secondary archive data obtained from CPVO (Community Plant Variety Office) UPOV (International Union for the Protection of New Varieties of Plants), and ISF (International Seed Federation) secondary data, information, reports, recommendations, interviews and evaluations in other open sources were used in 435 lettuce varieties. The situation among the lettuce varieties from the first registration year to the present has been examined over the years. The findings obtained with the required percentage (%) using the EXCELL program are discussed with other sources.

In the second stage of the research, the differences between the varieties based on qualitative, and pseudo-qualitative in registered varieties were tried to be revealed by using UPOV (TG/13/11 Rev. 2) variety characteristics. The total according to the UPOV descriptor characterization was done in terms of 11 characters for this purpose using measurements or observations. Among these characters, it has been tried to be evaluated in terms of diseases for Lettuce mosaic virus [*Lettuce mosaic potyvirus* (LMV)], *Tomato bushy stunt virüs* (TBSV), Downy mildew [*Bremia lactucae* (BI)], Lettuce leaf aphid [*Nasonovia ribisnigri* (Nr)], Lettuce root aphid [*Pemphigus bursarius* (Pb)], Fusarium wilt [*Fusarium oxysoprum* f.sp. Lactucae (Fol1/4)], which have never been investigated in our country until today. Varieties were also evaluated for leaf characteristics (anthocyanin coloration, hue of anthocyanin coloration, area covered by anthocyanin coloration, color, intensity of green color), growing methods and lettuce types.

Analyses (Exeter Software of Setauket, New York, NTSYS-PCv 2.01 computer package program) were used to determine the relationship and pattern of variation between cultivars (Rolf, 1993). First, the data has been normalized using the STANDARDIZATION module because it exhibits continuous variance. SIMINT module was used to compute a distance matrix. The similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average) to determine genetic relationships among the lettuce varieties studied. The COPH module was used to convert the tree matrix into a matrix of ultrametric distances (a matrix of distances implied by the cluster analysis), to evaluate how well a cluster analysis represents the distance matrix.

RESULTS AND DISCUSSION

According to data 435 lettuce varieties are registered in Turkey. Normally generally grouped into four main types ıceberg, cos, lollo, and butterhead. These varieties range in terms of their flavor, disease resistance, and aesthetic appeal. But in recent years, different types of lettuce are added to market because of variations in taste, shape and color as well as leaf textures. In UPOV test guidelines, butterhead type, novita type, ıceberg type, batavia type, frisée d'Amérique type, lollo type, oakleaf type, multi-divided type, frillice type, cos type, gem

type, stem type of lettuces divided according to growth types (UPOV, 2022). Although our country is included in this classification, it is known that many new lettuce varieties have been developed. Classification of types of lettuce is summarized in Table 1.

Table 1. Distribution of lettuce varieties based on the growing types

Types	Variety	Freq. (%)	Types	Variety	Freq. (%)
Cos	95	21.83	Butterhead (colored	4	0.91
Cos (crunch)	2	0.45	Lollo bionda	27	6.20
Cos mini	8	1.83	Lollo rosso	21	4.82
Cos midi	3	0.69	Oak leaf	9	2.06
Cos midi (colored)	1	0.22	Oak leaf (colored)	8	1.83
Cos mini (colored)	3	0.68	Baby oakleaf	2	0.45
Iceberg	103	23.68	Baby oakleaf (colored)	1	0.22
Batavia	113	25.98	Multi divided	10	2.29
Batavia (colored)	3	0.68	Multi divide(colored)	4	0.91
Baby Batavia	1	0.22	Frillice type	2	0.45
Baby Batavia (colored)	1	0.22	Frillice type (colored)	1	0.22
Butterhead	13	2.98			

Genetic resources for lettuce are highly morphologically variable (Krıstkova et. al., 2008; Walley et al., 2017). For this reason, lettuce types differ from year to year in varieties that apply for registration as a result of breeding programs. Farmers interest in growing lettuce landraces has recently increased as a result of rising consumer demand for natural, regional, and high-quality products that offer more convenience in their increasingly busy lives (Missio et. al., 2018). Pre-cut, ready-to-use veggie packs, practical or even creative ingredient mixtures in one bag or box, or convenient "snack-sized" packs for eating on the go. Retailers and consumers alike are continuously striving for taste, flavor, texture, and physical appearance as this sector matures. Baby leaf lettuce has been packaged and consumed in numerous nations in recent years as a result. In our country, mini and leaf lettuce varieties have been developed and registered in recent years. Especially mini, midi, and little gem types, baby oakleaf, and baby batavia types, and their colored types are registered in recent years. A new type of crunchy cos lettuce is also registered. It offers a very intriguing substitute for iceberg or cos lettuce in salad bags. Additionally, the iceberg lettuce's crisp and crunchy bite contributes to the excellent flavor. The other type is Frillice type is non-heading; thick, crispy leaves, sometimes weakly

divided. Its nice green, shiny leaves and excellent sweet taste. This type of lettuce accounts for 5.74 % of all registered lettuce in our country.

Variation in lettuce genotypes for disease parameters including Lettuce mosaic potyvirus (LMV), *Tomato bushy stunt virus* (TBSV), *Bremia lactucae* (BI 1-37), *Nasonovia ribisnigri* (Nr), *Pemphigus bursarius* (Pb) and *Fusarium oxysoprum* f.sp. *Lactucae* race and race 4 are presented in Table 2. Over the past 50 years, significant progress has been made in the sources of *Bremia lactucae*-resistant cultivated lettuce species (Sustar-Vozlic et. al., 2021). The results showed that nearly half of the varieties observed resistance to *Bremia* races BI:1-16 and BI:17-27 while the rest of the varieties did not have disease resistance. Among 435 varieties, It was determined that 209 cultivars were resistant to *Bremia* race 28-32 (Table 2). It has been found that 31 of the cultivars that are resistant to the new downy mildew strain Bl:37EU, which will be formally identified by IBEB EU in June 2021, have been registered (ISF, 2022).

The lettuce aphid *Nasonovia ribisnigri* is a pest responsible for major damage worldwide (Wu et. al. 2018). With regard to Lettuce leaf aphid, more than ⅓ of the varieties (36.55%) are resistant, but no information was found about other cultivars. In regions of the world where lettuce is grown, lettuce mosaic virus (LMV) is a serious disease of commercial crops. The resistance of LMV was found in106 cultivars among all 24.37% while the other important diseases TBSV and Pb were found in 435 cultivars only %8.97 and 6.21% respectively (Table 2). These morphological descriptors are the primary indicators that could be utilized to distinguish cultivars as a result.

Resistance to *Bremia lactucae* is frequently inherited as a monogenic trait, which forms the basis of the interaction between the host and the pathogen (Lebeda et. al., 2002). In the UPOV Lettuce test guidelines, *Bremia lactucae* races and *Nasonovia ribisnigri* are qualitative characters. Based on single dominant resistance genes or resistance factors, there is a classification scheme (Crute and Johnson, 1976). But resistance to *Fusarium oxysporum* f.sp. *lactucae* races are quantitative characters affected by the environment.

The varieties studied displayed wide phenotypic variation in the grouping utilizing 435 lettuce cultivars. On average, the varieties were characterized by, anthocyanin coloration, hue of anthocyanin coloration, area covered by anthocyanin coloration, color, intensity of green color on leaf, Plant growing type, *Bremia* races, *Nasonovia ribisnigri*, LMV, TSBV and Pb. Based on this initial grouping, a dendrogram was built by clustering using their 0.66 correlation coefficient. Cophenetic correlation between ultrametric similarities of tree and very high similarity matrix ($r = 0.8029$) was calculated ($P<0.01$). The 435 varieties of *L. sativa* were subdivided into discrete branches according to plant type with some exceptions. According to UPGMA dendrogram, the cluster was divided into 18 main groups with a similarity of 66%. In the current study, the first group, comprising 171 cultivars, constitutes the largest portion. The 9th group, which takes the second place, contains 90 varieties, and the 7^{th} and 14^{th} groups contain 63 and 30 varieties, respectively. The rest of the other groups all consist of 81 varieties (Figure 1). Some Batavia- and cos-type varieties were difficult to distinguish from one another, and they appeared to have a more varied genetic background than the other types. According to Yang et. al. (2007), the genetic system controlling the leafy type is either quantitatively controlled by several recessive genes. But the lollo type is more predominant than the Batavia

type. As a result, the tested varieties were divided into different leaf types based on their resistance to diease using clustering analysis by morphological traits.

Table 2. Distribution of lettuce varieties based on the resistance

Resistance	Race	Class	UPOV code	Number of varieties	Freq. (%)
Downy mildew [*Bremia lactucae* (BI)]	BI:1-16	Absent	1	261	60
		Present	9	174	40
	BI:17-27	Absent	1	254	58.39
		Present	9	181	41.61
	BI:28-32	Absent	1	226	51.95
		Present	9	209	48.05
	BI:29-36	Absent	1	389	89.43
		Present	9	46	10.57
	BI:37	Absent	1	404	92.87
		Present	9	31	7.13
Lettuce leaf aphid (Nr) [*Nasonovia ribisnigri*]	Nr:0	Absent	1	276	63.45
		Present	9	159	36.55
Lettuce mosaic potyvirus (LMV)	Pathotype II 1/4	Absent	1	329	75.63
		Present	9	106	24.37
Tomato bushy stunt virus	TBSV	Absent	1	396	91.03
		Present	9	39	8.97
Lettuce root aphid (*Pemphigus bursarius*)	Pb	Absent	1	408	93.79
		Present	9	27	6.21

CONCLUSIONS

It was concluded that registered lettuce varieties had a large variation in morphological parameters and resistance. The present study investigated the diversity of Lactuca sativa varieties registered in VRSCC from 1964 to the present, and the present findings revealed that morphological characterization systems can be used effectively However, there are no observations or results regarding resistance to disease between all varieties. Both morphological and molecular tests will be useful for future studies.

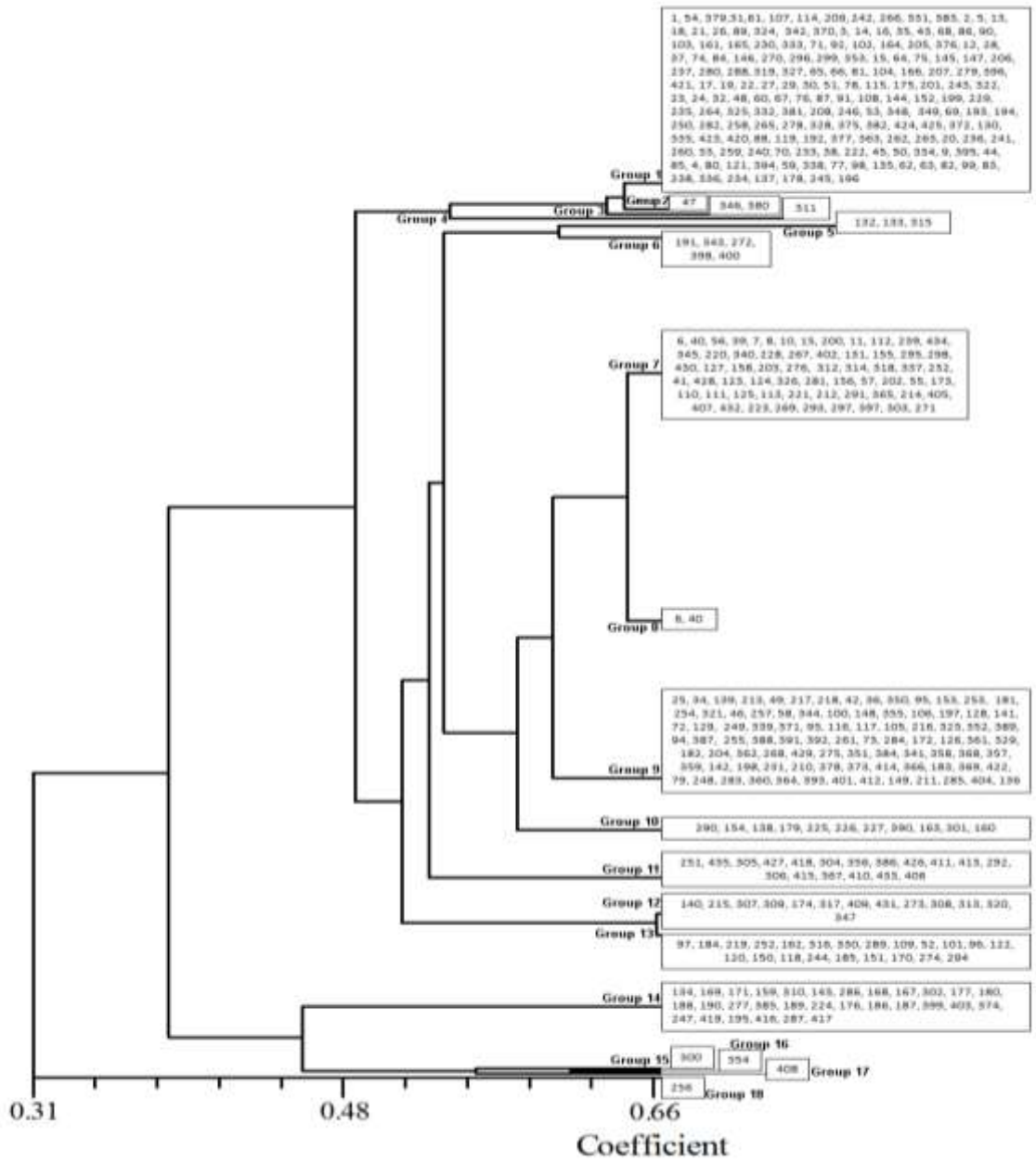

Figure 1. Dendrogram of lettuce varieties obtained from cluster analysis of phenotypic traits

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EFFECTS OF CLIMATE CHANGE ON DRYING OF AGRICULTURAL PRODUCTS AND FOODS WITH TRADITIONAL SUN-DRYING TECHNIQUE

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ABSTRACT

Drying is one the preservation methods applied for making agricultural products and foods more stable. Sun-drying technique, as the simplest and oldest of the drying techniques used, has been used for years in the traditional drying of agricultural products and foods. Fruits, vegetables, grains, herbs, meat and meat products and dairy products are commonly dried by sun in spreading over a sheet on the ground. The moisture present is removed from the product by joint effect of heat and mass transfer in all of the drying techniques. In sun-drying technique, the product is dried until it reaches a safe humidity level for storage by direct exposure to the radiation coming from the sun. There exist external and internal parameters that affects the drying rate of the product. Internal parameters are mostly product-dependent but external parameters that can be classified as solar radiation, temperature, wind velocity and relative humidity are mostly depend on environmental factors. These parameters can be practically traced by microclimatic measurement devices. Since sun-drying has a seasonal dependency, in general it is carried out in July to September when external factors are satisfactory. Reasonable average temperature and relative humidity together with rainfall-free and cloud-free conditions are crucial for successful sun-drying application. In recent years, adverse effects of climate change can be observed with extreme temperatures, temperature fluctuations, and untimely rainfall events etc. Therefore, in this study, the possible effects of climate change on sun drying technique will be evaluated.

Keywords: Sun-drying, climate change, drying of agricultural products, drying of foods.

INTRODUCTION

Foods can become sustainable and stable by application of drying. Drying is one the preservation methods applied for making agricultural products and foods more stable and dates back to the Middle East and oriental cultures. Ancient people belonging to different cultures in the world get used to climate by adapting themselves in habitual practices. They use nature for local food sources required for their preservation to stay alive and establish communities

(Anonymous, 2022a). Grains, marine and meat products and also fruits and vegetables are dried for reduction of water activity to safe limits avoiding microbial growth (Jangam, 2011). Open-sun dehydration is a widely used by local people in small scale and also by drying industry. Meats are cured by using sun and also wind in early times of ancient people. In regions without enough sunlight or wind, fruits, vegetables and spices are dried by fire heating these foods in still houses (Anonymous, 2022a). Sun drying is one of the renewable energy sources provided by the nature. It is a green kind of technology without any generation of green-house gases. However, since environmental factors affects its existence there is a strong relation between sun-drying and climatic events. In this context, in this study, the characteristics, limitations of sun drying technique and the effects of climate change will be discussed.

Drying of Agricultural Products

Agricultural products mostly contain high levels of moisture content so regarded as highly perishable products. These products should be kept under temperature controlled environment and require sometimes some additional atmospheric conditions also. Especially fruits and vegetables has high amount of moisture (above 80%) that bacteria and fungi can easily grow on (Mishra et al., 2022; El-Mesery et al., 2022). High amounts of product losses occur (40-50%) due to adverse control of temperature during storage (inadequate refrigeration or freezing) (Mishra et al., 2022). Industrial and scientific developments provide ultra-modern drying techniques such as freeze drying microwave drying/infrared drying. However, the sun drying technique still exists today due to the almost lack of investment cost and practicality of application.

Drying of foods supply many advantages (Mishra et al., 2022).

- Extended shelf-life
- Availability of the products being out-of-season
- New alternative forms of product for consumption
- Reduced packaging, storage, transportation cost

Sun Drying Technique

Sun drying technique is the simplest and the oldest of drying technique used in drying the fruits and other products. Fruits, vegetables, grains, herbs, meat and meat products and dairy products are commonly dried by sun by spreading over a sheet on the ground. In sun-drying technique, the product is dried until it reaches a safe humidity level for storage by direct exposure to the radiation coming from the sun. Sun drying is strictly dependent on weather conditions and not applicable in regions having longer periods of wet seasons (Tan et al., 2022). Sun drying is a traditional type of drying method so preferred mostly people living in rural areas and in industrial production so far. In the selection of sun drying technique, being a natural renewable energy source and abundancy of sun are the major concerns.

Sun drying methods can serve retaining of sensorial quality and nutritional composition but performed under uncontrolled conditions when compared with novel drying techniques (vacuum drying, microwave drying, freeze drying etc.).

Sun drying technique can be classified and applied in 5 different ways and given in detailed way in Table 1 (Mishra et al., 2022).

- Open Sun Drying
- Black Cloth Shade
- White Cloth Shade
- Conventional Solar Dryer
- Improved Solar Dryer

Table 1. Sun Drying Techniques

Sun Drying Techniques	Conditions	Consequences
Open Sun Drying (mean drying temperature : 26.8 °C)	Foods are left open under sun to dry	-
Black Cloth Shade (mean drying temperature : 26.7 °C)	The black cloth used absorbs greater heat due to radiation	Drying efficiency increases
White Cloth Shade (mean drying temperature : 24.5 °C)	White cloth does not absorb heat surrounding	Food is kept cooler due to white cloth White cloth acts as a barrier to dry Increased drying time
Conventional Solar Dryer (mean drying temperature : 32.6 °C)	Solar panels absorb the solar energy and give it to dry the foods	Quicker drying rate takes place
Improved Solar Dryer (mean drying temperature : 40.3 °C)	Hybrid passive mode of solar drying	-

Table 2. Advantages and Disadvantages of Sun Drying

Advantages	Disadvantages
Renewable energy source	Seasonal dependency
Simple and easy to apply	Contamination risk due to dust and rain Attracts birds and animals
Less investment costs	Non-uniformity in nutritional and sensorial quality, less sustainability in quality maintenance
Improved solar dryers help improvement in sensory quality	Excessive or inadequate drying is maintained

Sun drying efficiency depends on external and internal parameters. Internal parameters are mostly product-dependent. External parameters that can be classified as solar radiation, temperature, wind velocity and relative humidity are mostly depend on environmental factors. These parameters can be practically traced by microclimatic measurement devices. Since sun-drying has a seasonal dependency, in general it is carried out in July to September when external

factors are satisfactory. Reasonable average temperature and relative humidity together with rainfall-free and cloud-free conditions are crucial for successful sun-drying application. Advantages and disadvantages of sun drying technique is given in Table 2 **(**Indiatro et al., 2021; Mishra et al., 2022).

Climate Change and Sun Drying

The long-term more general weather conditions observed in larger areas are described by climate and weather is the short term behavior of atmosphere in a definite region. Temperature fluctuations and extreme rainfall events are indicators of climate change (Purwaningsih et al., 2022). Agricultural production is affected by temperature, rainfall distribution, wind speed and wind direction (FAO, 2015). Excessive long-term changes in temperature and precipitation trends are observed in climate change. Irregular weather conditions occur as a result of changing ambient pressure and humidity levels. Extreme conditions which are closely related with human life and natural systems are observed. Melting of the global ice sheets and sea level rise are results of climate change and affects the world with many international and local effects. Impacts of climate change in agricultural production is classified in Table 3 (Godde et al., 2021).

Table 3. Impacts of Climate Change on Agricultural Production

Direct impacts	Indirect impacts
Temperature levels	Pollinators
Rainfall distribution	Pests
Wind speed	Disease causing factors
Wind direction	Invasive and destructive pests or species

Food security is correlated with climate change in that; availability accessibility, utilization and stability of food is dependent on climate change. Technology uses raw material that is available. Climate change will hinder access to healthy nutritious food since ecosystem is adversely affected by climate change that limits access to many crops and other species. Increasing temperatures results in timeless precipitations events at the end floods occur in agricultural lands and hurricanes can occur as extreme events that destroys cold frames. As reported in Anonymous (2022b) floods also affect post-harvest practices as lastly flood occurred in Manisa, Turkey (August 2022) during sun drying of grapes caused significant losses in the crops.

CONCLUSION

Unfortunately, extreme temperatures, temperature fluctuations, and untimely rainfall events etc. are adverse effects of climate change observed in recent years. Climate change affects food production and therefore food availability. High temperatures in drying process causes loss of quality of foods. Since sun drying technique has seasonal dependency that is strictly correlated with climate change. Any adverse condition related to climate change would affect at first food availability and then food processing depending on natural resources. There are several drawbacks of sun drying such as infestation by micro-organisms, destruction by

animals or pests and over-drying or intermittent dehydration. Climate change will bring these negativities more effectively. Solar energy, a pioneer in renewable energy sources and an important way to reduce dependence on fossil fuels. In order to overcome these climatological problems, human should build their daily-life again. Less contribution to climatic changes as less release of green-house gases to the atmosphere and taking all preventive actions related to global warming. Use of renewable energy more, selecting green technologies in production and simplifying our lives positive contributions that should be concentrated on. Together with these preventive actions, use of enhanced form of sun drying that is solar drying systems that supplies higher drying air temperatures and higher drying rates in food drying is thought to be a promising method. Recent researches on solar drying systems and hybrid alternatives are also carried out. This can make drying with a renewable energy source possible even in adverse weather conditions.

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A PRELIMINARY RESEARCH ON THE USE OF MONOATOMIC SILVER SOLUTION IN TOMATO

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ABSTRACT

This study was carried out as a preliminary study to determine the effect of monoatomic silver solution on some fruit characteristics in tomato. Different doses of silver solution (50, 100 and 200 mL L-1) and control (0) application were used in the current study. The study was carried out in a randomized block design with 3 replications in open field. At the end of the study, fruit diameter (mm), fruit weight (g), dry matter content (%), pH and silver content (mg kg-1) properties of tomato fruits obtained from nanoparticle silver applications and control application were investigated. While only the amount of silver in the fruit was affected by the control and silver solution doses (p=0.0004), other properties were not affected. As a result of this study, it was determined that the application of monoatomic silver water did not make a significant difference in some fruit characteristics of tomato fruits. However, since this study is a preliminary study, it is recommended to conduct a comprehensive study and to deal with yield and quality characteristics in a comprehensive manner.

Keywords: Tomato, Monoatomic silver water, Fruit, pH

INTRODUCTION

Agriculture faces many challenges such as climate change, increasing population and limited arable land (Ditta and Arshad, 2016). Today, the growing pressure of the agricultural sector to increase crop productivity has led to heavy use of chemical fertilizers and pesticides on fields to grow crops quickly and maximize crop yields. Although the use of chemical

fertilizers and pesticides increases crop yield, it causes negative side effects such as environmental pollution (for example, increase in nitrate and phosphate amount in water), soil poisoning and decrease in soil quality (for example, soil surface erosion, erosion), and destruction of beneficial microorganisms. These chemicals are also toxic to many animals and are known to be harmful to human health (Atilgan et al., 2007; Singh et al., 2020).

Although nanoparticles are naturally found in the environment, there has been a significant increase in the production of engineered nanoparticles as nanotechnology has become a widespread application (Rizwan et al., 2017). It is estimated that more than 800 products are currently available on the market (Al-Halafi, 2014; Safiuddin et al., 2014; Rajapaksha et al., 2015; Zhou et al., 2015). The designed nanoparticles have a characteristic size of less than 100 nm and different properties from non-nanoscale particles with the same chemical composition (Auffan et al., 2009). Besides pesticide and dye applications, nanoparticles can enter the soil through direct release through industrial activities (Grieger et al., 2009; Zhang et al., 2010; Liu and Lal, 2015). Due to the interaction of the static structures of nanoparticles with soil and air, plants may be exposed to nanoparticles released into the environment. Direct exposure of plants to metal and metal oxide nanoparticles should not be ignored (Ertekin et al., 2020). In the last few years, both positive and negative effects of nanoparticles on plant growth have been reported. These effects varied depending on the type, source and size of nanoparticles, plant species, and the exposure time of plants to nanoparticles (An et al., 2008; Roghayyeh et al., 2010; Rico et al., 2014; Bandyopadhyay et al., 2015; Lalau et al., 2015). For example, silver nanoparticles (AgNP) increased ascorbate and chlorophyll in the leaves of asparagus (Asparagus officinalis L.), while iron nanoparticles (FeNP) improved plant biomass in soy (Glycine max L.) (An et al., 2008; Roghayyeh et al., 2010). Similarly, the application of silica nanoparticles increased the germination, root and shoot length, photosynthesis and dry weight of corn seedlings grown under field conditions (Suriyaprabha et al., 2012).

Jung et al. (2010) investigated the effect of AgNP solution application on green onions (*Allium fistulosum* L.) in a greenhouse environment. All of the different solutions applied (Used solution: 5,000-15,000 µg/ml; 40,000-50,000 µg/ml) increased the biomass and dry weight of green onions. Also, little change occurred in the various bacterial and fungal populations in the soil of green onions grown in the greenhouse. In addition, as a result of the chemical analysis of the soil, it was revealed that there was minimal change in the soil composition. Ejaz et al. (2018) were determined that AgNP application positively affected the growth of rice (*Oryza sativa* L.) plants and increased their biomass and dry weight. Elshahawy et al. (2018) showed that AgNP application significantly reduced the number of dead tomato plants due to root rot caused by *Pythium aphanidermatum* in greenhouse and field experiments.

On the other hand, various literature reports indicated that metal nanoparticles have negative effects on growth and physiological functions in important plants such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.) and soybean (*Glycine max*

L.). (Dimkpa et al., 2012; Mahmoodzadeh et al., 2013; Nair and Chung, 2014; Shaw et al., 2014; Antisari et al., 2015). In addition, metal and metal oxide nanoparticles have been reported to be more toxic to plants than coarser metals (Wang et al., 2011). It has been reported that plants exposed to nanoparticles can take nanoparticles and transport them to different plant parts (Du et al., 2011; Le et al., 2014; Rico et al., 2015). When we looked at the studies, it is seen that nanoparticles can have positive or negative effects on plant growth and yield, as well as have negative effects on humans and animals by being included in the food chain (Foltête et al., 2011; Atha et al., 2012; Servin et al., 2012; Vannini et al., 2013; Cui et al., 2014; Shaw et al., 2014; Thiruvengadam et al., 2014; Moon et al., 2014; Xiang et al., 2015). The data available in the literature showed that the positive / neutral / negative effects of Ag-NPs completely depend on the plant species applied, the sizes of the applied silver nanoparticles, the shape of the nanoparticles and the dose of application.

Tomato (*Solanum lycopersicum* L.) is one of the three most important horticultural crops worldwide, as well as the most important vegetable in the world in terms of consumption. Tomato production has increased continuously in the last 10 years. Tomato cultivation areas increased by about 2.3% compared to the previous year and reached 1.73 million da in 2019. 16.8% of the total cultivation area is the greenhouse production area (FAO, 2021). When tomato cultivation areas are examined on a provincial basis, Antalya ranks first with an area of 197 thousand hectares. 93-95% of tomato fruit is water and 5-7% of it contains inorganic compounds, organic acids (citric acid and malic acid), solids insoluble in alcohol (proteins, cellulose, pectin, polysaccharides), carotenoids and lipids (Petro- Turza, 1987). It is an important source of high levels of potassium, organic acids, vitamins A and C. In addition to the nutrients it contains, tomatoes are also rich in carotenoids such as lycopene and β-carotene (Sönmez and Ellialtıoğlu, 2014). With its rich nutritional content, the tomato plant provides benefits such as increasing digestion, stimulating blood circulation, and reducing cholesterol levels.

This study was carried out as a preliminary study to investigate the effects of monoatomic silver water application on tomato plants.

MATERIAL AND METHOD

This study was carried out in Antalya AKEV University Application Garden in 2021. Tomato (cv. Veyron F1) seedlings were obtained from a commercial seedling company. The study was carried out in field conditions according to the randomized blocks design. The experiment was designed in 3 repetitions and control (0) and 3 different doses (50,100,200 ml/L) of silver nanoparticles were applied to tomato seedlings. The plots were formed of 4 rows with a length of 2 meters and a distance of 1 meter was left between the plots. Silver nanoparticle application was applied at 3 different times; the first application was done by immersing the seedlings in the solution during transplanting to the field, the second application was made 1 month after the seedlings were planted in the soil, and the third application was

during the fruit setting period. At the end of the study, fruit diameter (mm), fruit weight (g), dry matter content (%), pH and silver content (mg kg^{-1}) properties of tomato fruits obtained from nanoparticle silver applications and control application were investigated. The land where the experiment was established is sandy loam and the organic matter content of the soil (0.076%) is quite low. In the experiment, silver water could not be applied to the control application, and 6 kg da-1 NPK fertilizer was applied to these plants.

All numerical data obtained from this research were subjected to ANOVA test in the JMP statistical package program. Among the examined features, those that were found to be statistically significant ($P<0.05$) were analyzed by TUKEY multiple comparison test and grouped.

RESULTS AND DISCUSSION

The data describing the effect of monoatomic silver water application at different doses on the fruit diameter, fruit weight, fruit dry matter ratio, fruit pH and fruit silver content properties of tomato plants are given in Table 1. The effects of monoatomic silver water application doses and control application on fruit diameter, fruit weight, dry matter ratio and fruit pH of tomato plant were not found statistically significant. However, the effects on fruit silver content were significant ($p=0.0004$) (Table 1).

Table 1. Effects of monoatomic silver water application at different doses on some fruit characteristics of tomato plant

Doses (ml/L)	Fruit diameter (mm)	Fruit weight (g)	Dry matter ratio (%)	pH	Fruit silver content (mg/kg)
0 (control)	48.71	62.31	4.95	4.16	0.006 C
50	49.29	68.21	5.06	4.14	0.023 B
100	46.44	59.52	5.50	4.09	0.028 AB
200	49.52	69.54	4.78	4.13	0.033 A
p values	0.7840	0.7635	0.2938	0.8088	0.0004

Values indicated with different letters in the same column are different from each other ($p<0.05$).

As the monoatomic silver dose increased, the silver contents of the fruits also increased (Table 1). A linearity was determined in the amount of silver accumulated in the fruit with the increase of the doses. As a matter of fact, some researchers have emphasized that the plant is more resistant to biotic stress factors that may occur from diseases and pests thanks to the silver

nanoparticle accumulated in the structures of different plants (Jung et al., 2010; Elshahawy et al., 2018). On the other hand, many researchers have reported that silver nanoparticles may be included in the food chain as a result of the presence of foods and may adversely affect human health (Foltête et al., 2011; Atha et al., 2012; Servin et al., 2012; Vannini et al., 2013; Cui et al., 2014; Shaw et al., 2014; Thiruvengadam et al., 2014; Moon et al., 2014; Xiang et al., 2015).

Table 2. The effects of monoatomic silver water application at different doses on some plant growth characteristics in tomato.

Doses (ml/L)	Plant height (cm)	Flower number (piece)	Leaf number (piece)
O (control)	64.41	14.04	15.70
50	55.06	10.32	11.70
100	57.20	12.93	12.39
200	55.37	13.11	14.46
p values	0.6229	0.5168	0.3993

The effects of monoatomic silver water application at different doses on plant height, flower and leaf number characteristics were found to be insignificant (Table 2). The highest plant height, number of flowers and leaves were obtained from the 0 (control) application. The lowest plant height, number of flowers and leaves was determined in the application of monoatomic silver water at a dose of 50 ml/L. According to these results, it was determined that monoatomic silver water application did not affect plant growth in tomato.

CONCLUSION

As a result of this study, it was determined that the application of monoatomic silver water did not make a significant difference in some fruit characteristics of tomato. However, since this study is a preliminary study, it is recommended to conduct a comprehensive study and to deal with yield and quality characteristics in a comprehensive way.

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FUTURE FRUITS: CURRANT AND GOOSEBERRIES

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ABSTRACT

Berries are an increasingly important fruit group in the world while have just known inTürkiye. Currant and gooseberry have an important place in this fruit group. Wild forms of these fruits also common in forest area in Turkey and in the world. These natural populations create a rich source of genes for scientific research and studies. Today, global warming is an increasing crisis that emerges as a strong situation day by day. It is necessary to spread the culture of resistant species, which can be succeeded to grow with more limited opportunities in the future. These berries are important for human nutrition with their rich vitamin C and antioxidant activities. It is important to provide the necessary level of knowledge in terms of breeding. For the future of these fruits, first, it is expected to be widely introduced, to spread modern cultivation methods and to solve marketing problems. Generally, large quantities are processed to frozen or dried fruit, or concentrated fruit juice. A very small amount is consumed as fresh. In the review article presented here, the studies on currant (black, red and white) and gooseberry fruits in Turkey and in the world were discussed, and the problems and solutions in the cultivation of these fruit species were evaluated.

Keywords: *Ribes rubrum, Ribes grossulara*, breeding, fruit, cultivation

INTRODUCTION

Gooseberry is a fruit is widely grown in temperate European countries, Asia, Australia and North America (Okatan et al., 2017). This fruit began to recognize in London in the 16th century and started to become famous in 19th century. Currant and gooseberry are botanically inserted in the group of true grapes and is a shrub structure plant. Some of the common gooseberry varieties are named as Green Globe, Invicta, Pax and Achilles. Gooseberry varieties are classified by their color. Common produced varieties have yellow, brown, green, or red fruits.

Blackcurrant is botanically included in the group of true grapes and is a bushy plant with a few stems. It is widely grown in temperate European countries, Asia, Australia and North

America (Okatan et al., 2017). There are color and taste differences between the gooseberry varieties grown in Asia, Europe and America. This situation is determined by difference of the soil and climate conditions.

PLANT PROPERTIES

Currant and gooseberry are a small shrub approximately 1.0-1.8 m. tall and 1.0-1.2 m. wide. Most of the gooseberry cultivars have spines on their branches and their leaves are small and hairy. Currant flowers are produced in racemes known up to 8 cm long containing 10–20 flowers. Each flower has a hairy calyx with yellow glands, which are longer than the petals (Anonyms 1). Its berries are shiny, hairless. When fruit ripen turned to red, white or dark purple in color, almost black, with glossy skins and calyxes at the apex., each containing many seeds. A mature currant shrub can produce about 4.5 kilograms of fruit each year (Anonyms 2).

Gooseberry inflorescence structure is a raceme. Flowers are green, sometimes with pink tendencies, and form clusters of two to three flowers, sometimes up to 5. The sepal is green to red, the petals are green, white-pink and small. The flowers are bisexual, hairy, sessile, do not have a pedicel, are bell-shaped. Fruit maturation varied from 1.5 to 2 months due to varieties. The fruit sepal is no falling. The fruit is a multi-seeded berry. Gooseberry seedlings produces fruit on 1-year-old perennial branches and one or two flowers appear in the leaf base. The branches are covered with light reddish shoots, the leaves are lobed, the edges are toothed, covered with hairs from below. Produces broad branches with round or grooved shoots, grows fast. Gooseberry fruits are oval, the color varies from white to yellow, green, or red, and they have a hairy or smooth surface (Heiberg 2003, Pluta 2018, Nordlander 2021). Gooseberry fruits are connected to the inflorescences with an individual flower stalk. The fruits are larger and heavier than the blackcurrant fruits. Longitudinal creamy-white lines run between the lower and upper poles of the fruit. Fruits are semi-transparent and there is visible light colored very small hairs on the fruit. Remains of flower organs on the flower tip makes the fruit taste a bit irritating. Fruits are strongly connected to stalk and fruit drop is occurred if only harvesting is late. The berries of the currant (8.69-9.37 mm wide and 8.30-9.35 mm length, respectively) are smaller than the gooseberries (16.45 mm wide and 16.40 mm length, respectively) (Okatan et al. 2015).

Phenological period is similar for gooseberry and currant fruits. Foliation is started at the first week of March and finalized the last form in a month, till to first week of April. First flowering is appeared at the beginning of April and completed end of the April. Blossoming lasts 7-10 days due to varieties. After end of flowering, fruit set is occurred in 7-8 days. Fruits reach maturity period at the end of June and first week of July and then harvested (Okatan 2017).

Temperature effected the flowering parameters. When the plants are grown under controlled temperatures, percent of flowering plants and opening flowers amount are increased with the suitable degrees. Optimum temperature depends on cultivars. According to studies, flowering performance is increased with the increasing of temperatures (from 12 ℃ to 24℃ is

the maximum) (Sønsteby 2022). Frost can damage both unopened and open flowers of currant when the temperature falls below −1.9 °C. The flowers at the base of the strig are more protected by the foliage and are less likely to be damaged (Anonyms 1). Blossom of gooseberry takes place at +7 - (+ 18) ℃ temperature. The flowering period depends on the variety, local conditions, the best flowering conditions are +15 - (+ 20) ℃ temperature, 60 -65% relative humidity, 1-3m / s wind speed.

Currant and gooseberry are a honey-producing variety, pollinated by bees. Invicta and Green bottle varieties of gooseberry stand out with high yields, providing a yield of 12.0-15.0 t / ha, respectively (Nortlander 2021).

SELECTION STUDIES ON CURRANT AND GOOSEBERRY

Ribes L. is a genus within the plant family *Grossulariaceae* where black currant (*R. nigrum*), red currant (*R. rubrum*) and gooseberry (*R. uva-crispa* or *R. grossularia*) belong. There are about 150 known species of currants and gooseberries, mainly found in temperate regions of Europe and North America. European gooseberry species *R. uva-crispa* (synonym *R. grossularia*) is more distributed across the world than the North American species *R. hirtellum* (Anastasiadi et al. 2016; Pluta 2018).

A limited number of varieties are used in the breeding studies of black currant which the European subspecies (*Ribes nigrum* ssp. europeanum Yancz) and the Siberian subspecies (*Ribes nigrum ssp* sibiricum Wolf.) of black currant, dikuscha currant (Ribes dikuscha Fisch. ex Turcz.), few-flowered currant (Ribes pauciflorum Turcz. ex Pojark), wild black currant (*Ribes americanum* Mill.) and nutmeg currant (*Ribes glutinosum* Benth) (Brennan 1991, Brennan 1996).

Currant and gooseberry fruit are very common in natural forest area and selection of new types have continued in different countries. A first record of *Ribes uva-crispa* L. (*Grossulariaceae*) – a new species of the Sicilian flora – from the Madonie Mts. is reported in 2010. The autochthonous and relict new population of *Ribes uva-crispa* L. (Grossulariaceae) is found on Mount Carbonara (Madonie Mts., North-Central Sicily), which is several hundred kilometers away from the Central Apenninic and the South Mediterranean locations of its distribution range. *R. uva-crispa* shows a distribution like other taxa extending from the Euro-Asiatic area to the mountains of North Africa with disjunct populations on the main Sicilian reliefs (Madonie and Nebrodi Mts.), testifying ancient phytogeoFigureical connections. This noteworthy record adds a new species – as well as a new genus and new family – to the vascular flora of Sicily. Based on the morphological characters, the population found is to be referred to *Ribes uva-crispa* L. subsp. *austro-europaeum* (Bornm.) Bech var. *glanduligerum* (Lindberg) Maire (Gianguzzi et al. 2010).

The cultivated gooseberry is a temperate shrub originated from European. However, American species, *R. hirtellum* Michx. and *R. divaricatum* Dougl. genes were transferred to European gooseberries to increasing the resistance against to the American gooseberry mildew

disease. Most cultivars have spines on shoots and trunk that make some difficulties in cultivation treatments.

Studies on breeding of new varieties that are compatible with new technologies and resistant to diseases and pests are important. The success of the breeding program is directly related to the choice of parents. At this point, it is important for new parents to be involved in the breeding process. Selection of the source material for the creation of black currant varieties with complex resistance to diseases and pests was combined. As a result of the study, high productivity donors were identified with complex resistance to biotic factors (Bakhotskaya and Knyazev 2021).

Gooseberry buds during floral initiation was investigated by scanning electron microscopy to determine the changes taking place in developmental phase (Sønsteby et al. 2022).

EVALUATION OF CURRANT AND GOOSEBERRY

Gooseberry has a taste similar but sour than currant fruits flavor. Their fruits are consumed as cake decoration and marmalade cooking in European countries. Fruits can be used make jam and preferred in wine making in some regions. Gooseberry is not very high in calories. Fresh fruit contain a 100 g portion is only 44 calories. 100 g of frozen gooseberry is 46 calories (Anonim 1). Bakery sector use its fruit for puddings and desserts preparing.

There are industrial uses of fruits on ice cream, confectionery, flavored mineral water and liquor (Brennan, 1996). Currant berries are suitable for freezing.

HEALTH BENEFITS

Currant and gooseberry, which has an important place in folk medicine, has been used as a natural remedy for various diseases. Their fruits show benefits most in fresh form, consume it immediately after collected. Fruits can be also dried. It would be a good idea to prepare herbal tea by drying the leaves of the gooseberry. Herbal tea has a protective effect against to winter diseases in cold. Currant and gooseberry have antioxidant properties and purify body from toxic substances. It is a candidate to be the fruit of the future. Their fruits come to regulate digestion, balance blood sugar and lead you to a healthier life.

Fruits are a source of vitamin C. Therefore, both of two species fruits have a great effect on strengthening human immunity system. Their fruits serve as protector agent to diseases such as cancer and infection by protecting immunity. It is a natural source of iron, phosphorus and calcium source and makes bones healthier and stronger. Gooseberry fruit helps to decrease the pains and cramps during the menstrual period with its antioxidant effect. It helps hair grow healthier and more vibrant. Fruit eaten in its fresh form or gooseberry juice applied to the hair will prevent graying of hair, supports eyesight and prevents eye problems. Another feature of gooseberry is that it is a diuretic. Thus, edema accumulating in the body is also excreted. It works the intestines and eliminates problems such as constipation. Gooseberry, which strengthens the capillaries, also heals blood pressure by balancing high blood pressure.

Gooseberry is also good for anemia. It increases the number of red blood cells and increases the blood value (Anonyms 3).

Currant fruits are rich in vitamins A, B and C. It is very healthy, also diuretic, digestive effected.

Orsavová et al. (2019) was found that the total amount of phenolic substance contents of Red currant is 8.66 g kg-1 GAE, the total amount of flavonoid substance 8.13 g kg-1 RE, and the amount of ascorbic acid 6.20 g kg-1. Comparing of gooseberry fruit antioxidant contents with wild currant properties show the value of gooseberry fruits (Erbil et al. 2021).

However, the antioxidant activity values were higher in alpine currant, could not determine a difference between the total phenolic, flavonoid, and ascorbic acid values of gooseberry and wild alpine currant. Antibacterial effects were determined on the test bacteria in both gooseberry and mountain currant. When the antibacterial activity was examined, wild currant extract exhibited higher activity against the tested bacteria (Erbil et al. 2021).

Volatiles of gooseberries were isolated by means of vacuum-headspace-extraction, and the obtained concentrates were analyzed via capillary gas chromatoFigurey–mass spectrometry. Esters were shown to be responsible for the green and fruity character of fresh gooseberries (Hempfling et al. 2013).

Gooseberry fruit aqueous and leaf methanol extracts is a new promising source to produce anti-RSV drugs (Dogan 2020). Gooseberry fruit residues which generated in processing in the food industry suggested as an effective material for the efficient removal of iron(III) from wastewater and improving water quality. Its fruit waste effectively removes Fe(III) ions from aqueous solutions due to the content of appropriate functional groups in its composition and favorable physical and chemical properties (Kalaka et al. 2020).

MARKETING OF GOOSEBERRY AND CURRANT FRUITS

Edible gooseberry species has tasty berries (Kendir et al., 2019). This fruit are usually collected from the wild natural populations in forest areas. Recent days, people have started to make small plantation as ornamental plant or fruit production in their gardens. Increasing awareness has affected of these fruits attention on human health and has improve their production. It is a little difficult to find the gooseberry fruit in the local markets while currant a bit more common. In season, big markets and organic fruit shops sell as a special fruit. Frozen fruit is sold out of the season.

Currant and gooseberry is widely used in organic agriculture. The harvested berries can be stored for 7-10 days at a temperature of 0-0.50C and 80-85% relative humidity in fruit refrigerators. All these varieties and the local one has a multifunctional nature, are resistant to spotting. The green bottle variety stood out with the highest yield, and the round red local variety with the lowest. The round red local variety is sweet and more importantly the ripe fruits of this variety stay on the bush for a long time without falling, the Invicta and the Green Bottle

varieties stand out with pleasant acidity. The sugar content of gooseberry varieties between 8-10%, acidity between 0.5-1.0% (Gabrielyan et al. 2022).

CONCLUSION

Studies in different fields are continuing. Currants and gooseberries are increased their importance as the fruits of the future. Selection of wild productive genotypes and with contribution of this newly selected gene sources breeding studies will continue. Currant and gooseberry production amount and uses areas of these fruit will increase in near future.

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UTILIZATION OF WASTE BIOMASS FROM ANIMAL PRODUCTS - STATUS AND PROSPECTS

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ABSTRACT

The standard production of energy in power and heat plants is based on the burning of fossil fuels such as coal, oil and natural gas. A major disadvantage of these fuels is the fact that natural reserves are decreasing and energy needs are increasing. Harmful emissions released into the atmosphere during the burning of fossil fuels are also a serious problem, which creates significant environmental problems. These are some of the reasons to look for possible suitable energy alternatives to fossil fuels. One of them is the use of biomass for the production of so-called biofuels. For this, in the present study, an evaluation of the possibility of processing waste from animal waste and its use in the biogas sector was made. The results of the research show that agriculture has enough potential to ensure sustainable biogas production.

Key words: biomass, biogas, anaerobic digestion system, bioeconomy

INTRODUCTION

One of the main environmental problems of modern society is the generation of increasing amounts of animal waste. The fight against the reduction of biological waste becomes a top priority. A large share of the total volume of waste is generated in agriculture - manure. Environmental protection standards (Directive 86/278/ EES; Directive 1999/31/ES; Directive 75/442/EES) are becoming more and more strict and past practices such as unregulated landfilling, dumping or incineration of waste are not applicable in these days.

One of the methods for the utilization of animal waste is through a System for Anaerobic Decomposition of Biological Waste, in short - biogas installations. As a result of the processing, useful products are obtained - biogas, electricity and thermal energy, fertilizers and carbon dioxide (for use in industry). Biological biogas production combines renewable energy production and organic farming. Both are important for sustainable development. The production of biogas through anaerobic digestion (anaerobic fermentation) is considered as an optimal method for the treatment of liquid and solid manure, as well as for a wide range of suitable organic waste, as it converts this type of substrates into renewable energy and organic

fertilizer suitable for use in agriculture. The production and utilization of the resulting biogas was first documented in the United Kingdom in 1985 (Metcalf and Eddy, 1979). Subsequently, the process was further developed and widely applied for the purpose of wastewater treatment and sludge stabilization. The energy crisis of the 1970s prompted a new understanding of the use of renewable fuels, including biogas produced by anaerobic digestion. In recent times, interest in biogas has been increasing due to global efforts to replace fossil fuels with renewable ones, as well as due to the need to find environmentally sustainable solutions for the treatment and recycling of manure and organic waste.

The aim of the present research is the definition of the main categories and types of the residual biomass from animal husbandry, which could be used as an energy source and tentative determination of their potential in Bulgaria. On the topic related to the utilization of biomass from plant and animal substances for energy purposes, the authors worked as: Georgiev, 2018; Marinova S., Banov M., 2002; Persson, M., 2007; Simeonov I, Denchev D., Baykov B, 2006.

RESEARCH METHODS AND SOURCES OF INFORMATION

There is no officially published data on the amounts of solid agricultural substances from animal husbandry. For this reason, the analysis of the potential opportunities for providing raw materials for energy use from them was made on the basis of an expert assessment. For the purposes of the research, officially published data from the Directorate "Agrostatistics", Ministry of Agriculture and Rural Development were used. The spatial parameters of the scientific research are determined normatively by the National long-term program for promoting the use of biomass for the period 2008-2020, which regulates the criteria for evaluating the unused amounts of solid agricultural waste from animal husbandry tons/year. The time frame 2011-2020 includes the changing conditions after Bulgaria's accession to the European Union.

In addition to the method of expert evaluation, others such as: comparative analysis, statistical methods were used. For processing and visualization of the obtained results, Figureic images (Figures and tables) were created using appropriate software (Excel 2013). The map in the present study was prepared with ArcMap software (ArcGIS ESRI), version 10.5.

RESULTS AND DISCUSSION

Livestock wastes are also raw materials that can be used for energy purposes. The utilization of animal waste is based on anaerobic decomposition. The products that are obtained from this process are: biogas (mixture of methane and carbon dioxide), acidic decomposition residue and lye. Of these three products, biogas is of interest from an energy point of view. It can be used as a fuel for the production of electricity, heat or steam, or as an automobile fuel, and its use helps to reduce harmful emissions into the atmosphere, as it is a relatively clean fuel. The raw material used for biogas production is biomass including animal waste. Since there is no official data on waste from animal husbandry, the analysis will be made on the basis of the

calculations of the amounts of waste from the most mass-raised farm animals, and the calculations were made on the basis of daily waste from the respective animal (Table 1). The assessment of annual amounts of fertilizer refers only to those of large farms - cow farms, pig farms, sheep farms, goat farms and poultry farms.

Table 1. Quantity of solid waste from livestock in large farms for 2011-2020

№	Animals	Number of animals on large farms		Average daily amount of manure per animal, kg.	Annual amounts of manure released per animal, tons/year.	Annual quantities of manure released on large farms, tons / year.	
		Years				Years	
		2011	2020			2011	2020
1.	Cattle	557 600	568 726	40,00	14,6	8 140 960	8 303 399
2.	Sheep	1 454 600	1 307 771	1,00	0,365	530 929	477 366
3.	Goats	341 400	253 401	1,00	0,365	124 611	92 491
4.	Pigs	608 300	592 097	4,5	1,6	973 280	947 355
5.	Hens	13 150 000	12 411 000	0,17	0,06	789 000	744 660
	Total:					10 558 780	10 565 271

Source: MAF, „Agrostatistics" Directorate and own calculations.

Annual volumes of solid waste from one animal:

- cow – 40 kg x 365 days = 14,600 kg/year. ≈ 14.6 tons/years;
- sheep or goat - 1 kg x 365 days = 365 kg/year. ≈ 0.365 tons/years;
- pig – 4.5 kg x 365 days = 1,642 kg/year. ≈ 1.6 tons/years;
- hen – 0.17 kg x 365 days = 62.05 kg/year. ≈ 0.06 tons/years.

From the obtained results, it can be seen that at the beginning and at the end of the research period 2011-2020, the values of the generated waste from animal husbandry are almost identical - 10.5 million solid waste, with cattle breeding being the largest contributor (Table 1) Summarizing the results of the calculations, it can be concluded that agriculture has a sufficient amount of biomass from animal substances to be used for energy purposes.

Utilization of livestock waste is based on anaerobic decomposition, from which the biogas product is obtained. The concept of "animal unit" has been introduced for the production of biogas.

Table 2. Number of farms by species in Bulgaria in 2011 – 2020

№	Animals	Number of animals on large farms		Total biogas in m^3 per day	
		Years		Years	
		2011	2020	2011	2020
1.	Cattle	557 600	568 726	836 400	853 089
2.	Sheep	1 454 600	1 307 771	272 737	245 207
3.	Goats	341 400	253 401	64 012	47 512
4.	Pigs	608 300	592 097	152 075	148 024
5.	Hens	13 150 000	12 411 000	78 900	74 466
Total:				1 404 124	1 368 298

Source: MAF, „Agrostatistics" Directorate and own calculations.

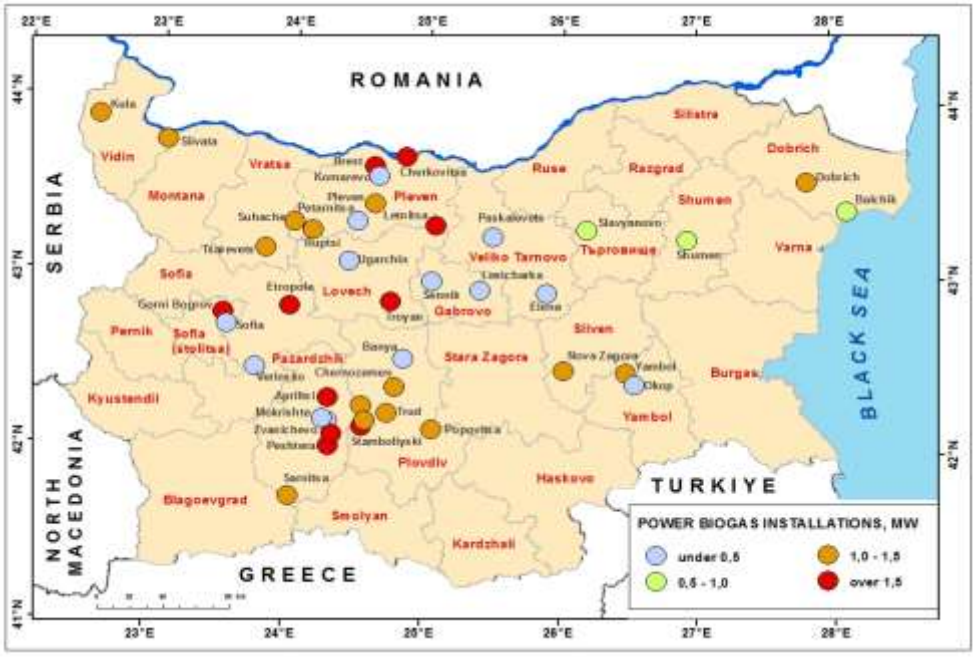
One animal unit produces waste (feces) per day, from which about 1.5 m^3 of biogas can be produced. It equals: 1.5m^3 biogas = 1 cow = 6 pigs = 8 sheep = 8 goats = 250 hens.

For the period 2011-2020, the obtained results show that the biggest contribution to biogas production can be made by cattle breeding, followed by sheep breeding and pig breeding (Table 2). According to Georgiev (2018), the biogas yield achieved by production from manure alone is too low. Therefore, plant waste is also added to the process, most often silage corn, from which the quantities of gas produced increase several times.

Table 3. Biogas composition (average values)

Component	Formula	Concentration
Methane	CH4	50-75% by volume
Carbon dioxide	CO2+	25-45% by volume
Steam	H2O	2-7% by volume
Sulphide	H2S	0.002-2% by volume
Nitrogen	N2	< 2% by volume
Ammonia	NH3	< 1% by volume
Hydrogen	H2	< 1% by volume
Gases in microconcentration		< 2% by volume

Source: Fachagentur Nachwachsende Rohstoffe (2009):Basisdaten Biienergie Deutschland.35.
Biogas is a flammable mixture of gases obtained from the natural fermentation of wet biomass in the absence of oxygen (anaerobic decomposition/fermentation). The main combustible component, namely methane, makes up about 50-75% of biogas by volume.



Source: RIEW data and maping Rositza Mikova, IAE

Fig. 1. Built biogas plants by 2020, working with biomass from plant and animal substances from agriculture.

Carbon dioxide, hydrogen sulphide, oxygen and water vapor are also present in biogas (Table 3). Biogas is formed naturally in wetlands, where biological matter is decomposed by anaerobic bacteria, the so-called "swamp gas".
As of 2020, after an inquiry and summarized data from RISW, there are more than 40 biogas plants operating with plant and animal substances of various capacities built in Bulgaria (Figure 3). It is noteworthy that the largest number of biogas plants have been built in North and South Central Bulgaria, i.e. they are territorially located close to the raw material sources.

CONCLUSION

The production and use of biogas from the anaerobic digestion of organic waste leads to a number of environmental, socio-economic benefits for society and for participating farmers. Using a domestic asset chain for biogas production increases local economic opportunities,

creates jobs in rural areas and increases regional capacity provision. It improves living standards and contributes to economic and social development.

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MAF, Agrostatistics

The National Long-Term Program for the Promotion of the Use of Biomass for the Period 2008-2020

DEVELOPMENT OF ORGANIC AGRICULTURE IN THE WORLD AND IN TURKEY

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ABSTRACT

The rapid increase in the world population has increased the need for food and intensive chemical inputs have been used in agricultural enterprises in order to obtain more output in a short time. Although this situation causes the deterioration of the ecological balance and the reduction of natural resources, the fact that approximately 1/3 of the population experiences hunger has not been able to solve the food security problem. Therefore, it is necessary to develop alternative agricultural systems that will produce enough to meet the needs of the population without disturbing the ecological balance, without polluting the environment. Organic (ecological) agriculture, which is one of the alternative production systems developed within this framework, has started a new period of structural change in the production process. While this structural change is gaining momentum due to the high level of healthy living awareness in countries, the development process of organic agriculture needs to be analyzed in order to improve the process and make better use of the opportunities. In this study, it is planned to reveal the difference of the organic agriculture sector from other agricultural systems and to compile the developments and emerging opportunities in the process. Thus, by discussing the opportunities that arise in response to the developments in organic agriculture, suggestions will be developed to increase the competitiveness of the new world order and to ensure the sustainability of production.

Keywords: organic agriculture, ecological agriculture, sustainable agriculture

INTRODUCTION

The rapid increase in the world population has increased the need for food and has led the producers to use intensive chemical inputs in order to obtain more output in a short time in agricultural enterprises. However, although this situation leads to the deterioration of the ecological balance and the decrease of natural resources, the hunger problem of about 1/3 of the population has not been able to provide a solution to the food security problem. Therefore, it is necessary to develop alternative agricultural systems that will produce enough to meet the needs of the population without disturbing the ecological balance, without polluting the

environment. One of the alternative production systems developed within this framework is organic (ecological) agriculture.

In this direction, organic agriculture, which is an environmentally friendly production method that does not harm the ecological system and supports the sustainability of production; "Production, which eliminates the use of artificial production inputs completely or as much as possible, instead uses crop rotation, animal manure, green manure and non-agricultural organic wastes, uses biological control methods in the control of diseases, pests and weeds, and constitutes the organism activity in the soil in order to increase soil fertility. system". Organic agriculture has different definitions in many studies, and these definitions include organic agriculture; It is defined as an agricultural system that takes human and animal health into account, approaches soil, water and other environmental factors with a holistic view, prohibits the use of chemical inputs, contributes to the rebuilding of the ecosystem, and ultimately protects the ecological balance. At the same time, organic agriculture is defined as "certified production" because it is certified and controlled within the framework of international and national legislation. (Kirazlar, 2001; Demir ve Gül, 2004; Çakmakçı ve Erdoğan, 2005; Turhan, 2005; Ayla, 2011; Merdan ve Kaya, 2013; Demiryürek, 2016; Süzer, 2020; Bayramoğlu ve ark., 2021).

As can be understood from the definition, the period of structural change that started with organic agriculture has accelerated due to the high level of healthy life awareness in developed countries. Developing countries have generally been involved in this process by meeting the increasing foreign demand for organic agricultural products. Turkey, which is among the developing countries, has turned to the organic agricultural products market in order to meet the demand in other countries and increase its exports in this direction, and has started to give more importance to organic agriculture due to the increasing domestic demand in today's conditions. In this process, organic farming practices are becoming widespread day by day, depending on the protection of the ecological balance throughout the world and meeting the consumers' demand for healthy products. In this context, the institutional and commercial development of organic agriculture has been examined in the study, and the historical developments of organic agriculture have been shown in many studies to date. In these studies, the development/potential of organic agriculture in the world and in Turkey has been examined, and research has been conducted on theoretical definitions, legislation structure, organic agriculture production, foreign trade, its impact on rural development and its development. (Kırmacı, 2003; Kurtar ve Ayan, 2004; Turhan, 2005; Olhan ve Ataseven, 2006; Uzun, 2006; Avcı, 2007; Bayram ve ark., 2007; Demiryürek ve Bozoğlu, 2007; Gündüz ve Kaya, 2007; Ataseven ve Güneş, 2008; Yolcu ve ark., 2008; Gülçubuk, 2010; Ayla, 2011; Demiryürek, 2011; Aydın, 2012; Baysel, 2013; Merdan ve Kaya, 2013; Kurt, 2016; Dalbeyler ve Işın, 2017; Deviren ve Çevik, 2017; Okudum ve ark., 2017; Marangoz ve Kumcu, 2018; Ünal ve Can, 2018; Merdan, 2019; Yılmaz, 2019; Boz ve Kılıç, 2021). In these studies, the principles, advantages and problems of organic agriculture were also examined, and the necessity of making the necessary arrangements and scenarios by periodically examining them was emphasized because the expansion potential of organic agriculture areas is high and it is important to provide competitive advantage in organic agriculture trade in terms of creating an entrepreneurial opportunity.

MATERIAL AND METHOD

Within the scope of the study, secondary data was used and the data used in the research were taken from all kinds of printed and internet sources. Statistics were collected from various institutions and organizations, especially the Organic Agriculture Research Institute (FIBL), the International Federation of Organic Agriculture Movements (IFOAM) and the Turkish Statistical Institute (TurkStat). In addition, all kinds of printed and electronic articles, papers, reports, theses, etc., under the headings of organic agriculture, ecological agriculture and biological agriculture. By examining the publications, suggestions were prepared in order to guide the decision makers.

RESULTS AND DISCUSSION

The history of organic farming dates back to the 20th century. Before this process, a wide variety of methods were developed, and even schools that included the effect of the moon and stars in production by adding astrological dimensions to these methods emerged. the basic philosophy obtained when all these schools are examined; It is to carry out plant and animal production together in the form of family business by preserving the organic balance. Therefore, it is to ensure self-sufficiency by establishing short chains from production to consumption. Due to this feature, the importance of organic agriculture increased more during World War I. (Merdan, 2014). Especially in this period, organic agriculture gained importance with the use of nitrogen used in the agricultural sector as an explosive material during the war and the negative effects of this substance on public health started to be noticed. As a result, organic agriculture was examined philosophically and ideologically for the first time. Rudolf Steiner and Lady Eve Balfour, the pioneers of this current of ideas, based organic agriculture on ideas that have no scientific basis. A course on Biodynamic Agriculture Method was organized in 1924 and an institute with the same name was established in 1928. However, in response to these developments, another scientist, Sir Albert Howard, who is considered to be one of the pioneers of organic agriculture, emphasized in his work titled "An Agricultural Test" that Steiner and Balfour's thoughts were not suitable for the ecosystem of nature and that what they said was not applicable. (Yılmaz, 2019).

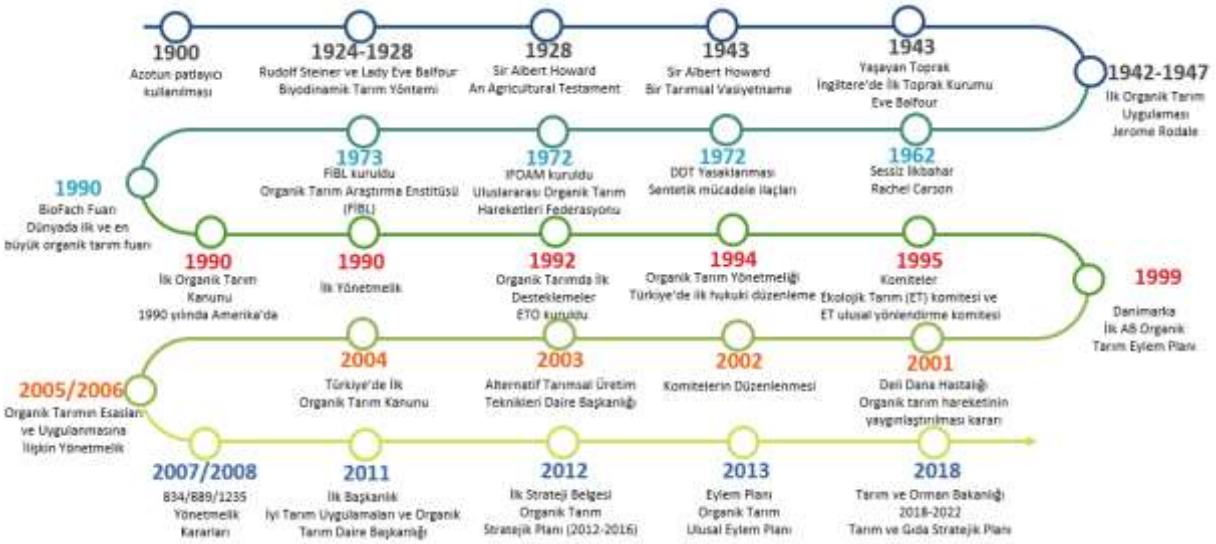

Figure 1. Development of Organic Agriculture in the World and in Turkey

Parallel to the increase in the world population after the 1930s, the concern of supplying the needs of the population and the Second World War in the 1945s. Organic agriculture lost its importance again due to the presence of hormones and antibiotics in World War II. The most important work on organic farming during this period is the book written by Howard in 1943. In the book titled "Bir Agricultural Testament", it is emphasized that the inputs used to increase plant and animal production reduce the fertility of the soil, and it is mentioned that increased production causes soil erosion, diseases and deterioration of the natural ecosystem. It has been stated that the soil fertility will be improved again with humus to be obtained from plant and animal wastes and the physical, biological and chemical capacity of the soil will be increased with beneficial fungi-bacteria. In addition, this book revealed the advantages of organic agriculture over traditional agriculture in the country of Bengal between 1905-1924. Inspired by this book written by Howard, Eve Balfour published her book "Living Soil", in which she compared organic and traditional products for the first time in 1943, and led to the establishment of the Soil Association in England. In addition, in this period, the certification process with the "Bioland Organic Label" was started for the first time in the world by Hans Muller, and the first organic farming application was made in the USA by Jerome Rodale, who was influenced by Howard's ideas. In 1940, Rodale conducted many trials in its own field and established the Rodale company, which currently operates in 67 countries around the world. This company first published a magazine under the name of "Organic Agriculture and Gardening" in 1942 and has made many publications over the years. The second magazine was published in 1947 and organic farming began to become popular. However, after this period, a period called the "Green Revolution" and a period in which intensive input was used has passed. In this period, an international campaign under the name of Green Revolution was started in Mexico for the first time under the leadership of American-based companies, and subsequently, hybrid seed production, chemical and synthetic fertilizers and pesticide production, irrigation in large areas and improved mechanization practices began to be widespread. Especially II. After the World War II, synthetic combat drugs (dichloro diphenyl trichloroethane (DDT) etc.) developed for

the military began to be used in the agricultural sector. Until 1978, organic and non-organic businesses experienced polarization.

In this period, which is described as the green revolution, the problem of hunger in the world could not be solved against the increasing amount of production, and the deterioration in the agricultural ecosystem, natural resources and public health showed that the green revolution practices did not give correct results. Therefore, there were important discussions on the subject in the 1970s and 1980s, and in 1972, the use of DDT group chemical inputs was banned in many countries, especially the USA (in Turkey in 1980), due to increasing pressure. The book "Silent Spring" written by Rachel Carson in 1962 was especially influential on this decision. In his book, Carson revealed the effects of DDT and other pesticides on human and environmental health. This book has been accepted as the beginning of environmentalism and the importance of organic agriculture has started to increase again. Especially in the USA, the law that obliges the use of organic agricultural products in the production of 0-2 age group children's foods has made a significant contribution to this commercial dimension.

In parallel with these developments, IFOAM was established in France in 1972 in order to gather and direct the organic agriculture movements in the world under one center, to raise awareness of the society and to prepare the necessary legislation for organic agriculture. IFOAM organized activities for the dissemination of the organic agriculture movement and pioneered many scientific researches on the subject. IFOAM, which has more than 500 member organizations in more than 75 countries in the current period; has provided global leadership in the development of organic production, processing and trading standards. The main objective of IFOAM is; Although 20% of the world's food consumption was produced by organic methods until 2000, the share of organic farming lands in 2000 was 0.27%. (FİBL, 2022). Another institution established following this period is the Organic Agriculture Research Institute (FIBL). The institute was established in 1973 with the support of the Swiss government and is still considered the largest organic agriculture research center in the world. The institute's periodic reporting and publication of statistics have been effective in maintaining this importance. In this period, the actions towards organic agriculture increased in various countries. For example; The book "One Straw Revolution" was published in Japan, the Ecology and Agriculture Foundation (SOEL) was established in Germany, and "BioFach", the first and still the biggest organic agriculture fair in the world, was held in Germany in 1990. After this date, organic farming movements with different names were organized in many countries of the world and necessary legislation was prepared.

In 1990, the "Organic Agriculture Law" was enacted in the USA in order to make organic products subject to trade, to make legal regulations regarding control and certification, and to regulate organic farming activities. Following this, the European community published regulation 2092/91 on 24 June 1991. After this period, the first agricultural supports were given to the enterprises engaged in organic farming with the EU regulation numbered 2078/92 in 1992 and these supports still continue in line with the regulation numbered 1257/1999. In this period, the first EU organic agriculture action plan, which is considered as the milestone of organic agriculture in Europe and started in Denmark, and supports per decare for those who want to switch to organic agriculture have started (Babaoğlu, 2006).

While the first published regulations only included expressions for plant production, provisions on organic livestock were included in the legislation in 1999. In 2001, the importance of organic agriculture increased with the "Mad Cow" disease in the UK, and the Environment Ministers of the countries that are members of the Organization for Economic Development

and Cooperation (OECD) took decisions to expand the organic agriculture movement for sustainable development. In this direction, the Council Regulation No. 2092/91 (ECC) dated 24 July 1991 giving "Indicators for Organic Production and Agricultural Products and Foodstuffs", which had a total of 44 amendments until 2009, was repealed and 834/2007, 889/2008 and 1235 Organic production activities were organized by taking the /2008 regulations into effect. The regulation numbered 834/2007 explains the organic production and product labeling rules, the regulation numbered 889/2008 explains the application rules of organic agriculture and the regulation numbered 1235/2008 explains the organic product import rules. After this date, the EU made its first regulation on 1 July 2010 and made it mandatory to use the EU's organic product logo in organic agriculture. Therefore, the EU contributed to the trust of all stakeholders, especially consumers, on organic agriculture.

According to FIBL and IFOAM data, while the organic agriculture area in the world was 14,980,99 thousand hectares in 2000, it increased to 74,926.51 thousand hectares in 2020 with a 4-fold increase, while Australia has the most important share with 47.63%. Over the years, Australia has increased its organic agricultural lands by approximately 5 times, reaching an area of 35,687,80 thousand hectares and has the largest organic agricultural lands in the world. The main reason for having the largest organic agricultural land is organic livestock activities. Organic animal husbandry is very common in the country and a significant part of organic farming areas are divided into organic pasture and grazing areas. In addition, the share of organic foods in the food consumed in Australia is increasing by 17-20% annually. (Hatunoğlu ve Açma, 2010). This country is followed by Argentina with 4,453.64 thousand hectares and Uruguay with 2,742.37 thousand hectares, while increasing health concerns in recent years, the self-sufficiency of countries and the increase in input costs have led to an increase in organic farming areas (FİBL, 2022). Oceania ranks first in organic farming areas by continent, while Europe, which has taken important steps in combating climate change, food security and healthy food concerns, ranks second. The fact that organic farming areas will be increased within the scope of the zero carbon emission vision targeted within the scope of the Paris Agreement and the Green Agreement in European countries will create new opportunities in these areas, while at the same time it will be possible to meet the demand of the consumer. As a matter of fact, while there are 3.4 million producers in the world, the organic agricultural food market has exceeded 120 billion € due to the increase in consumer demand. In this situation, both producer and consumer planning should be done. While the countries with the most important organic food markets in the world are the USA, Germany and France, the countries with the fastest market growth are respectively Canada, China and Germany. The support provided by the central governments of these countries to organic agricultural operators, the fact that they have the best systems for labeling, packaging and certification of organic agricultural products, and their high purchasing power increases the demand for organic agricultural products and increases production in the domestic market. For this reason, organic

farming areas should be increased and made sustainable in order to achieve the expected transformation in agricultural production.

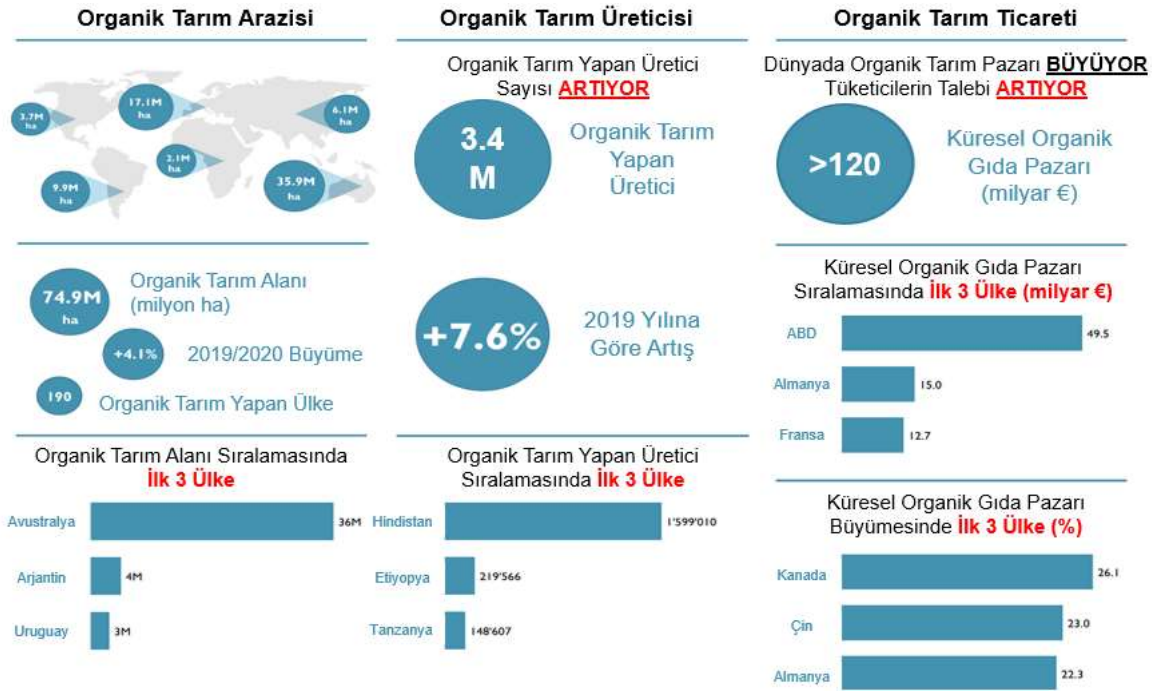

Figure 2. Organic Agriculture in the World

In Turkey, the development of organic agriculture can be examined under two headings, institutional and commercial. The first institutional development was experienced in 1992, and the Ecological Agriculture Organization Association (ETO) was established in order to realize organic agriculture more accurately, to strengthen financial-social aspects by bringing businesses together, and to support research on organic agriculture. After this date, organic agriculture in Turkey has shown legal development in accordance with the EU's Council Regulation 2092/91. In Turkey, legal development started with the "Organic Agriculture Regulation", which was first published in the Official Gazette No. 22145 on 18 December 1994, with the cooperation of various institutions and organizations, then the Ministry of Agriculture and Rural Affairs (TKB) and the current name TOB. This regulation was issued in parallel with the regulation published by the European Union Committee in 1991. After this period, the first amendments to the regulation were made on 29 June 1995, and the Ecological Agriculture (ET) committee and the ET national steering committee were established. After this regulation, the second regulation was made on July 11, 2002 and Organic Agriculture (OT) committee, OT National Steering Committee, OT National Trade Committee, OT Research and Projects National Committee were established. After this date, on 22 July 2003, the Alternative Agricultural Production Techniques Department was established for the first time under TOB, and the traceability and inspections of organic farming activities began. After the establishment of the Presidency, the “Organic Agriculture Law” was adopted and published on 03 December 2004. The law brought along many developments such as organic agriculture principles, principles, penalties and the regulatory authority of the relevant institutions, and the regulation of the law was published on 10 June 2005 as the "Regulation on the Principles and

Implementation of Organic Agriculture". This regulation; It has been prepared in order to be included in the list of "Third Countries Exporting Organic Products to the European Union", which is largely in line with the Organic Agriculture EU Council Regulation No. 2092/91. The marketing of organic agricultural products in demanded varieties and quantities in foreign markets became possible with this regulation. In order to internalize the additional changes in the EU related legislation, the regulation amending various articles of the "Regulation on the Principles and Implementation of Organic Agriculture" published in the Official Gazette dated October 17, 2006 and numbered 26322 has entered into force. After this date, the EU's legislation on organic agriculture was repealed and the directives 834/2007 and 889/2008 were put into effect. In Turkey, the "Regulation on the Principles and Implementation of Organic Agriculture" has been renewed in accordance with the said legislation, and in the last regulation, the obligation to use organic product logos has been introduced for companies that produce and sell organic products. In addition, the appropriateness of logo use is also included in the regulation. (Anonim, 2020).

Under current circumstances, TOB is the responsible organization for the general direction and control of organic agriculture in Turkey. The "Department of Good Agricultural Practices and Organic Agriculture", operating under the General Directorate of Plant Production (BÜGEM) established within the body of the Ministry in 2011, determines the priorities and national strategies for the development of organic agriculture and is also responsible for the legal development of the issue. In this context, BÜGEM first published the "Organic Agriculture Strategic Plan" covering the years 2012-2016, and with this plan, it was aimed to develop, expand and ensure the sustainability of organic agriculture. In addition, the desired result could not be achieved due to the lack of objective, measurable and achievable targets, despite the detailing of the practices that should be done for the targets to be realized in this strategy plan. Following this, in the "Agriculture and Food Strategic Plan" of TOB covering the years 2013 and 2017, it was aimed to expand environmentally safe and healthy production systems in the production of plant, animal and aquatic products that require inspection and certification within the framework of certain rules at all stages of consumption. aimed to increase. However, the absence of objective and time-bound targets, as in the previous strategy document for organic agriculture, did not contribute to the progress of the process. At the same time, the "National Action Plan for Organic Agriculture" was prepared in 2013, and it was aimed to increase the number of organic agriculture enterprises, improve the certification process, develop institutional capacity, and increase training and extension activities for organic agriculture. In this plan, the main actions related to the supports and corporate responsibilities that should be given are specified and the target calendars are explained. This plan has been the most remarkable document among the target, policy and strategy documents announced and published so far in terms of content. Ministry of Development's "10. Development Plan" and TOB's "2018-2022 Agriculture and Food Strategic Plan". Therefore, in line with this latest strategic plan, it is aimed to increase the number of organic farming enterprises to 110,000 by 2022. However, this target could not be achieved, as the number of enterprises engaged in organic farming in Turkey is 52,600 in 2020 (TOB, 2018).

Organic agriculture commercial activities in Turkey started in 1986 in line with the wishes of exporting companies, unlike the developments in Europe. The production, which was carried out in accordance with the legislation of the countries to which the export was made, continued in line with the regulation of the European Community after 1991. Later, in the annex numbered 94/92 published on 14 January 1992 to the regulation numbered 2092/91 regulating

organic farming activities; The issues that countries that will export organic products to the European Community have to comply with are specified in detail. In this direction, countries are obliged to implement their own legislation and apply to the European Community with a file containing various technical and administrative issues, including this legislation. (Merdan, 2014).

Table 1. Organic Agriculture Production Statistics by Years in Turkey

	Product Number	Farmer Number	Area (Ha)	Production (Ton)
2002	150	12.395	89.827	310.125
2003	179	14.754	113.621	323.981
2004	174	12.751	209.573	377.616
2005	205	9.427	203.811	421.934
2006	203	14.256	192.789	458.095
2007	201	16.276	174.283	568.128
2008	247	14.926	166.883	530.224
2009	212	69.735	501.641	983.715
2010	216	42.097	510.033	1.343.737
2011	225	42.460	614.618	1.659.543
2012	204	54.635	702.909	1.750.127
2013	213	60.797	769.014	1.620.387
2014	208	71.472	842.216	1.642.235
2015	197	69.967	515.268	1.829.291
2016	238	67.878	523.777	2.473.600
2017	214	75.067	543.033	2.406.606
2018	213	79.563	626.885	2.371.612
2019	213	74.545	545.870	2.030.466
2020	235	52.600	382.665	1.631.943

While products such as dried apricots, raisins and dried figs were exported as raw materials or primary production in the early stages of organic agriculture trade, it is known that processed organic agricultural products were traded over time and added value was created in this regard. In this context, more than 200 organic agricultural products are traded in Turkey under current conditions, which is insufficient to meet the market demand in terms of both product variety and quantity. While the demand for organic agricultural products is increasing in developed countries, especially in Europe and America, this situation creates important entrepreneurship opportunities in Turkey. As a matter of fact, factors such as ecological and climatic factors, land availability/productivity, labor potential, institutional structure, national legislation, geopolitical position, renewable energy resources and biodiversity of Turkey carry the country to an important potential in organic agriculture.

The first statistics on organic agriculture in Turkey belong to 2002, and a total of 12,395 operators grew 310,125 tons of organic products on 89,827 hectares of land and 150 types of products during the period. In the last 18 years, significant differences have been observed in the number of farmers, production area and, accordingly, the amount of production, especially in the number of products. The reason for the decreased production depending on the number of farmers is the structural problems in the organic agriculture sector. Insufficient supports,

limited grant and incentive applications, low consumer awareness, deterioration in the supply chain and increasing costs have caused the shrinkage of organic farming areas.

Table 2. Production Amount in Organic Agriculture by Products (Thousand tons)

Crops	2002	2005	2010	2015	2016	2017	2018	2019	2020
Olive	11,16	10,7	38,66	262,74	563,88	458,74	277,35	210,36	299,48
Wheat	19,75	36,75	243,15	308,81	334,43	258,97	256,57	170,85	139,89
Apple	69,19	51,61	49,76	101,19	123,9	145,42	153,11	193,25	127,36
Barley	2,07	8,4	35,54	57,53	84,26	68,34	74,86	82,14	99,95
Fig	9,47	6,82	19,17	94,5	111,04	121,96	111,38	106,69	98,57
Corn	0,95	9,24	40,39	53,73	39,57	47,59	51,68	62,72	96,1
Peach	5,94	9,67	18,9	36,22	122,03	79	88,25	87,82	77,93
Alfaalfa	0	1,56	367,83	242,26	338,57	261,19	172,28	145,6	61,77
Pomergrante	0,18	1,61	8,38	26,12	29,71	38,97	67,85	43,9	54
Oat	0	1,46	0,69	3,39	5,15	18,89	54,62	57,45	42,57
Hazelnut	7,67	5,11	13,37	17,72	21,51	31,28	39,87	41,95	33,7
Cheery	1,33	1,16	1,94	21,42	36,18	25,55	32,15	43,55	32,73
Sainfoin	0	0,42	87,5	122,25	113,57	111,3	87,73	74,38	31,74
Tomato	82,81	25,76	15,33	20,63	25,54	31,82	21,69	24,11	29,78
Vetch	0	0,3	45,73	73,68	80,25	76,73	53,91	56,01	28,03
Tea	0,06	0,06	3,45	34,48	73,08	21,85	27,75	31,22	27,31
Grass	0	0	147,66	53,91	49,93	58,29	51,88	41,9	25,9
Chickpea	3,1	4,75	6,88	3,52	5,86	7,23	10,82	14,17	21,34
Sileage Corn	0	0	5,31	26,63	16,61	14,7	12,44	18,96	21,32
Lentil	17,01	14,66	9,84	10,44	16,05	34,31	13,84	13,73	21,11
Pistachio	2	0,46	1,13	5,15	9,69	17,4	55,75	38,63	20,11
Sourcherry	6,58	2,48	5,42	15,45	27,64	20,38	27,93	26,37	19,83
Cotton	21,79	35,35	51,65	36,47	29,48	40,43	48,37	53,07	17,95
Sugarbeet	0	0	0,05	3,79	2,34	4,72	7,5	3,89	12,2
Rasberry	3,29	4,62	4,15	12,44	28,57	13,03	12,13	12,41	11,08
Triticale	0	0,05	0,04	3,46	1,01	1,43	3,86	10,69	10,2
Almond	0,78	0,15	0,21	6,25	7,11	18,57	11,91	9,68	8,85
Chessnut	0,83	0,36	1,59	11,07	12,4	14,37	15,33	12,01	8,34
Walnut	0,51	0,32	1,44	8,29	8,86	11,96	12,1	11,07	8,24
Orange	0,69	1,67	5,15	3,04	6,63	10,05	7,82	8,36	7,64
1st 30 crops	267,18	235,51	1.230,31	1.676,61	2.324,85	2.064,47	1.862,72	1.706,93	1.495,00
Others	42,94	53,57	113,43	152,68	148,75	342,14	508,89	323,54	136,94
Total	310,12	289,08	1.343,74	1.829,29	2.473,60	2.406,61	2.371,61	2.030,47	1.631,94

CONCLUSIONS

Sustainability goals are at the heart of organic agriculture. The importance of organic agriculture has gradually increased in order to maintain global food security and ecosystem

balance, which was initially seen as an innovative production system. In order to ensure the sustainability of agricultural enterprises, natural resources and rural development, improved agricultural practices can be realized with organic agriculture, which considers the balance of plant-animal-community health. Organic agriculture, which is also expressed as protective agriculture, is a necessary production system in terms of quality production of products, sustainable use of renewable resources, rural development and income increase, as well as public health and protection of natural resources. For this reason, the development of organic agriculture has been examined under two headings, institutional and commercial.

Within the scope of the study, it was observed that organic agriculture production and consumption increased due to increasing health concerns and climate change in the world. As a result, organic farming areas have gradually decreased. In addition, the fact that consumers are not knowledgeable and conscious about organic products, the way they are presented to the market is insufficient, their purchasing power is insufficient, there is a problem of trust and the price difference between organic products and traditional products has caused the domestic market of organic agriculture to not develop sufficiently. In this context, the necessary recommendations for the institutional and commercial development of organic agriculture are given below:

1. While making regional production plans, natural factors, especially soil, water and climate, and many micro and macro factors such as marketing, workforce, technology, export structure should be taken into consideration.

2. Grants and incentives, especially supports, are insufficient in organic agriculture. For this reason, support should be increased for both producers and consumers, a contracted production model should be adopted, and marketing support should be provided by increasing the level of organization.

3. Institutional arrangements for organic agriculture should be made and these regulations should be designed to cover the sector. In the production and marketing phase, it is necessary to develop applicable methods, especially by regulating legal gaps. First of all, legal regulations regarding the description of organic agriculture should be made both institutionally and commercially and sanctions should be applied.

4. The Organic Agriculture Information System (OTBİS), in which the records of organic agriculture are kept, should be developed and its function should be increased and the GTIP code should be provided.

5. The efficiency of agricultural cooperatives, associations or other non-governmental organizations in the organic agriculture sector should be increased. In this context, organic farming areas can be increased by providing production and marketing supports, especially investment supports.

6. Within the scope of the EU Green Deal, an action plan for increasing organic agricultural lands should be prepared and field studies should be started actively.

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EFFECTS OF DIFFERENT NITROGEN DOSES ON FORAGE YIELD AND QUALITY OF SOME ANNUAL RYEGRASS CULTIVARS

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ABSTRACT

This study was carried out as a field trial in the vegetation period of 2017-2018, at the Mustafa Kemal University Telkaliş Research and Application Center (Reyhanlı/Hatay) under the ecological conditions of Hatay-Turkey. The field trial was laid out according to randomized split block experimental design with 3 replications. Accordingly, there were 5 different nitrogen doses [0 (control), 5, 10, 15 and 20 kg/da)] in the main parcel and 3 different annual ryegrass cultivars (Hellene, Trinova and Devis) in the sub-parcel. In order to determine the effect of different N doses on yield and quality of perennial ryegrass cultivars; fresh forage yield, dry forage yield, rate of crude protein, crude ash content, neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were examined. An increase in fresh and dry forage yields were observed due to the increased nitrogen doses. Crude protein ratios increased with increasing nitrogen application as well, and the highest values were reached with a dose of 15 kg/da N application. It has been also determined that nitrogen fertilizers have no effect on the crude ash contents, NDF and ADF contents of the annual ryegrass cultivars. It has been concluded from research data that nitrogen fertilizers increase yield and quality of the Italian ryegrass up to a certain level, but higher nitrogen fertilizer doses (especially N dose at 20 kg/da) decrease the forage yield and quality. Therefore, 5-10 kg/da N dose can be recommended in Italian ryegrass production under Hatay/Turkey ecological conditions also by considering economic reasons. Besides, cv. Devis of Italian ryegrass was recommended for the region in terms of plant height, stem diameter, fresh and dry forage yield, crude protein yield and ratio, and NDF and ADF contents. The cv. Trinova can be the second choice for the region.

Key Words: *Lolium multiflorium*, Nitrogen fertilizer, Forage yield, Forage quality, Hatay

INTRODUCTION

It is known that human beings need about 1 g of protein per 1 kg of body weight in order to be fed in a healthy and stably. It is very important that amino acids, which are absolutely necessary for the body and cannot be synthesized by the body, are taken from the outside. Half of the protein taken for these amino acids must be obtained from foods of animal origin. However, even though nutrition with animal-derived proteins around the world is around 44 g per day, especially in developed countries, this amount is around 27-30 g in Turkey.

In our country, plant food sources are included in the consumption of basic nutrients. Therefore, plant food sources are one of the most consumed products. Although meat and meat products are among the most important foods to meet people's protein needs, the consumption of meat and meat products is only 3% of the total foods consumed in Turkey. One of the factors in the emergence of this situation is that meat and meat products are expensive (Alçiçek et al., 2010).

Our roughage resources obtained from forage crops cultivated together with our roughage resources obtained from meadows and pastures are having difficulty in meeting the roughage needed by our current animal existence. As a matter of fact, some researchers have provided important information on this subject (Alçiçek et al., 2010). The inability to provide cheap roughage sources to livestock causes price instability in meat and meat products in our country. Therefore, the spreading of forage crops culture is of great importance in terms of animal production in our country. On the other hand, the quality of the roughage obtained will reduce the intensive feeding needs of the animals.

It is reported that annual ryegrass, which is among the poaceae family, has an important potential in terms of roughage production (Açıkgöz, 2001). Although the annual ryegrass plant is one of the oldest cultivated plant species, it has become widespread in our country in recent years. In spite of scientific research on annual ryegrass has already started in our country, the production statistics of this plant were shared by TUIK in 2014 for the first time. The production of this plant in our country is increasing day by day (TUIK, 2022).

In order to obtain adequate roughage yield from plants that are generally considered as forage crops, it is important to provide the plant nutrients needed in the appropriate amount and time. With appropriate fertilization, sufficient yield and quality can be obtained from the forage plant, and the yield and health of the animals fed with these forages can be good (Serin and Tan, 1999). Nitrogen fertilization program is very important especially in forage crops culture where it is aimed to obtain roughage. Nitrogen fertilization of grasses grown for the purpose of obtaining roughage is very important compared to legumes (Bakır, 1985; Serin et al., 1996).

Annual ryegrass is an important forage plant that grows fast and can give 2-3 cutting in a year depending on ecological conditions. This plant species is an extremely convenient source of roughage, especially in dairy farming. Annual ryegrass is a plant that can be used both as fresh and dry feed and as silage feed for livestock (Baytekin et al., 2009). It has been emphasized that nitrogen fertilization is very important to increase the forage yield and quality of annual ryegrass cultivars (Serin et al., 1996; Çolak and Sancak, 2016).

In this study, the effects of different nitrogen doses on the forage yield and quality of some annual ryegrass varieties were investigated.

MATERIAL AND METHOD

In this study, three different annual ryegrass cultivars (Devis, Hellene and Trinova) were used as plant material. Urea (46% N) fertilizer was preferred as a nitrogenous chemical fertilizer source.

Table 1. Relative humidity, temperature and precipitation data for the growing season (2017-2018) and long-term averages (LTA) of the field where the experiment was conducted

Months	Relative humidity (%)		Temperature(°C)		Rainfall (mm)	
	LTA	2017-2018	LTA	2017-2018	LTA	2017-2018
November	66.5	72.1	13.6	14.6	34.6	35.0
December	82.0	85.8	8.6	9.5	25.5	28.7
January	84.9	86.4	7.5	9.1	95.2	120.2
February	76.0	83.1	9.7	11.8	58.5	75.2
March	70.4	73.7	13.2	15.8	54.6	10.2
April	64.8	64.1	17.5	19.2	39.7	15.0
May	60.0	61.2	22.0	23.8	15.0	18.0
June	56.1	62.2	26.2	26.5	3.8	3.8
Total	-	-	-	-	366.9	306.1
Averages	70.1	73.6	14.8	16.3	-	-

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The soil of the trial area, which was established to determine the effect of different fertilizer doses on the yield and quality of some annual ryegrass varieties, has a clay-loam structure. Its pH is slightly alkaline and the organic matter content of the soil is quite low (Yılmaz et al., 2018). The climatic characteristics of the experimental site (Hatay Mustafa Kemal University, Faculty of Agriculture, Telgaliş Research and Application Site) for 2017-2018 and long-term averages (LTA) of the experimental site where the study was carried out are given in Table 1. When the climate data in Table 1 is examined, the climatic conditions of a typical Mediterranean climate are observed. As a matter of fact, it is seen in Table 1 that the winter and early spring periods are rainy. While a total of 306.1 mm precipitation fell in the region during the vegetation period of the annual ryegrass plant,

this data was recorded as 366.9 mm in long years. It was determined that the region has less precipitation compared to long years. On the other hand, it is observed that there is an increase in temperature data compared to the long-term averages. This indicates that a temperature increase has occurred in the region.

This study was laid out according to split plots of randomized blocks design in the field with three replications. In the experiment, the sub-plots were varieties (Devis, Hellene, Triniva) and the main plots were fertilizer doses (0, 5, 10, 15, 20 kg/da). Assuming that the annual ryegrass plant will be cut 2 times in our region, fertilizer doses were applied by sowing and after the first form dividing into two equal parts. There was a 3 m among the blocks and 1 m among the parcels. Seven rows of seeds were sown in each plot and the row length was 4 m while the spacing between rows was 25 cm. The plants were harvested when they reached the full flowering period. At the beginning of the rows, 50 cm sections and side rows were removed and 5 rows of 3 m length were harvested in total. The first cutting was made on April 17, 2018, and the second cutting was on June 15, 2018. After harvesting, the fresh forage yields of the herbages obtained from the plots were weighed and the results were presented as kg/da. Some of the herbages obtained from the plots were separated and dried under controlled conditions. From this point of view, dry forage yields of the treatments were calculated. Dry forage samples taken from ryegrass dried under controlled conditions were ground in the mill for forage quality analysis. Crude protein and crude ash analyzes in ground dry samples were according to AOAC (1990), while NDF and ADF analyzes were performed by Van Soest et al. (1991).

All data obtained from the study were subjected to variance analysis in the SAS JMP 13.0 statistical package program. Among the examined features, those found to be significant at the 5% probability level were grouped with the TUKEY comparison test.

RESULTS AND DISCUSSION

FRESH FORAGE YIELD

Mean values of fresh forage yield and comparison test results are given in Table 2. While there was no difference ($0.05<P$) in terms of fresh forage yield (FFY) among cultivars, a difference ($P\leq0.05$) was found among nitrogen doses and the interactions of cultivars and nitrogen doses. Among the nitrogen doses, the highest FFY was found in 20 kg/da nitrogen application, while the lowest was found in control (0 kg/da). It was determined that there was no difference among the nitrogen treatments of 5, 10 and 15 kg/da. On the other hand, the highest FFY of interaction was obtained when 20 kg/da nitrogen was applied to cv. Devis. However, in general, it was determined that there was no difference in terms of FFY among 10, 15 and 20 kg/da nitrogen treatments in all cultivars. Control doses gave the lowest FFY value in all cultivars. The results obtained from the nitrogen dose studies conducted in different ecological regions of our country

contradict the FFY results obtained from this study. It is thought that the most important reason for the emergence of these differences is that the varieties can give different yield results (Parlak et al., 2007; Akgün et al., 2008; Çolak and Sancak, 2016; Çetin, 2017; Pak-Örün, 2019; Lale, 2020).

Table 2. Mean values of fresh forage yield and formed groups

Nitrogen doses (kg/da)	Cultivars			Mean
	Hellene	Trinova	Devis	
0 (control)	3235.6 D	3415.6 CD	3397.8 CD	3349.6 b
5	4530.0 BCD	4550.0 BCD	4805.6 ABC	4628.5 ab
10	5351.1 AB	4948.9 AB	5337.8 AB	5212.6 ab
15	5315.6 AB	5342.0 AB	5469.8 AB	5375.8 ab
20	5886.7 AB	4842.2 ABC	6246.7 A	5658.5 a
Mean	4863.8	4619.7	5051.5	

DRY FORAGE YIELD

Mean values of dry forage yield and comparison test results are given in Table 3. While the effect of nitrogen doses on dry forage yield (DFY) was not significant, the effects of cultivars and cultivars and nitrogen doses interactions were found to be significant. Among the cultivars, the highest DFY was obtained from the cv. Devis, while the cv. Hellene was also in the same group. Among the interactions, the highest DFY was obtained with 20 kg/da nitrogen application to Devis cultivar similar to the results of fresh forage yield. The lowest DFY was determined in the control treatment in all cultivars. DFY values obtained from previous nitrogen dose studies support the results obtained from this study. As a matter of fact, Kuşvuran et al. (2014) obtained similar dry forage yield values in their study.

Table 3. Mean values of dry forage yield and formed groups

Nitrogen doses (kg/da)	Cultivars			Mean
	Hellene	Trinova	Devis	
0 (control)	664.2 C	763.4 ABC	744.4 BC	724.0
5	957.7 ABC	877.5 ABC	1080.8 AB	972.0
10	1015.2 AB	997.4 ABC	1042.0 AB	1018.2
15	928.5 ABC	1016.3 AB	1049.8 AB	998.2
20	1081.9 AB	827.4 ABC	1101.8 A	1003.7
Mean	929.5 ab	896.4 b	1003.8 a	

CRUDE PROTEIN RATIO

Mean values of crude protein ratio and comparison test results are given in Table 4. While there was no difference among cultivars in terms of crude protein ratio, a difference

was found among nitrogen doses and interactions of cultivars and nitrogen doses. Among the nitrogen doses, the highest crude protein was determined in 15 kg/da nitrogen treatment. Between cultivars and nitrogen doses interactions, the highest crude protein ratio was determined with 18.3% in 15 kg/da nitrogen application to cv Devis. The fact that the 15 kg/da nitrogen application results in a higher crude protein rate than the 20 kg/da nitrogen application is due to the fact that the excess plant height in the 20 kg/da nitrogen application and the problem of lying in these plots and a poor quality weed is obtained. Many researchers reported that crude protein content in plants increased with the increase in the applied nitrogen dose (Sönmez et al., 2008; Çolak, 2015; Aktar, 2019; Lale, 2020).

Table 4. Mean values of crude protein ratio and formed groups

Nitrogen doses (kg/da)	Cultivars			Mean
	Hellene	Trinova	Devis	
0 (control)	8.5 B	8.6 B	9.3 AB	8.8 d
5	9.9 AB	11.8 AB	8.5 B	10.1 cd
10	11.3 AB	12.1 AB	12.8 AB	12.1 bc
15	14.5 AB	16.2 AB	18.3 A	16.3 a
20	12.4 AB	13.6 AB	13.9 AB	13.3 b
Mean	11.3	12.5	12.6	

CRUDE ASH RATIO

Mean values of crude ash ratio and comparison test results are given in Table 5. While there was no difference among cultivars in terms of crude ash content, a difference was found among nitrogen doses and interactions of cultivars and nitrogen doses. Among the nitrogen doses, the highest crude ash ratio was determined in the nitrogen treatment of 5 kg/da. Among the interactions, the crude ash ratio generally decreased as the nitrogen dose treatment increased in all cultivars. The highest crude ash ratio was obtained when 5 kg/da nitrogen was treated to Hellene cultivar. Similar to the results obtained from our study by Şeker (1992), it was determined that the crude ash ratio decreased with the increase of the nitrogen dose.

Table 5. Mean values of crude ash ratio and formed groups

Nitrogen doses (kg/da)	Cultivars			Mean
	Hellene	Trinova	Devis	
0 (control)	12.7 A	11.3 AB	10.1 AB	11.4 ab
5	12.9 A	12.4 AB	12.2 AB	12.5 a
10	10.2 AB	10.8 AB	7.6 B	9.5 b
15	11.4 AB	8.3 AB	9.8 AB	9.8 b
20	9.8 AB	10.0 AB	12.3 AB	10.7 ab
Mean	11.4	10.6	10.4	

NDF RATIO

Mean values of NDF ratio and comparison test results are given in Table 6. While the effects of nitrogen dose treatments and the interactions of cultivars and nitrogen doses on NDF ratio were not significant, the effect of cultivars was significant. The highest NDF ratio among the cultivars was obtained from cv. Trinova and cv. Devis with 66.3%. The lowest NDF ratio was determined in cv. Hellene. The NDF ratios were detected between 55.0% and 63.4% in different annual ryegrass cultivars by Lale (2020). These data are similar the results obtained from our study.

Table 6. Mean values of NDF ratio and formed groups

Nitrogen doses (kg/da)	Cultivars			Mean
	Hellene	Trinova	Devis	
0 (control)	66.5	67.0	63.6	65.4
5	66.3	67.2	65.8	66.4
10	62.8	65.5	67.4	65.2
15	62.8	66.0	68.1	65.6
20	58.4	65.8	66.5	63.6
Mean	63.2 b	66.3 a	66.3 a	

ADF RATIO

Mean values of ADF ratio and comparison test results are given in Table 7. While there was no difference in terms of ADF ratio among cultivars and nitrogen doses, the effect of the interactions of cultivars and nitrogen doses on ADF ratio was found to be significant. Among the interactions, the highest ADF ratio was determined with 15 kg/da nitrogen treatment to cv. Devis. The lowest ADF ratio was found in 20 kg/da nitrogen treatment to cv. Hellene. In a study conducted by Lale (2020), it was determined that and ADF ratios of different nitrogen doses and cultivars treatments varied between 38.3% and 40.7%. In our study, it was determined that lower ADF results occurred. The reason for the emergence of this is considered as a variety of species.

Table 7. Mean values of ADF ratio and formed groups

Nitrogen doses (kg/da)	Cultivars			Mean
	Hellene	Trinova	Devis	
0 (control)	37.2 AB	37.3 AB	35.8 AB	36.8
5	37.9 AB	37.5 AB	39.3 AB	38.3
10	35.1 AB	37.0 AB	38.6 AB	36.9
15	36.1 AB	38.1 AB	39.6 A	37.9
20	33.3 B	36.9 AB	38.6 AB	36.3
Mean	35.9	37.4	38.4	

CONCLUSIONS

As a result of this study, it was determined that the forage yield and quality of annual ryegrass increased with the increase of nitrogen doses. However, this increase continued till a certain nitrogen dose. Especially, there was no difference between the treatment of nitrogen dose of 15 kg/da and the treatment of 20 kg/da nitrogen dose. On the other hand, with the application of 20 kg/da nitrogen dose, the cultivars lay dormant, especially in the spring, and the crude protein ratios of the forages obtained from these cultivars were lower than the nitrogen application at 15 kg/da. As a result, it can be said that nitrogen treatment of 15 kg/da would be suitable for these cultivars. Among the cultivars, cv. Devis was found to be superior to the others.

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IMPORTANCE OF COMMERCIAL SILAGE INOCULANTS

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ABSTRACT

Fermentation of fresh fodder is a traditional forage storage method that gains importance over hay production and feeding green roughage directly to animals. This fermentation technology is simple and based on the compression and storage of green fodder in an airtight environment. In this airtight environment, lactic acid bacteria convert the free sugars into lactic acid. The addition of some lactic acid bacteria to the surface of the silage material at the beginning of fermentation affects the fermentation process at a high level. Most biological additives used for silage contain the highest possible amount of homo-fermentative lactic acid bacteria for silage fermentation. On the other hand, there is a tendency towards hetero-fermentative lactic acid bacteria as well as homo-fermentative lactic acid bacteria that promote silage fermentation. *Lactobacillus buchneri* is the most preferred bacterium among hetero-fermentative lactic acid bacteria. In addition, among the homo-fermentative lactic acid bacteria, *Lactobacillus plantarum* is the most preferred bacteria. In this review, various information about *L. buchneri* and *L. plantarum* bacteria is presented.

Key words: Silage, Fermentation, Lactic acid bacteria, inoculant

INTRODUCTION

Silage making refers to the preservation of fresh fodder or vegetable by-products by fermenting them in an aerobic environment. Many plant species, especially corn, are protected in the world based on this method. Thanks to this protection method, the freshness of the fresh fodder is preserved to a great extent, and livestock can be fed with fresh feed at all times of the year. Storage as silage has many advantages over storage as hay. Some of these can be summarized as follows. For instance, more feed can be stored per unit area, dry matter loss in feeds can be reduced and there is less dependence on weather conditions. In recent years, the use of lactic acid bacteria as inoculants in silage making technology has increased. Among these bacterial species, *Lactobacillus plantarum* and *Lactobacillus buchneri* are the most studied microorganisms and their commercial use has become widespread.

Within the scope of this review, the effectiveness of these bacterial species is discussed based on literature reports and our current knowledge.

PROTECTIVE EFFECT OF LACTIC ACID IN THE SILO

Lactic acid fermentation contributes to the preservation of food and feed by inhibiting spoilage-causing pathogens such as yeasts, molds, enterobacteria and clostridia. This protective effect is mostly due to acid production and pH decrease in the environment, but it is also the result of lowering the oxidation-reduction potential and competition for nutrients. In addition, this effect may be due to the production of inhibitory compounds (Bonestroo et al., 1993).

During homo-fermentative lactic acid fermentation, the predominant product is lactic acid, which has a limited preservative effect depending on the pH value. The inhibitory effect on the growth of degrading microorganisms mainly depends on the unresolved acid molecules (Bonestroo et al., 1993). However, some other metabolites may be more effective in inhibiting degrading microorganisms in the silage fermentation process. For example, short-chain fatty acids have antifungal activity and can therefore inhibit yeasts and molds during aerobic respiration in silage (Moon, 1983). The antimicrobial effect of lactic acid and acetic acid in silage has been reported (Adams and Hall, 1988). Unlike mineral acids, which can be used as additives to lower the pH, lactic acid and acetic acid have special antimicrobial properties (Bonestroo et al., 1993).

SİLAGE MAKING PROCESS

Conventional ensiling without silage-starting inoculants relies on naturally occurring epiphytic lactic acid bacteria. Various lactic acid bacteria can be found on plant surfaces, indicating that plants have a habit of certain types of bacteria. The exact role of lactic acid bacteria on living plants is unknown (Daeschel et al., 1987; McDonald et al., 1991). However, lactic acid bacteria are believed to protect plants against pathogenic microorganisms by producing antagonistic compounds such as organic acids, bacteriocins, and antifungal agents, because they are present in greater numbers in damaged plant parts compared to uninjured plants. These compounds limit the proliferation of degrading microorganisms in the damaged plant surface. It was believed that homo-fermentative lactic acid bacteria inhibited spoilage microorganisms, mainly by lowering the pH. However, the lactic acid produced is easily broken down by yeasts and molds when exposed to oxygen, and this theory is therefore questioned. After the plant material has been chopped, combined and compacted for silage, *Streptococcus faecalis* and *Leuconostoc mesenteroides* usually initiate fermentation. These microorganisms are replaced by microorganisms such as *L. brevis*, *L. plantarum* and *L. buchneri*, which are more resistant to acid, in the further process of fermentation. Until 1995, hetero-fermentative lactic acid bacteria were thought to have little positive effect on silage quality. For example, Bucher (1970) reported that these microorganisms convert lactic acid to acetic acid in the presence of oxygen in silage. However, in recent years, these microorganisms have been of great importance in the silage making process. Müller et al. (1991) emphasized that mostly hetero-fermentative strains that are not believed to produce enough lactic acid to obtain well-preserved silage are important.

LACTOBACILLUS PLATARUM AS A SILAGE INOCULANT

Homo-lactic acid fermentation represented by *Lactobacillus plantarum* has been one of the most used methods in silage making technology in recent years. This type of bacteria can rapidly produce lactic acid in the early stages of fermentation and quickly lower the pH value. Thus, the fermentation system can quickly create an acidic environment. This acidic environment prevents the proliferation and growth of harmful microorganisms in the silo and the preservation of the feed is ensured in a healthy way. Currently, researchers have evaluated *L. plantarum* as an inoculant in corn silage (Wang et al., 2018), sugarcane silage (Robelo et al., 2019), and *Moringa oleifera* leaf silage (Wang et al., 2019). On the other hand, this bacterial species is extensively used commercially in whole plant corn silage. The use of the *L. plantarum* bacterial strain as inoculant increases the relative density of Lactobacillus species in silages and decreases pH, acetic acid, and ammonia nitrogen while increasing lactic acid concentration in silages compared to unused silages (Mu et al., 2020). The effectiveness of the *L. plantarum* bacterial strain is still being investigated on many plant species.

LACTOBACILLUS BUCHNERI AS A SILAGE INOCULANT

Lactobacillus buchneri is a preferred microorganism as an inoculant to prevent aerobic deterioration in silages. Cooke (1995) was the first scientist to report that *L. buchneri* bacteria inhibit the growth of yeast and mold in the silo. Weinberg and Muck (1996) tested *L. buchneri* bacteria on corn silage and reported that it improved aerobic stability in corn silage. If the aerobic stability is not controlled, with such a degradation mechanism, the lactic acid concentration of the silage will decrease and it will be lost as carbon dioxide. However, dry matter loss in silage can be prevented with *L. buchneri*. In some studies, it was reported that a very high rate of aerobic stability was achieved in silage with the use of *L. buchneri* as an inoculant (Weinberg et al., 1993; Driehuis et al., 1999; Oude-Elferink et al., 2001; Kung and Ranjit, 2001; Salawu et al., 2001). Several articles suggest the use of *L. buchneri* in combination with enzymes. For instance, Taylor et al. (2000) reported that the combined use of *L. buchneri* and enzymes in high moisture corn silages improved aerobic stability. A similar effect was described by Kung and Ranjit (2001) for barley silage. However, another study reported that enzymes alone did not provide much improvement in aerobic stability in corn silage compared to *L. buchneri* (Ebling, 2002).

The combination of *L. buchneri* with other lactic acid bacteria (especially bacteria with homo-fermentative effect) is used extensively. In addition, scientific research on many plants continues today.

CONCLUSION

L. plantarum as homo-fermentative lactic acid bacteria and *L. buchneri* as hetero-fermentative lactic acid bacteria have intensively been the subject of scientific research and eventually started to be used as inoculants in ensiled plants during silage making. In addition,

various combinations of these bacterial strains are commercially available as commercial inoculants. We would like to express the opinion that these bacterial species should be effectively included in the silage production process in our country and the fermentation quality of the silages should be increased.

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EFFECTS OF KITCHEN WASTE COMPOST AND CHEMICAL FERTILIZER APPLICATION ON LETTUCE (*LACTUCA SATIVA* L.) PLANT GROWTH

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ABSTRACT

New approaches in plant production gain importance due to the accumulation of organic residues and the problems caused by agricultural inputs that are used extensively in traditional methods. For this purpose, materials such as plant and animal wastes and compost are widely used. In this study, the effects organic and chemical fertilizer source on the essential nutrient content, nitrate accumulation and the growth of lettuce plant (Lactuca sativa L.) were investigated. The experiment was carried out according to the randomized plots experimental design under greenhouse conditions, with 2 chemical fertilizer applications (with or without chemical fertilizer), 1 compost material (kitchen waste compost), four different doses (0, 8, 16, 24, 32 g kg^{-1}) and 3 replications. For 15 kg pure N da^{-1} as chemical fertilizer, 15-15-15 ($N:P_2O_5:K_2O$) fertilizer was used. Overall, kitchen waste compost (KWC) applications increased plant growth and nutrient content. However, the most significant increases occurred in the trial subjects where chemical fertilizer and KWC were applied together. The use of KWC together with chemical fertilization increased the plant height, wet weight and dry weight, as well as nitrate (NO_3) accumulation, nitrogen (N), calcium (Ca) and magnesium (Mg) content in the plant more than KWC. While the highest plant height values were found in 32 g kg^{-1}, 24 g kg^{-1} of chemical fertilizer and KWC medium in wet and dry weight, the highest N content and nitrate (NO_3) accumulation were obtained at 16 g kg^{-1} dose. As a result, compost applications were effective in nutrient concentrations when used with chemical fertilizers and nitrate accumulation remained at acceptable values.

Key words: Lactuca sativa, kitchen waste, compost, plant growth, fertilizer, nutrition

INTRODUCTION

Lettuce (Lactuca sativa L.) is an annual cool climate vegetable belonging to the Lactuca genus of the Compositae (Asteraceae) family (Kibar, 2018). Lettuce is among the vegetables whose leaves are usually consumed the most in the world (Eşiyok, 2012; Alromian, 2020; Wardany and Anjawati, 2020). Lettuce, which has been cultivated in the world for many years and consumed fondly, can be found in markets throughout the year (Taha, 2017). Lettuce can be grown in open field conditions when climatic conditions are favorable. Good results are obtained in terms of yield and quality in under cover in winter months when climatic conditions are not favorable, and in high plateau sections in summer months (Eşiyok, 2012). Lettuce is a rich supply of antioxidants in addition vitamins A and C besides phytochemicals that are anti-

cancer. Lettuce provides also with a number of dietary fiber, carbohydrates, protein and fat in small amounts (Taha, 2017). The lettuce is fairly tolerant of soil type, and will do well on soils varying from light sand to heavy clay, provided the nutritional and water status is good (Taha, 2017). Lettuce is very fond of organic matter. Lettuce grows rapidly in soils rich in organic matter and comes to harvest maturity in a short time (Kibar, 2018). Lettuce is a very sensitive species to fertilization, especially nitrogen fertilization, and fertilization is the most important factor affecting yield and quality when other conditions are equal. However, excessive and unconsciously used nitrogenous chemical fertilizers increase the nitrate accumulation in the plant, which is harmful to human health (Kibar, 2018). Similarly unconscious use of inorganic fertilizers affects the soil texture and structure, decreases the soil organic matter content and hinders the microbial activities in soil (Nirgude et al., 2019). New approaches in plant production gain importance due to environmental problems caused by agricultural chemicals used extensively in traditional methods (Özenç and Şenlikoğlu, 2017). Sustainable agriculture is a system that use of environmentally friendly organic inputs such as "compost" (Rahman and Akter, 2017). It has been stated that the combined application of inorganic and organic fertilizers to the vegetables does not only increase the yield, but also can be a method used to prevent environmental problems (Yusheng et al., 2005). Furthermore due to the high cost of imported agricultural inputs, there is a need for stable and quality material produced locally. The use of composts has been recognized generally as an effective means for improving soil aggregation, structure and fertility, increasing microbial diversity and populations improving the moisture-holding capacity of soils, increasing the soil cation exchange capacity and increasing crop yields (Marinari et al. 2000). The compost can meet the nutrient demand of crops and significantly reduce the use of synthetic fertilizers (Hernández et al., 2010). The destinations of urban sludge and other biosolids provoke environmental problems, their agricultural reuse as compost is essential to avoid those problems (Reis et al., 2014). Materials which are typically suitable for composting includes kitchen waste and biodegradable municipal waste, plus other enriched organic waste streams, such as sewage sludge, agricultural, and food processing wastes (Rahman and Akter, 2017).

Economic development and population growth have significantly increased food consumption and thus the generation of kitchen waste in recent years (Hafid et al., 2017). Kitchen waste is commonly mixed with other municipal wastes and ends by landfill but food waste could be converted for beneficial use (Kadir et al., 2016; Ghinea and Leahu, 2020). Recently, studies on the evaluation of kitchen waste for its sustainable management have been increasing (Yang et al., 2019).

In this work the effects of organic versus combined inorganic with organic sources of nutrients on the growth, and the accumulation of nitrates in the leaves of a crispa lettuce was compared. Composted kitchen waste is used for organic fertilization whereas standard 15-15-15 fertilizer applied by farmers in the area is used for the inorganic fertilizer material. Fertilization from both sources is applied at a different dose during the autumn season in the same soil. Effects of compost and cumulative fertilization on plant are studied. The aim of this study carried ut in the greenhouse conditions is to evaluate the effects of chemical fertilizer use

and KWC applications on the development of lettuce (Lactuca sativa L.) plants. To recommend the KWC dose in order to increase vegetable yield.

MATERIAL AND METHODS

1.1. Material

The pot experiment was carried out in the greenhouse of Bursa Uludağ University, Faculty of Agriculture (40° 13' 36" N latitude, 28° 51' 35" E longitude), Department of Soil Science and Plant Nutrition during the autumn of 2021. The soil used in the experiment was taken from the land of Bursa Uludag University, Faculty of Agriculture, Agricultural Application and Research Center at 0-20 cm depth. The soil used in the experiment is in the class of Vertisol (Typic Haploxerert) according to the soil classification system and Eutric Vertisol according to the FAO/Unesco classification system (Aksoy et al., 2001). It has a sandy clay loam texture, is slightly alkaline, non saline and is in the low class in terms of organic matter content. In terms of total nitrogen is low, available phosphorus and potassium are moderate (Table 1).

Kitchen waste compost (KWC) as a source of organic matter prepared by keeping it in container under greenhouse conditions after the 121 days process waited in bulk. The 15:15:15 NPK [15% (ww^{-1}) N:15% (ww^{-1}) P_2O_5:15% (ww^{-1}) K_2O] chemical fertilizer was used as inorganic nitrogen, phosphorus and potassium source. In order to define the soil and compost material used in the experiment, some basic physical and chemical analysis results are given in Table 1 and 2. The Crispa variety from Asteraceae family was selected as lettuce seed.

1.2. Methods

1.2.1. Experiment set up

The experiment was conducted in randomized plats experimental design. Two fertilizer applications (chemical fertilizer applied, no chemical fertilizer applied), one compost material (KWC), five different mixing ratios (0, 8, 16, 24, 32 g kg^{-1}), one plant variety, according to the randomized plots trial design and established in three replications (30 pots in total). While determining the ratios to be mixed into the soil, the addition of 2 t of organic material to the soil per 1 decare was taken into account. Calculations were made by accepting approximately 250.000,00 kg of soil in 1 decare area and doses were determined. The media prepared by mixing the soil and compost materials sieved through a 4 mm sieve separately in the determined proportions were filled in 16 cm x 21 cm x 20 cm polyethylene pots were used and grouped for each application. 2.5 kg of soil per pot was weighed by a scale. Then, after KWC was prepared separately at the determined rates and filled into the pots. The 15-15-15 NPK chemical fertilizer was calculated from the application of 15 kg nitrogen, phosphorus and potassium fertilizers per decare and applied to the pots in the group of chemical fertilizer application was done. So that the 15-15-15 compound fertilizer was given to fifteen pots before planting and other fertilization processes were also carried out. The lettuce seeds were planted in the pots at a depth of 2-3 cm at intervals and the pots were watered with pure water up to 40 % of the field capacity. The 3 plants showing the best emergence and germination were left in pots and

thinned out. All the planting procedures of the experiment were completed in one day. Until the end of the experiment, the necessary cultural processes were carried out in lettuce cultivation. The trial was harvested approximately 90 days later.

1.2.2. Analysis methods

Before the plants were harvested, the leaf color of each plant was determined by a portable chlorophyll meter (Konica Minolta Spad-502; Konica Minolta Sensing, Inc., Japan) and given as SPAD value. The harvesting process of the plants was carried out from the root collar part (Kacar and İnal, 2008). The leaf lengths of the harvested plants were measured with the help of a ruler by carefully separating the leaves from the knuckle without wasting time. The fresh weights of plants were taken by a micro scale after this measurement. The plants were washed first with tap water, 0.01 M HCl solution, then with distilled water respectively. The leaf samples were oven-dried at 65 °C for 48 hours (Kacar and İnal, 2008). The dry weights were also taken after the plants were dried. The leaf samples were grounded in stainless steel mill to pass through a 0.5 mm sieve (Kacar and İnal, 2008). The leaf samples were digested with 2 ml hydrogen peroxide (H_2O_2, 30%) and 8 ml nitric acid (HNO_3, 65%) in closed pressured vessels in a microwave oven (Anonymous 1994). The plant samples were dissolved for total element analysis, microwave-assisted acid decomposition was performed at high temperature and pressure (Berghof speedwave MWS-2). The Fe, Zn, Cu, Mn, and Mg contents in digest were analyzed with an inductively coupled plasma emission spectrometer (PE Optima 8300 ICP-OES). Total N was determined by the Kjeldahl method reported by Bremmer (1965). Total P was determined by the vanado molybdo phosphoric acid method (Kacar and İnal, 2008). Total K, Ca and Na was determined as reported by Kacar and İnal (2008) by flame photometer (Eppendorf Elex 6361). Care was taken to prevent contamination at all steps in the process. The nitrate (NO_3^-) accumulation in the plant was determined colorimetrically by nitriding of salicylic acid method according to Cataldo et al. (1975).

In order to determine the properties of the trial soil, soil samples taken from 0-20 cm depth were air-dried and then sieved from the 2 mm sieve. The soil texture was determined using the hydrometer method (Bouyoucos, 1951) and identified using the U.S. Department of Agriculture soil taxonomy (USDA, 2013), bulk weight and field capacity according to Tan (2005), The soil reaction (pH) and EC was measured in a 1:2.5 soil to water ratio mixture (Jackson, 1958). The soil organic carbon was determined by the wet oxidation method of Walkley-Black (1934). Exchangeable cations [calcium (Ca), magnesium (Mg) and potassium (K)] were determined after extraction with 1 M ammonium acetate (NH_4OAc) at pH 7.0 (Kacar, 2009). The extract was analyzed for Ca and K by Eppendorf Elex 6361 model flame photometer and for Mg by PE Optima 8300 ICP-OES. Available phosphorus was extracted by 0.5 M sodium bicarbonate ($NaHCO_3$) at pH 8.5 (Olsen and Dean, 1965) and concentration was measured colorimetrically after developing the blue color with ascorbic acid. Available Fe, Zn, Cu and Mn contents of the soils were extracted by 0.005 M diethylene triamine penta acetic acid (DTPA) extractant (Lindsay and Norvell, 1978). The concentration was measured by PE Optima 8300 ICP-OES. Some physical and chemical soil characteristics are given in Table 1.

Some properties of the KWC was determined after maturation. Samples were oven-dried at 65 °C for 48 hours (Kacar and Kütük, 2010). The dry weights were also taken after the plants were dried. The leaf samples were grounded in stainless steel mill to pass through a 0.5 mm sieve (Kacar and Kütük, 2010). The KWC samples were digested with 2 ml hydrogen peroxide (H_2O_2, 30%) and 8 ml nitric acid (HNO_3, 65%) in closed pressured vessels in a microwave oven (Anonymous 1994). The humic acid and humic matter was determined according to Swift et al. (1996), pH and EC was determined in a 1:10 (w/v) compost to water ratio suspension by using WTW 3110 pH meter and WTW 3310 EC meter respectively (Kacar and Kütük, 2010). The amount of organic carbon was determined according to the modified Walkley-Black method (Kacar and Kütük, 2010). Also the organic matter content was determined by multiplying the amount of organic carbon with the Van Bemmelen factor (Kacar and Kütük, 2010). Ammonium (NH_4) was determined by Indophenol Blue Method (Solorzano, 1969) and Nitrate (NO_3) was determined colorimetrically according to nitration of salicylic acid in the presence of sulfuric acid (Robarge et al.,1983). Total nitrogen (N) was determined by the Kjeldahl method as reported by Bremner (1965). The total phosphorus content was determined by using a Bausch&Lomb brand Spectronic 20 model spectrophotometer according to the vanadomolybdophosphoric yellow color method (Kacar and İnal, 2008). The total Na, K and Ca amounts of the KWC samples were determined according to the dry burning method, with the Eppendorf Elex 6361 model flamephotometer device. Total Mg was determined with the Perkin Elmer Optima 2100 model ICP-OES device (Pratt, 1965). The Iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) amounts of KWC samples were determined by, Perkin Elmer Optima 2100 model ICP-OES device (Kacar and İnal, 2008).

The data obtained at the end of the experiment were analyzed with the analysis of variance according to the randomized parcels trial design in the "JUMP" package program, and the LSD multiple comparison test was applied at 1% and 5% significance levels to determine the difference between the applications in the statistically significant results. The results are expressed as letters next to the averages.

Table 1. Some physical and chemical properties of the soil used in the research.

Analysis	**Soil**	**Analysis**		**Soil**
Clay, %	27.85	Total Nitrogen (N), %		0.09
Silt,%	18.75	Excangeable (me 100 g^{-1})	Potassium (K)	0.46
Sand, %	53.46		Calciyum (Ca)	19.75
Texture	Sandy clay loam		Magnessium (Mg)	2.41
Reaction (pH)	8.25		Sodium (Na)	0.49
EC, µS cm^{-1}	90.64	Available (mg kg^{-1})	Phosphorus (P)	8.43
Organic material, %	1.10		Iron (Fe)	9.54
$CaCO_3$, %	1.55		Copper (Cu)	2.25
Bulk density, g cm^3	1.35		Zinc (Zn)	1.12
Field capacity, %	36.10		Manganese (Mn)	19.48

Table 2. Some physical and chemical properties of the KWC used in the research.

Analysis		KWC	Analysis			KWC
Reaction (pH)		6.05			Nitrogen (N)	5.64
EC, mS cm^{-1}		5.81			Phosphorus (P),	0.15
C/N ratio		8.61		%	Potassium (K)	1.37
	Dry weight	45.65			Calciyum (Ca)	0.44
	Organic material	83.63			Magnessium (Mg)	0.16
	Organic carbon	48.51			Sodium (Na)	0.11
%	Humic matter	9.25			Iron (Fe)	165.00
	Humic acid	3.16		mgkg^{-1}	Copper (Cu)	17.00
	NH_4-N	0.20	Total		Zinc (Zn)	26.00
	NO_3-N	0.299			Manganese (Mn)	125.00

RESULTS AND DISCUSSION

1.3. Leaf length

Plant height is an important component that helps to determine plant growth (Sanni, 2016). Application of KWC to soil at increasing rates caused significant differences in leaf length of lettuce plant. Likewise, the application of chemical fertilizers with KWC was also effective on plant leaf growth. The leaf length parameter of lettuce were significantly influenced ($P<0.05$) by the combined application of KWC and chemical fertilizer treatments (Table 3).The most statistically significant increase in leaf length of lettuce plant was obtained with the application of chemical fertilizer with 32 g kg^{-1} (% 3.2) KWC into the soil (Table 3). Farag et al. (2013) reported that they had the highest vegetative gowth on lettuce by using 150 mg kg^{-1} nitrogen level combined with % 4 compost. It was stated that the yield elements of lettuce plants enhanced with the increase in the doses of nitrogenous and organic fertilizers (Kavak et al., 2003; Wanga and Lia, 2004; Hernández et al., 2010; Brito et al., 2014; Karataş and Büyükdinç, 2017). This increment could be due to the presence of phytohormones in organic fertilizers that incite plant growth (Hernández et al., 2010). Although the application of KWC to the soil enhanced the leaf width of the lettuce plant, there were no significant differences determined between KWC treatments with means of leaf width.

Figure 1. Plant growth in KWC compost at different growth stages.

1.4. Plant weight

The data indicated that fresh weight and dry weight of lettuce enhanced with the application of KWC by increasing doses (Table 3). Compost and chemical fertilizer applications and their different doses created statistically significant ($P<0.01$) differences on fresh weight and dry weight of lettuce (Table 3). The addition of chemical fertilizers to the KWC increased the fresh and dry weight of lettuce plant leaves. The highest fresh weight and dry weight results were obtained when 24 g kg^{-1} KWC was applied with 15-15-15 chemical fertilizer (15 kg N da^{-1}). Some authors reported that lettuce require soils with high organic content and adequate mineral nutrients favoured the production of higher plant fresh and dry weight (Hernández et al., 2010; Brito et al., 2014; Hossain and Ryu, 2017; Taha et al., 2017; Alromian, 2020). Farag et al., (2013), reported that the increasing nitrogen level up to 150 mg kg^{-1} significantly increased plant fresh and dry weights and yield of lettuce. Tsiakaras et al. (2014) indicated that nitrogen rates as 300 and 450 mg L^{-1} resulted in higher fresh weight but lower dry weight, respectively.

1.5. SPAD values

The SPAD (Chlorophyll meter) values of the leaves, measured before harvesting the experiment (Table 3). The results showed an constantly decrease with the increase of the KWC applied. Similar results were also reported in other studies (Ceglie et al., 2011; Naderi and Ghadiri, 2013). The measured values were not statistically different, despite the low SPAD values measured in the KWC treatment which added the chemical fertilizer and in KWC alone treatments. Cozzolino et al. (2020) reported an increase in SPAD index when the lamb's lettuce plants are grown under the diffuse light cover film and with increased N dose.

1.6. Total nitrogen

Nitrogen (N) is an essential element required for successful plant growth. In many agricultural conditions, nitrogen is a nutrient limit for high plant growth and yield (Marschner, 1995). While the combine application of chemical fertilizer and KWC made a significant difference on the total nitrogen content of the lettuce plant, the effect of the KWC used was statistically significant ($P<0.01$) depending on the application doses. The average N content of lettuce leaves was determined as 3.86 %. The highest leaf N concentration was found 4.36 % and observed by 16 g kg^{-1} KWC combined with 15-15-15 fertilizer. The N contents of the lettuce leaves were considered normal for lettuce according to the nutrient sufficiency range of N (3.50 - 4.50 %) for lettuce proposed by Jones et al., (1991). They showed statistically significant differences as compared to non 15-15-15 NPK fertilizer added KWC. It is noteworthy that the total N amount in the lettuce plant significantly increased in pots where inorganic fertilizer was added. Plants without the addition of chemical fertilizer to the KWC showed less development, and it is considered that there is a less nutrient accumulation due to the low dry matter content in these plants. Nutrient balance in a composted mixture is achieved by the C/N ratio (Musa et al. 2020). Fricke and Vogtmann (1993) reported that compost must have a C/N ratio of 18 or less for production purposes and to prevent N competition in plants and soil microorganisms. In this study, a 121 days decomposition process was used for KWC

with C/N ratios of 8.61 at near the suggested range for KWC and food waste which should promote slow N mineralization. In similar studies, the C/N ratio was found by Seo et al. (2004), 10.4 -15.5, and Adhikari et al. (2008), 10-15 for food waste compost. In addition, compost is considered as a slow release nitrogen fertilizer because it only mineralizes a fraction of total N, estimated at 2% in a crop cycle (Hernández et al., 2010; Reis et al., 2014). Brito et al., (2014) reported that the average increase in N accumulation of lettuce with 15 t ha^{-1} and 30 t ha^{-1} of compost was 48 and 99 mg N plant $^{-1}$ respectively, these values are 40 % less than the available nitrogen of the compost, and this loss may be due to leaching.

1.7. Nitrate (NO_3-N) accumulation in leaves

In the leaf of the lettuce samples, the concentration of nitrate (NO_3-N) ranging between 310,33 and 1143,80 mg kg^{-1}. A remarkable increase in nitrate accumulation was observed in the KWC with chemical fertilizer treatments. Among the compost doses added to the soil, the highest nitrate content was obtained with an average of 1143,80 mg kg^{-1} in the environment where enriched compost with nitrogen fertilizer was applied. The 16 g kg^{-1} KWC with 15-15-15 NPK fertilizer treatment showed the highest nitrate concentration compared to all fertilization treatments. While the nitrate content of the lettuce plant, which was applied chemical fertilizers with KWC, increased significantly compared to the KWC application patterns without chemical fertilizer, it created statistically significant ($P<0.05$) differences (Table 3). As can be seen from the table, nitrogen added with chemical fertilizer application caused the nitrate content in the plant to increase considerably. Nitrogen fertilization facilitates nitrate accumulation as a result of reduced nitrogen consumption in plant tissues (Van der Leij et al., 1998). Accumulation of nitrates results from an imbalance between the uptake and translocation of nitrates by the xylem, and the reduction of these nitrates to ammonia which is subsequently rapidly incorporated into amino acids (Maynard et al., 1976). Accumulation of nitrates in lettuce has been shown to be affected by the soil texture and the source of fertilizer-N, the NH_4-N-to-NO_3-N fertilizer-N ratio, the timing of fertilizer-N release, the low light intensity and duration, crop season, lettuce type and cultivar. In most types of lettuce, including the romaine type, the highest concentration of nitrates is normally observed in the external leaves (Pavlou et al., 2007).

The nitrate content of lettuce was found as 65-330 mg kg^{-1} by Lyons et al. (1994) from Australia; 2782 mg kg^{-1} by Dejonckheere et al. (2001) from Belgium; 1945 mg kg^{-1} by Ximenes et al. (2000) from Brazil; 580-1454 mg kg^{-1} by Zhou et al. (2000) from China; 625-3209 mg kg^{-1} by Ysart et al. (1999) from England; 428-810 mg kg^{-1} by Santamaria et al. (1999) from Italy. From Türkiye, Işık et al. (1996) and Artık et al. (2002) were reported the nitrate as 0.20-11304 mg kg^{-1} and 317.36-1117.77 mg kg^{-1} for lettuce respectively. The European Commission has specified nitrate levels for spinach and lettuce as the maximum acceptable limit of 3000-4500 mg kg^{-1} for the winter season, and 2500-3500 mg kg^{-1} for the summer season (MAFF, 1999). According to the Turkish Food Codex, the maximum allowable nitrate amount for spinach and lettuce is 3500 mg kg^{-1} fresh weight (TFC, 1997). The nitrate content of lettuce samples was consistent with the values referred to above. On the other hand the nitrate content of the lettuce samples of this study determined lower than the values reported in the codex.

1.8. Total phosphorus, potassium and sodium

Increasing the rates of KWC above to 8 g kg^{-1} was seems to associated with an increase in P, K, and Na concentrations in the lettuce plants. However, the effects of the applications and their doses on the total P, K and Na content of the lettuce plant were not found statistically significant (Table 3). Phosphorus content of plants was found at the highest level with 0.28 %. This value is quite low compared to the limit values (0.45 - 0.80 %) of the P content of the lettuce plant (Jones et al., 1991). Potassium content of plants was found between 5.19-6.50 % except for control pots. This value is in the limits compared to the limit values (5.50 - 6.20 %) of the K content of the lettuce plant (Jones et al., 1991). Özenç and Şenlikoğlu (2017) determined a similar result in their study with the spinach plant. Some researchers (Çıtak et al., 2011; Barral et al., 2011) reported that differences in phosphorus mineralization rate among organic fertilizers due to different fixation and microbial immobilization. Mupondi et al. (2006) stated that, leaf N, P and K concentrations were affected depending on the characteristics of the compost they used in the vegetable seedlings they were working with, but the inorganic fertilization used did not affect the leaf P concentration. On the other fand Farag et al., (2013) reported that the interaction effect between N levels and compost showed that using 150 mg kg^{-1} N combined with 4% compost increased P and K. These results are in agreement with that of Taha et al. (2017) reported that lettuce plants that organic fertilizing by compost (15 $m^3 fed^{-1}$) produced the maximum values of N, P and K percentages and its uptake by lettuce leaves.

1.9. Total calcium and magnessium

The effect of the KWC with chemical fertilizers (15-15-15) on the total Ca and Mg content of the lettuce plant was found to be statistically significant ($P<0.05$). A remarkable difference in Ca content was observed in the KWC combined with chemical fertilization treatments compared to KWC results. Application of KWC merely with the increasing doses to the soil as a source of organic matter decreased the Ca content of the lettuce plant (Table 3). Increasing the rates of KWC with commercial fertilizer was seems to associated with an increase in Ca concentrations in the lettuce plants. On the other hand, increasing the KWC ratios with commercial fertilizer seems to be associated with a decrease in Mg concentrations. Farag et al. (2013) reported that the interaction effect between nitrogen levels and compost showed that using 150 mg kg^{-1} nitrogen combined with 4% compost increased Ca and Mg. Contrastly some researchers reported that application high rates of N decreased the absorption of P, K, and Ca in plants, which is presumably due to the dilution on these nutrients in plant tissues (Stagnari et al., 2007; Awaad et al., 2016). The highest leaf Ca and Mg encountered in this study was 1.59 and 0.41 g kg^{-1} respectively. The upper limit of the Ca and Mg range was below the sufficiency limit (2.0 - 2.8 %) for Ca and (0.6 - 0.8 %) for Mg reported by the reference (Jones et al., 1991). On the other hand, Hochmuth et al., (1991), reported the sufficiency limit of the Ca (2.0 - 2.8 %) and Mg (0.25 - 0.40 %). Accordingly, lettuce leaves are considered to have low Ca content and sufficient Mg content.

1.10. Total micronutrient contents

The impact of different doses of KWC with/without 15-15-15 NPK fertilizer on the concentration of micronutrients like Fe, Cu, Zn and Mn in a lettuce plant has been shown in Table 3. It is concluded that there is not a statistically significant impact of KWC on the concentration of metals. However depending on the application doses, slight increases were detected in the iron and zinc contents of lettuce leaves. According to the nutrient sufficiency range for lettuce proposed by Jones et al., (1991), concentration ranges for Fe (40-100 mg kg^{-1}), Zn (20-50 mg kg^{-1}), Cu (5-20 mg kg^{-1}) and Mn (11-250 mg kg^{-1}) were considered normal for lettuce. When the micronutrient contents of lettuce leaves are evaluated, it is understood that these elements are at normal levels.

Table 3. The effects of KWC and chemical fertilizer applications on some parameters on the leaf of lettuce plant.

	g kg^{-1}	cm	g		cm		mg kg^{-1}	%						mg kg^{-1}			
Fertilizer Application	KWC Doses	Leaf length	Fresh weight	Dry weight	Leaf width	Spad	NO_3^-	N	P	K	Ca	Mg	Na	Fe	Cu	Zn	Mn
Fertilized	0	11.28 d	37.35 de	2.06 cd	10.00	23.06	895.53 a	4.04 abc	0.25	5.13	1.56 a	0.41 a	0.36	99.31	1.95	25.63	144.96
	8	11.89 cd	40.94 cde	2.27 bc	9.36	21.36	873.36 ab	4.32 a	0.26	5.42	1.50 a	0.38 ab	0.34	80.75	1.86	34.53	128.65
	16	13.80 ab	45.73 bcd	2.41 bc	10.96	21.30	1143.80 a	4.36 a	0.28	5.70	1.50 a	0.37 ab	0.39	67.02	1.79	30.99	124.32
	24	13.35 abc	56.15 a	3.30 a	11.00	21.13	984.20 a	4.24 ab	0.26	5.54	1.59 a	0.35 bc	0.36	134.60	2.32	28.74	126.72
	32	14.78 a	51.13 ab	3.00 ab	11.00	20.83	979.76 a	4.28 a	0.27	6.50	1.51 a	0.35 bc	0.40	89.51	1.90	32.56	130.36
Not fertilized	0	9.44 e	24.78 f	1.46 d	8.66	22.43	434.46 bc	3.46 de	0.27	5.02	1.40 ab	0.37 ab	0.35	63.61	2.09	19.68	125.32
	8	11.00 de	33.39 ef	2.06 cd	9.00	21.36	310.33 c	2.98 e	0.28	5.35	1.49 ab	0.35 bc	0.36	60.40	1.77	25.26	117.45
	16	12.33 bcd	42.49bcde	2.65abc	10.00	21.30	434.46 bc	3.46 de	0.26	5.19	1.41 ab	0.36 ab	0.38	78.75	1.66	21.35	122.72
	24	12.55 bcd	44.50 bcd	2.81abc	10.00	21.13	913.26 a	3.77 bcd	0.28	5.63	1.28 bc	0.34 bc	0.37	77.46	1.72	22.19	116.85
	32	14.27 a	48.90 abc	3.27 a	11.83	20.83	766.96 ab	3.73 cd	0.24	5.32	1.19 c	0.31 c	0.41	67.72	2.03	30.53	117.59
		**	**	**	n.s.	n.s.	*	**	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.
		P<0.001	P<0.001	P<0.001			P<0.005	P<0.001			P<0.005	P<0.005					

CONCLUSIONS

Kitchen waste compost can be used as an organic fertilizer. Using it together with chemical fertilizers increases the yield values even more. Applying kitchen waste compost with using commercial source such as 15-15-15 NPK fertilizer added a rate of 24 g kg (6 t da^{-1}) was sufficient to obtain optimal leaf nutrient concentrations and growing aspects of lettuce var except for the P content. One of the factors affecting the nitrate content of vegetables is the excessive use of nitrogen fertilizers to increase yield. At this study the nitrate content of the lettuce plant, which was combine applied chemical fertilizers with KWC, increased significantly. However, this increments determined lower than the values reported in the codex. For this reason, fruit and vegetable producers should be trained and supervised on the use of appropriate fertilizers. In order to reduce the nitrate content in lettuce, which has a high nitrate content among vegetables, it is necessary to develop low nitrate-containing cultures and harvest them at the appropriate time. It should be taken into account that the outer leaves of lettuce contain more nitrates. Future works should investigate the effects of both organic and inorganic fertilizers on leaching of nitrate. Regarding mineral composition of lettuce leaves, their micronutrient content showed no statistical differences between treatments with compost. The results have confirmed the validity of the hypothesis that proper fertilization with the KWC can increase lettuce growth and yield in a sandy clay loam texture soil. The KWC did not show weed contamination. Weed number increased under lower doses of the compost, indicating that compost was not contaminated with weed seeds and, nevertheless its maturity, compost exhibited some control of weed germination.

Disclosure statement

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QUALITY OF AGRICULTURAL LANDS FOR GLOBAL FOOD SUPPLY SECURITY

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ABSTRACT

Population growth, unequal distribution of resources, climate change, waste, food losses, etc. These issues have become a global problem by threatening food supply security. Ending poverty and solving the problem of hunger are among the top priorities in the United Nations' Sustainable Development Goals. With these goals, the importance of ensuring the self-sufficiency of the country is emphasized. Self-sufficiency in terms of food; It is the balancing of production and consumption in the country (without external dependence). However, there are some obstacles in the countries' inability to provide food sufficiency. These; climate change, population growth, international migrations, limited agricultural lands and water resources, migration of the population working in agriculture to the city due to low income and price fluctuations in the agricultural sector, loss of productivity, high input prices, high risks, implementation of wrong agricultural policies. Agricultural lands and water availability, which are among these factors, are the main production factors that affect the agricultural production potential. In this direction, it is aimed to compare the food supply security of 10 countries (including Turkey) with the highest agricultural production value according to FAO data. In the comparison, evaluations will be made by considering the products with the highest production amount in terms of product groups in Turkey. Production potentials of selected products in countries, foreign trade statistics, land structures, water resources and self-sufficiency in food supply are discussed. In the study, secondary data were used, and policies were developed to improve the quality of agricultural lands in ensuring the food supply security of the countries.

INTRODUCTION

Food security of the United Nations Food and Agriculture Organization (FAO); It defines it as “continuous access, both physically and economically, to adequate, safe, nutritious and healthy food in order to meet the nutritional needs of people”. Food supply security is important not only for consumers, but also for producers who carry out production activities in the agricultural sector and workers employed in the sector. However, population growth, unequal distribution of resources, climate change, waste, food losses, etc. These issues have become a global problem by threatening food supply security. In this direction, among the "Sustainable Development Goals", ending poverty in the first place, solving the hunger problem in the second place, healthy individuals in the third place, access to clean water and public health in the sixth place, reducing the inequalities between and within the country in the tenth place, and responsible in production and consumption in the twelfth place. and the dissemination of sustainable models was emphasized (UN, 2022). For this reason, in the study,

food safety is defined as "providing the quality of supply of agricultural products, which are the raw materials of food necessary for the healthy sustainability of human life, and the hygienic accessibility of the processing and marketing organization for all income groups".

While the world population was 7.6 billion in 2018, it is estimated that it will reach 9.7 billion in 2050 and 10.87 billion in 2100. While 18.71% of the current population belongs to China and 17.72% to India, Turkey ranks 18th with 1.08% (FAO, 2022a). While births and prolongation of life expectancy cause population growth throughout the world, international migrations are also effective based on countries. Therefore, this situation increases the need for food supply. Increasing population and limited access to food cause malnutrition (~815 million people) and threaten the lives of approximately 52 million children worldwide. especially food safety: It is seen in parts of Sub-Saharan Africa, Southeast Asia and Western Asia (UN, 2017). As a matter of fact, countries aim to meet the needs of society in terms of quantity and quality in food production. While meeting the food needs of the citizens in the country in terms of quality allows the society to access healthy foods, meeting them quantitatively increases the self-sufficiency of the country.

Self-sufficiency in terms of food; It is to meet the nutritional needs of the population in the country without processing or by processing the agricultural products produced within the borders of the country. In other words, it is the balancing of production and consumption in the country (without external dependence). the country's attainment of qualification; It contributes to the increase of social welfare, prevention of hunger, ensuring fair accessibility of the society to products, development of the country, healthy living of individuals, access to clean-safe food and ensuring stability/sustainability in production. For this reason, it is necessary to ensure food adequacy in countries. However, it is not available under certain conditions. Among the obstacles that countries cannot provide food sufficiency are climate change, population growth, international migration, limited agricultural lands and water resources, migration of the population working in agriculture to the city due to low income in the agricultural sector and price fluctuations, loss of productivity, high input prices, risks. There are factors such as the fact that it is too much, implementation of wrong agricultural policies (Kıymaz and Şahinöz, 2010).

The availability of land and water is one of the basic factors that restrict agricultural production, and it is not possible to realize production without land. Even if it seems possible with alternative production systems such as soilless agriculture and vertical agriculture, there is a need for space/space for production to be carried out with these systems. Therefore, land is one of the important production factors affecting agricultural food supply security. Water resources, on the other hand, are an important production factor in terms of providing productivity increase in agricultural production and enabling product differentiation (cereals, vegetables, fruits, industrial plants, etc.) to meet the various food needs of the society. Although these production factors are limited in the world and in Turkey, population growth increases the pressure on these factors. As a matter of fact, there are 4.91 million hectares (ha) of agricultural land (ITB, 2014) and 1.4 billion km3 million m3 of water resources in the world. 2.5% of the water resources consist of fresh water and 23.9 million km3 is in the form of glaciers and cannot be used (Kara and Çiftçi, 2010; Tuncer and Kaya, 2010). Therefore, water resources should be used in a controlled manner to reduce the risk in the production of agricultural products that will meet the needs of the population. For controlled use, it is necessary to reduce water losses, prevent illegal use, use pressurized irrigation systems and prepare an irrigation program by taking into account the need for plants. However, since the main purpose in agricultural enterprises is profitability, the damages to soil, water, human beings, living things and the environment are not considered. This situation increases the demand for irrigation water. The amount of precipitation, temperature values, geoFigureical location and strategic importance of the country or region are also effective in the increase in demand. In this

direction, within the scope of the study, it is aimed to determine the agricultural production potentials, foreign trade structures and food supply adequacy of the countries that are in the first place in agricultural production value, considering the land characteristics.

MATERIAL AND METHOD

In the study, statistics, reports, scientific publications etc. By examining the documents, the importance of the subject was revealed with the help of secondary data. In this direction, 10 countries with the highest agricultural production value were determined by using FAO data. These countries include China, India, the United States of America (USA), Brazil, Indonesia, Iran, Russia, France, Japan, and Turkey, according to 2020 data (Table 1). In the food safety comparisons of the countries, evaluations were made by considering the products with the highest production in terms of product groups in Turkey. Among these products, in the grains group; wheat, barley and corn in the legumes group; chickpeas, lentils and beans, oil crops group; sunflower, cotton and olive in the vegetables group; tomatoes, peppers and onions in the fruit group; apple, grape and watermelon, in the group of hard-shelled fruits; hazelnuts, pistachios and walnuts, and potatoes in the root crops group (Table 2). Production potentials of selected products, foreign trade statistics were examined and countries' land structures, water resources and self-sufficiency in food supply were compared. In addition, the amount of water needed for the products currently grown in the countries, the total precipitation amount of the country, the effective precipitation amount, the net irrigation water need, and the total irrigation water need were calculated. In this direction, the situation of meeting the water demands of the products produced with precipitation has been determined. The following formulas were used to determine these parameters. In the calculation of effective precipitation, 80% of the total precipitation was considered and the guide named "Plant Water Consumption of Irrigated Plants in Turkey" was used to determine this value. In irrigation efficiency, T.C. The 56% value stated in the “Technical Support Service Procurement Work for the Preparation of the Konya Closed Basin Sectoral Water Allocation Plan-Draft Water Demands Analysis Report” carried out by the Ministry of Forestry and Water Affairs General Directorate of Water Management in 2017 was considered.

Table 1. Agricultural Production Value by Countries (2020-thousand US$)

	Country	**Value of Agricultural Production**
1	Çin	1.555.973.863
2	Hindistan	403.454.686
3	ABD	307.388.822
4	Brezilya	135.846.342
5	Endonezya	107.181.525
6	İran	91.691.266
7	Rusya	81.510.584
8	Fransa	67.047.015
9	Japonya	66.996.997
10	Türkiye	54.405.852

Source: FAO, 2022b.

Table 2. Products with the Highest Production in Turkey by Product Groups (2020- Tons)

Product Group	Crops	Production Quantity	Product Group	Crops	Production Quantity
Cereals	Wheat	20.500.000	Vegetables	Tomatoes	13.204.015
	Barley	8.300.000		Pepper	2.636.905
	Sweetcorn	6.500.000		Onion, dry	2.280.000
Pulses	Noah	630.000	Fruits	Apple	4.300.486
	Lentil	370.815		Grape	4.208.908
	beans, dry	279.518		Watermelon	3.491.554
Oil Seeds	sunflower	2.067.004	Nuts	Hazelnut	665.000
	Cotton	1.773.646		pistachios	296.376
	olives	1.316.626		Walnut	286.706
Edible Roots and Tubers	potatoes	5.200.000			

Source: TÜİK, 2022.

$$Product\ Water\ Requirement = Area\ of\ the\ product\ in\ question\ x\ Average\ water\ consumption\ of\ the\ product\ in\ question$$

$$Total\ Rainfall = Production\ area \times Annual\ precipitation$$

$$Effective\ Rainfall = Total\ precipitation \times 0.80$$

$$Net\ Irrigation\ Water\ requirement = Amount\ of\ water\ needed - Effective\ precipitation$$

$$Total\ Irrigation\ Water\ Requirement = Net\ irrigation\ water\ demand\ /\ Irrigation\ Efficiency\ (0.56)$$

RESULTS AND DISCUSSION

Risks in the production of foods, which are the basic building blocks of human life, threaten food safety. Among these risks, during the production phase; While there are factors such as the use of wrong tillage techniques, insufficient use of mechanization, limited water availability, insufficient use of pesticides and fertilizers, not preferring quality seeds / seedlings / saplings, not using qualified labor, application of wrong harvesting techniques, climatic conditions. There are also risks in the transportation, processing, storage and marketing of the product. In addition to these, the stages of cooking and keeping the product after purchasing by consumers also threaten food safety. As can be seen, food safety can be adversely affected by many factors. These negativities, on the other hand, lead to a decrease in the annual production amount and prevent the food needs of the society from being met regularly and healthily. While the world population was 7.26 billion people in 2014, it increased to 7.76 billion people in 2020. In the said years, the number of undernourished people increased from ~607 million people to 768 million people, and the highest increase was detected in 2020. The pandemic in 2020, which affected the whole world, also has a share in this rapid increase in the number of undernourished people. In the pandemic conditions, countries have managed the process by applying curfews and restrictions on national/international trade. In particular, the restrictions on trade caused the

deterioration of products such as fresh fruits and vegetables and reduced people's access to food. A similar change is observed in people with severe food insecurity problems. The number of these people increased from 604 million people in 2014 to 927.6 million people in 2020 (Figure 1).

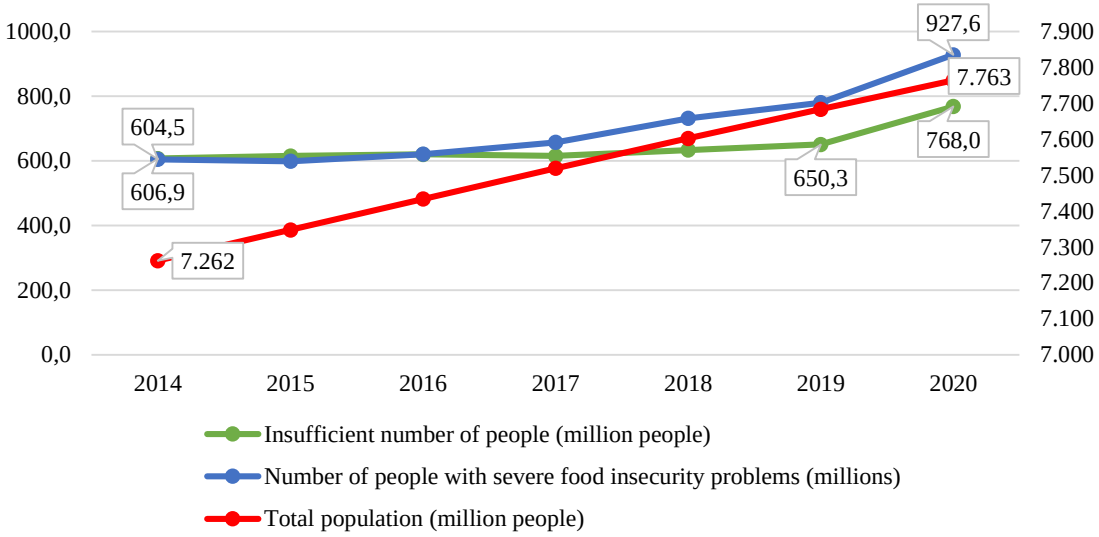

Figure 1. World Population, Number of Undernourished and Severely Food Insecure People (millions). (Source: FAO, 2022a; World Bank, 2022)

In food safety, not only the population and production amount are focused, but also the purchasing power of consumers, access to food, the quality of food products, the existence of natural resources (soil, water, etc.) needed to produce these products and the durability of these resources should also be considered. In line with these criteria, the Global Food Security Index (GFSI) is calculated in 113 countries by the Economist Intelligence Unit. As seen in Figure 2, from the countries covered in the study; While China, USA, Brazil, France, Russia, Japan, and Turkey have sufficient level of food security, moderate sufficiency is seen in India and Indonesia. Iran cannot be interpreted due to lack of data.

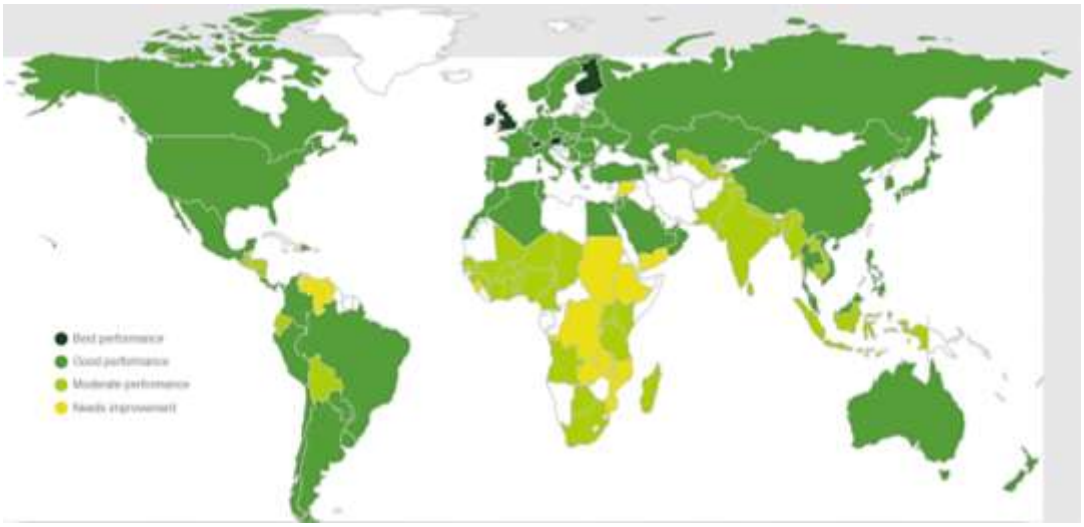

Figure 2. Country Performance by Global Food Security Index (Source: GSFI, 2022)

The existence of agricultural lands and irrigation status are important factors in ensuring food security, and they differ according to the borders of the countries and land use patterns (housing, workplace, agriculture, etc.). Table 3 shows the agricultural land assets of the selected countries as of 2000-2020. As seen in the table, there are 4.74 billion hectares of agricultural land in the world in 2020, and the 10 selected countries account for 36.80%. When the changes in the agricultural land assets of the countries are examined over the years, it is seen that there is a decrease in general (except for Indonesia, Brazil and China). It was determined that the highest decrease was in Iran with 25.24% and Japan with 16.85%, and the highest increase was in Indonesia with 32.06%. On the world average, it has been determined that agricultural lands have decreased by 2.74%.

In addition to the reduction of agricultural lands, the irrigation potential also has an impact on the amount of agricultural production. As can be seen in Table 4, the irrigation potentials, total areas equipped for irrigation and irrigated areas equipped for irrigation are not sufficient. Although it varies according to the countries, the existence of areas with irrigation potential constitutes 26.98% of the total agricultural lands, and the presence of lands equipped for irrigation constitutes 17.38%. Water stress, which is another criterion in the table; It represents the ratio between the total freshwater withdrawn by all major sectors and the total renewable freshwater resources, after considering environmental flow requirements. The highest water stress by countries; It is experienced in Iran with 81.29%, India with 66.49%, Turkey with 45.38% and China with 43.22%. Water use efficiency: It defines the added value per unit of water used and is \$82.82/m^3 in France, \$56.71/m^3 in Japan, \$43.66/m^3 in the USA. Water use efficiency in irrigated agriculture; It represents the agricultural added value per unit water used for the agricultural sector (crop production, livestock, and aquaculture). This value is \$2.19/m^3 in China, \$1.71/m^3 in France and \$0.73/m^3 in Japan. In Turkey, water use efficiency is 14.06 \$/m3, and in irrigated agriculture it is 0.29 \$/m^3. In water stress and water use efficiency, water availability, water management strategy, consumer behavior, climate characteristics, agricultural production pattern, technology use, geoFigureical location, etc. factors are influential. According to 2018 data of FAO, renewable water assets are 8.65 million hm^3 in Brazil, 4.52 million hm^3 in Russia, 3.07 million hm^3 in the USA, 2.84 million hm^3 in China and 2 in Indonesia. 02 million hm^3, 1.91 million hm^3 in India, 430 thousand hm^3 in Japan, 212 thousand hm^3 in Turkey, 211 thousand hm3 in France and 137 thousand hm^3 in Iran (Aquastat, 2022). In the study conducted by the United Nations, it was estimated that 40% of the civil wars that took place in the last 60 years were related to natural resources (UN, 2017).

Table 3. Agricultural Land Presence by Countries (1,000 Hectares)

Ülkeler	2000	2005	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
China	523.731,00	526.682,22	528.916,80	528.885,10	528.799,50	528.801,50	528.695,00	528.633,40	528.552,90	528.512,10	528.509,70	528.509,20	528.508,10
India	180.975,00	180.126,00	179.573,00	179.670,00	179.642,00	179.698,00	179.728,00	179.675,00	179.578,00	179.235,60	179.115,56	179.044,95	179.045,08
USA	414.399,00	412.939,60	406.991,70	405.036,40	403.081,05	403.627,00	404.172,90	404.718,70	405.264,60	405.810,35	405.810,35	405.810,35	405.810,35
Brazil	228.323,50	228.842,00	231.834,20	232.555,00	233.275,80	233.996,60	234.717,40	235.438,20	236.159,00	236.878,60	236.878,80	236.878,80	236.878,80
Indonesia	47.177,00	51.846,00	55.600,00	56.500,00	56.500,00	57.000,00	57.000,00	57.300,00	60.200,00	62.300,00	62.300,00	62.300,00	62.300,00
Iranian	62.884,00	47.631,00	46.539,40	46.392,60	46.245,80	45.954,00	45.954,00	45.954,00	45.954,00	46.435,00	46.915,00	47.013,00	47.013,00
Russia	217.162,00	215.680,00	215.494,00	215.494,00	215.494,00	215.494,00	215.494,00	215.494,00	215.494,00	215.494,00	215.494,00	215.494,00	215.494,00
France	29.807,40	29.390,40	28.926,30	28.878,60	28.844,80	28.773,70	28.766,50	28.726,90	28.718,02	28.697,50	28.660,10	28.621,19	28.553,75
Japan	5.258,00	4.692,00	4.593,00	4.561,00	4.549,00	4.538,00	4.518,00	4.496,00	4.471,00	4.444,00	4.420,00	4.397,00	4.372,00
Turkey	40.479,00	41.223,00	39.012,00	38.247,00	38.407,00	38.423,00	38.554,00	38.551,00	38.328,00	37.964,00	37.802,00	37.716,00	37.762,00
World	4.878.013,49	4.820.422,03	4.794.148,17	4.813.488,69	4.766.469,46	4.757.162,18	4.756.178,97	4.731.572,46	4.728.790,95	4.764.972,05	4.749.674,20	4.751.292,48	4.744.459,54

Source: FAO, 2022a.

Table 4. Irrigation Potentials and Water Use Efficiency of Countries (2018)

	China	İndia	USA	Brazil	Indonesia	Iran	Russia	France	Japan	Turkey
Water Stress (%)	43,22	66,49	28,16	1,42	29,70	81,29	4,12	23,64	36,46	45,38
Water Usage Efficiency ($/m3)	23,54	3,00	43,66	22,52	3,94	4,62	18,91	82,82	56,71	14,06
Water Usage Efficiency in Irrigated Agriculture ($/m3)	2,19	0,41	0,18	0,45	0,24	0,36	0,05	1,71	0,73	0,29
Irrigation potential (1000 hectares)	70.000,00	139.500,00		29.350,00	10.886,00	15.000,00	29.000,00			8.500,00
Area equipped for irrigation: total (1000 ha)	69.863,0	70.400,0	26.708,0	5.645,0	6.722,0	8700,0	3.830,0	2.811,44	2.500,0	5.353,0
Area equipped for irrigation: actually irrigated (1000 ha)	58.449,0	66.103,0	22.387,0	4.454,0		6.423,0	1.245,0	1.377,46	2.893,37	5.280,0

Source: Aquastat, 2022.

Factors such as increasing world population, deterioration in soil structure, decrease in agricultural areas, insufficient water resources and inefficient use lead countries to foreign trade in meeting food products. While foreign trade causes the transfer of capitals of countries to abroad, it also increases their dependence on other countries. This situation may constrain the determination of the country's agricultural policies. Within the scope of the study, the export (Table 5) and import (Table 6) values of the products of the selected countries are given. As seen in Table 5, the highest export value of these products in 2020 is USA with 22.45 billion $, Russia with 10 billion $, France with 9.96 billion $, Brazil with 6.48 billion $, 5.70 billion $. with China. When the values of the last 20 years are analyzed, Brazil has increased its export value by 48 times, Russia by 38 times, and Japan by 22 times. In Turkey, the rate of increase in the products in question was determined as 3.68 times, and exports worth $2.97 billion were realized in 2020. In terms of products, China exports mostly grapes and apples, India wheat, maize and onions, USA corn and wheat, Brazil maize, Indonesian pepper, Iran pistachio, Russia wheat and barley, France wheat, maize and barley, Japan apples and Turkey hazelnuts.

When the import values are analyzed, China ranks first with $11.28 billion. China is followed by the USA with $7.80 billion, Japan with $5.82 billion and Turkey with $4.45 billion. India is in the first place with an increase of approximately 22 times in the changes in the import values of the products in question over time (between the years 2000-2020). Turkey ranks second with an increase of 9.03 times. In terms of products, maize, wheat, barley, grapes in China, lentils, dry beans in India, tomatoes, grapes in the USA, wheat in Brazil and Indonesia, corn in Iran, tomatoes, apples, grapes in Russia, sunflower, tomato in France, corn, wheat in Japan and wheat in Turkey are the products with the highest import value (Table 6).

Table 5. Export Value by Products ($1,000)

Product Groups	Years Crops	China		İndia		USA		Brazil		Indonesia		Iran		Russia		France		Japan		Turkey	
		2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020
Cereals	Wheat	244	14	92.433	243.067	3.387.502	6.318.111	204	111.731	12		52		41.940	7.918.294	2.059.201	4.528.591	7		196.308	39.492
	Barley	78	231	1	860	122.502	47.156		4					44.593	898.888	575.256	1.359.114		65	20.108	2.603
	Maize	1.051.816	4.358	6.076	389.280	4.682.565	9.575.254	9.366	5.853.003	4.984	16.516		673	25	395.244	1.195.758	1.711.436		12	4.096	121.037
Pulses	Beans, dry	79	162	1.016	140.663	19.739	105.910	13	155		1	8.128		895	116.252	3.099	12.147			33.132	148.848
	Lentil	4.117	7.591	90.844	18.615	33.242	179.978	1	101			11	100	14	28.559	2.716	9.405	11		57.526	305.511
	Haricot bean	199.557	426.837	238	53.443	190.851	413.191	2.233	123.049	2.307	51.702	2.322	964	15	2.043	9.838	24.068	140	238	9.393	111.880
Oil Seeds	Sunflower	17.651	648.463	1.059	1.110	97.130	236.627		230	4	63	25		166.661	562.510	121.949	442.620	25	3.579	4.850	193.404
	Cotton	449	87	238	3.338	52.434	94.576	606	6.526	193	668		9			2	796		13	941	8.320
	Olives				8		808		1				4		3	651	934				114
Vegetables	Tomatoes	3.354	274.458	234	35.568	182.431	312.886	5.202	1.495	655	590	1.192	28.657	198	6.784	79.385	445.137	5	779	37.502	310.058
	Pepper	18.305	18.892	70.617	66.661	15.382	37.537	69.275	185.322	221.090	160.388	185	198	2.375	4.339	6.768	31.859	384	461	208	6.430
	Onion, dry	42.404	500.228	61.512	346.640	104.781	249.935			8		3.983	12.068	70	7.580	12.395	50.561	374	14.648	11.666	51.409
Fruits	Apple	115.344	1.494.445	929	14.067	388.028	855.305	30.757	41.270	165	45	14.289	150.000	394	4.296	425.992	478.210	5.611	100.334	5.394	110.912
	Grape	52.763	1.591.151	18.468	286.118	454.802	822.385	14.618	108.992	370	1	258	5.571	19	892	18.906	26.662	213	38.607	28.841	157.876
	Watermelon	2.393	37.429	225	8.924	42.081	126.287	1.809	44.365	49	153		27.937	1.326	3.927	3.010	40.177	2	350	1.351	19.990
Nuts	Hazelnut	9.797	6.129		37	25.966	98.227	1	5	40	17	78	201	1.213	198	8.357	21.140		7	367.916	1.112.540
	pistachios	37.524	108.710	1	762	63.124	1.478.561		22	1		314.223	798.051	63	58	2.074	7.084	51	3	1.576	152.885
	Walnut	25.612	287.968	24.481	5.727	171.039	1.247.970	1.770	5.254	22	4	7.089	2.369	5	267	47.658	91.733	4		1.199	94.031
Edible Roots And Tubers	Potatoes	6.526	289.806	2.926	71.637	93.385	244.468	11	2.983	4.495	3.335	3.389	25.615	1.357	50.469	183.975	681.452	552	449	24.956	24.453

Sources: FAO, 2022a.

Table 6. Import Value by Products ($1,000)

Product Groups	Years Crops	China		İndia		USA		Brazil		Indonesia		Iran		Russia		France		Japan		Turkey	
		2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020	2000	2020
Cereals	Wheat	344.018	2.664.763	640	67	244.768	467.784	981.191	1.459.354	502.406	2.616.037	860.046	299.287	277.731	64.839	70.129	76.777	1.029.642	1.524.539	126.143	2.334.510
	Barley	331.279	1.890.291		18.273	83.027	38.677	19.435	180.508	3	116	158.366	171.772	48.845	7.545	12.006	16.873	243.878	303.110	5.207	163.400
	Maize	567.395	3.384.566	3.284	64.701	174.319	337.389	201.960	218.996	157.949	172.649	146.600	1.223.417	124.886	132.704	81.594	311.590	1.887.315	3.293.212	146.887	485.439
Pulses	Beans, dry	208	5.552	20.654	162.239	8.931	47.085	2.826	7.531	24	325	807	15.365	10	963	7.954	8.097	522	2.516	4.278	91.704
	Lentil	582	12.144	9.033	580.893	5.178	50.805	3.321	14.578	8	64	6.045	40.229	2.009	862	13.761	27.185	220	1.142	59.143	311.790
	Haricot bean	19.156	238.436	18.280	440.904	48.394	260.343	22.131	78.197	7.805	102.071		7.910	4.994	28.497	39.746	66.676	95.071	150.487	11.890	120.362
Oil Seeds	Sunflower	13.683	87.306		2.016	18.920	151.699	3.291	7.560	2.696	19.262		50.140	8.148	332.704	27.807	267.849	4.275	9.768	103.324	628.373
	Cotton	832	1.579		1.369	49.837	6.507	85		41			1.339		64	96	10	27.847	27.854	23.104	3.726
	Olives		41			1.785	3.990				13		43			1.314	4.988		14		
Vegetables	Tomatoes	3.670	22.940			718.068	2.918.319	51	254	223	4		190	42.572	525.128	290.683	718.440	26.863	34.982		4.346
	Pepper	32.413	83.631	13.391	85.919	263.317	258.384	564	782	2.655	1.986	504	8.000	7.646	23.878	37.733	47.218	40.954	41.558	869	12.299
	Onion, dry	10.858	55.755	0	67.668	148.248	471.342			3.438	65.512		165	40.758	86.006	23.989	83.049	71.835	97.666	57	172
Fruits	Apple	156.040	573.859	4.695	201.305	114.017	181.181	23.947	100.955	42.421	326.231			82.248	476.821	45.701	93.153	855	16.829	1.265	622
	Grape	206.288	1.274.973	26	9.914	687.210	1.887.640	10.136	11.387	10.961	273.268		283	48.813	360.625	131.875	218.821	26.320	132.487	118	249
	Watermelon	27.999	39.907	5	288	60.220	379.368	1		34				13.323	21.621	22.514	171.163	3.057	1.441	22	4.757
Nuts	Hazelnut	15.596	57.656	75	1.259	17.519	31.087	7.949	30.509	117	206		1.002	5.012	85.714	48.237	185.419	1.700	9.105	1.498	10.431
	pistachios	60.406	799.985	16.318	163.243	1.224	14.570	903	2.972	140	149		5.834	1.702	2.844	32.580	85.287	15.177	26.491	544	97.625
	Walnut	7.181	51.846	140	85.258	654	6.702	9.064	25.428	49	621		73.210	4.439	34.901	18.583	72.689	37.207	136.947	3.541	159.538
Edible Roots And Tubers	Potatoes	7.598	37.374	107	33	85.864	285.759	2.285	9.879	2.112	17.649	1.448	3.879	45.477	125.654	63.343	101.113	86	13.336	4.727	17.194

Sources: FAO, 2022a.

Tablo 7. Production Areas and Yields by Products (2020)

Crops	China		İndia		USA		Brazil		Indonesia		Iran		Russia		France		Japan		Turkey	
	Area Harvested (ha)	Yield (kg/ha)	Area Harvested (ha)	Yield (kg/ha)	Area Harvested (ha)	Yield (kg/ha)	Area Harvested (ha)	Yield (kg/ha)	Area Harvested (ha)	Yield (kg/ha)	Area Harvested (ha)	Yield (kg/ha)	Area Harvested (ha)	Yield (kg/ha)	Area Harvested (ha)	Yield (kg/ha)	Area Harvested (ha)	Yield (kg/ha)	Area Harvested (ha)	Yield (kg/ha)
Wheat	23.380.000	5.742	31.357.000	3.431	14.870.740	3.342	2.434.703	2.607			7.584.358	1.978	28.864.312	2.976	4.512.420	6.680	212.600	4.465	6.914.632	2.965
Barley	260.000	3.462	618.085	2.783	863.200	4.170	104.383	3.709			2.062.429	1.746	8.267.448	2.533	1.972.270	5.209	63.600	3.486	3.092.442	2.684
Maize	41.260.000	6.318	9.865.000	3.057	33.373.570	10.795	18.253.766	5.696	3.955.340	5.689	205.372	6.817	2.731.870	5.081	1.691.130	7.935	62	2.645	690.553	9.413
Beans, dry	3.056	5.356	10.948.882	1.012	106.390	1.822					514.284	441	335.261	868					511.493	1.232
Lentil	65.446	2.512	1.353.912	872	208.010	1.616					130.582	534	128.583	899					247.642	1.497
Haricot bean	735.843	1.742	13.006.503	420	678.460	2.204	2.686.870	1.130	196.292	1.082	82.819	2.051	4.035	1.558			34.000	1.671	102.963	2.715
Sunflower	900.000	2.639	320.000	666	674.090	2.007	49.051	1.624			61.000	1.000	8.392.310	1.587	778.400	2.065			728.368	2.838
Cotton	3.250.000	9.077	12.864.576	1.378	3.521.410	2.765	1.633.091	4.329	2.898	103	56.114	2.591							359.220	4.938
Olives					14.570	4.216	1.894	1.400			40.644	2.664			17.620	1.716			887.077	1.484
Tomatoes	1.107.485	58.482	812.000	25.336	110.439	110.716	51.960	72.240	57.304	18.934	129.058	44.841	80.765	36.843	5.950	112.706	11.400	61.930	181.879	72.598
Pepper	734.961	22.655	8.396	8.454	19.668	36.365			314.772	8.808	6.305	14.642			1.160	26.379	3.160	45.190	91.491	28.822
Onion, dry	1.083.959	21.827	1.434.000	18.646	53.742	71.100	47.487	31.495	186.900	9.714	52.762	39.125	59.908	29.014	17.680	39.855	25.610	49.319	70.275	32.444
Apple	1.911.656	21.186	308.000	8.877	119.504	38.917	32.468	30.284			112.270	19.656	215.258	9.480	50.150	32.301	35.108	20.520	170.903	25.163
Grape	765.038	19.305	140.000	22.321	372.311	14.474	73.726	19.472			158.467	12.564	72.438	9.414	759.060	7.752	16.500	9.903	400.998	10.496
Watermelon	1.397.658	42.989	110.000	25.336	39.133	44.504	98.205	22.248	33.417	16.767	100.732	27.164	100.082	15.830	1.010	17.396	9.446	32.755	78.179	44.661
Hazelnut	12.093	2.006			24.290	2.652					24.307	552			5.540	1.749			734.538	905
pistachios	27.158	2.954			150.543	3.149					162.960	1.166							381.847	776
Walnut	284.375	3.868			153.781	4.601	3.712	1.126			59.920	5.952			24.990	1.429			141.790	2.022
Potatoes	4.215.534	18.547	2.158.000	23.772	369.930	50.793	117.253	32.134	65.621	19.548	131.073	34.140	1.178.098	16.643	214.500	40.522	72.306	31.456	147.965	35.143

Sources: FAO, 2022a.

The production areas and yields of the products selected in the countries are shown in Table 7, the average water willingness of the products in question in Table 8, and the annual average precipitation amounts of the countries in Table 9 are shown. As seen in the tables, while hazelnuts with 1.100.0 mm, walnuts with 912.3 mm, cotton with 832.0 mm have the highest water consumption, wheat, barley, chickpea, lentils, dry beans and onions have the lowest values (Table 8) . In addition, Iran is the country with the lowest precipitation with 228 mm/year in precipitation amounts. Iran is followed by Russia with 460 mm and Turkey with 593 mm. The highest precipitation occurs in Indonesia, Brazil, Japan and India.

Table 8. Average Water Requirements of Products (mm)

Country	Water Demand of Products	Country	Water Demand of Products	Country	Water Demand of Products
Wheat	526,5	Sunflower	659,7	Apple	733,2
Barley	460,1	Cotton	832,0	Grape	669,3
Maize	630,6	Olives	700,0	Watermelon	558,2
Chickpeas	500,9	Tomatoes	668,7	Hazelnut	1.100,0
Lentil	436,2	Pepper	627,5	pistachios	775,0
Beans, dry	549,4	Onion, dry	499,0	Walnut	912,3
				Potatoes	637,1

Source: Zaman, 2004; Anonymous, 2007; TOB, 2017; Engin, 2019; Anonymous, 2022

Table 9. Annual Average Rainfall Amounts of Countries (mm)

Country	Annual Precipitation Amount	Country	Annual Precipitation Amount
Chinese	645,00	Iranian	228,00
India	1.083,00	Russia	460,00
USA	715,00	France	867,00
Brazil	1.761,00	Japan	1.668,00
Indonesia	2.702,00	Turkey	593,00

Source: Aquastat, 2022

Using the data in Tables 7, 8 and 9, the irrigation water needs of the countries in selected crops and the level of meeting these needs with precipitation were calculated and are shown in Table 10. As seen in the table, olives in China, olives, hazelnuts, pistachios and walnuts in India, chickpeas, lentils, peppers, hazelnuts and pistachios in Brazil, wheat, barley, chickpeas, lentils, sunflowers, olives, apples, grapes in Indonesia. , hazelnuts, pistachios and walnuts, cotton in Russia, cotton, olives, peppers, hazelnuts, pistachios and walnuts, in France chickpeas, lentils, dried beans, cotton and pistachios, in Japan chickpeas, lentils, sunflowers, cotton, olives, hazelnuts , pistachios and walnuts are not grown. All of the selected products are grown in the USA, Iran and Turkey. There is no need for irrigation water in countries where crops with high water demand are not grown and average rainfall is high. Therefore, the calculated values in India, Brazil, Indonesia, France and Japan were negative.

Table 10-Continued. Total Irrigation Water Need of Selected Crops Grown in Countries (million m^3)

Crops	China					İndia				
	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement
Wheat	12.309,57	15.080,10	12.064,08	245,49	438,37	16.509,46	33.959,63	27.167,70	-10.658,24	-19.032,58
Barley	119,63	167,7	134,16	-14,53	-25,95	284,38	669,39	535,51	-251,13	-448,44
Maize	26.018,56	26.612,70	21.290,16	4.728,40	8.443,56	6.220,87	10.683,80	8.547,04	-2.326,17	-4.153,87
Beans, dry	1,53	1,97	1,58	-0,05	-0,08	5.484,29	11.857,64	9.486,11	-4.001,82	-7.146,10
Lentil	28,55	42,21	33,77	-5,22	-9,33	590,58	1.466,29	1.173,03	-582,45	-1.040,09
Haricot bean	404,27	474,62	379,69	24,58	43,89	7.145,77	14.086,04	11.268,83	-4.123,06	-7.362,61
Sunflower	593,73	580,5	464,4	129,33	230,95	211,1	346,56	277,25	-66,14	-118,11
Cotton	2.704,00	2.096,25	1.677,00	1.027,00	1.833,93	10.703,33	13.932,34	11.145,87	-442,54	-790,25
Olives										
Tomatoes	740,58	714,33	571,46	169,11	301,99	542,98	879,4	703,52	-160,53	-286,67
Pepper	461,19	474,05	379,24	81,95	146,34	5,27	9,09	7,27	-2,01	-3,58
Onion, dry	540,9	699,15	559,32	-18,43	-32,91	715,57	1.553,02	1.242,42	-526,85	-940,81
Apple	1.401,63	1.233,02	986,41	415,21	741,45	225,83	333,56	266,85	-41,03	-73,26
Grape	512,04	493,45	394,76	117,28	209,43	93,7	151,62	121,3	-27,59	-49,28
Watermelon	780,17	901,49	721,19	58,98	105,32	61,4	119,13	95,3	-33,9	-60,54
Hazelnut	13,3	7,8	6,24	7,06	12,61					
pistachios	21,05	17,52	14,01	7,03	12,56					
Walnut	259,44	183,42	146,74	112,7	201,25					
Potatoes	2.685,72	2.719,02	2.175,22	510,5	911,61	1.374,86	2.337,11	1.869,69	-494,83	-883,62
TOTAL	**49.595,83**	**52.499,30**	**41.999,44**	**7.596,39**	**13.564,99**	**50.169,40**	**92.384,62**	**73.907,69**	**-23.738,30**	**-42.389,81**

Crops	USA					Brazil				
	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement
Wheat	7.829,44	10.632,58	8.506,06	-676,62	-1.208,25	1.281,87	4.287,51	3.430,01	-2.148,14	-3.835,96
Barley	397,16	617,19	493,75	-96,59	-172,49	48,03	183,82	147,05	-99,03	-176,84
Maize	21.045,37	23.862,10	19.089,68	1.955,69	3.492,31	11.510,82	32.144,88	25.715,91	-14.205,08	-25.366,22
Beans, dry	53,29	76,07	60,86	-7,56	-13,51					
Lentil	90,73	148,73	118,98	-28,25	-50,44					
Haricot bean	372,75	485,1	388,08	-15,33	-27,38	1.476,17	4.731,58	3.785,26	-2.309,10	-4.123,39
Sunflower	444,7	481,97	385,58	59,12	105,57	32,36	86,38	69,1	-36,74	-65,61
Cotton	2.929,81	2.517,81	2.014,25	915,57	1.634,94	1.358,73	2.875,87	2.300,70	-941,97	-1.682,08
Olives	10,2	10,42	8,33	1,86	3,33	1,33	3,34	2,67	-1,34	-2,4
Tomatoes	73,85	78,96	63,17	10,68	19,07	34,75	91,5	73,2	-38,46	-68,67
Pepper	12,34	14,06	11,25	1,09	1,95					
Onion, dry	26,82	38,43	30,74	-3,92	-7,01	23,7	83,62	66,9	-43,2	-77,15
Apple	87,62	85,45	68,36	19,26	34,4	23,81	57,18	45,74	-21,94	-39,17
Grape	249,19	266,2	212,96	36,23	64,69	49,34	129,83	103,87	-54,52	-97,36
Watermelon	21,84	27,98	22,38	-0,54	-0,96	54,82	172,94	138,35	-83,53	-149,17
Hazelnut	26,72	17,37	13,89	12,83	22,9					
pistachios	116,67	107,64	86,11	30,56	54,57					
Walnut	140,29	109,95	87,96	52,33	93,45	3,39	6,54	5,23	-1,84	-3,29
Potatoes	235,68	264,5	211,6	24,08	43	74,7	206,48	165,19	-90,48	-161,58
TOTAL	**34.164,48**	**39.842,50**	**31.874,00**	**2.290,48**	**4.090,15**	**15.973,80**	**45.061,47**	**36.049,18**	**-20.075,37**	**-35.848,88**

Table 10-Continued. Total Irrigation Water Need of Selected Crops Grown in Countries (million m^3)

Crops	Indonesia					Iran				
	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement
Wheat						3.993,16	1.729,23	1.383,39	2.609,78	4.660,32
Barley						948,92	470,23	376,19	572,74	1.022,74
Maize	2.494,24	10.687,33	8.549,86	-6.055,63	-10.813,62	129,51	46,82	37,46	92,05	164,37
Beans, dry						257,6	117,26	93,81	163,8	292,5
Lentil						56,96	29,77	23,82	33,14	59,18
Haricot bean	107,84	530,38	424,3	-316,46	-565,11	45,5	18,88	15,11	30,39	54,28
Sunflower						40,24	13,91	11,13	29,12	51,99
Cotton	2,41	7,83	6,26	-3,85	-6,88	46,69	12,79	10,24	36,45	65,09
Olives						28,45	9,27	7,41	21,04	37,57
Tomatoes	38,32	154,84	123,87	-85,55	-152,77	86,3	29,43	23,54	62,76	112,07
Pepper	197,52	850,51	680,41	-482,89	-862,31	3,96	1,44	1,15	2,81	5,01
Onion, dry	93,26	505	404	-310,74	-554,89	26,33	12,03	9,62	16,7	29,83
Apple						82,32	25,6	20,48	61,84	110,43
Grape						106,06	36,13	28,9	77,16	137,78
Watermelon	18,65	90,29	72,23	-53,58	-95,68	56,23	22,97	18,37	37,86	67,6
Hazelnut						26,74	5,54	4,43	22,3	39,83
pistachios						126,29	37,15	29,72	96,57	172,45
Walnut						54,67	13,66	10,93	43,74	78,1
Potatoes	41,81	177,31	141,85	-100,04	-178,64	83,51	29,88	23,91	59,6	106,43
TOTAL	**2.994,05**	**13.003,49**	**10.402,80**	**-7.408,74**	**-13.229,90**	**6.199,44**	**2.662,00**	**2.129,60**	**4.069,83**	**7.267,56**

Crops	Rusya					Fransa				
	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement
Wheat	15.197,06	13.277,58	10.622,07	4.574,99	8.169,63	2.375,79	3.912,27	3.129,81	-754,03	-1.346,47
Barley	3.803,85	3.803,03	3.042,42	761,43	1.359,70	907,44	1.709,96	1.367,97	-460,53	-822,37
Maize	1.722,72	1.256,66	1.005,33	717,39	1.281,05	1.066,43	1.466,21	1.172,97	-106,54	-190,25
Beans, dry	167,93	154,22	123,38	44,56	79,56					
Lentil	56,09	59,15	47,32	8,77	15,66					
Haricot bean	2,22	1,86	1,48	0,73	1,31					
Sunflower	5.536,41	3.860,46	3.088,37	2.448,04	4.371,49	513,51	674,87	539,9	-26,39	-47,12
Cotton										
Olives						12,33	15,28	12,22	0,11	0,2
Tomatoes	54,01	37,15	29,72	24,29	43,37	3,98	5,16	4,13	-0,15	-0,26
Pepper						0,73	1,01	0,8	-0,08	-0,14
Onion, dry	29,89	27,56	22,05	7,85	14,01	8,82	15,33	12,26	-3,44	-6,14
Apple	157,83	99,02	79,21	78,61	140,38	36,77	43,48	34,78	1,99	3,55
Grape	48,48	33,32	26,66	21,83	38,97	508,04	658,11	526,48	-18,45	-32,94
Watermelon	55,87	46,04	36,83	19,04	33,99	0,56	0,88	0,7	-0,14	-0,24
Hazelnut						6,09	4,8	3,84	2,25	4,02
pistachios										
Walnut						22,8	21,67	17,33	5,47	9,76
Potatoes	750,57	541,93	433,54	317,03	566,12	136,66	185,97	148,78	-12,12	-21,64
TOTAL	**27.582,92**	**23.197,97**	**18.558,38**	**9.024,54**	**16.115,25**	**5.599,95**	**8.714,98**	**6.971,98**	**-1.372,03**	**-2.450,05**

Table 10-Continued. Total Irrigation Water Need of Selected Crops Grown in Countries (million m3)

Crops	Japan					Turkey				
	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement	Amount of Water Needed	Total Rainfall	Effective Rainfall	Net Irrigation Water Requirement	Total Irrigation Water Requirement
Wheat	111,93	354,62	283,69	-171,76	-306,71	3.640,55	4.100,38	3.280,30	360,25	643,31
Barley	29,26	106,08	84,87	-55,61	-99,3	1.422,83	1.833,82	1.467,05	-44,22	-78,97
Maize	0,04	0,1	0,08	-0,04	-0,08	435,46	409,5	327,6	107,86	192,61
Beans, dry						256,21	303,32	242,65	13,55	24,2
Lentil						108,02	146,85	117,48	-9,46	-16,89
Haricot bean	18,68	56,71	45,37	-26,69	-47,66	56,57	61,06	48,85	7,72	13,79
Sunflower						480,5	431,92	345,54	134,97	241,01
Cotton						298,87	213,02	170,41	128,46	229,39
Olives						620,95	526,04	420,83	200,12	357,37
Tomatoes	7,62	19,02	15,21	-7,59	-13,55	121,62	107,85	86,28	35,34	63,11
Pepper	1,98	5,27	4,22	-2,23	-3,99	57,41	54,25	43,4	14,01	25,01
Onion, dry	12,78	42,72	34,17	-21,39	-38,2	35,07	41,67	33,34	1,73	3,09
Apple	25,74	58,56	46,85	-21,11	-37,69	125,31	101,35	81,08	44,23	78,98
Grape	11,04	27,52	22,02	-10,97	-19,6	268,39	237,79	190,23	78,15	139,56
Watermelon	5,27	15,76	12,6	-7,33	-13,09	43,64	46,36	37,09	6,55	11,7
Hazelnut						807,99	435,58	348,46	459,53	820,58
pistachios						295,93	226,44	181,15	114,78	204,97
Walnut						129,36	84,08	67,27	62,09	110,87
Potatoes	46,07	120,61	96,49	-50,42	-90,03	94,27	87,74	70,19	24,07	42,99
TOTAL	**270,42**	**806,97**	**645,57**	**-375,15**	**-669,91**	**9.298,96**	**9.449,01**	**7.559,21**	**1.739,74**	**3.106,69**

CONCLUSIONS

GeoFigureical locations, land assets, agricultural areas, soil structures, water assets, irrigation potentials, water management decisions, climate characteristics, population structures, technology levels of countries differ. These differences threaten food security. For this reason, countries aim at sustainable production to meet the needs of societies. Soil and water resources are indispensable elements of environmental sustainability, which is one of the components of agricultural sustainability. As a matter of fact, the inadequacy of the land assets of the countries and the inability of the irrigation facilities to meet the country's needs are the most important obstacles in front of the food supply. When countries cannot meet the food needs of their communities with their own resources, they meet the demand by supplying them from other countries. This situation not only contributes to the country's food security, but also contributes to the continuation of people's lives. Therefore, environmental sustainability brings with it social and economic sustainability. While doing this, some problems may be encountered. In this respect, the main problems regarding food safety in the world are stated below.

Problems

- Inadequate planning of use of agricultural lands
- Soil pollution
- Population growth
- Climate change
- Restriction on access to nutritious food
- High costs
- Limited resources such as water
- Fluctuations in food prices
- Abandonment from agriculture
- Lack of organization
- Unconscious use of input
- Internal and external wars
- Economic crises

Suggestions

- Land planning should be done and misuse of lands should be prohibited.
- Producers should be made aware of input usage.
- Population planning should be done and public awareness should be raised accordingly.
- Factors causing climate change should be improved
- Saving-based approaches should be followed in the use of water resources.
- Production planning should be done
- Policies should be developed to increase the income of producers operating in agricultural production.
- Due to the fact that crop production takes place in certain periods during the year, alternative job opportunities should be developed for producers where they can use their spare time.
- Producers operating in line with common goals should be brought together and an organization should be established.
- Organized producers should be guided to produce both in terms of quantity and quality.
- Conscious agricultural production should be carried out and producers should be trained in this regard.
- Intelligent agriculture technologies should be used
- Peace environment should be provided
- Global measures should be taken to end hunger and malnutrition and activities should be carried out in line with these.

In addition to the problems mentioned above, geolocation is an unsolvable problem. For this reason, countries should ensure food security by supplying agricultural products that they cannot produce with their natural resources from other countries through foreign trade.

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FOAM-MAT DRYING METHOD AND ITS APPLICATIONS IN DAIRY TECHNOLOGY

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ABSTRACT

Foam-mat drying method is perhaps the simplest of the dehydration methods that has been studied recently to preserve food quality. It is simple since the material to be dried is mixed with a foaming agent that is coupled with a foam stabilizer. By the help of whipping, bubbling and/or shaking, stable foam is created and foamed material is spread on a tray with a definite thickness (3-5 mm) and dried by hot air or various hybrid drying methods. The foam-mat drying method has the potential to be used for drying a wide range of products such as fruit juices, beverages, milk, fruits, vegetables and pharmaceuticals and the method is advantageous since it is cost effective, rapid and also enables drying at lower temperatures. In foam-mat drying of the liquid, semi-liquid and solid foods, a honey comb like porous structure is obtained and this sheet like material is ground into powder easily. Foam-mat dried materials are of good quality in terms of powder characteristics and reconstitution properties when compared to many other drying methods. Yogurt, cheese and whey are some of dairy products dried by foam-mat drying technique. These reported studies differ in foaming agent concentrations and types, drying procedure and conditions. In overall assessment of the method, foam-mat drying technique gave successful results in dried dairy products. Future studies in dairy can be conducted about using alternative foaming agents, foam stabilizers and hybrid drying techniques that can be applied together with the foam-mat drying technique.

Keywords: Foam-mat drying, foaming agents, foam stabilizers, hybrid drying techniques, yogurt, cheese, dairy products.

INTRODUCTION

Foam-mat drying (FMD) method is perhaps the simplest of the dehydration methods that has been studied recently to preserve food quality. In the method the material to be dried is mixed with a foaming agent that is coupled with a foam stabilizer (Malik and Sharma, 2019). Stable foam is created by agitation as whipping, bubbling and/or shaking also injection of gas and supersaturation (Huppertz, 2010). After then foamed material is spread on a tray with a definite thickness (1-5 mm) and dried by hot air or various hybrid drying methods (Thippeswamy, Joshi and Sethi, 2021). FMD enables high drying rates in the product due to open structure created by foaming and also better flavor characteristics related to retention of volatile components (Hardy and Jideani, 2017) and also rehydration properties in terms of powder characteristics when compared to many other drying methods (Fournaise et al., 2020). Dairy powders and dried dairy products have potential use as

ingredients and/or as an alternative form of product in food industry. In commercial production, dairy powders are mostly spray dried (Fournaise et al., 2020). Foam-mat dried powders serve technologically good powder properties with honey-comb structure created by foam-mat drying when compared with spray dried and drum dried powders (Malik and Sharma, 2019).

Principle of Foam-mat Drying Method

In foam-mat drying the liquid, semi-liquid and solid food material is transformed into a stable foam by adding foaming agent and a foam stabilizer by incorporation of air in any means into the product. The foam created is spread on a tray and dried as a thin layer. The resulting honey-comb like porous structure of the dried material is ground into powder easily. During drying the porous structure (capillaries formed) in the foam stimulates evaporation of water from the system so the method is an energy saving drying method. The technology was patented by Campbell in 1917 with drying of foamed evaporated milk. Viscous and high sugar food matrices as melon, cherry, pineapple etc. are suitable for drying by this method (Thippeswamy, Joshi and Sethi, 2021). Fruit juices, beverages, milk, fruits, vegetables and pharmaceuticals can be dried by this technique (Hardy and Jideani, 2017). Advantages and some limitations of FMD method is given in Table 1 (Hardy and Jideani, 2017; Thippeswamy, Joshi and Sethi, 2021).

Table 1. Some advantages and limitations of Foam-mat Drying Method in general

Advantages of FMD	Limitations of FMD
Drying at lower temperatures	Since material is spread on a tray as a thin layer, lower amounts of material is dried at a time
Lower drying times are required	Thermal conductivity of foamed material decreases as thickness of the spread material increases.(0.1-0.5 cm is suggested)
Serve ready-to-reconstitute beverages	Mechanical and thermal stability of foam is required during handling and drying
Low cost of operation when compared with spray drying, freeze drying and drum drying	Whipping time is limiting factor determining foam stability.
Higher energy efficiency	
Better protection of heat-sensitive and light-sensitive components	
Porous structure obtained supplies easier grinding of dried material	
Fruits with high-sugar content can be dried efficiently without sticking	

Foam is the colloidal mixture of the two phases. Continuous phase is the liquid and the dispersion phase is the air can be called as internal and external phases, respectively. The dispersed phase (gas) is present in larger amounts than continuous phase (Thippeswamy, Joshi and Sethi, 2021). A gas, a surfactant and energy is required for foam formation. The surfactant lowers the surface tension between the gas and water for the formation of gas bubbles. In many systems that the surfactants used are proteins exist in the system (Hardy and Jideani, 2017; Huppertz, 2010).

Foaming and Stabilizing Agents

In foam stability foam volume is measured as function of storage time. Huppertz, (2010) expressed the foam volume decreases by three ways as;

1) Liquid present in the foam is drained off from the foam.
2) Coalescence of gas bubbles
3) Gas bubbles takes part in a reaction (disproportionation)

Foam stabilization requires reduction of surface tension on the bubble surface and proteins and fats exist in the system behave differently. Fat-rich products are not suitable for drying by FMD since fat molecules increase surface tension. Protein molecules reduce the surface tension so used as foaming agents. Egg albumin, pea protein, soy protein and also whey can be used for foaming agents in different ratios (Thippeswamy, Joshi and Sethi, 2021).

Carbohydrates principally the gums carboxymethyl cellulose (CMC), xanthan gum, guar gum etc. are used as foam stabilizers in ratios of 0.5-2.0 %. Their function in foam stabilization is enhancing the viscosity of continuous phase and forming three dimensional network with proteins (Thippeswamy, Joshi and Sethi, 2021).

Foam Formation

There are 3 methods used in foam formation (Huppertz, 2010).

1) Injection: gas is injected as cold aeration or as steam.
2) Agitation: mechanically energy is transferred to the gas-water interface by stirring as a result bubbles are formed by high-shear mixers and/or kitchen blenders. Intensity and duration of agitation and also the temperature of the material affects the agitation efficiency. Whipping, shaking and also bubbling are mechanical means of air incorporation to the system.

As reported by Hardy and Jideani (2017), whipping is supplied by blenders, vortex mixers. Gas (air) is introduced into the liquid, the speed of the agitator determines the geometry and also the size of bubbles. Shaking is the agitating the liquid vigorously, low foam volumes are obtained. Bubbling is supplied by injection of gas through a narrow opening, uniform bubbles are obtained. The amount of foaming agent used in the liquid determines the foam volume.

3) Supersaturation: Gas used is dissolved in liquid under pressure.

Dairy Applications of Foam-Mat Drying Method

Yogurt, cheese, whey and liquid milk are some of dairy products dried by FMD. In reported studies drying by FMD differed in foaming agent used and their concentrations and also in drying method used. Use of FMD in dairy products is summarize in Table 2.

Table 2. Use of FMD in dairy products

Product & Reference	Foaming agent and ratio (%)	Foam Stabilizer	Foaming Temperature and method	Drying method & conditions	Optimum Conditions
Cheese powder (white cheese) Varying in amounts 40%, 50% and 60% (Izadi et al., 2020)	Whey protein (1%, 3% and 5%)	-	30°C, 55°C and 80°C	-Cabinet drying -Thickness of the foam: 1, 3 and 5 mm -Drying at 50°C, 65°C and 80°C.	Cheese content 60%, 3% WPC and 55°C
Whey (de Paula et al., 2020)	Emustab® (8.0%)	-	Mixing for 20 min with the first minimum speed and tehn 15 min at full speed.	Tray dryer at 40, 50, 60, 70 and 80°C Thickness of the foam: 1, 40 and 50 cm	-
Yogurt (Gallardo-Rivera et al., 2021)	Albumen (3%)	Guar gum 0.25, 0.375 and 0.5%)	Homogenizer for 5 min	Hot air tunnel dryer (47±2°C for 4 hours), vacuum dryer (47±2°C at a pressure of 65 mm Hg for 4 hours and freeze drying.	-
Whey (Baptestini et al., 2021).	Emustab® (5.0%)	-	Whipping	Forced air circulation dryer at 40, 45, 50, 55 and 60°C Thickness of the foam:1 cm	Forced air circulation dryer at 60 °C for speed of 5.6 m/s.
Yogurt (Malik and Sharma, 2019)	Soy lecithin 0% , 0.2%, 0.4%, 0.6%,	-	Whipping (700 rpm) Whipping	Electric tray dryer at 58-63 50°C for 3 hours	0.4 % soy lecithin with a mixing

	0.8% and 1.0%)		time : 1.0-1.5 min.		time of 1.5 minutes
Yogurt (Sulaksono et al., 2013.)	Tween 80 (1.25%)	Maltodextrin (%20)	Homogenizer, for 10 minutes	Drying in baking -pan with aluminum foil at 52°C.	-
Probiotic yogurt (Ayu et al., 2019).	Egg albumin (15, 20, 25 %), Tween 80 (0.75, 1.25 and 1.75 %)		Whipping for 12 minutes	cabinet dryer at 50°C for 3 hours	20 % egg albumin meet the standards.
Yogurt (Krasaekoopt and Bhatis, 2012	Methylcellulose (0.5, 1.0, 1.5 %) and egg albumin (2%)		Whipping for 12 minutes	Oven drying at 50,60 and 70 °C for 3 hours	3 % egg albumin, 60°C for 3 hours
Milk powder (Febrianto et al., 2012)	Tween 80, as emulsifier 1% (v/v) of 2 mL fresh milk	Maltodextrin (5,10 and 15%) and Gum arabic (2, 4 and 6%)	Whipping for 5 minutes	vacuum dryer at 70 °C for 7 hours	1 % emulsifier with % 15 maltodextrin.

Foam stability of dairy-originated foams depend on two types of surface active agents exist in milk as proteins and also low molecular weight surfactants as polar lipids (monoglycerides and diglycerides) free fatty acids and phospholipids (Huppertz, 2010).

- Buttermilk has poor foaming ability due to presence of high level of phospholipids. Foaming property of milk is damaged by addition of phospholipids, free fatty acids and polar lipids. A visco-elastic film was formed on the surface of foam by proteins during stabilization of foam.

- Temperature is also effective on foam stability; skim milk foams have maximum stability at 40-50°C. The lowest amount of non-micellar casein existence and also the lack of denaturation of whey proteins are the reasons of stability at that temperatures.

- Acceptable lactic acid bacteria (LAB) viability is obtained dried yogurt by FMD (Gallardo-Rivera et al., 2021).

CONCLUSIONS

Ready-to-eat and ready-to-cook food alternatives are very promising developments that consumer demand in daily life. Dairy products are nutritious food components but besides that highly perishable. Drying enables longer shelf-life, convenience in use, easy handling and transportation

and above all dried dairy products does not strictly require refrigeration. FMD is applicable at low drying temperatures that supplies retention of vitamins, heat-sensitive components in functional foods such as bioactive components, lactic acid bacteria and probiotics in dairy products (Izadi et al., 2020). In overall assessment of the method, FMD gave successful results in dried dairy products. Future studies in dairy can be conducted about using alternative foaming agents, foam stabilizers and hybrid drying techniques that can be applied together with FMD.

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SEASONAL FRUIT GROWING OF KIWIFRUIT

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ABSTRACT

Hayward cultivar is one of the most important commercial kiwifruits (*Actinidia deliciosa*). It is a woody fruit that grows in shrub or tree form in tropical and subtropical regions. It is widely grown in all countries that have favorable ecological conditions, namely Italy, France, the United States, South Africa, Chile, Italy, and Turkey. In its homeland, China, the average winter temperature is 0 °C, the annual average is 20–22 °C, and annual precipitation is 750 mm. Ideal conditions are an average daytime temperature of 4.5 °C in winter and 13.30–13.9 °C in summer, 130–163 mm average annual precipitation, and 76–78% relative humidity. This study was carried out to establish the seasonal growth curves of kiwifruit. Together with, kiwi vines age effect was also evaluated. The trial was carried out in an orchard in Kocaeli, Turkey, 2.8 km from the sea at an altitude of 6 m (35°27.6′ N). Fruit measurements (width, thickness and length) were taken throughout the growing season and draw growing lines. The kiwifruit growth curve was characteristically a double sigmoid that reflects two growing phases, fast and slow. Kiwifruit development was affected by many factors such as temperature, light. Plant age was also a factor that affects fruit quality based on how the accumulation of dry substance and sugar vary with age. Age also affected the fruit size and weight.

Keywords: Kiwifruit, growing curve, Kocaeli, fruit growing

INTRODUCTION

Kiwi fruit is one of the most important fruit species. It belongs to *Actinidiaceae* family that grows in tropical and subtropical regions. Kiwifruit grown in too many countries all around the world, including Italy, France, the United States, South Africa, Chile, Italy and Turkey. Hayward variety has a high commercial value because of this, we used this cultivar in our study.

Kocaeli province has a place in kiwifruit production with its favorable climate. It is popular for farmers because of long-storage life and less careful needs in cultivation. It is suitable for ecological agriculture too (Drzewiecki et al., 2016). Kiwifruit growth curve was characterized as a double sigmoid that has a two growing phase as fast and slow growing phase

(Bebbington et al., 2009). The age of the tree effects the fruit growing line (Andrzejewska et al., 2015).

To determine the seasonal growing period help producer in kiwifruit cultivation. It is therefore the aim of the study was to determine the draw fruit growing curves in a season.

2. MATERIAL AND METHOD

The study was conducted in the kiwi orchard of Kocaeli Provincial Directorate of Agriculture and Forestry for 3 years, between 2017 and 2019. All measurements were made in the laboratory of Horticulture Department of the Kocaeli University Faculty of Agriculture.

"Hayward" female kiwi variety vines 12 and 25 years old and pollinator was Matua variety was planted at intervals of 4x4 m, a T-wire training system was applied. Applications such as tillage, irrigation, fertilization, and pruning are carried out regularly. Winter pruning is carried out.

The climate of Kocaeli has a transitional climate between the Mediterranean climate and the Black Sea climate. When the climate data for many years are assessed, the annual average temperature is 14.8°C. There has been a rainfall of 668.8 mm. as total annual rainfall. The average relative humidity is 79.2%.

In March 2017 before flowering, 6 Harvard kiwi trees (three for each 25 and 12 -year-olds) were selected to best reflect the condition of the garden. In each selected trees, two branches were selected in each direction, north, south, east and west. The directions of branches were marked by tying raffia in different colors. Observations were made in the same trees in each working year.

RESULTS AND DISCUSSION

Fruit set in kiwi plants occurred in the first week of June. A rapid development was recorded in the first 60 days after fruit set that fruit width, thickness and fruit length increased rapidly in this period. Fruit enlargement was showed slowly but continuous line until harvest time. Fruit size increased in 2018 compared to other years, while fruits remained smaller in 2017. 12-year-old plants fruit length increased in 2017 while fruit width increased in 2018

Rounded shaped fruits were obtained in 2019 because of increasing thickness. Larger fruits were harvested from 25-year-old kiwi plants in 2018 and from 12-year-old kiwi plants in 2019. 25 years old kiwi fruit length was statistically larger than 12 years old ones (66,17 cm and 61,26 cm, respectively) (Figure 1).

The biggest fruit were harvested in 2017 from younger kiwi plants. In this year, fruit length curve showed two tap point and double sigmoidal type clearly. This growth character was also defined in previous studies (Snelgar et al, 2005). Fruit growing parameters were

affected with the climatical conditions, while the length of fruit more changeable parameter compare with thickness.

Fruits were completed 70% of size in the first 30 days of maturation period (69%, 68% and 76% for fruit width, thickness and length respectively). Increasing of fruit width and thickness was clear (approximately 20%) until 60th day of growing period, while was slower for fruit length (about 10%). Fruits were reached their final size (95%) on 110-120 days after full blooming. In the last 4-6 weeks of growing period, fruits were completed the last 5% part of their final size (Figure 2). A regular size increasing intervals was observed until harvest.

In previous studies, throughout the growth period, the fruit weight of Hayward showed a single S shape (standard sigmoidal growth) (Burdon et al., 2017). In the first rapid growth period (first 60 days) fruits completed 70% of their size. In the second part of growth 10-20% of fruit growing was completed, so finally stagnant growth period was observed until harvesting time. It was found that fresh weight was increased especially in the first 30 days (Yu-Fei et al. 2021) while was suggested a rapid development with a rapid longitudinal diameter increase of fruit in first 20-35 days intervals after anthesis. In this study, kiwifruit development followed a sigmoidal pattern with two rapid growing phases that in agreement with previous studies (Richardson et al. 2011 Bebbington et al 2009).

Similarly, Hayward kiwi fruits was completed 62,46% of their development in the fast-growing period in the 75 days after fruit set in this study. According to our observations, the last 4-6 weeks were the most affective period on fruit quality parameters that irrigation was the best treatment increasing the fruit quality. Fruits have reached to commercial harvesting maturity at the first half of November. Fruits had been harvested on 23rd of November in 2017, 9th November 9 in 2018, and 13rd of November in 2019 at commercial harvesting criteria. Harvesting date, kiwifruit shape and quality has affected by ecological conditions. In 2018, fruit width increased and more rounded fruits were obtained as a characteristics of Hayward cultivar (Hosseinzadeh et al.,2013). In 2019, the thickness of the fruit increased, and the fruits became flat. Larger fruits were harvested from 25-year-old kiwis in 2018 and from 12-year-old kiwis in 2019. It was found that fruit size was increased especially in the first 30 days.

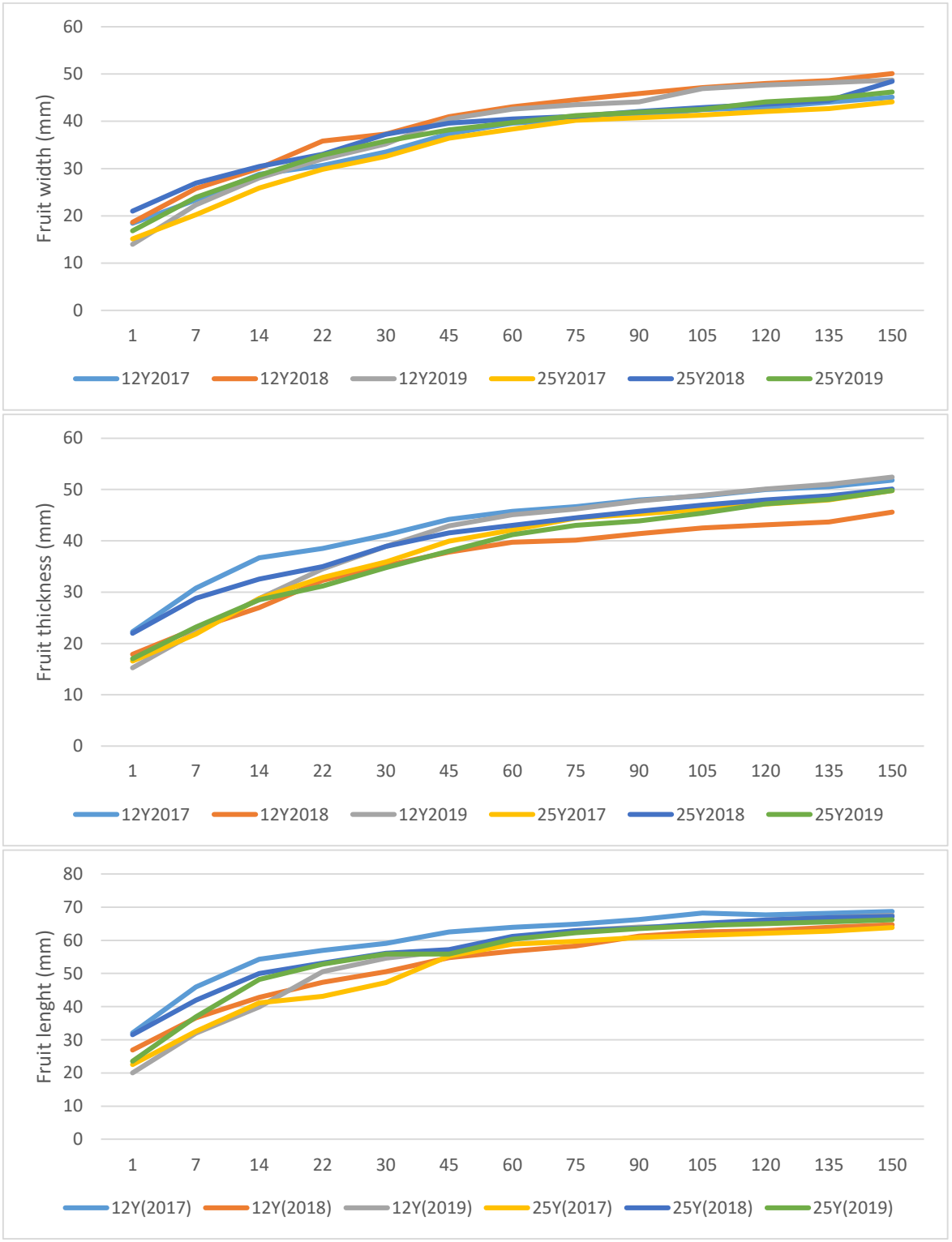

Figure 1. Fruit size variation of kiwi fruits during the 3 years growing period

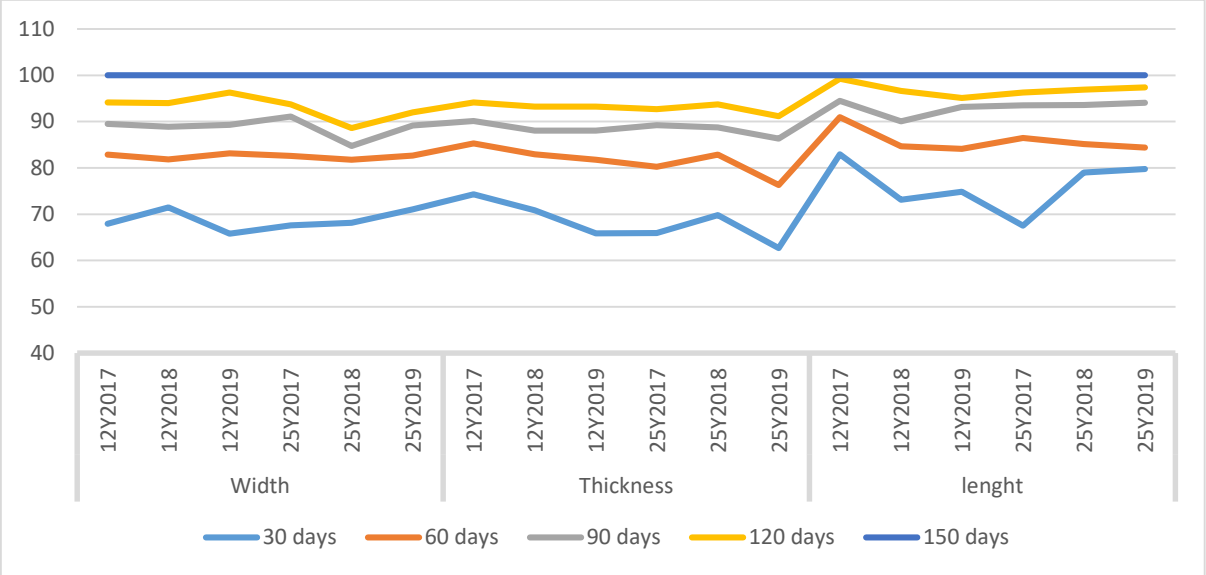

Figure 2. Enlargement of fruit (%) during the growing period after fruit setting

CONCLUSIONS

Kocaeli has a temperate climate influenced by the sea and mountains. Kiwi vines showed double sigmoid growing curves and the first peak was more prominent. The first 30 days after fruit set are very important in terms of yield. During this period, fruits complete nearly 70% of their growth. Fruits continue to grow and weight until harvest day, it is important to pay attention to cultural processes. The results of the study will support the cultural practices in kiwi plantations.

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COLD TOLERANCE-RELATED MIRNA EXPRESSION PATTERNS IN CULTIVATED AND WILD TOMATO GENOTYPES

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ABSTRACT

The cultivated tomato (*Solanum lycopersicum L.*) is one of the popular vegetables in the world that has been used as a target plant in genetic and molecular breeding studies. As this plant originated from the tropics, it is susceptible to chilling stress. Chilling stress can easily damage its growth and development. In the present study, The expression level of miRNA 167, miRNA 169, miRNA 172, miRNA 393, and miRNA 397 in wild genotypes of *Solanum habrochaites* (LA 1777) and *Solanum pimpinellifolium* (LA 1579) and cultivated genotypes of *Solanum lycopersicum* cv. Anit F1 and *Solanum lycopersicum* cv. Lice were assessed at 4, 6, and 8 hours after chilling treatment (4°C) via Real-time quantitative PCR to determine if these miRNAs are responsible for controlling the expression of the genes associated with chilling tolerance. Significant upregulation of miRNA 167, 169 and 172, and 397 were observed at different levels of chilling stress in the tolerant genotypes of *S. habrochaites* (LA 1777) and *S. lycopersicum* cv. Anit F1. and also, in *S. pimpinellifolium* (LA 1579) while this level of up-regulation was not seen in *S. lycopersicum* cv. Lice. The observed levels of cold-related miRNA expression in *S. pimpinellifolium* (LA 1579) indicated the tolerance of *S. pimpinellifolium* (LA 1579) and susceptibility of *S. lycopersicum* cv. Lice to chilling stress.

Keywords: miRNA, chilling stress, Real-time PCR, gene expression, tomato

INTRODUCTION

The plants originating from tropical areas such as tomato (S. lycopersicum L.) lack the ability to acclimatize to low temperatures (Ou et al., 2015). Tomato (S. lycopersicum L.) is a popular and essential vegetable crop around the world because of its nutritional, economic, and therapeutic benefits (Gerszberg et al., 2015). It is quite vulnerable to low temperatures (Weiss and Egea-Cortines, 2009). Cultivated tomato (S. lycopersicum L.) is vulnerable to chilling stress in all steps of its growth process (Liu et al., 2012). Low temperatures can lead to a deleterious effect on several molecular, physiological and morphological elements of tomato (S. lycopersicum L.) development (Wu et al., 2022; Mesa et al., 2022). This condition can

reduce tomato seed germination and disturb vegetative growth by degrading hydration status and photosynthesis (Foolad and Lin, 2000, 2001; Venema et al., 2005). Furthermore, the low temperature has a negative impact on tomato reproductive development, which reduces the fruit set (Lozano et al., 1998).

Assessment of the gene expression regulation system of cold-responsive genes is critically important to decipher chilling stress tolerance mechanisms. There have been a large number of studies regarding the importance of the genes encoding chilling-regulated proteins such as anti-chill proteins in the tomato plant's reactions to chilling stress (Kyu et al., 2019; Ma et al., 2018; Zhang, 2020; Wang et al., 2020).

MiRNAs are one of the different types of small non-coding RNA molecules that are considered vital molecules in plant responses to environmental stimuli (Wang et al., 2017). The intial miRNA was explored in plants less than 20 years ago (Millar, 2020). There has been a wealth of knowledge released on the origin of miRNAs since the research of Reinhart et al. (2002) on plant miRNAs. Following their discovery, evidence of the existence of more than 2000 plant miRNAs was published (Alptekin et al., 2017). MiRNAs are abundant and variable and many biological processes in plants require the interference of miRNAs at various phases of growth (Voinnet, 2009). Recent genetic studies aimed at determining the origin, synthesis, and modes of action of plant miRNAs (Rogers and Chen, 2013). The mature miRNA performs its function during the post-transcriptional regulation (Michlewski et al., 2019). The nucleus is the first site for the primary miRNA (pri-mRNA) to be transcribed. Following this process, the precursor miRNA (pre-mRNA) is generated by cleaving the pri-mRNA. The cleavage of pri-mRNA results in a duplex of mature miRNA. The mature miRNA needs to be loaded in an RNA-induced silencing complex (RISC) for mRNA degradation or translation inhibition (Cardoso et al., 2018).

The up or down-regulation of some miRNAs under environmental stresses was proven in many crops (Khraiwesh et al., 2012). The miRNAs that are involved in chilling defense mechanisms have recently been discovered in various plants including Arabidopsis thaliana Populus tomentosa, Oryza sativa, Prunus persica, Brachypodium, and Zea mays, and Astragalus propinquus (Sunkar and Zhu, 2004; Abla et al., 2019; Chen et al., 2012; Lv et al., 2010; Barakat et al., 2012; Zhang et al., 2009; Yang et al., 2011). The predicted target genes of mentioned miRNAs probably mediate several physiological and biochemical responses to chilling stress. It is also found that many conserved and novel miRNAs were associated with the different levels of heat stress in S.lycopersicum L. and it elucidates the miRNA-mediated regulatory mechanism in this plant in abiotic stresses (Zhou et al., 2016).

High-throughput sequencing was used to find many conserved and new miRNAs implicated in the chilling response in a tolerant genotype of tomato (S. habrochaites LA 1777), and degradome sequencing was used to examine the target genes. Significant up and down-regulation of a number of these novel and conserved miRNAs have been approved (Cao et al.,

2014). In addition, some chilling-stress-related miRNA expressed differentially in tomato (S.lycopersicum L. var. H-2274) under chilling stress and their important effect on chill-related genes and chilling tolerance was approved (Koc et al., 2015).

Having said that, there is little research about the chilling-responsive miRNAs in different species of tomato treated by various levels of chilling stress at different stages of growth. It seems that more detailed information is required for a better understanding of the miRNA differential expression in the wild and cultivated species and its correlation with morpho-physiological traits. Also the level of sensitivity to chilling stress in the wild tomato of Solanum pimpinellifolium (LA 1579) and cultivated tomato of S. lycopersicum cv. Lice had not been determined in the previous studies. The present study provided some comparable information to extend and complete previous data regarding the expression profile of these miRNAs and the relation of their expression profile with the tolerance of the mentioned genotypes.

MATERIAL AND METHOD

Plant Growth

The accessions used for this study are wild genotypes; Solanium haibrochaits (LA 1777), Solanium pimpinellifolum (LA 1579) and cultivated genotypes S. lycopersicum cv. Anit F1, and the susceptible genotype of S. lycopersicum cv. Lice. The seeds were sterilized by soaking in 2% NaClO for a minute and rinsed in sterile warm water (55°C) 3 times, and then were sown in the 48-cell seedling trays. There were four genotypes, four treatments, and three replicates (12 seedlings for each genotype) and irrigated with distilled deionized water from the bottom of the seedling trays. To manage the of heterogeneity of germination and consequently plant growth stage and rate, before starting the main experiment, a group of all genotypes was sown once before the primary trial and the accurate germination date of each of them was recorded. The seeds were then sown on different dates and all germinated in the same week. Three seeds were sown in each cell and two seedlings per cell were removed leaving one seedling per cell. Also, KNO3 of 0.2% was sprayed regularly and evenly on all soil surfaces from the first day of sowing to the day of germination in order to enhance the germination of the seeds. The seedling trays were kept in the growth chamber which had a 16h of lightness and 8 hours of darkness photoperiod at 25± 1ºC. The peat material (Klasmann-Deilmann corporate group) was used. The seedlings at 2 leaves stage were transferred to pots (18 × 15.5 × 14 cm) and then kept at 25± 1ºC for a week with regular cultivation management for environmental acclimatization. The plants were fertilized twice a week with a 20:20:20 NPK fertilizer (1g. / lit.) according to Zucco et al, (2015) and were sufficiently irrigated with tap water every day until the plants began the flowering phase. Then the 3-leave-stage plants in the pots were treated by chilling treatment (at 4 ºC for 4, 6, and 8 h) (Fig. 1). 8 hours after chilling treatment, plants were held at 25°C±1 till the flowering stage. The control plants of each genotype were kept at 25°C±1 to compare with chilling-treated samples.

Figure 1. Plant Growth Stages, 1) Seed cultivation in the seedling trays, 2) Seedling growth, 3) Creation of protective chamber around the seedling trays, 4) Seedling transfer to the pots, 5) pots arrangement 6) flowering stage

Sampling

3 leaflets were collected (9 leaflets in total) from 3 randomly selected plants at 3 leaves stage in both chilled condition (chilled treated plant) and optimum condition (control plant)) at the 4^{th}, $6^{th,}$ and 8^{th} hours after chilling treatment. The leaflets were washed with distilled water, wrapped in an aluminum foil, and stored at -80ºC immediately using cooling box till used for RNA analysis.

Preparation of Total RNA

Lysate Preparation

Total RNA isolation was performed using the Plant/Fungi Total RNA Purification Kit (Cat. 25800) of Norgen Biotech Corp. (Fig. 3). The plant leaf tissue was placed in a mortar with enough liquid nitrogen to completely cover the sample. The samples were ground and turned into a fine powder by using a pestle (Fig. 2). A centrifuge tube containing 50 mg of powder was filled with 600 µL of lysis buffer and intensively vortexed. After that, it was incubated for 5 minutes at 55°C. Additionally, during incubation, the tube was inverted two or three times to mix the lysate. Then, using one of the supplied collecting tubes, a filter column was put assembled. After being pipetted into the filter column, the lysate was spun for two minutes at 20,000 x g (about 14,000 RPM). A pipette was used to transfer the crystal-clear supernatant from the flow-through into an RNAase-free microcentrifuge tube. The lysate obtained above

was mixed with an equal volume of 96–100% ethanol (100 µL of ethanol is added to every 100 µL of lysate) via vortexing.

Figure 2. Grinding initial samples 1) Nitrogen container and other equipment required for grinding the initial samples 2) Grinding the tomato leaf with liquid nitrogen by using a mortar and pestle

Binding to Column

One of the given collecting tubes was used to collect the content of a spin column. Using ethanol as a solvent, 600 µL of the cleared lysate was put into the column and centrifuged for one minute at 3,500 x g (around 6,000 RPM). The spin column and collection tube were put back together after the flow through was discarded.

Column Wash

After adding 400 µL of wash solution A to the column, it was centrifuged for one minute at 20,000 x g (about 14,000 RPM). After removing the flowthrough, the spin column and collection tube were put back together. To wash the column a second time, these procedures were repeated. A further 400 µL of wash solution A was added, and the column underwent a third round of washing before being centrifuged for one minute. After removing the flowthrough, the spin column and collection tube were put back together. To completely dry the resin, the column was spun for 2 minutes at 20,000 x g (approximately 14,000 RPM). The collecting tube was thrown away.

RNA Elution

The column was placed into a fresh 1.5 mL Elution tube provided with the kit. 50 µL of Elution Solution A was added to the column and centrifuged for 2 minutes at 200 x g (~2,000 RPM), followed by a 2-minute spin at 20,000 x g (~14,000 RPM). Then the extracted RNA was transferred to an elution tube and stored at -80°C (Fig. 4).

Measurement of RNA concentration and integrity

Nanodrop 2000c spectrophotometer of Thermofisher scientific corporation was used for RNA concentration measurement. An agarose gel of 1% was used to evaluate the RNA sample integrity. The gel was run at 100 V until the dye was 80% of the way down the gel. A UV transilluminator was used to visualize the RNA integrity of the samples. The ladder in the first lane was used as a guide to check the size of each band (Fig. 3).

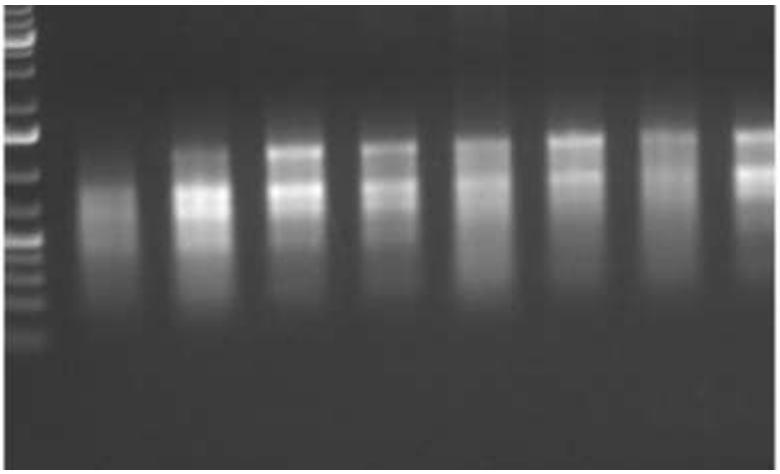

Figure 3. RNA integrity results in the gel electrophoresis

cDNA Synthesis and Quantitative Real-Time PCR

For the reverse transcription of microRNA from either Total RNA preparations or enriched microRNA preparations, Norgen's microScript microRNA cDNA Synthesis Kit is an all-in-one, ready-to-use product. The 2x Reaction Mix and the microScript microRNA Enzyme Mix are both included in the kit.1 µg of total RNA was used for cDNA synthesis. The First-Strand miRNA cDNA was set up for synthesis reaction in the heat block (Techne Dri-Block – DB3D) (Fig.4). cDNA reaction contained 1 µg RNA, 5 µL 2x Reaction Mix, 0.5 µL microScript miRNA Enzyme Mix, and Rnase-free water was added into the mixture up to 10 µL per sample. First-Strand miRNA cDNA Synthesis reaction was incubated in a heat block manually as described in Table 1.

Table 1. cDNA Synthesis Conditions at different temperatures

Temperature Time	**Time**
37ºC	30 minutes
50ºC	30 minutes
70ºC	15 minutes
4ºC	Hold

Figure 4. cDNA Synthesis process, 1) cDNA synthesis kit (Norgen corp.) 2) heat block used as a thermocycler.

The cDNA which had been synthesized in the earlier stage can be used as a template in a PCR reaction. The cDNA was diluted 2-fold using nuclease-free water. 1 µL of the diluted cDNA was used in a 20 µL PCR. For quantitative PCR, 10µl of 2x PCR Master, 1 µl miRNA-specific Forward Primer (5 µM) Mix was used. The PCR reaction was set up with the Universal PCR Reverse Primer and a miRNA-specific forward primer, as indicated in Table 2.

Table 2. The PCR Reaction components volume for miRNA

Components	**Volume per Reaction**
2x PCR Master Mix	10 µL
miRNA-specific Forward Primer (5 µM)	1 µL
Universal PCR Reverse Primer	1 µL
miRNA cDNA	2 µL
Nuclease-Free Water	8 µL
Total Volume	20 µL

The real-time PCR amplification was performed in a Real-time PCR cycler (LightCycler 480 II, Roche) as described in Table 3.

Table 3. Reaction Protocol miRNA Real-time PCR

Temperature	**Time**	**Cycles**
94ºC	3 minutes	1 Cycle
94ºC	15 seconds	40 Cycles
60ºC	30 seconds	40 Cycles
72ºC	45 seconds	40 Cycles

The primer sequences used have been mentioned in Table 4. The prepared reaction was run on a 96-well plate of a real-time PCR machine.

Table 4. Designed primers (Koc et al. 2015).

Name		**Sequence**
Reference gene (Actin)	**F**	GCCGGGCGTGATCTTACTGA
Reference gene (Actin)	**R**	AGCTACTCCTGGCGGTCTCC
Universal primer		GTGCAGGGTCCGAGGT
miR167	**F**	TCGCGTGAACCTGCCAGCAT
miRI67	**RT**	GTCGTATCCAGTGCAGCGTCCGAGGTATTCGCACTGGATACGACTAGATC
miR169	**F**	GCGGCGGCAGCGAAGGATGACT
miRI69	**RT**	GTCGTATCCAGTGCACGGTCCGAGGTATTCGCACTGGATACGACTCGGCA
miR172	**F**	CGGCGCAGAATCATGATGATG
miRI72	**RT**	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATGCAG
miR393	**F**	GCGCGGTCCAAAGCGATCGCA
miR393	**RT**	GTCGTATCGAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGATCAA
miR397	**F**	CGGCGTCATAGAGTGCAGCG
miR397	**RT**	GTCGTATCCAGTGCACGGTCCGAGGTATTCGCACTGGATACGACCATCAA

Real-time Data Analysis

The double delta Cp analysis and the relative standard curve approach are the two basic methods for analyzing qPCR data (Pfaffl method). The data in this study was calculated by the double delta Cp analysis method. There are five steps in this analysis. First, the Cp values were added from all technical replicates together. The first step was to average the Cp values from each sample's technical replicates. As a result, when performing qPCR in duplicate, these data must first be averaged. Each sample was run twice in the plates. Then the delta Ctp was calculated for each sample. The next step was to calculate delta Cp (ΔCp) for each sample by using the Cp values which was created in the previous step. The formula to calculate delta Cp is presented below.

$$\Delta Cp = Cp \text{ (gene of interest)} - Cp \text{ (housekeeping gene)}$$

After that a reference sample was selected to calculate delta delta Cp. Whichever sample, or group of samples, can be used as the calibrator (reference). Because this was consistent throughout the analyses and was reported in the results so it is clear. The results produced at the end were relative gene expression values.

Delta delta Cp values were calculated for each sample. Delta delta Cp values were relative to the untreated/control group in this experiment. The formula to calculate delta delta Cp is presented below.

$$\Delta\Delta Cp = \Delta Cp \text{ (Sample)} - \Delta Cp \text{ (Control average)}$$

Finally, to work out the fold gene expression it was needed to do the primer efficiency (which was 1.98 in this case) to the power of negative ΔΔCp. The formula for this can be found below.

$$\text{Fold gene expression} = 1.98\text{^-}(\Delta\Delta Cp)$$

The significant fold change was ≥1.5 and the q value was 0.001 in this analysis. The analysis was done with two methods. First, the data was calculated manually on excel and then the final fold change was checked using REST software (Pfaffl et al., 2002).

RESULTS

MiRNA Differential Expression Analysis

S. habrochaites (LA 1777)

The expression patterns of miRNAs in *S. habrochaites* (LA1777) were given in Fig. 5. The expression level of miRNA167 increased 5 times at 4 hours, 4 times at 6 hours and 10 times at 8 hours after chilling treatment. While the miRNA 169 expressed almost 7 folds at 4 hours and nearly 10 folds at 8 hours, the transcript amount of miRNA 169 at 6 hours had a level as

that of control. The miRNA 172 was highly expressed over 8 folds at 4 hours and also had a noticeable over 5-fold expression profile at 8 hours. The miRNA 393 did not show any significant differential expression in *S. habrochaites* during chilling treatment. The transcript accumulation of miRNA 397 upsurged 8 folds at 4 hours, then decreased up to the same level of control at 6 hours, and finally, re-increased over 5-folds at 8 hours.

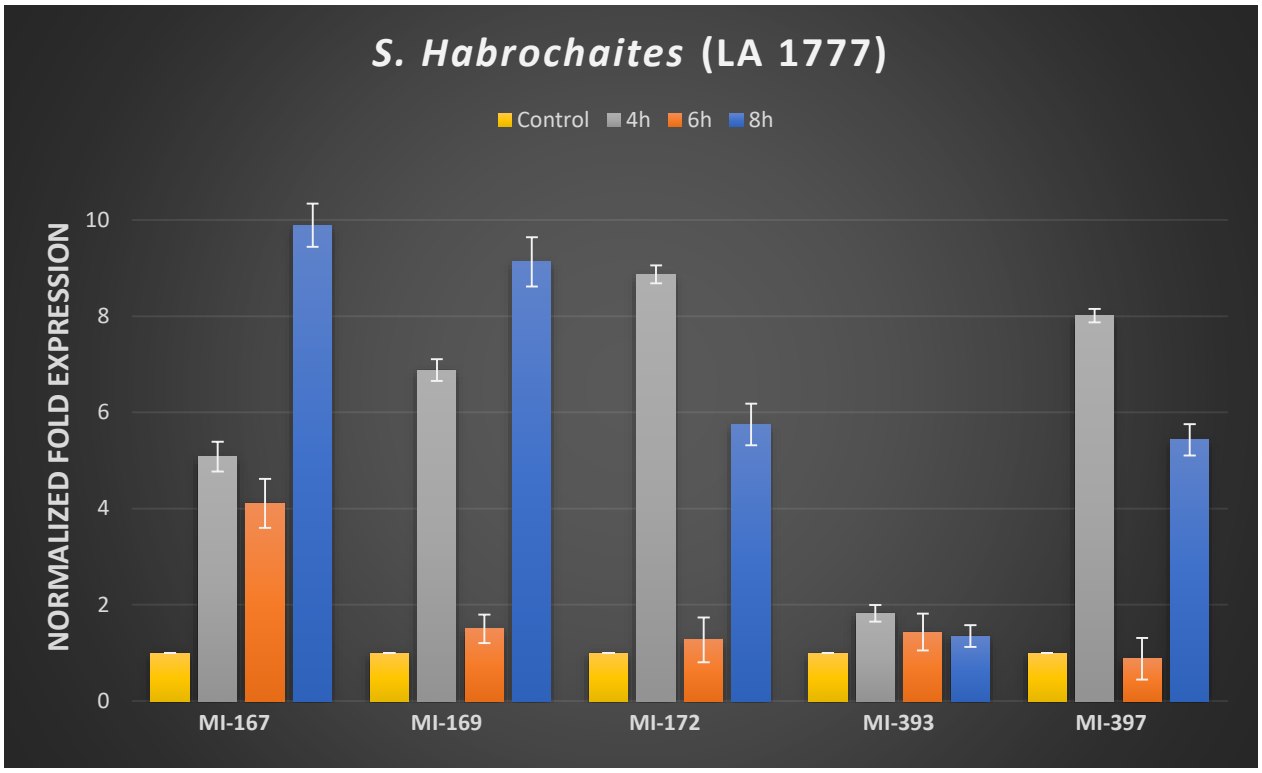

Figure 5. *S. Habrochaites* (LA 1777) miRNA expression profile.

S. pimpinellifolium (LA 1579)

As is shown in the illustration (Fig. 6), the expression of miRNA 167 was induced at 6 hours (7 folds) and at 8 hours (8 folds) after chilling treatment but there was no up-regulation of miRNA 167 at 4 hours. A noticeable differential expression of miRNA 169 was clearly seen at 6 hours and 8 hours after chilling stress, but there was no significant change in expression of miRNA 169 at 4 hours. A significant increase in the expression profile of miRNA 172 was determined at 6 hours (3 folds) and 8 hours (8 folds) after chilling treatment. The transcript abundance of miRNA 393 had a considerable rise up to 6 folds at 6 hours and then decreased at 8 hours (almost 2 folds) after chilling treatment. The expression of miRNA 397 increased at 6 hours of chilling stress by over 7 folds while any upsurged transcript accumulation was not seen at 4 hours and 8 hours and control.

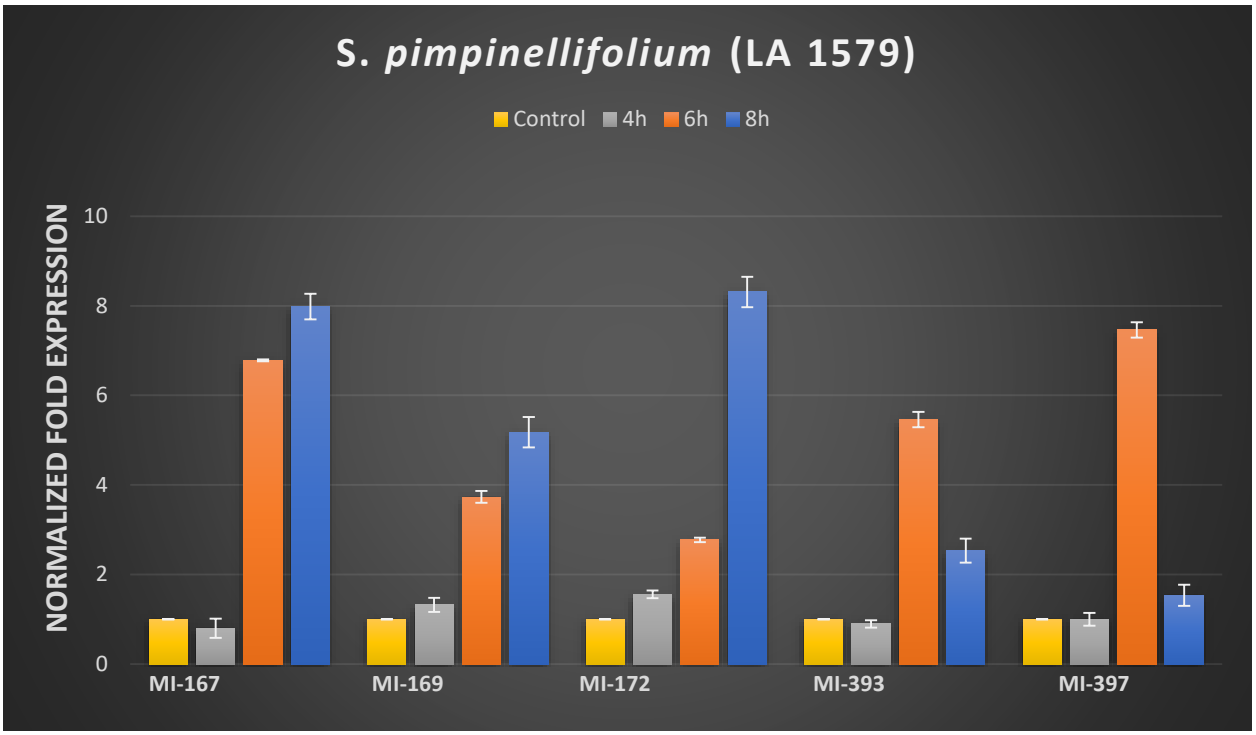

Figure 6. S. *pimpinellifolium* (LA 1579) miRNA expression profile

S. lycopersicum cv. Anit

The expression pattern of miRNAs in *S. lycopersicum* cv. Anit was given in Fig. 7. The expression level of miRNA167 increased 8 times at 8 hours, and 4 times at 4 hours, and a slight change was seen at 6 hours after chilling treatment. MiRNA 169 expressed 4 folds at 6 hours and over 2 folds at 4 hours. MiRNA 172 was extremely transcribed at 4, 6, and 8 hours by over 5, 6, and 8 folds respectively. The miRNA 393 did not show any significant differential expression in *S. lycopersicum* cv. Anit during chilling treatment. Likewise, the expression level of miRNA 397 at 4 and 8 hours changed slightly. Yet, the transcript abundance of this miRNA at 8 hours soared over 2.5 folds.

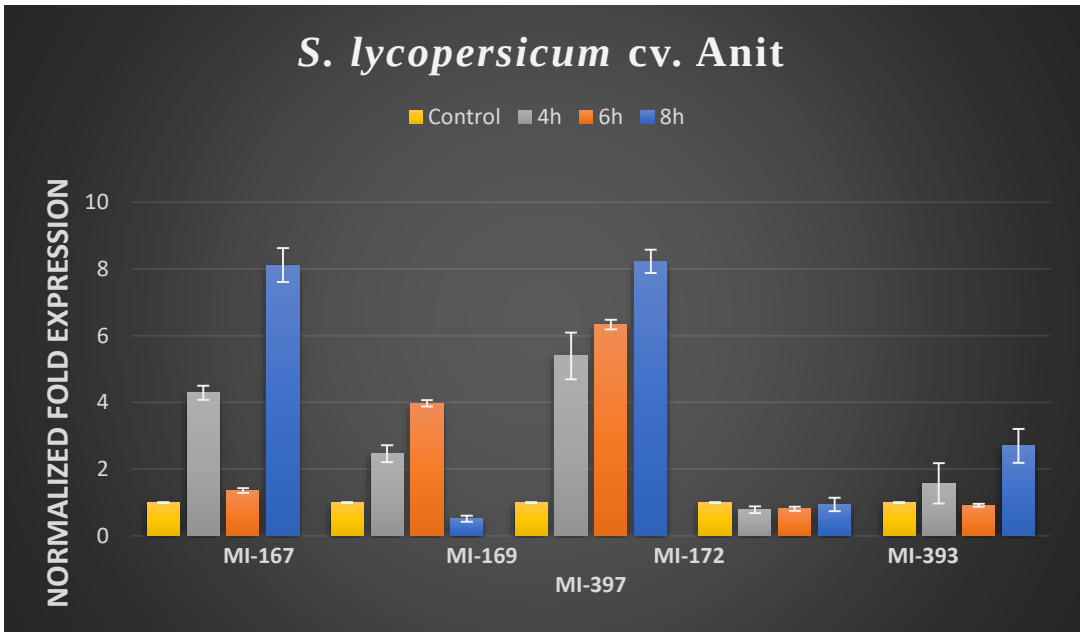

Figure 7. *S. lycopersicum* cv. Anit miRNA expression profile

S. lycopersicum cv. Lice

As is shown in Fig. 8, the expression of miRNA 167 was induced at 4 hours (2 folds) and at 8 hours (1.5 folds) after chilling treatment but there was no up-regulation of miRNA 167 at 6 hours. A noticeable differential expression of miRNA 169 was clearly seen at 8 hours (over 2 folds) after chilling stress, but there was no significant change in expression of miRNA 169 at 4 and 6 hours. A significant increase in the expression profile of miRNA 172 was determined at 4 hours (over 4 folds) and 8 hours (over 2 folds) after chilling treatment. The transcript abundance of miRNA 393 had a considerable increase at 8 hours (over 2 times) but the expression changes at other times were insignificant. The expression of miRNA 397 increased slightly at 8 hours of chilling stress but any upsurged transcript accumulation was not seen at 4 hours and 6 hours.

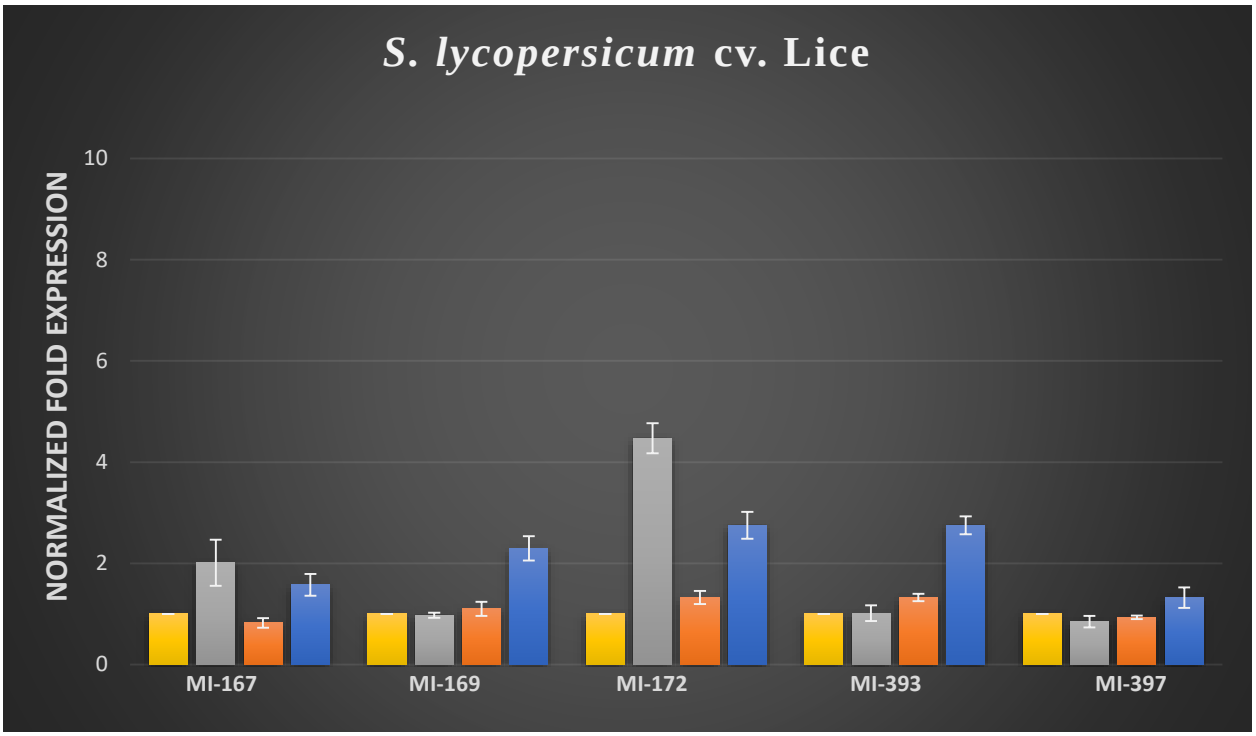

Figure 8. *S. lycopersicum* cv. Lice miRNA expression profile

DISCUSSION

Chilling stress is one of the key environmental constraints in the occurrence of molecular, physiological, biochemical, and morphological changes (Khan et al., 2019; Mesa et al., 2021). Numerous tomato varieties have been shown to be susceptible to chilling stress and heating stress in previous investigations (Liu et al., 2012). Future environmental stresses are anticipated to increase in severity and frequency (Hatfield and Prueger, 2015), therefore, it's important to comprehend how they could impact plant improvement and the molecular and physiological defenses that plants employ. The main objective of the present study was to investigate the role of a group of miRNAs which determined to be related to abiotic stress in former literature, under chilling stress in the wild (S. habrochaites LA 17777 and S. pimpinellifolium LA1579) and cultivated tomato (S. lycopersicum cv. Anit and cv. Lice) genotypes. Therefore, the expression profile of the miRNA 167, miRNA 169, miRNA172, miRNA393, and miRNA 397 were determined in the leaves of tomato genotypes under chilling stress of 4 ° C at 3 different stress levels (4h, 6h, and 8h).

In LA7777 (S. habrocraites), which is cold tolerant (Chen et al., 2015), the expression of miRNA 167, 169, 172 and 397 upregulated up to 9 folds as duration of the chilling stress increased, but miRNA393 down regulated. In LA1579 (S. pimpinellifolium), while the

expression of mi RNA 167, 169 and 172 started to upregulate at 6 hours and then continuous to upregulate up to 8 folds, the expression pattern of miRNA397 and 393 showed an increase at 6 hours after chilling treatment then decrease. The expression pattern of miRNA 167 and 172 in Anıt F1, determined as cold tolerant (Tepe and Kabaş, 2019) started to increase from 4 hours after chilling treatment. In Lice, the mi RNA 167, 169 and 172 slightly upregulated but not like that of LA777. The expression level of the miRNA397 had no change during chilling stress in cultivated tomato, cv Lice and Anıt F1. While the expression of miRNA 393 in Anıt F1 had similar level as control plants, its expression level in Lice upregulated at 8hours after chilling stress. The increase the expression of mi RNA 167, 169, 172 and 397 showed that, these miRNAs could be related to chilling stress tolerance by upregulation their expression (Koc et al., 2015). In contrast to this, the upregulation of the expression of miRNA393 determined only in cultivated tomato genotypes Lice, which is non tolerant to chilling stress. Therefore, it can be stated that miRNA393 may not be considerably related to chilling stress at this stress and growth stages in the studied cultivated tomato. This conclusion was also made in another study reported by Koc et al, (2015) in which miRNA 393 did not show a high level of expression at early stages of growth under short period of chilling stress in tomato (S. lycopersicum). However, the expression of miRNA393 increased at 0°C for 24 h in leaf, stem and root of Arabidopsis (Sunkar et al, 2004) and at 4°C for 24 hours in the leaf of Brachypodium (Zhang et al, 2009). No significant change in expression was seen at the five-leaf stage in LA1777 (S. habrochaites) in miRNA 167 and 169 the study done by Cao et al., (2014) under chilling condition (at 1 hour, 4 hours, 8 hours, 12 hours, 24 hours and 48 hours after subjecting stress), suggesting that these miRNAs may be specific to species or the growth stages. Additionally, the level of expression in miRNA 397 increased in the mentioned study that indicating the importance of this miRNA in three and five-leaf stages of growth under short chilling conditions. MiRNA 167 and other conserved miRNAs had been down-regulated under short periods of chilling stress (2 hours, 6 hours, 12 hours, and 24 hours after treatment) in a study in another member of the Solanaceae family (S. aculeatissimum) at the 3-true-leaf stage in study conducted by Yang et al., (2017). The findings of that study and what is seen in the expression level of miRNA 167 in the current study revealed that conserved miRNAs may have comparable roles across plant species by concentrating on homologous genes involved in the chilly stress response. According to the results of the present study miRNA 172 was overexpressed noticeably in S. pimpinellifolium (LA 1579) at all levels of low-temperature stress. S. pimpinellifolium is a wild relative of the cultivated tomato and it has a lot of breeding potential for desirable features including abiotic stress tolerance according to a study done by Razali et al., (2018). According to a study carried out by Zhao et al., (2017) miRNA 172 was upregulated in S. pimpinellifolium LA1375 under high-temperature stress. Therefore, this miRNA is a prominent factor in the transcription regulation in defense mechanisms of this species against temperature stresses.

CONCLUSION & RECOMMENDATION

This study analyzed some miRNAs (miR167, miR169, miR172, miR393, and miR397) associated with chilling stress during young seedling stage in wild and cultivated tomato genotypes. The outcome revealed that this variation between the expression levels may be connected to tomato genotypes' ability to tolerate chill. Among the miRNAs evaluated, miRNA 167, 169, 172 and 397 were upregulated in the genotypes (S. habrochaites LA 1777 and S. lycopersicum cv. Anit) which already determined tolerant to low temperatures in previous literatures (Venema et al., 1999; Foolad and Lin, 2000; Venema et al., 2005; Tepe et al., 2019). Interestingly, miRNA 167 showed the highest transcript accumulation at 6 hours of chilling exposure in S. pimpinellifolium, 4 and 8 hours in S. lycopersicum cv. Anit, and in all levels of chilling stress in S. habrochaites. It is also worth noticing that miRNA 169 was up-regulated the most at 6 hours of chilling application in S. lycopersicum cv. Anit compared to the control samples. Another important finding in this study is that miRNA 172 can be over-expressed regardless of stress level and tolerability or susceptibility of the plant under chilling stress. It seems that mentioned miRNA is one of the integral parts of tomato plants' defense mechanism against chilling stress.

To sum up the findings of this study demonstrated that chilling-susceptible tomato species can be adversely affected by short-term exposure to chilling stress during the early stages of growth. The molecular responses of the tomato plant to stress treatment at this growth stage seem to be trustable markers for the evaluation process of plant breeding schemes of newly bred lines. In conclusion, the high level of expression in miRNA 167, 169, 172, and 397 could be related to chilling tolerance in tomato genotypes.

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OPPORTUNITIES FOR ENERGY USE OF STRAW AND OTHER AGRICULTURAL PRODUCTS IN BULGARIA

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ABSTRACT

One of the main goals of the energy strategy of the Republic of Bulgaria is to reduce the emissions of harmful greenhouse gases released into the atmosphere. It is necessary to increase the share of renewable energy sources, including energy obtained from biomass from agriculture, thereby contributing to environmental protection and sustainable development of society. Therefore, the present study examines the raw material potential for the production of straw and other agricultural products in the six planning regions in Bulgaria. The following scientific methods were used in the research: comparative analysis and statistical analysis, expert evaluation. The results of the analysis of the conducted research show that a sufficiently large amount of solid waste is generated annually in agriculture in our country, and its incineration turns out to be a rational solution for the production of thermal energy, both for individual consumers and in larger plants.

Key words: biomass, energy, bioeconomy, straw, sunflower stalks, corn stalks

INTRODUCTION

The issue of the biomass potential of wheat, rye, barley, corn and sunflower in Bulgaria has been particularly relevant in recent years. Their waste biomass is straw, sunflower and corn stalks, which are a completely ecological renewable energy source. Their use as renewable energy sources leads to a drop in carbon dioxide, a reduction in energy dependence, and the development of new industries. Emission levels are significantly lower when burning ecological biomass.

Straw, sunflower and corn stalks are generally accepted as biofuels, and burning them turns out to be a rational solution for the production of thermal energy, both for individual users and in larger plants. Their advantages as fuels are: their wide distribution and easy accessibility in agricultural areas; the neutrality with regard to emitted carbon oxides, due to which they are considered an ecologically clean source of energy; the fact that they are a relatively cheap fuel compared to traditional fuels, since in most cases they are obtained as by-products from the production of grain and sunflower seeds.

The aim of the present research is the definition of the main categories and types of the residual biomass from crop production, which could be used as an energy source and tentative determination of their potential in Bulgaria.

On the topic related to the utilization of biomass from plant substances for energy purposes, the authors worked as: Kehayov, Komitov, 2013; Nikolova, Simeonov, 2011; Jansen, Peter et al.,2002., Zlateva, 2012.

RESEARCH METHODS AND SOURCES OF INFORMATION

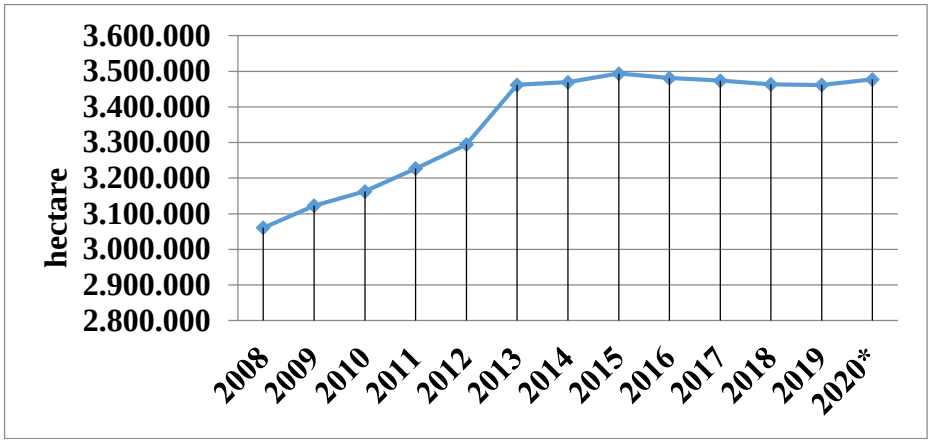
There is no officially published data on the quantities of solid agricultural substances from wheat, rye, barley, corn and sunflower in Bulgaria. For this reason, the analysis of the potential opportunities for providing raw materials for energy use from them was made on the basis of an expert assessment. The spatial parameters of the scientific research are determined normatively by the National long-term program for the promotion of the use of biomass for the period 2008-2020, which regulates the criteria for evaluating the unused amounts of solid agricultural waste from plant and animal husbandry. The time frame of the study covers the period 2008-2020, including the changing conditions after Bulgaria's accession to the European Union. For the purposes of the research, officially published data from the Directorate "Agrostatistics", Ministry of Agriculture and Rural Development were used. Based on them, an expert assessment was made of the available free quantities of straw, corn and sunflower stalks. In cases where certain quantities have to be calculated during the assessment, the following proportions have been used:

- It is assumed that the total amount of straw represents 61% of the yield of wheat, rye and barley (tons/year). The proportion of straw that cannot be harvested from the field is estimated as 20% of the total amount.
- It is assumed that the total amount of corn stalks represents 128% of the corn yield (tons/year). The portion of corn stalks that cannot be harvested from the field is estimated to be 60% of the total amount.
- It is assumed that the total amount of sunflower stalks represents 2000 kg/ha of the harvested areas (ha). The share of sunflower stalks that cannot be harvested from the field is estimated as 60% of the total amount.

In addition to the method of expert evaluation, others such as: comparative analysis, statistical methods were used. For processing and visualization of the obtained results, Figureic images (Figures and tables) were created using appropriate software (Excel 2013).

RESULTS AND DISCUSSION

After the accession of Bulgaria to the EU, a permanent trend of increasing the arable land has been outlined, which includes the areas of fields where crop rotation is applied, temporary meadows with wheat and leguminous grasses, fallows and greenhouses.

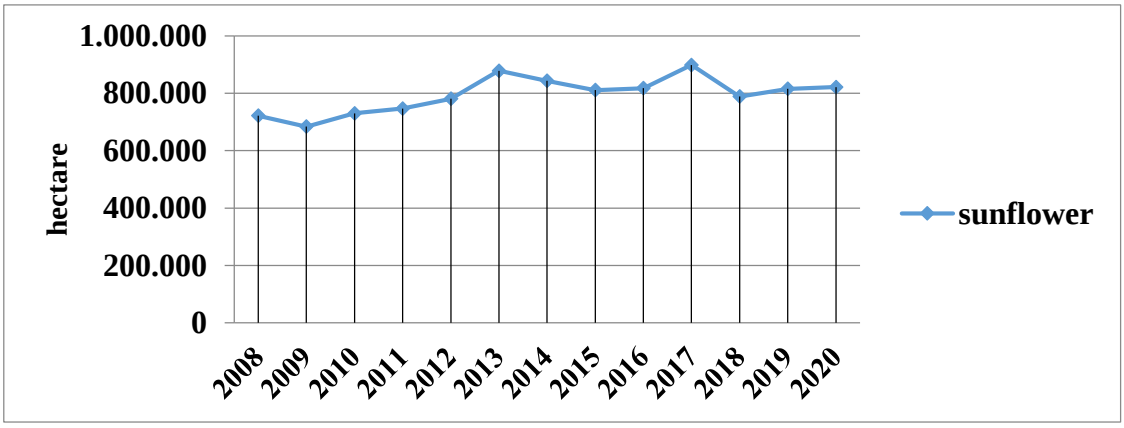


Source: MAF, „ Agrostatistics" Directorate

Fig. 1. Arable land in Bulgaria, ha

As a result of the activation of direct payments, cultivated areas increased from 3 million ha in 2008 to 3.4 million ha in 2013, after which their size remained relatively constant until 2020 (Fig.1).

In Fig. 2 shows the change in the harvested areas of sunflower. In the period 2008-2020, a permanent trend of increasing sunflower areas was observed, with a slight increase in 2013 and 2017, and after 2018, their size remained constant. The significant improvement in the price market, as well as the looming serious decrease in the production of the largest exporter, Ukraine, contributes to having an incentive for our country's sunflower harvested areas to be stable.

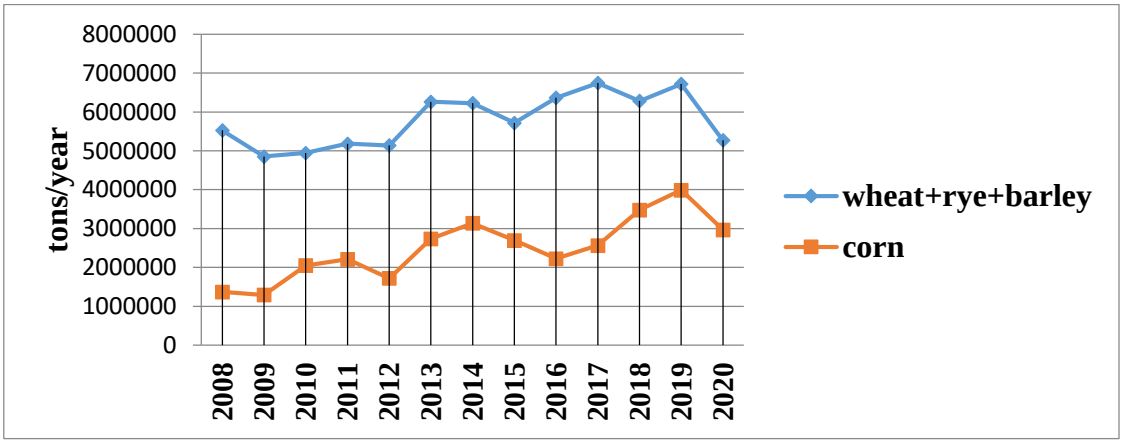


Source: MAF," Agrostatistics" Directorate

Fig. 2 Harvested sunflower areas in Bulgaria for the period 2008-2020.

Wheat, rye, barley and corn are strategic crops and continue to occupy a major place in the structure of agricultural production. The food stability of the country, the trade balance and strategic opportunities for the export of wheat and corn and processed products depend on their level of yields. The yields of wheat, rye, barley and corn in individual years fluctuate because the climatic conditions in the country have a significant impact on them (Fig. 3). For the studied period 2008-2020, a positive trend

is observed in the yields of wheat, rye and barley. They are permanently increasing from 5 million t/year. in 2008 to 6 million t/year. in 2013. The introduction of foreign high-yielding varieties (mostly French) and the increased use of nitrogen fertilizers can be cited as the main reason for the increased yields, especially for wheat. Certain fluctuations in the yields of wheat, rye and barley continue to be observed in the years with pronounced droughts (2015 and 2020). The climatic conditions in Bulgaria are suitable for growing corn.



Source: MAF, „ Agrostatistics" Directorate

Fig. 3 Production of wheat and corn in Bulgaria for the period 2008-2020.

As it is the most important cereal crop after wheat and has established itself as the main fodder cereal and silage crop. In many regions of the country, in some years, prolonged droughts occur, combined with extremely high temperatures, which have an extremely unfavorable effect on the yield of corn. For the research period 2008-2020, fluctuations in corn yields are observed (Fig. 3). This is due to extreme weather conditions, which are often the cause of a strong reduction in its yields (especially in 2009, 2012, 2016 and 2020). Taking into account yields and harvested areas of cultivated crops, the shares of solid agricultural waste from wheat, rye, barley and corn can be calculated, and they are: straw, corn and sunflower stalks. They are a significant potential for waste biomass that can be used for energy purposes. Straw is solid agricultural waste, which in the country is mainly used in crop and animal husbandry. About 20% of it can be utilized for energy needs. The corn and sunflower stalks have no other use and the proportion of the quantities used for energy purposes is consistent with the maximum possibilities for their collection.

Table 1 Available unused amounts of straw for the period 2008-2020, tons/year

	Tons/years			
areas	2008	2012	2016	2020
Northwest	132387	124230	168098	183595
North Central	156211	141366	167258	148762
Northeast	184203	154971	204577	94458
Southeast	144386	133422	151407	115613
South Central	44631	52764	61056	74823
Southwest	12228	20207	24470	25635
Bulgaria	674047	626960	776865	642927

Source: *NLPTPPB* and own calculations.

From the calculations made based on an expert assessment for the period 2008-2020, it can be seen that the waste biomass of wheat, rye and barley can provide for energy purposes a large amount of straw. The highest values of straw production were reported in 2008 and 2016, probably due to the favorable weather conditions in the crop development process (Table 1). In 2020, Northern Bulgaria has the largest amount of straw - about 66%, and only 29% in the South-Eastern and South-Central regions. Traditionally, a larger part of wheat production is in Northern Bulgaria, because the climatic conditions from sowing to harvesting are the most suitable. Besides straw, there are other types of agricultural by-products that could be used for energy production. Here should be taken: corn stalks and cobs, sunflower stalks and pits. The available free quantities, as above for straw, are estimated based on statistical information (table 2 and table 3). As with straw, the share of by-products that cannot be harvested from the field, as well as the currently harvested and beneficially used quantities, is reported for them.

Table 2 Available unused amounts of corn stalks for the period 2008-2020, tons/year

	Tons/years			
Areas	2008	2012	2016	2020
Northwest	297 412	466 032	630 326	943 841
North Central	324 021	291 667	494 508	771 620
Northeast	335 030	495 173	496 076	403 705
Southeast	20 069	27 898	26 105	41 196
South Central	38 396	24 461	33 746	52 150
Southwest	35962	14029	28879	67842
Bulgaria	1 050 890	1 319 259	1 709 640	2 280 353

Source: *NLPTPPB* and own calculations.

From the calculations made during the period 2008-2020, it can be seen that the annual quantities of agricultural by-products from corn have increased from 866,027 tons/year in 2008 to 986,306 tons/year in 2020 (table 2). During the entire study period of 2008-2020, the data indicate that the Northwest and North Central regions can produce the

largest amounts of corn stalks. Taking into account the harvested areas of sunflower, calculations were made on the basis that it can be seen that they can provide 986 306 tons/year of sunflower stalk waste by 13% more than in 2008.

Table 3 Available unused amounts of sunflower stalks for the period 2008-2020, tons/year

	Tons/years			
Areas	2008	2012	2016	2020
Northwest	256235	204691	258324	259084
North Central	186934	217417	204061	166578
Northeast	194104	235525	226117	223248
Southeast	149275	179402	162546	183030
South Central	68483	76446	99136	111128
Southwest	10997	23424	30829	43238
Bulgaria	866027	936906	981013	986306

Source: *NLPTPPB* and own calculations.

From table 3 it can be seen that the Northwest, Northeast, Northcentral and Southeast planning regions can provide the largest amounts of waste from sunflower stalks. Summarizing the results of the calculations, it can be concluded that agriculture has a sufficient amount of biomass from plant substances that can be used for energy purposes. For the production of electricity from agricultural waste biomass, the following technologies are applied - direct combustion, pyrolysis, gasification, anaerobic decomposition. Of all the listed technologies, direct burning is the most effective, and mainly for straw. Since solid agricultural wastes have different calorific value and combustion behavior, an evaluation of their quality characteristics, at a certain humidity, is also included here, which is essential.

Table 4. Quality indicators of solid agricultural waste,

	Types of solid agricultural waste	Humidity (%)	Carbon content of the work table (%)	Lower heat of combustion, kcal / kg.
1.	Straw	10 – 20	42	3 400
2.	Corn stalks	40-60	24	1 800
3.	Sunflower stalks	30-40	30	2 200

Source: *NLPTPPB*

Of all organic waste, only straw is easier to burn directly compared to the others (Table 4). For example, the moisture content of corn stalks (about 40-60%) is much higher compared to the moisture content of straw (10-20%).

Straw is a biofuel providing a relatively high potential for heat production. The energy equivalent of the unused amount of straw (with a calorific value of 3 400 kcal/kg) amounts to 5,8 x 10^6 Gcal/year. (about 642 thousand tons per year in our country). For comparison, this is about 50% of the final consumption of diesel fuel and gas oil in the country. It is also worth noting the significant ecological effect that would result from the burning of straw as an energy fuel in connection with the reduction of CO2

emissions. These are the largest amounts of greenhouse gas emissions of concern for global climate change.

The burning of straw turns out to be a rational solution in the production of thermal energy both for individual users and in larger plants. The straw is used for the production of biobriquettes, biopellets or for direct burning in special installations to obtain thermal and electrical energy.

The use of straw as an energy source can be summarized in the following advantages:

- costs are minimal for straw production - only baling, transport and storage;
- high yield of straw - for example in rapeseed - about 1.5 tons per hectare.
- mechanization of the straw collection and processing process;
- straw ash can be used as a soil conditioner;
- gives off heat like wood and coal;
- lowering the percentage of harmful emissions released into the atmosphere during combustion;
- possibility to receive subsidies and financing from EU funds.

Territorially, the greatest concentration of the available quantities of straw exists in the following districts: Dobrichka, Razgradska, Varna, Plevenska, Silistrenska, Yambolska, Shumenska, Velikotarnovska and Starozagorska.

The corn and sunflower stalks represent a more difficult type of biomass to burn directly, due to their high moisture values (Table 4). For the burning of corn and sunflower stalks, no suitable technology has yet been used in Bulgaria. At the same time, the energy equivalent of the free amounts of corn cobs (3,5 x10^6 Gcal/year) and sunflower stalks (2,0 x 10^6 Gcal/year) in Bulgaria is significant - total for both type of products is equal to the energy equivalent of the straw (5,8 x 10^6 Gcal/year). From this point of view, further research and development of different types of technologies for energy use of similar products is of serious interest.

CONCLUSION

The presented results show the great importance of agricultural activity, which is accompanied by the formation of large amounts of residues of straw, corn and sunflower stalks possessing good energy characteristics and a high potential for energy production. The advantages of this waste biomass from agriculture as an energy source is through the application of technologies for its conversion, which is one approach to moving the country's economy towards sustainable development, because in this way it moves away from the traditional use of fossil fuels. As a domestic energy source, biomass can significantly reduce the economy's dependence on imported crude oil. Moreover, biomass is spread over the globe more evenly than other limited energy sources and therefore offers opportunities for local, regional and national energy independence. The use of agricultural waste will stimulate the development of crop agriculture and related service industries by creating new products, markets and jobs.

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The National Long-Term Program for the Promotion of the Use of Biomass for the Period 2008-2020

ESSENTIAL OIL COMPOSITION AND ANTIOXIDANT ACTIVITIES OF FOUR CULTIVARS OF LAVENDER FROM EDIRNE

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ABSTRACT

The research is the first study to compare the chemical compositions of 3 different lavender cultivars, including Raya, Sevtopolis, Yubileina and Hemus, after adaptation in Edirne. The essential oil composition, polyphenol content and antioxidant activities of these cultivars were evaluated. The essential oils of these varieties were basically characterised by β-Linalool (47-35, %), Linalyl acetate (22- 29, %), α-Terpineol (5-0.46, %), Lavandulyl acetate (5-4, %), 1-Terpinen-4-ol (0.09-8.12, %), 3-Octanone (0.78-1.89, %), Limonene (0.35-0.27, %), Lavandulol (0.00-2.49, %), β-Cadinene (0.00-0.24, %) and camphor (0.26-0.72, %). The essential oils from these varieties have also significant differences for total phenolic and antioxidant contents. Hemus and Raya oils possess the highest total phenolic contents (16.98 µg GAE/mg (10µL) and 16.22 µg GAE/mg (10µL), respectively), followed by Yubileina and Sevtopolis (12.77 µg GAE/mg (10µL) and 9.17 µg GAE/mg (10µL), respectively). The antioxidant activities as well as chemical composition of from essential oils of lavanders were compared. The highest antioxidant activity was calculated by the essential oil from Sevtopolis (18.403 mg/mL) while the oil from Hemus, Yubileina and Raya had lower antioxidant activity (14.666 mg/mL, 8.208 mg/mL and 4.639 mg/mL, respectively). This research has importance for agriculture and commercial production of Lavender in Edirne. Because these results belong to the cultivars that the first adaptation in Edirne, which formerly grown in Bulgaria.

Key words: Essential oil, Lavender, Lavender cultivars, β-Linalool, Linalyl acetate.

INTRODUCTION

It is known that the genus *Lavandula L. (Lamiaceae)* have lots of medicinal and technological values (Canavagh ve Wilkinson, 2002; Kim ve Lee, 2002; Gülçin vd., 2003; Karık ve Öztürk, 2010; Yang vd., 2010; Aslan, 2012). Essential oils, obtained from medicinal and aromatic lavenders by using different methods, have important technological properties with including terpenes, alcohols, aldehydes, phenols and esters (Messaoud et al., 2012; Kıvrak, 2018). These properties depend on the biological characteristics of the plant. The essential oils of the different Lavender varieties have the same chemical composition including different proportions. The basic properties, which indicator for oil quality, are camphor, linalool and

linalyl acetate contents of essential oil. Besides, it has differences between lavender and lavandin essential oils. In generally, component ratios can be expressed as linalool (25–38%), linalyl acetate (25–45%) and camphor (0.5–1.0%) for lavender essential oils, and linalool (24–35%), linalyl acetate (28–38%) and camphor (6–8%) for lavandin essential oils (Baydar and Kineci, 2009; Kıvrak, 2018).

The results presented in the study are the data belong to the first year of the adaptation of these varieties in Edirne in 2016. Therefore, it has a special value for interpreting the change of adaptation in the following years. Besides, it has importance for the observation of the changes in chemical compositions, technological properties, and economical values.

MATERIAL AND METHOD

Materials

Plant materials, *L. angustifolia* (cvs Raya, Sevtopolis, Yubileina and Hemus) cultivars were provided from the cultivation areas of Trakya Agricultural Research Institute (Edirne, Turkey). The first-year-old plants were harvested for each cultivar at the flowering time in cloudless sunny weather at midday in 2016. The extraction was performed by Neo-Clevenger apparatus by hydro-distillation and 100 g dry flower samples of each cultivar were used in essential oil extractions. Essential oil samples kept in dark vials at -20°C until analyzing.

Methods

Determination of total phenolic compounds

Total phenolic contents of essential oils were determined using the Folin-Ciocalteu reagent assay (Aliakbarlu and Shameli, 2013) with gallic acid standard. Briefly, 500 µL of the essential oils in methanol (1 mg/mL) was mixed with 2.25 mL distilled water and then 250 µL of Folin-Ciocalteu reagent was added. The mixture was vortexed for a minute and was allowed to react for 5 minutes. Then, 2 mL of Na_2CO_3 solution (7.5%) were added. After incubation at room temperature for 120 min, the absorbance of each mixture was measured at 760 nm. The same procedure was also used to the standard solution of gallic acid, and a standard curve was obtained. Total phenolic contents were expressed as mg of gallic acid equivalent per g of the essential oils. All tests carried out in triplicate.

Radical Scavenging Assay

Determination of free radical scavenging capacity (DPPH) of the extract was performed based on the method of Sanchez-Moreno et al. (1998). The essential oil dissolved in methanol was prepared in various concentrations (50–1500 µg/mL). 1 mL of the extract solutions mixed with 3 mL of DPPH solution ($6x10^{-5}$ M) dissolved in methanol. After incubation at room temperature for 30 min, absorbance of samples at 517 nm was read against a methanol blank.

All the experiments were conducted in triplicate. Inhibition of free radical, DPPH, in percent (I %) was calculated:

I%=(Ablank - Asample/Ablank) x 100

IC50 (50% inhibition) values for the essential oils were calculated.

Determination of aroma compounds

Aroma compounds in essential oils were analyzed by gas chromatography combined with a triple quadrupole mass spectrometry system (GC-MS/MS) Scion TQ 456 (Bruker, Billerica, Massachusetts, USA) coupled with a triple quadrupole (TQ) mass spectrometer Scion TQ MS/MS (Bruker Daltonics, Billerica Massachusetts, USA) equipped with a DB -1MS column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness; Agilent Technologies, Santa Clara, California, USA). The GC–MS/MS electron impact source was operated in multiple reaction monitoring (MRM) mode with the MS source temperature of 250 °C, the manifold temperature of 40 °C, transfer line temperature of 280 °C and collision-induced dissociation on argon as collision cell gas with pressure 267 Pa. The injector temperature was maintained at 250 °C with a constant flow rate of 1.0 ml min-1 of helium. Injection was splitless with a hold of 1 min. The electron energy was –70 eV. The oven temperature program consisted of a 3 min hold at 50 °C, followed by a 8 °C·min-1 ascent to 100 °C and a 30 min hold at 250 °C. The samples were diluted with methylene chloride (Sigma-Aldrich, St. Louis, Missouri, USA). The concentrated extract was im- mediately injected to the GC device.

RESULTS AND DISCUSSION

Total Phenolic Compounds and free radical scavenging capacity (DPPH)

The amounts of the total phenolic compounds were calculated from gallic acid standard curve, and the equation was determined as,

$y = 0.0107x + 0.1718$ $(R^2 = 0.9998)$

The amounts of the total phenolic compounds of the essential oils are shown in Table 1. The total phenolic contents significantly varied among methanol extracts of the studied species. Total phenolic contents varied from 9.17 μg/10 μL in Sevtopolis to 16.98 μg/10 μL in Hemus.

Antioxidant properties of Lavender essential oils were assessed using DPPH scavenging (Table 1). When the DPPH the radical-scavenging potential of the essential oils, different concentration of essential oils caused a 50% inhibition (IC50) of the free radical for samples. The IC50 values was determined as 4.639 mg mL^{-1} in Raya, 8.208 mg mL^{-1} in Yubileina, 14.660 mg mL^{-1} in Hemus and 18.403 mg mL^{-1} in Sevtopolis.

There are lots of factors influenced to the antioxidant activities of essential oils from lavenders, such as cultivars, fresh/dried flowers, aerial parts, climacteric conditions, adaptation conditions, plant year etc. (Ghasemi Pirbalouti et al., 2013). Smigielski et al. (2018) were compared to the antioxidant activities as well as chemical composition of essential oils from fresh and dried flowers and aerial parts of lavender (*Lavandula angustifolia*). The highest antioxidant activity was exhibited by the essential oil from fresh aerial parts (IC50 =77.11 mg/mL) while the oil from dried flower displayed the weakest activity (IC50 = 22.1 mg/mL). In another study, the IC50 values of essential oil of *L. coronopifolia, L. multifida, L. stoechas* were found as 162.2, 201.6 and 2321.7 µg/mL, respectively (Messaoud et al. 2012).

Table 1. Total phenolic content and antioxidant activity values of essential oils of lavenders.

Cultivars	TPC (µg/10 µL)	IC50 (mg extract /mL)
Hemus	16.98	14.660
Yubileina	12.77	8.208
Raya	16.22	4.639
Sevtopolis	9.17	18.403

Adaptation and growing conditions are the effective factors on essential oil composition. As predominant components of lavender essential oils are linalool, linalool acetate, linalyl acetate, 1,8-cineole, borneol, fenchon, camphor, menthol and α-pinene (Nurzyńska-Wierdak and Zawiślak, 2016). The share of above-mentioned components undergoes is subject to, among others, ontogenetic variability. It was also demonstrated that the manner of adaptation conditions may affect the chemical profile of essential oil (Nurzyńska-Wierdak and Zawiślak, 2016).

Aroma compounds

The chemical compositions of four essential oils, obtained from lavander cultivars were analyzed using GC/MS. Essential oil composition percentages of varieties are displayed in Table 2. Especially, linalool and linalyl acetate are the most important compounds to determine the perfume quality. Besides, according to ISO 3515:2002 lavender oil quality standards, it is required to contain at least 25% in lavender oil to be used in the perfume industry (Kara, 2011). The highest linalyl acetate content was found in Hemus (29.28%), while the lowest linalyl acetate content determined in Sevtopolis (22.97%). Sevtopolis had a slightly lower value than it should have been by International Standard ISO 3515. It belongs to the first adaptation year and the linalyl acetate value of Sevtopolis must be compared in future studies.

Previous studies showed that the ranges of linalyl acetate and linalool in essential oil of Raya, Yubiliana, Sevtopolis and Hemus are respectively 22.45-60.81% and 18.74-47.29% (Kıvrak 2018; Zagorcheva et al.,2013; Milina et al., 2012). These studies (Kıvrak 2018; Zagorcheva et al.,2013; Milina et al., 2012) were completed with same cultivars (Raya, Yubiliana, Sevtopolis and Hemus), which firstly cultivated in Bulgaria, and collected from Turkey and Bulgaria. We detected linalyl acetate 26.04%, 28.43%, 22.97% and 29.28% in respectively Raya, Yubiliana, Sevtopolis, Hemus for first adaptation in Edirne. Besides, linalool in our samples were calculated as 45.69%, 47.94%, 39.83% and 35.68% in respectively Raya, Yubiliana, Sevtopolis, Hemus for first adaptation in Edirne. Alkan et al. (2021) studied for these varieties cultivated in Edirne in 2019 (three years after first harvest). They found the highest linalyl acetate in Raya with 30.0%, followed by Yubiliena and Hemus with 24.2 and 23.1% contents, respecgtively. It was found that Raya essential oils contained 42.5% linalool whereas Yubileina and Hemus essential oils contained 36.0% and 28.5% linalool, respectively. Kıvrak (2018) was studied with the same cultivars adapted in Fethiye, Mugla and harvested in 2016. The linalyl acetate contents were determined as 33.92%, 29.09%, 32.12%, 46.19% for Raya, Yubiliana, Sevtopolis, Hemus respectively. Also, linalool was found as 35.49%, 30.46%, 28.10% and 29.34% for Raya, Yubiliana, Sevtopolis, Hemus respectively. They found higher values for linalyl acetate and lower values for linalool than Edirne. Zagorcheva et al. (2013) and Milina et al. (2012) were evaluated to the same lavender varieties but cultivated and harvested in Kazanlak, Bulgaria. Besides, both studies performed in 2012. However, there were some differences in compounds contents.

Camphor is another and the most emphasized compound in lavender oils in terms of quality and market value. Although it is found in low rates, they are sold at high prices. Among the lavender varieties, lavender oils are generally of higher quality as they have a lower camphor content than lavandin oils. According to ISO 3515:2002 lavender oil quality standards, the camphor ratio, which should be at most 0.5% in high quality lavender oil, is between 0.5-1% in the essential oil of lavender varieties and 5-10% in lavandin oils. Camphor values were determined between 0.26-0.72% for lavender essential oils. The data are in accordance with International Standard ISO 3515 (2002) Oil of Lavender (*Lavandula angustifolia Mill.*). Besides, data are similar with other studies (Alkan et al., 2021; Kıvrak 2018; Zagorcheva et al.,2013; Milina et al., 2012).

Other significant component α-terpineol was indicated in lavender essential oils between 0.46-5.57% (Raya 5.57%, Hemus 5.26%, Sevtopolis 5.12% and Yubiliana 0.46%). On comparison of the present results with those reported from samples of other studies, β-myrcene, limonene, oct-1-en-3-ol, borneol, β-caryophyllene were marked slighter less in these samples. Also, it is quite evident that the contents of α-terpineol were higher.

Table 2. Essential oil composition percentages of lavender varieties.

	Ret. T. (min)	RAYA	SEVTOPOLIS	YUBILEINA	HEMUS
1-Hexanol	6.097	0.00	0.10	0.00	0.00
α-Thujene	7.75	0.00	0.00	0.00	0.00
α-Pinene	7.918	0.00	0.10	0.00	0.16
Camphene	8.373	0.16	0.32	0.17	0.22
β-Pinene	9.245	0.00	0.00	0.00	0.00
Oct-1-en-3-ol	9.356	0.00	0.14	0.08	0.00
3-Octanone	9.578	0.78	1.30	1.89	1.13
β-Myrcene	9.713	0.37	0.50	0.43	0.56
3-Octanol	9.888	0.00	0.49	0.17	0.00
Hexyl acetate	10.425	0.27	0.76	0.27	0.52
m-Cymene	10.646	0.00	0.00	0.00	0.68
o-Cymene	10.729	0.12	0.18	0.09	0
Limonene	10.857	0.32	0.27	0.32	0.35
Eucalyptol	10.936	1.78	3.06	1.11	0.36
(E)-β-Ocimene	11.17	0.58	0.29	0.91	0.42
(Z)-β-Ocimene	11.486	0.48	0.41	0.57	0.12
Linalool Oxide	12.235	0.30	0.77	0.00	0.56
Linalool Oxide	12.717	0.33	0.65	0.27	0.69
β-Linalool	13.068	45.69	39.83	47.94	35.68
1-Octen-3-yl-acetate	13.468	0.68	0.83	1.14	1.25
3-Octyl acetate	13.876	0.10	0.30	0.17	0.00
Perillol	14.369	0.00	0.00	0.00	0.00
camphor	14.585	0.41	0.72	0.26	0.43
Hexyl isobutyrate	14.774	0.00	0.00	0.00	0.00

Borneol	15.419	1.65	2.66	1.03	1.97
Lavandulol	15.512	0.42	2.49	0.49	0.00
1-Terpinen-4-ol	15.932	0.60	0.72	0.09	8.12
p-Cymen-8-ol	16.138	0.00	0.05	0.00	0.00
Crypton	16.311	0.61	0.73	0.60	0.00
α-Terpineol	16.492	5.57	5.12	0.46	5.26
Hexyl butyrate	16.599	0.31	0.10	0.02	0.00
Verbenone	17.207	0.00	0.00	0.00	0.00
Carveol	17.557	0.00	0.04	0.00	0.00
Geraniol	17.873	0.61	0.67	0.52	0.55
Cuminal	18.241	0.41	0.45	0.41	0.00
Carvone	18.387	0.00	0.09	0.00	0.00
Linalyl acetate	18.772	26.04	22.97	28.43	29.28
Bornyl acetate	19.618	0.50	0.35	0.72	0.50
Lavandulyl acetate	19.752	5.65	4.41	4.71	5.70
Neryl acetate	21.505	1.04	0.87	1.08	1.01
Geranyl acetate	21.917	2.10	1.83	1.83	2.16
β-Caryophyllene	22.717	0.89	1.26	1.97	0.69
(Z)-β-Farnesene	23.37	0.22	0.75	0.56	0.00
β-Cubebene	23.88	0.00	0.00	0.24	0.00
Caryophyllene oxide	25.65	0.85	3.20	1.05	1.61
β-Cadinene	26.509	0.15	0.24	0.00	0.00

CONCLUSION

All cultivars may be considered as a natural raw material source for pharmaceuticals, cosmetic, and food products. These results verified industrial usage of these plants and it has importance for agriculture and commercial production of Lavander in Edirne.

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STUDIES ON THE NEW PEST CEUTORHYNCHUS SUTURALIS FABRICIUS (COLEOPTERA: CURCULIONIDAE) IN ONION FIELDS IN BURSA AND BALIKESIR PROVINCES

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ABSTRACT

In this study, distribution of *Ceutorhynchus suturalis* Fabricius and larvae population density were investigated under laboratory and field conditions in Bursa and Balıkesir provinces in 2004-2005. Surveys were carried out once a week or two weeks from April to August. *C. suturalis* was known onion pest in some European countries. First time it was determined on *Prunus domestica* in Tekirdağ but for the first time it was determined in onion field in Balıkesir and Bursa provinces in 2004. In field, first larvae were seen at the mid of April and begining of May and larvae were seen at the end of June. In Manyas and Susurluk (Balıkesir) larvae population density was 3.6, 2.6 larvae / plant and in Yenişehir and Karacabey (Bursa) 1.8, 1.6 larvae/ plant, respectively.

Key Words: *Ceutorhynchus suturalis*, new pest, onion, population density

INTRODUCTION

Onion (*Allium cepa* L.) is a herbaceous plant that has been shown in the Liliaceae family until now, but it belongs to the Amaryllidaceae family, with its sharp smell, tuber and green leaves in recent records. The composition of onion contains essential and fixed oils, sugars, vitamins, minerals, ferments and amino acids. It is an extremely beneficial product for human health, especially since it shows antiseptic and antibiotic properties and the loss of vitamins when cooked is low (Karahocagil, 2003). The world dry onion production area is constantly increasing. The largest producing country is India. When the dry onion export amounts of the world countries are analyzed, it is seen that the largest exporting country was the Netherlands as of 2001, followed by India, China and the USA. Turkey, on the other hand, ranks 7th in onion exports with a 3% share (Karahocagil, 2003).

In Turkey, especially since 1996, dry onion production areas and production amounts have been increasing. As of 2005, Turkey ranks 4th in dry onion production after China, India and the USA. In 2005, 2.000.000 tons of dry onions were produced in our country (Anonim, 2005). In Turkey, which has different climatic conditions, onions are grown in almost every region except the Eastern Anatolia Region, and it is grown intensively in Thrace Region, Balıkesir, Bursa,

Bandırma, Amasya, Çorum, Tokat, Kastamonu, Hatay and Denizli (Vural ve ark., 2000). The province with the highest dry onion production is Amasya, which provides 13% of the total dry onion production. Other important provinces after Amasya are Ankara-Polatlı (12%) and Bursa-Karacabey (7%) (Karahocagil,2003). According to 2001 data; Dry onion production in Bursa is 148.300 tons, and fresh onion production is 3.997 tons. Dry onion production in Balıkesir is 46,580 tons and fresh onion production is 3,099 tons (Anonim, 2001). In both provinces, more than one variety is produced in the regions where onions are grown; The most commonly grown variety is Aki. Production in the form of table or seeded onions is made for two purposes.

As in every plant, some problems are encountered in onion cultivation. In recent years, complaints about pests on onions have increased. However, there are few studies on onion pests in our country. Akkaya and Uygun (1996) mentioned a few onion pests in their study on the insect fauna of Diyarbakır and Şanlıurfa summer vegetable ecosystems. Studies on onion pests in the world are carried out in Pakistan, Egypt and Brazil (Malik et al., 2004, Elbolok et al., 1990, Ciociola et al., 2002, Gonçalves et al.,2003). In addition, although no studies on *Ceutorhynchus suturalis* were found in our country, from European countries Germany (Schrameyer, 1991; 2001), Poland (Luczak and Wiewiora, 2001, 2002; Szwejda, 2005a, 2005b; Wiewiora and Luczak, 2003) and Lithuania (Duchovskiene, 2003) are in progress.

MATERIAL AND METHOD

The main material of the research is the larvae and adults of *C. suturalis* collected from onion fields in Bursa and Balıkesir provinces in 2004-2005.

Laboratory Studies

Obtaining Adults of Pest Species

In 2004, onion plants found to be infected by the larvae of the pest were brought to the laboratory. After planting in pots, they were covered with a chiffon cloth and cultured and placed in a climate room at 25±5ºC and 65±5% relative humidity.

Larval Measurements

After the detection and locations of the pest were determined, the counts of the larvae found in the onion samples brought from the field in 2005 were made in the laboratory by visual control method. An average of 8 leaves were examined in an onion plant.

Field Studies

In 2005 field studies, in Bursa province Karacabey district center and Şahinköy; Yenişehir district, in Koyunhisar village; in Boğazpınar village of Manyas district of Balıkesir; in Tuzakçı village of Gönen district and Söve village of Susurluk district was carried out periodically (10-14 days). Based on the size and location of the field, 5 or 10 onion plants were randomly

removed from each plot. After the samples were wrapped in newsprint, they were brought to the laboratory for larval enumeration in polyethylene bags.

RESULTS AND DISCUSSION

Distribution and Damage Type

A new pest, *Ceutorhynchus suturalis* F. was detected in the surveys carried out in the onion cultivation areas of Bursa and Balıkesir provinces in 2004-2005 (Table 1). This species was first reported in Turkey by Lodos et al. (1978), in their study on the determination of harmful insect fauna of Aegean and Marmara regions, reported that it was detected in *Prunus domestica* in Tekirdağ-Center, but this species caused damage especially on onions and was an economic pest in Europe, Bulgaria and Yugoslavia. Sert (2005) identified 20 species belonging to the genus *Ceutorhynchus* in the Mediterranean and Central Anatolia regions, but *C. suturalis* is not among these species. Studies on onion pests in our country have not found any data on this species (Akkaya ve Uygun, 1996). *C. suturalis* lays its eggs in the inner part of the onion leaves in groups of 3-4, while the larvae are in the inner part of the onion leaves. They begin to cause damage in the early stages of the onion, and the larvae emerge at the beginning of April and continue to feed on the leaves. The larvae cause damage by eating the fleshy part of the leaf and only the membrane part of the leaf remains. Adults cause damage by making external defeats on the leaves, albeit a little. Schrameyer (1991) reported that *C. suturalis* is a potential pest of onions in Germany and emerged after a mild winter. Luczak and Wiewiora (2001) emphasized that *C. suturalis* is an important pest of onions in Poland.

Table 1. Locations of *Ceutorhynchus suturalis* F. detected in Bursa and Balıkesir in 2004-2005

ORDER	FAMILY	SPECIES	LOCATION		
			PROVINCE	DISTRICT	COUNTRY
Coleoptera	Curculionidae	*Ceutorhynchus suturalis* F.	Bursa	Karacabey	Merkez
					Şahinköy
				Yenişehir	Koyunhisar
			Balıkesir	Susurluk	Merkez
					İclaliye
					Söve
				Bandırma	Külefli
				Manyas	Boğazpınar
				Gönen	Tuzakçı

Larval Population Density of Pest

Larvae Population Density of *C. suturalis* in Bursa Province Yenişehir and Karacabey District in 2005

In the studies conducted in Yenişehir and Karacabey districts, 7 samplings were made. The first emergence of the larvae of *C. suturalis* was seen on May 5 in the onion field in Koyunhisar village of Yenişehir, the number of larvae continued to increase after this date and a peak was formed with 1.9 larvae/plant on May 31. Afterwards, the number of larvae gradually decreased and ended on July 21 (Figure 1). In Yenişehir, on 27-31 May, when the larva peaks in 2005, the pentate precipitation is 0 mm and the relative humidity is 60%. In addition, the pentate temperature on the same dates is 17.8 ºC (Figure 2).

In the onion fields of Karacabey district Merkez and Şahinköy, the first hatching of C. suturalis larvae was seen on the same date (on April 16) in both places, the number of larvae determined after this date continued to increase and on May 24, with 1.2 larvae/plant was a peak in Merkez, a peak was formed in Şahinköy with 1.7 larvae/plant. After this date, the number of determined larvae decreased and ended on June 15 (Figure 3, 4). In Karacabey, on 21-25 May 2005, the pentate precipitation amount was 1.2 mm and the relative humidity was 78.8%. In addition, the pentate temperature on the same dates was 17 ºC (Figure 5).

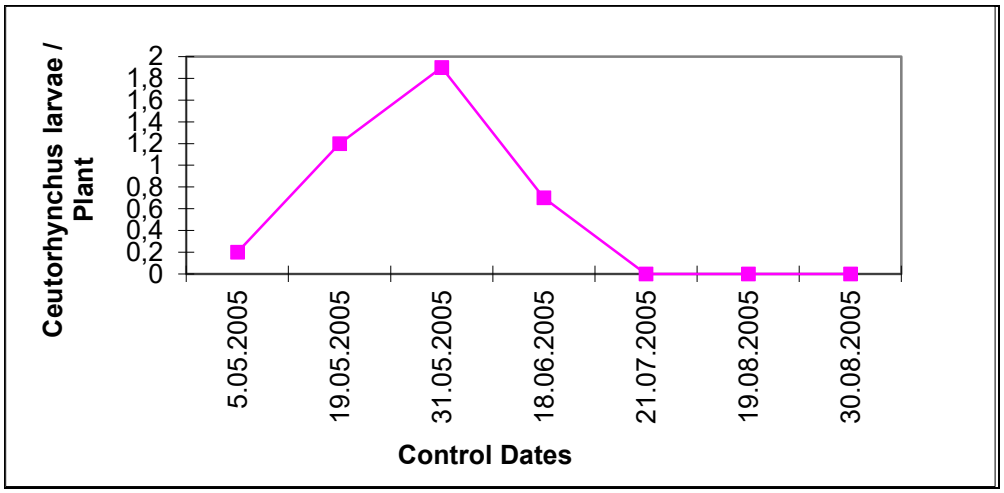

Figure 1. Larval population density of *Ceutorhynchus suturalis* in Yenişehir-Koyunhisar village in 2005

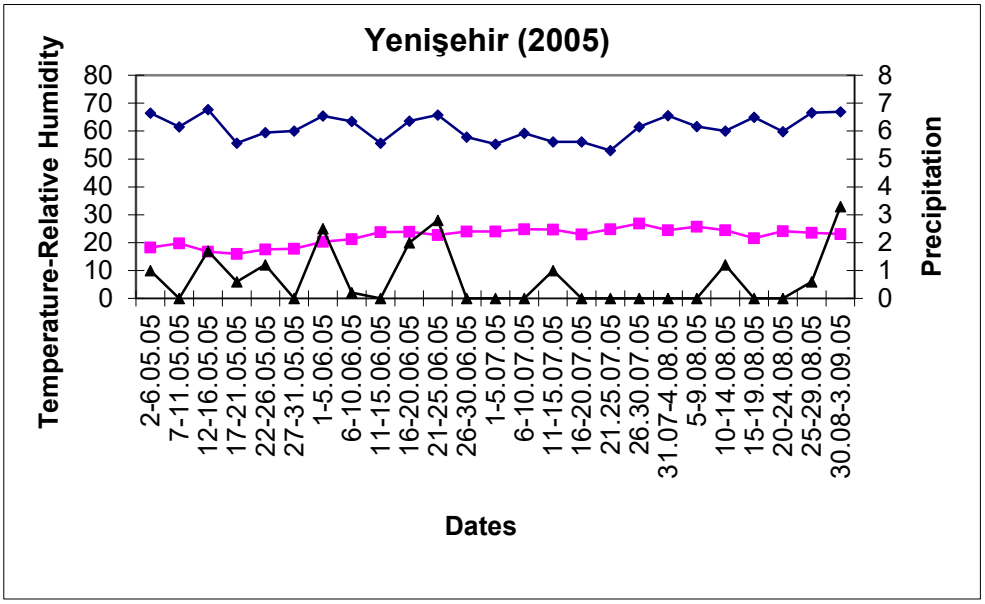

Figure 2. Climate data for 2005 in Yenişehir district

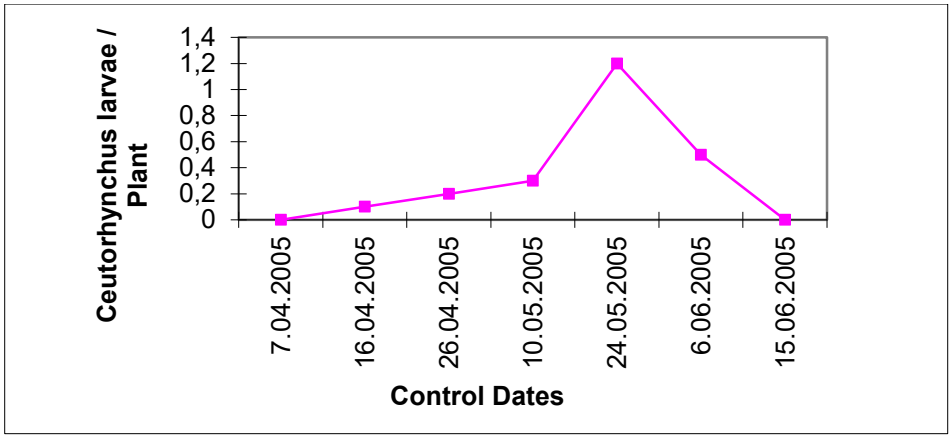

Figure 3. Larval population density of *Ceutorhynchus suturalis* in Karacabey-Center in 2005

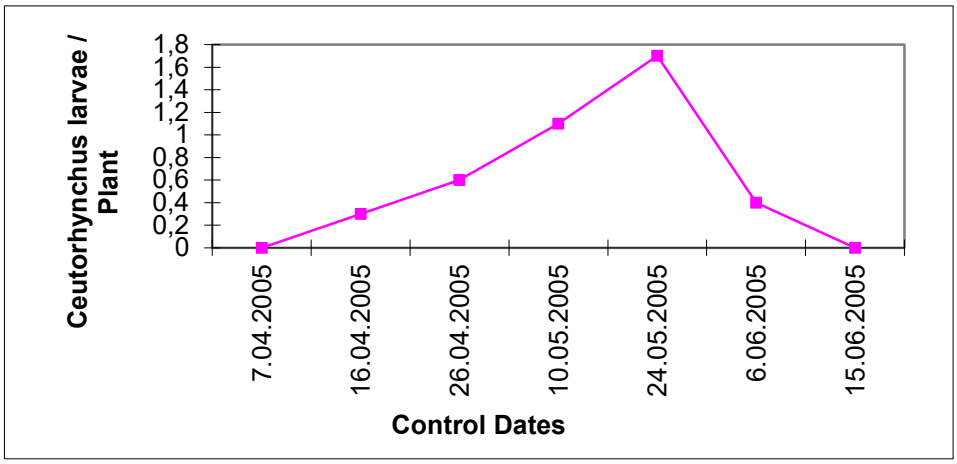

Figure 4. Larval population density of *Ceutorhynchus suturalis* in Karacabey-Şahinköy in 2005

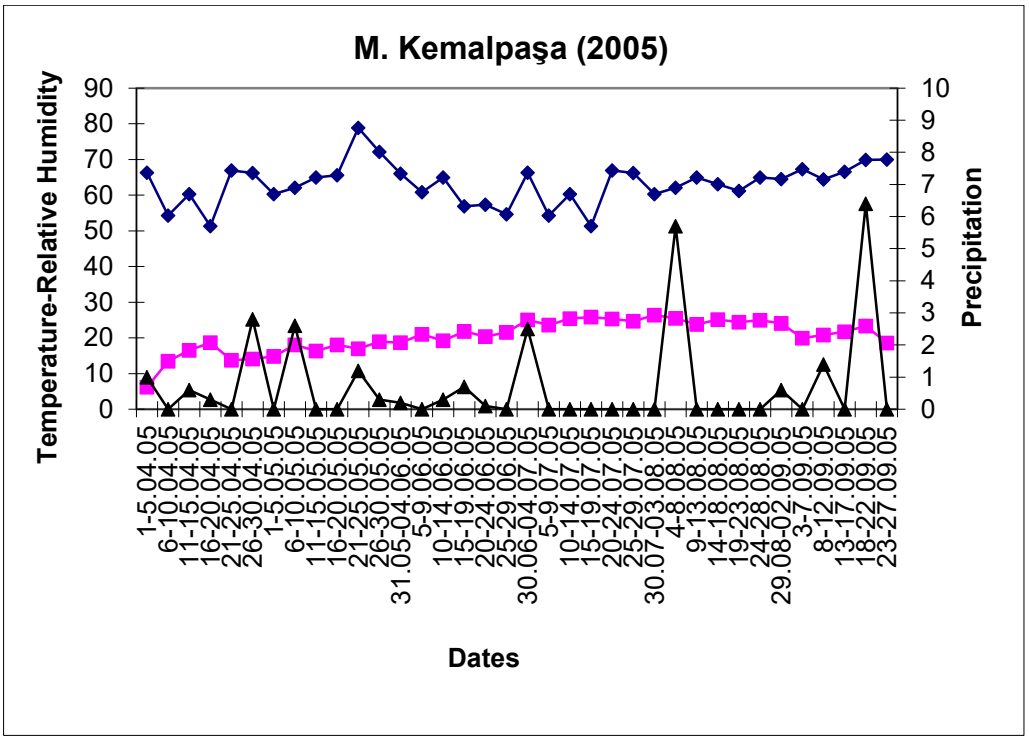

Figure 5. Climate data for 2005 in M. Kemalpaşa, Karacabey and Susurluk districts

Larva Population Density of *C. suturalis* in Balıkesir Province Susurluk, Manyas and Gönen Districts in 2005

The onion field in Söve village of Susurluk district was controlled by sampling method 8 times in 2005 and the population density of *C. suturalis* was followed. The first emergence of the larvae was seen on April 17, the number of larvae determined after this date continued to increase and a peak was formed with 2.6 larvae/plant on May 20. Afterwards, the number of larvae decreased and ended on 20 June (Figure 6). Pentate precipitation amount was 0 mm and relative humidity was 65.6% in Susurluk on May 16-20 in 2005. In addition, the pentate temperature on the same dates was 18 ºC (Figure 5).

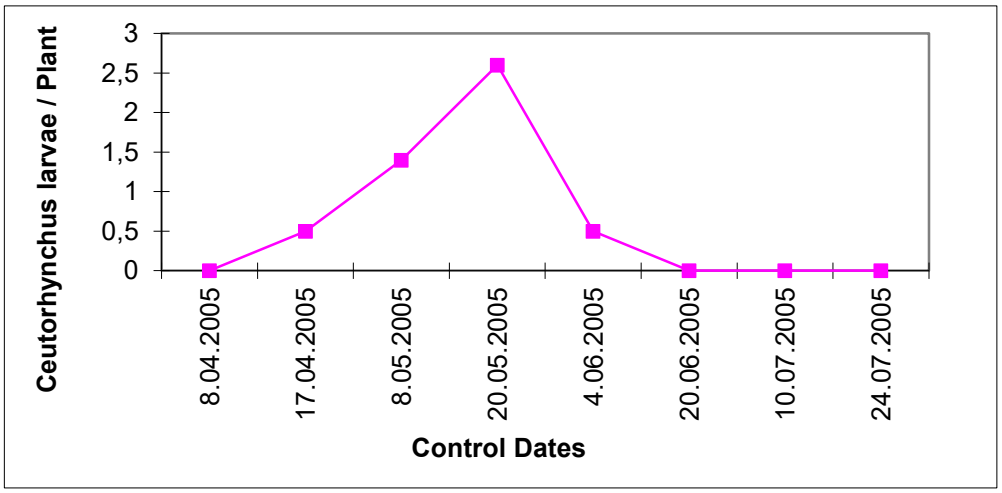

Figure 6. Larval population density of *C. suturalis* in Söve village of Susurluk district in 2005

Onion field in Boğazpınar village of Manyas district was sampled 12 times in 2005 and population density was followed. The first hatching of *C. suturalis* larvae was observed on 17 April, after this date the number of larvae detected increased and a peak was formed with 3.6 larvae/plant on 1 May. After this date, the number of larvae detected for the first time started to decrease and it is thought that the reason for this was the rainfall (3mm) that occurred on May 6-11. Then it started to increase again and on May 15, a second peak was formed with 3 larvae/plant. Afterwards, the number of larvae continued to decrease and ended on 5 June (Figure 7). Pentate precipitation amounts on May 1-5 and May 11-15 in 2005 in Manyas were determined as 1.8 mm, 0 mm and relative humidity as 76% and 79.6%, respectively. In addition, the pentate temperatures on the same dates were 14.4 and 16.4 ºC, respectively (Figure 9).

Onion field in Tuzakçı village of Gönen district was controlled 9 times in 2005 by sampling method, and the first emergence of larvae was seen on 17 April. After this date, the number of determined larvae continued to increase and a peak was formed with 1.7 larvae/plant on 22 May. Later, the number of larvae decreased and ended on 29 June (Figure 8). The pentate precipitation amount was 1.5 mm and the relative humidity was 88.9% in Gönen on May 21-25 in 2005. In addition, the pentate temperature on the same dates is 17.6 ºC (Figure 9).

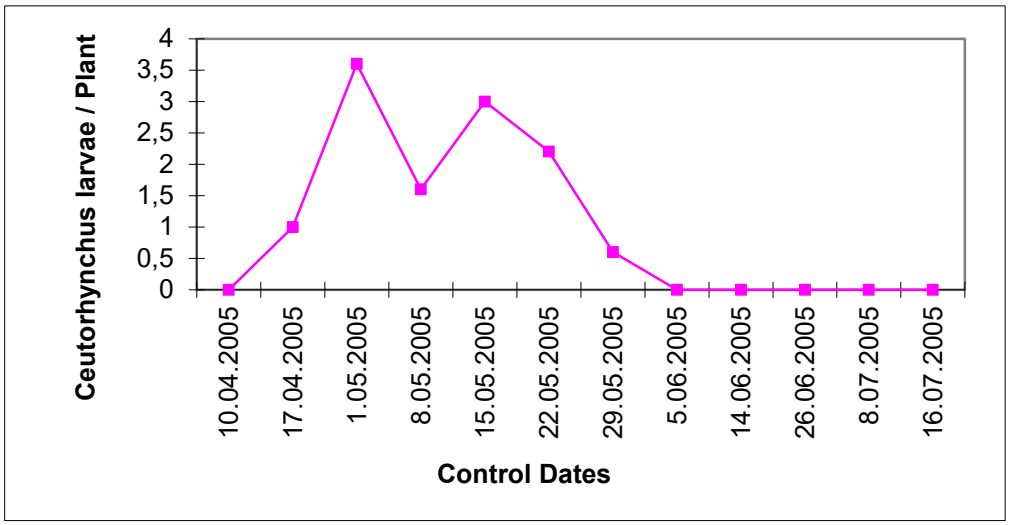

Figure 7. Larval population density of *C. suturalis* in Boğazpınar village of Manyas district in 2005

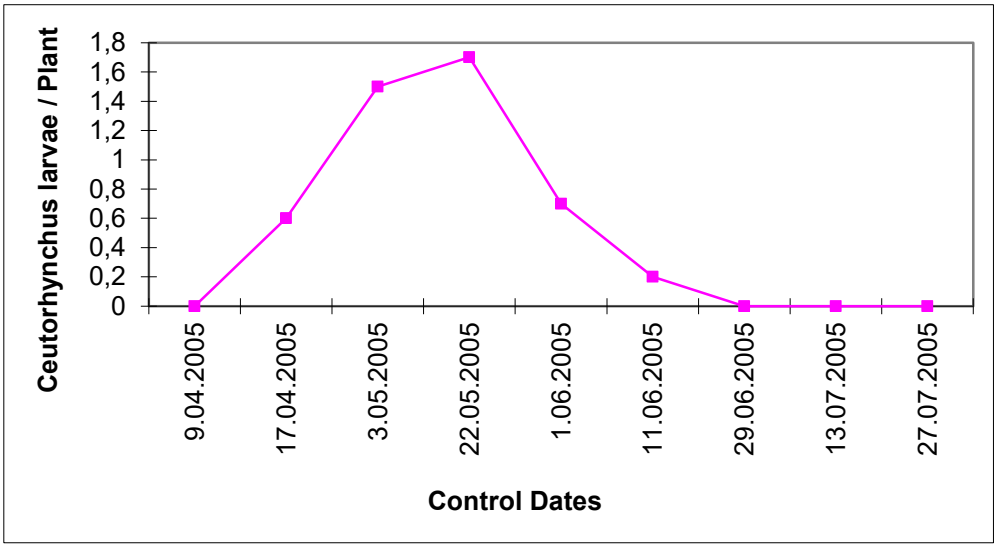

Figure 8. Larval population density of *C. suturalis* in Tuzakçı village of Gönen district in 2005

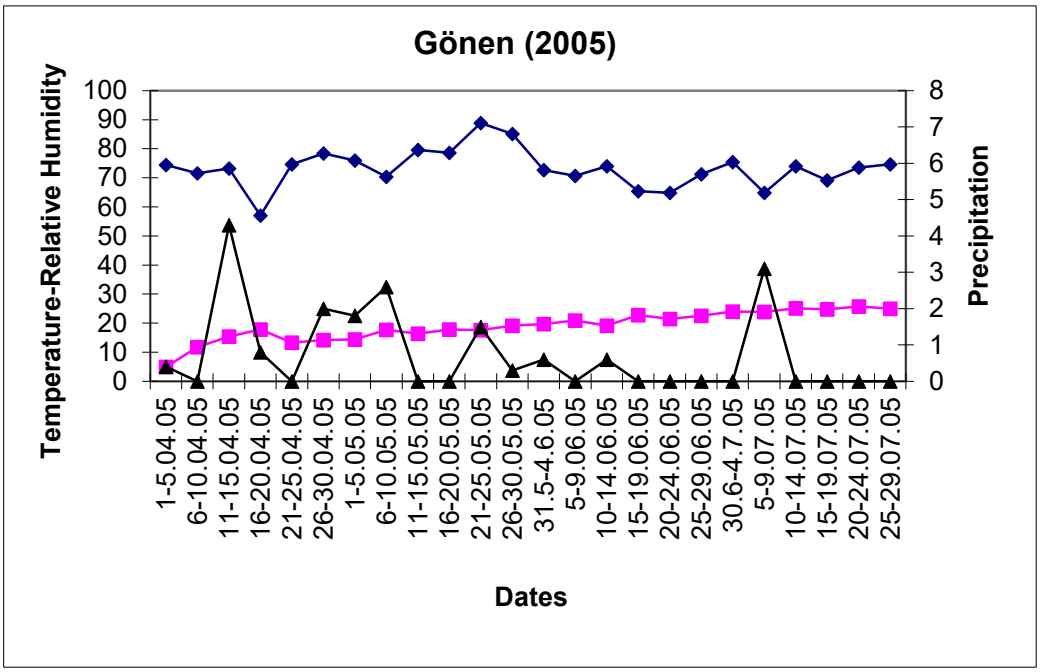

Figure 9. Climate data for 2005 in Gönen and Manyas districts

CONCLUSIONS

When the larval population density of C. suturalis was examined, it was determined that the pest was detected once a year in Karacabey, Yenişehir, Manyas, Gönen and Susurluk districts. Although these results need to be confirmed by biological studies, considering that each peak corresponds to one generation, it can be accepted that C. suturalis gives one generation per year in onions. According to the information given by EPPO, the pest gives 1 generation per year (20). It has been determined that the pest emerges in April in all regions where the pest has been detected, the air temperature is above 10ºC at the time the first larvae are seen, the population peaks in the second half of May and the air temperature is about 17ºC during these dates.

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THE EFFECTS OF ADDITIONS ORGANIC ACIDS AND SACCHAROSE TO DRINKING WATER BEFORE WITHDRAWAL ON MEAT AND LIVER pH, COLOR VALUES, AND NUMBER OF MICROORGANISMS

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ABSTRACT

This study aimed to determine the effects of adding organic acids and saccharose to the drinking water of broiler chickens on the number of small intestine coliform bacteria, and meat and liver color during the pre-slaughter feed withdrawal period. Broilers were housed individually and divided into four groups before slaughter. All groups provided drinking water ad libitum during the 10-hour feed withdrawal period before slaughter. The experimental groups were 7.5 pH drinking water (control group), 7.5 pH drinking water and saccharose (1%), pH 4.0 drinking water and organic acids addition, and pH 4.0 drinking water and saccharose (1%) addition. Organic acids used a mixture of formic acid and citric acid, while granulated sugar was used as a source of saccharose. The measurement was carried out at the time of slaughter and 24 hours after slaughter. The addition of organic acids and saccharose to the drinking water increased the breast meat b*, thigh meat L*, and b* values and decreased the pH values of thigh and liver.At 24 hours after slaughter, the addition of organic acids and saccharose increased the L* and b* color values of the liver. The total number of coliform bacteria in the small intestine was significantly affected by the addition of a combination of organic acids and saccharose (pH 4.0 and 1% saccharose).

Keywords: Broiler, wıthdrawal perıod, meat and liver color, pH, organic acid, sucrose

INTRODUCTION

For the supply of quality and healthy products to the consumer, a pre-slaughter feed withdrawal period is applied to prevent microbial contamination. Thus, the digestive tract is cleaned of residues and microbial contamination originating from crops and intestines is prevented (Northcutt, 2010). Businesses use water acidifiers 72 hours in advance as standard practice during this feed withdrawal period. Lactic acid, fumaric acid, citric acid, propionic acid, butyric acid, formic acid, acetic acid, and sorbic acid are the most commonly used organic acids for this purpose. Acidifiers are generally used in combinations of two or more, rather than alone (Dhama et al., 2014; Krisham and Narang, 2014). Pre-slaughter starvation ensures that the digestive tract is cleaned, minimizing contamination during processing and reducing the amount of undigested feed. Carcass contamination is increased due to the emergence of more excrement during transport and pre-

slaughter holding, and crop and intestinal rupture/leakage during processing (Harris et al., 2019). On the other hand, prolongation of the fasting period may cause the body's reserve reserves to be spent and increase the possibility of intestinal rupture. As the crop empties during feed withdrawal, the development of lactic acid bacteria decreases, and the pH of the crop rises, and this increase increases pathogens such as Salmonella. Most pathogenic bacteria are Gram-negative and sensitive to acidic environments with a bacteriostatic effect. At pHs below 5, many pathogens remain stable, and when the pH rises above 5, pathogenic bacteria begin to multiply. Birds can tolerate pH levels of 4-8 in drinking water and it is important to acidify the drinking water in the right amount to achieve the ideal level of 4-4.5pH. Studies have proven that pathogenic bacteria such as Salmonella, Campylobacter and Clostridium show minimal growth at this pH (Byrd et al., 2001; Biomin, 2022). Lactic or formic acid (0.5%) reduces salmonella in the crop (Bryd et al. 2001), adding citric acid (especially 4.5 and 6% levels) to drinking water for 8 hours in pre-slaughter feed withdrawal reduces the pH of gizzard and cecum content and reduces fecal contamination.

In this study, the effects of organic acids and sucrose addition on the color and pH values of carcass meat and liver and the number of small intestine microorganisms will be emphasized.

MATERIAL AND METHODS

This study was carried out at Ondokuz Mayis University, Faculty of Agriculture, Animal Husbandry Unit. Forty-eight mixed-sex ROSS 308 broiler chickens were used. In addition to the mixed genders within the groups, the body weight distributions were kept uniform. Formic acid (CH_2O_2) and citric acid (lemon salt, $C_6H_8O_7$) combinations and sucrose (granulated sugar, $C_{12}H_{22}O_{11}$) combinations were used for drinking water additions in the study.

When the broilers completed the 42nd day, a 10-hour fasting period was started at 11:30 p.m. The animals were divided into four groups before slaughter. Water was given ad libitum to all groups, with and without treatment, during the 10-hour feed withdrawal period before slaughter. Experimental groups, 7.5 pH water (control group), 7.5 pH and 1% sucrose added water, 4.0 pH and organic acid water, and organic acid (pH 4.0) and 1% sucrose mixture added quenched groups.

The pH of the drinking water is determined by the automatic pH meter available, and the acidification of the water is determined by adding organic acid to the pH. It is set to 4.0. The amount of water needed was acidified by estimating the water consumption according to the feed consumption amount of the animals. 10g/L sucrose (tea sugar produced from sugar beet) was added to the same water. The analyzes of the samples taken from the small intestine were made in the Poultry Laboratory of Samsun Veterinary Control and Research Institute. The samples value was diluted with FTS (physiological saline) solution and the 7th dilution was read.

RESULTS and DISCUSSION

The color (L*, a*, b*) values of the breast, thigh, and liver obtained at the end of the experiment are shown in Table 1, pH data in Table 2, and the total number of coliform bacteria in the small intestine are shown in Table 3. Breast, thigh, and liver color (L*, a*, b*) values obtained 24 hours after slaughter are shown in Table 4 and pH data are shown in Table 5.

Table 1. Breast, thigh, and liver color values (L*, a*, b*)

		C*	OA 4.0	SW7.5	OAS 4.0	SEM	P-value
	L*	54.92	52.33	56.16	55.87	0.666	0.160
Breast	a*	1.7	1.49	1.29	1.49	0.148	0.830
	b*	3.75[b]	2.59[a]	2.34[a]	2.52[a]	0.188	0.024
	L*	59.25[a]	62.52[b]	62.70[b]	62.53[b]	0.470	0.016
Thigh	a*	4.04	3.17	3.12	3.03	0.204	0.271
	b*	5.14[b]	3.92[ab]	2.52[a]	3.49[a]	0.285	0.007
	L*	35.20[a]	38.73[b]	39.04[b]	40.05[b]	0.609	0.022
Liver	a*	16.33	17.61	17.29	16.79	0.226	0.202
	b*	6.19[a]	7.41[ab]	7.89[ab]	8.62[b]	0.359	0.011

* Control (Water pH 7.5), OA 4.0 (organic acids and pH 4.0), SW 7.5 (sucrose1% and water pH 7.5), OAS 4, 0 (organic acids and sucrose 1%, and water pH 4.0). a b Values within a column with different letters differ significantly. SEM: Standard error of the means

When Table 1 is examined, the addition of organic acids and sucrose decreased the b* value in breast meat. Leg meat and liver L* (lightness) values were found to be lighter in the experimental groups than in the control group. Accordingly, Karaçay et al., (2007) are in line with their study. Karacay et al. reported that the addition of sucrose to the drinking water of broiler chickens increased the brightness of liver and breast meat, and lighter-colored thigh meat was obtained.

Table 2. Breast, thigh, liver, and small intestine pH values at the slaughter

	C*	OA 4.0	SW7.5	OAS 4.0	SEM	P-value
Breast	6.65	6.59	6.56	6.56	0.025	0.577
Thigh	6.49[b]	6.31[a]	6.37[a]	6.30[a]	0.025	0.016
Liver	6.33[b]	6.17[a]	6.16[a]	6.15[a]	0.025	0.025
Small intestine	6.34	6.26	6.41	6.33	0.032	0.435

* Control (Water pH 7.5), OA 4.0 (organic acids and pH 4.0), SW 7.5 (sucrose1% and water pH 7.5), OAS 4, 0 (organic acids and sucrose 1%, and water pH 4.0). a b Values within a column with different letters differ significantly. SEM: Standard error of the means

At the end of the experiment, breast, thigh, liver, and small intestine pH measurements were made and the results are shown in Table 2. It was observed that the pH values of the thigh and liver were lower in the experimental groups. It was understood that as the pH of the thigh meat decreases, the L* (lightness) value increases, and as the pH increases, the L* (lightness) value decreases and shows darker tones. Von Lengerken et al. (2002) reported that a high L* value will result in the appearance of PSE meat (pale, tender, and juicy). The data obtained in the study showed that the meats were in the normal class, as the PSE meat pH was not as low (pH<5.8).

Table 3. Small intestine total coliform bacteria count(log10cfu)

	C*	OA 4.0	SW7.5	OAS 4.0	SEM	P-value
Small intestine	7,06[b]	7,12[b]	7,07[b]	6,62[a]	0.055	0,001

* Control (Water pH 7.5), OA 4.0 (organic acids and pH 4.0), SW 7.5 (sucrose1% and water pH 7.5), OAS 4, 0 (organic acids and sucrose 1%, and water pH 4.0). a b Values within a column with different letters differ significantly. SEM: Standard error of the means

The combination of organic acids and the addition of sucrose significantly reduced the total number of small intestinal coliform bacteria (Table 3). This result by Alzawgari et al. (2013) is compatible with their work. Alzawqari et al. (2013) reported that the addition of acetic acid at different levels (1.5, 3, 4.5, and 6%) to the water in the pre-slaughter period (8 hours) significantly inhibited the colony of Bacillus, Clostridium, Coliform and facultative aerobic microorganisms in the gizzard, caecum, and feces of male chickens.

Table 4. Breast, thigh, and liver color values 24 hours after slaughter

		W 7,5	OA 4.0	SW 7.5	OAS 4.0	SEM	P-value
Breast	L*	60.96	59.97	61.49	60.83	0.391	0.581
	a*	1.97	1.78	1.82	2.24	0.157	0.719
	b*	6.07	6.35	6.73	6.83	0.227	0.620
Thigh	L*	62.60	62.66	62.45	62.86	0.375	0.990
	a*	3.68	3.53	3.46	3.84	0.139	0.765
	b*	4.60	4.83	4.45	5.59	0.171	0.152
Liver	L*	35.62^{a}	40.03^{b}	39.83^{b}	40.31^{b}	0.735	0.053
	a*	18.38	18.37	18.92	19.72	0.307	0.350
	b*	8.72^{a}	11.25^{b}	11.01^{b}	11.65^{b}	0.433	0.051

* Control (Water pH 7.5), OA 4.0 (organic acids and pH 4.0), SW 7.5 (sucrose1% and water pH 7.5), OAS 4, 0 (organic acids and sucrose 1%, and water pH 4.0). a b Values within a column with different letters differ significantly. SEM: Standard error of the means

Color measurements of breast, thigh, and liver after 24 hours are presented in Table 4. It was understood that the effects of organic acids and sucrose additions on liver L* (clearance) values continued. It was observed that organic acids and sucrose additions did not affect breast, thigh, and liver pH values 24 hours after slaughter (Table 5).

Table 5. Breast, thigh, and liver pH values 24 hours after slaughter

	W 7,5	OA 4.0	SW 7.5	OAS 4.0	SEM	P-value
Breast	5.94	3.88	5.92	5.83	0.017	0.089
Thigh	6.18	6.14	6.22	6.13	0.018	0.278
Liver	6.27	6.17	6.08	6.15	0.029	0.114

* Control (Water pH 7.5), OA 4.0 (organic acids and pH 4.0), SW 7.5 (sucrose1% and water pH 7.5), OAS 4, 0 (organic acids and sucrose 1%, and water pH 4.0). a b Values within a column with different letters differ significantly. SEM: Standard error of the means

CONCLUSION

As a result of the experiment, the addition of organic acids and sucrose to the drinking water of broiler chickens increased breast meat b* value, thigh meat L* and b* values, and decreased thigh and liver pH. In the data examined 24 hours after slaughter, it was determined that the addition of organic acids and sucrose increased the liver L* and b* color values. The addition of a mixture of organic acids and sucrose (pH 4.0 and 1% sucrose) significantly reduced the number of coliform bacteria in the small intestine. In this context, it can be argued that the co-administration of organic acids and sucrose gives positive results.

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THE EFFECTS OF ORGANIC ACIDS AND SACCHAROSE ADDITION TO THE DRINKING WATER OF BROILERS DURING THE PRE-SLAUGHTER WITHDRAWAL PERIOD ON THE WEIGHT OF CARCASS, HEARTH, LIVER, GIZZARD, AND SMALL INTESTINE

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ABSTRACT

This study aimed to determine the effects of adding organic acids and sucrose to the drinking water of broiler chickens on weight loss, carcass yield, heart, liver, gizzard, and small intestine weight during the pre-slaughter feed withdrawal period. Broilers housed individually were divided into four groups before slaughter. Water was given ad libitum to all groups, with and without the treatments, during the 10-hour feed withdrawal period before slaughter. The experimental groups consisted of 7.5 pH water (control group), 7.5 pH and 1% sucrose added group, 4.0 pH water and organic acids added group, and organic acids (4.0 pH water) and 1% sucrose mixture. Formic acid and citric acid mixture were used as organic acids, and granulated sugar was used for sucrose addition. Analyzes were made twice, firstly at the time of slaughter and 24 hours after slaughter. Adding organic acids and sucrose increased the gizzard weight and the ratio of gizzard to pre-slaughter live weight compared to the control group. Consequently, adding organic acids and sucrose to the drinking water of broiler chickens increased the gizzard weight and percentage (compared to the pre-slaughter period) without affecting the body weight and carcass weight loss and the weight of heart, liver, and small intestine.

Keywords: Formic acid, citric acid, sucrose, broiler, feed withdrawel, performance, edible inner organs, small intestine

INTRODUCTION

To supply healthy products to the consumer, companies apply feed withdrawal to broilers before slaughter to prevent microbial contamination. Thus, the digestive tract is cleaned of residues and microbial originating from crops and the intestine, preventing contamination (Northcutt, 2010). Many companies use water acidifiers starting 72 hours before feed withdrawal to acidify the broiler crop and reduce the bacterial population. Lactic acid, fumaric acid, citric acid, propionic acid, butyric acid, formic acid, acetic acid, and sorbic acid are the most commonly used organic acids for this purpose. Acidifiers are often used in combinations of two or more rather than alone (Dhama et al., 2014; Krisham & Narang, 2014). Pre-slaughter starvation ensures that the digestive tract is cleaned, minimizing contamination during processing and reducing the amount of undigested feed. Carcass contamination is increased due to more excrement during transport, pre-slaughter waiting, and crop and intestinal rupture/leakage during processing (Harris et al., 2019). It has been reported that 8-12 hours of fasting is appropriate to obtain contamination and maximum carcass yield, meat quality, and edible visceral weight (Bilgili, 2002; Ocak et al., 2005).On the other hand, prolonging

the fasting period may cause the body's reserve reserves to be spent and increase the possibility of intestinal rupture. Also, the crop empties during feed withdrawal, the development of lactic acid bacteria decreases, and the crop's pH rises, which increases pathogens such as Salmonella. Most pathogenic bacteria are Gram-negative and sensitive to acidic environments with a bacteriostatic effect. If pH is below 5, many pathogens remain stable, and when pH rises above 5, pathogenic bacteria begin to multiply. Birds can tolerate pH levels of 4-8 in drinking water, and 4-4.5 is ideal. Acidifying the drinking water correctly is crucial to reaching the pH level. It proved that pathogenic bacteria such as Salmonella, Campylobacter, and Clostridium show minimal growth at this pH level (Byrd et al., 2001; Biomin, 2022). Also, it is claimed that drinking water acidification is one method to eliminate these adverse effects (Harris et al., 2019).

In this study, we will focus on the loss of carcass, thigh and breast meat, and edible visceral weights caused by feed withdrawal, and to what extent the small intestine total coliform bacteria count can be improved with organic acids and sucrose additions.

MATERIAL AND METHOD

This study was carried out at Ondokuz Mayis University, Faculty of Agriculture, Animal Husbandry Unit. Forty-eight mixed-sex ROSS 308 broiler chickens were used. In addition to the mixed genders within the groups, the body weight distributions were kept uniform. Formic acid (CH_2O_2) and citric acid (lemon salt, $C_6H_8O_7$) combinations and sucrose (granulated sugar, $C_{12}H_{22}O_{11}$) combinations were used for drinking water additions in the study.

When the broilers completed the 42nd day, a 10-hour fasting period was started at 11:30 p.m. The animals were divided into four groups before slaughter. Water was given ad libitum to all groups, with and without treatment, during the 10-hour feed withdrawal period before slaughter. Experimental groups, 7.5 pH water (control group), 7.5 pH and 1% sucrose added water, 4.0 pH and organic acid water, and organic acid (pH 4.0) and 1% sucrose mixture added quenched groups.

The pH of the drinking water is determined by the automatic pH meter available, and the acidification of the water is determined by adding organic acid to the pH. It is set to 4.0. The amount of water needed was acidified by estimating the water consumption according to the feed consumption amount of the animals. 10g/L sucrose (tea sugar produced from sugar beet) was added to the same water. The analyzes of the samples taken from the small intestine were made in the Poultry Laboratory of Samsun Veterinary Control and Research Institute. The samples value was diluted with FTS (physiological saline) solution and the 7th dilution was read.

RESULTS and DISCUSSION

At the end of the trial data on weighing and analysis of the pre-slaughter period and 24 hours after slaughter are shown in Tables 1 and 2, and the results of the small intestine total coliform bacteria count are shown in Table 3.

Table 1 is examined the body weights, slaughter weight, carcass, liver, and heart weight parameters when taken into the experimental groups that were not affected by the addition of organic acids and sucrose. While this result is consistent with the study of Kop- Bozbay and Ocak (2014), Menconi et al. (2014) and Farhat et al. (2002) are inconsistent with his work. Kop - Bozbay and Ocak (2014) reported that the addition of carbohydrates (glucose, sucrose, and starch) during the pre-slaughter feed withdrawal period had no effect on the decrease in body weight loss and the increase in meat quality. Menconi et al. (2014) reported that the addition of an organic acid mixture (lactic, acetic, tannic, propionic, and caprylic acids) to drinking water during the pre-slaughter feed

withdrawal period generally reduced body weight loss in broilers. Farhat et al. (2002) reported that the addition of energy product maltodextrin and energy + protein (maltodextrin + dehydrated egg white) to chickens in the pre-slaughter feed withdrawal period significantly reduced body weight loss (g and %) and increased carcass yield significantly.

Table 1. Live weight loss, carcass yield, the weight of edible inner organs, and weight and length of small intestine

	C*	OA 4.0	SW 7.5	OAS 4.0	SEM	P-value
Live weight[1]	3185	3146	3144	3171	28.052	0.952
Slaughter live weight[1]	3165	3137	3111	3193	33.314	0.850
Live weight loss[1]	-20	-9.25	-33.25	22.75	10.194	0.255
Live weight loss[2]	-0.63	-0.30	-1.19	-0.71	0.342	0.259
Carcass weight[1]	2563	2519	2504	2577	27.786	0.781
Carcass yield[2]	81.00	80.30	80.50	80.68	0.002	0.641
Abdominal fat[1]	44.44	42.26	39.66	41.26	2.122	0.892
Abdominal fat[2]	1.4	1.4	1.3	1.3	0.068	0.955
Liver weight[1]	57.46	65.51	64.20	62.74	1.319	0.143
Liver weight[2]	1.82	2.09	2.08	1.96	0.044	0.087
Heart weight[1]	14.34	12.76	13.83	14.89	0.413	0.318
Heart weight[2]	0.45	0.41	0.44	0.47	0.011	0.265
Gizzard weight[1]	29.81[a]	33.08[ab]	35.19[b]	36.63[b]	0.922	0.043
Gizzard weight[2]	0.94[a]	1.06[ab]	1.14[b]	1.14[b]	0.028	0.032
Small intestine weight[1]	113.7	112.1	112.4	115.1	3.589	0.992
Small intestine weight[2]	3.59	3.56	3.60	3.61	0.100	0.999
Small intestine length[1]	192.4	186.6	199.0	192.9	2.956	0.553
Small intestine length[2]	6.08	5.96	6.41	6.04	0,086	0.280

* Control grup, [1]Grams,[2] Percent every 100g of live weight, 7.5 (Water pH 7.5), OA 4.0 (organic acids and water pH 4.0), SW 7.5 (sucrose (1%) and water pH 7.5), OAS 4, 0 (organic acids and sucrose (1%), and water pH 4.0). SEM: Standard error of the means, [a,b] Values within a column with different letters differ significantly

In our study, no adverse effects were found in carcass, breast, and thigh weights with the addition of organic acids and sucrose. However, the gizzard weight and proportional value are compatible with the study of Kayan and Açıkgöz (2020). Kayan and Açıkgöz (2020) reported that adding organic acid to the water in different pre-slaughter fasting periods in male chickens adversely affected carcass, breast and thigh weights, carcass, and breast yields but increased the gizzard proportional value and serum glucose level.

According to the values 24 hours after slaughter, carcass, heart, liver and gizzard weights were not affected by the addition of organic acids and sucrose (Table 2). With the combination of

organic acids and sucrose supplementation, the number of coliform bacteria in the small intestine was significantly reduced (P<0.001, Table 3).

Table 2. Weighs of carcass, heart, liver, and gizzard 24 hours after slaughter (g)

	C*	OA 4.0	SW 7.5	OAS 4.0	SEM	P-value
Carcass	2555.36	2520.57	2491.28	2606.33	27.94	0.605
Heart	13.14	12.32	13.15	14.30	0.386	0.380
Liver	55.63	62.12	61.63	61.85	1.290	0.235
Gizzard	30.32	32.37	34.03	35.73	0.904	0.153

* Control grup (water pH 7.5), OA 4.0 (organic acids and water pH 4.0), SW 7.5 (sucrose (1%) and water pH 7.5), OAS 4, 0 (organic acids and sucrose (1%), and water pH 4.0). SEM: Standard error of the means

Table 3. Small intestine total coliform bacteria count (log10cfu)

	C*	OA 4.0	SW 7.5	OAS 4.0	SEM	P-value
Small intestine	$7,06^{b}$	$7,12^{b}$	$7,07^{b}$	$6,62^{a}$	0.055	0,001

* Control grup (water pH 7.5), OA 4.0 (organic acids and water pH 4.0), SW 7.5 (sucrose (1%) and water pH 7.5), OAS 4, 0 (organic acids and sucrose (1%), and water pH 4.0). ^{a,b} Values within a column with different letters differ significantly, SEM: Standard error of the means

CONCLUSION

It was determined that adding organic acids and sucrose to the drinking water of broiler chickens before slaughter did not affect the loss of body weight and carcass weight, heart, liver, and small intestine weight but increased the percentage of gizzard weight and body weight. The combined administration of organic acids and sucrose addition significantly reduced the number of coliform bacteria. In this context, it has been seen in our research that the combination of organic acids and sucrose addition will yield positive results.

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THE USE OF THE NITRIFICATION INHIBITOR 3, 4 DIMETHYLPYRAZOLE PHOSPHATE (DMPP) ON PHYSIOLOGIC CHARACTERS IN BEAN (Phaseolus vulgaris L.)

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ABSTRACT

The experiments were established during the 2017 and 2018 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey. The experiment was designed as factorial arrangement in the complete randomized block design with four replications. In this study, rhizobia and non-rhizobia were investigated at different nitrogen fertilizer types and doses (control, 25 kg ha^{-1} AS, 25 kg ha^{-1} DMPP, 50 kg ha^{-1} AS and 50 kg ha^{-1} DMPP). The effects of year, rhizobia and nitrogen fertilization were significantly for all of the investigated characters. All of the investigated characters were higher in the 2017 than the 2018 except for the canopy temperature. Rhizobia application and 50 kg ha^{-1} DMPP were positively affected all investigated characters. The most likely reason for the increase in crop yield was that N loss through soil NH_3 volatilization was not increased by DMPP application. The benefits for the nitrification inhibitors are increases in yields and reduced fertilizer use.

Key Words: Bean, DMPP, physiological properties

INTRODUCTION

The world population is rapidly increasing, and world food production needs to be increased due to human consumption (Ohyama 2017). We are required to increase crop production per area because the growth in the agricultural region is difficult. (Hatono et al. 2019). Nitrogen fertilizer was important in protecting plant health, producing high-quality plants, and obtaining high crop yields in agriculture (Li et al. 2019). There is increasing anxiety in the world about the large nitrogen losses due to excessive organic and synthetic fertilizer use. When viewed environmentally, ammonia (NH_3), nitrate (NO_3^-), and nitrous oxide (N_2O) gaseous emissions are injirious N contaminants. They may lose from agroecosystems and also contribute to lower nitrogen use efficiency (NUE) (Sutton et al. 2011). Besides the economic loss due to this unproductive process, the N infiltration into the environment is a serious reason for contamination causative water eutrophication. It is important to contribute to climate change because of the emission of greenhouse gases such as N_2O (Huérfano et al. 2016).

N losses to the agricultural systems were reduced that the use of nitrification inhibitors in the world (Chen et al. 2010; Luo et al. 2016; Li et al. 2016). Microbial activity such as nitrification, denitrification, and nitrifier denitrification was the main source of N_2O loss in agricultural systems (Balafoutis et al. 2017). Nitrification inhibitors can very effective at immobilization microbial nitrification and denitrification. Because they can delay the oxidation of NH_4 to nitrite (NO_2^-) for several weeks or months. (Weiske et al. 2001; Zerulla et al. 2001). Therefore, nitrification inhibitors have become an increasingly important strategy in recent years to help reduce N_2O emissions (Simith et al. 2015). Many research have shown that nitrification inhibitors reduced N_2O emissions by a mean of 30–45 %. (Akiyama et al. 2010; Gilsanz et al. 2016; Thapa et al. 2016).

3,4-dimethylpyrazole phosphate (DMPP) is one of the most common NIs. It is inhibiting the activity of the ammonia monooxygenase enzyme and therefore delays the bacterial oxidation of ammonium (NH_4) (Ruser and Sculz 2015). DMPP may also reduce N loss through denitrification by potentially inhibiting denitrifying enzyme activity and reducing the accumulation of NO_3N (Weiske et al. 2001; Zhu et al. 2005).

The use of biological nitrogen fixation in the form of rhizobia inoculants in legumes can be an alternative to nitrogenous fertilizer. Most legume crops can use symbiosis methods to fix atmospheric nitrogen (N_2). For example rhizobia is a soil microorganisms and it is fix atmospheric nitrogen. The most important N source in agriculture is still legume-rhizobia symbiosis (Ohyama 2017). Bean, like other legumes develops soil fertility by fixing atmospheric nitrogen through symbiosis and makes nitrogen (N) available to plants. Inoculation with rhizobia is increasing N uptake by plants. Thus it reduces the use of nitrogen fertilizer. (Choudhury and Kennedy 2004; Kennedy et al. 2004; Mia et al. 2005). Biological N_2 fixation can play an important role in replacements for commercially nitrogen fertilizers. Also they can reduce the environmental problem to some extent. Sustainable agriculture is important in the world. Nitrogen fixation and plant growth enhancement by rhizosphere bacteria might be helping achieving sustainable agriculture in the future (Baset Mia and Shamsuddin 2010).

Akiyama et al. (2010) reported that nitrification inhibitors were efficient in reducing N_2O emissions from nitrogen fertilizers. The aim of this study is to investigate of the effects of rhizobia and different nitrogen fertilizer types and doses on yield and some physiological parameters for the bean.

MATERIAL AND METHOD

The experiments were established during the 2017 and 2018 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey (39º48' N; 30º31' E, 798 m above sea level). Climatic data for experimental years and long term are submitted in Figure 1. Long-term annual total precipitation is 104.1 mm. Experimental years were 143.4 and 170.2 mm, respectively. The mean temperature was 19.64 ºC in 2017 and 20.1 ºC in 2018. The soil of the experimental area was organic matter content of 1.44%, with lime at 2.50% and pH of 7.61. Corresponding available P_2O_5, K_2O and N contents were 108.9 kg ha^{-1},1944.6 kg ha^{-1} and 0.07% in the first year, respectively. In the second year, it was organic matter at 1.65%, with lime at 7.56%, pH of 7.71, available P_2O_5 177.5 kg ha^{-1} K_2O 2450.0 kg ha^{-1} and N 0.08%.

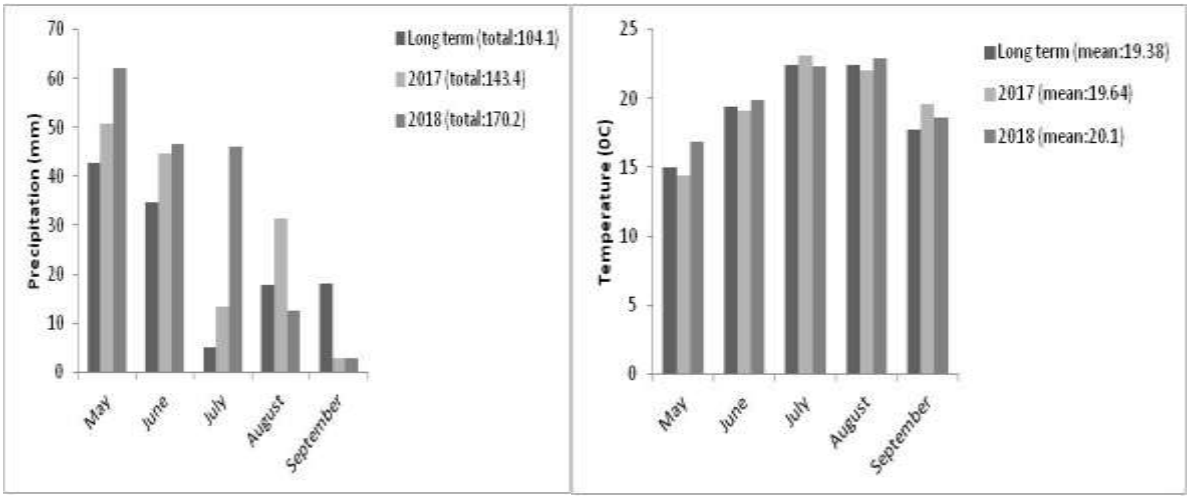

Figure 1. Climatic data of the research

The experiment was designed as factorial arrangement in the complete randomized block design with four replications. In this study, rhizobia and non-rhizobia were investigated at different nitrogen fertilizer types and doses (control, 25 kg ha^{-1} AS, 25 kg ha^{-1} DMPP, 50 kg ha^{-1} AS and 50 kg ha^{-1} DMPP). Bean varieties Göynük-98 was used as research material. Each plot was 7.2 m^2 (4 m x 1.8 m) and bean was sown 45 cm row spacing and seeding rate was 26 seeds m^{-2}. The sowing time was 03 May and 04 May in 2017 and 2018, respectively. All of the nitrogen fertilizers were applied at sowing time. Seeds were inoculated with *Rhizobium endophyticum* (formed colonies at 10^{-8} level) bacteria at the recommended rate (100 kg seed to 1 kg peat inoculant) before sowing in rhizobia plots. Application of the peat inoculant on the seeds was carried out by water, which contains 2% sugar. Peat inoculation was provided by the Soil, Fertilizer and Water Central Research Institute. It was kept in refrigerator at + 4ºC until use**.** 60 kg ha^{-1} TSP (triple super phosphate (43-44 P_2O_5 %) was given to each plot at the sowing as basal fertilization. The harvest time of chickpea was on 13 September 2017 and 27 August 2018 in the first and second year, respectively. The leaf area was measured with a portable area meter Sunscan in the middle two rows. Normalized difference vegetation index and canopy temperature were measured in the middle of each plot by a hand-held optical sensor with Greenskeer and Mastech GM-320, respectively. Chlorophyll amounts were measured on 5 randomly selected plants in each plot with Spad 502 Plus chlorophyll meter. Each plot was harvested, blended and biological yield (kg ha^{-1}) and seed yield (kg ha^{-1}) were estimated.

The variance analysis was subjected based on General Linear Model using the Statview package (SAS Institute). Means were compared by Least Significant Differences (LSD) test.

RESULTS AND DISCUSSION

The effects of year, rhizobia and nitrogen fertilization were significantly for all of the investigated characters (Table 1). While all of the values showed superior performance for leaf area index, normalized difference vegetation index, chlorophyll amount, biological yield and grain yield in first year, the values were lower in second year. For this reason, year x rhizobia x nitrogen fertilization interaction was significantly (Figure 2A, 2B, 4A, 4B, 5A). Canopy temperature showed superior performance in second year but the values were lower at first year. For this reason, year x nitrogen fertilization interaction was significantly (Figure 3A). While canopy temperature showed superior performance in Rh^+, the values were lower at Rh^-. For this reason, rhizobia x nitrogen fertilization interaction was significantly (Figure 3B).

All of the investigated characters were higher in the 2017 than the 2018 except for the canopy temperature (Table 1). Leaf area index was 2.55 at first year but it was 1.99 at second year (Table 1). The flowering occurs at June in our research and there was a very low temperature for June in first experimental year (Figure 1). Maximum leaf area index (LAI) is formed before flowering (Müjdeci et al. 2005). Öner and Sezer (2007) reported that leaf area increases with increasing light intensity at low temperatures. Therefore LAI might be higher in first experimental year. There is a positive significant relationship between LAI and grain yield (Müjdeci et al. 2005; Kızılgeçi et al. 2016). The estimation of leaf area index and fraction of photosynthetically active radiation absorbed by the vegetation from atmospherically corrected is defined normalized difference vegetation index (NDVI) (Myneni et al. 1997). While NDVI was 0.67 at first year, it was 0.59 at second year (Table 1). There is a significant positive correlation between NDVI and LAI (Kızılgeçi et al. 2016) and between NDVI and grain yield (Aparicio et al. 2000). Canopy temperature was 27.01 at first year but it was 27.55 at second year (Table 1). Low canopy temperature during the grain filling period is important to withstand high temperature stress (Munjal and Rana 2003). The temperature is higher in first experimental year but canopy temperature is lower (Figure 1, Table 1) This shows that the variety might be withstand high temperatures. Canopy temperature is negatively correlated with biological yield (Öztürk et al. 2017). High biological yield might be

reduced canopy temperature. Also grain yield might be affected positive. Kızılgeçi et al. (2016) reported that there is a negative significant relationship between canopy temperature and grain yield. High temperature stress reduces photosynthetic activity because of damage to chloroplasts and chlorophyll accumulation is decreases (Xu et al. 1995). The temperature was higher than long term and first experimental year in second experimental year. High temperature might be negative affected the chlorophyll amount in second year (Figure 1, Table 1). Biological yield and grain yield were lower due to total high temperature especially grain filled period in the second experimental year (Figure 1, Table 1). High temperatures is reduced the total leaf area and net assimilation amount and therefore plant growth is decreased (Rodríguez et al. 2005; Ashraf and Hafeez 2004). Warland et al. (2006) reported that grain yield was reduced when temparature is increased 1.5 ^{o}C.

Rhizobia application was positively affected all investigated characters (Table 1). While leaf area index was 1.76 at Rh^{-}, it was 2.77 at Rh^{+} (Table 1). Cytokinin may have caused the increase in LAI following rhizobia. Cytokinin is an important growth hormone. As a reason for leaf growth is its effect on cell division and cell expansion (Allen et al. 1981). Large leaf area is to keep large amount of solar radiation which indirectly increases the dry matter production (Shibles and Weber 1966). Therefore biological yield was higher at second year (Table 1). The large leaf area which high photosynthetic activity has a positively relationship with seed yield (Chandrababu 1990). Grain yield was higher at second year (Table 1).

Table 1. Effects of rhizobia and different nitrogen fertilization on some traits of bean

Treatments	LAI	NDVI	CT (^{o}C)	CA (spad)	BY (kg ha^{-1})	GY (kg ha^{-1})
2017	2.55 A	0.67 A	27.01 B	45.81 A	4687 A	1885 A
2018	1.99 B	0.59 B	27.55 A	43.25 B	3703 B	1425 B
Mean	2.27	0.63	27.28	44.53	4244	1655
Rh^{-}	1,76 B	0.57 B	26.39 B	42.78 B	3417 B	1312 B
Rh^{+}	2.77 A	0.69 A	28.17 A	46.29 A	5072 A	1999 A
Mean	2.27	0.63	27.28	44.53	4244	1655
Control	1.59 E	0.56 E	25.41 D	42.36 E	3025 E	1162 E
25 kg ha^{-1} AS	1.85 D	0.60 D	26.30 C	43.36 D	3694 D	1446 D
25 kg ha^{-1} DMPP	2.25 C	0.64 C	27.73 B	44.06 C	4392 C	1693 C
50 kg ha^{-1} AS	2.48 B	0.66 B	27.63 B	45.57 B	4547 B	1783 B
50 kg ha^{-1} DMPP	3.16 A	0.71 A	29.33 A	47.29 A	5565 A	2193 A
Mean	2.27	0.63	27.28	44.53	4244	1655
Year	**	**	**	**	**	**
Rhizobia	**	**	**	**	**	**
Nitrogen fertilization	**	**	**	**	**	**
Year x rhizobia	**	**	ns	**	**	**
Year x nitrogen fertilization	**	**	*	**	**	**
Rhizobia x nitrogen fert.	**	**	**	**	**	**
Year x rhizobia x nitrogen fert.	**	**	ns	**	**	**

ns: non-significant, *: p≤0.05, **: p≤0.01. Means in the same column with different letters are significant. LAI: Leaf area index NDVI: Normalized Difference Vegetation Index CT: Canopy temperature CA: Chlorophyll amount BY: Biological yield GY: Grain yield

Vaishnavi and Jeyakumar (2016) and Barroa et al. (2018) reported that leaf area index was increased with rhizobia inoculation. The increase in leaf area is due to rhizobia inoculation which also improves the chlorophyll content in the leaves (Anjum et al. 2006). The reason for the

increases of chlorophyll content with rhizobia inoculation might be increase in enhanced plant growth, stomatal conductance, transpiration, photosynthesis. (Sampath and Ganesh 2003; Rajasekaran et al. 2006) and large and more chloroplasts (Krishna and Bagyaraj 1984). Chlorophyll amount 42.78 spads at first year but it was 46.29 spad at second year (Table 1). Vaishnavi and Jeyakumar (2016) reported that chlorophyll content was increased with rhizobia inoculation. The biological yield and grain yield were higher at Rh^+ than Rh^-. Biological yield was 3417 kg ha^{-1} at first year but it was 5072 kg ha^{-1} at second year. While grain yield was 1312 kg ha^{-1} at first year, it was 1999 kg ha^{-1} at second year (Table 1). Karadavut and Özdemir (2001) and Christy Kala et al. (2011) found that rhizobia inoculation significantly increased biological yield as compared to control. Çakmakçı (2005), Bayraklı et al. (2017) and Barros et al. (2018) indicated that grain yield was increased with rhizobia inoculation.

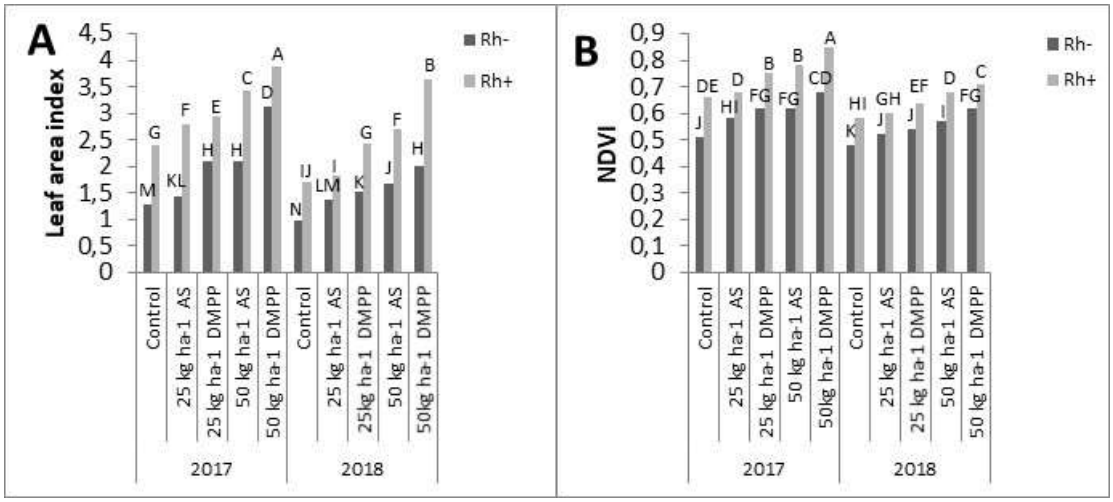

Figure 2.The interaction between rhizobia and different nitrogen fertilization on leaf area index (A) and normalized difference vegetation index (B) of bean. Letters on each bar represent significance level at $P < 0.05$

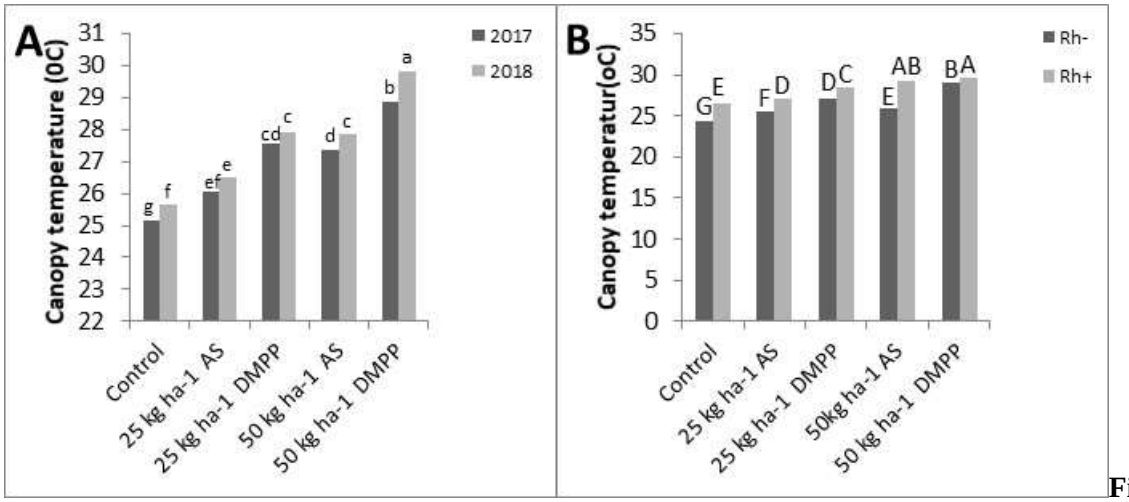

Figure 3.The interaction between rhizobia and different nitrogen fertilization on canopy temperature (A,B) of bean. Letters on each bar represent significance level at $P < 0.05$

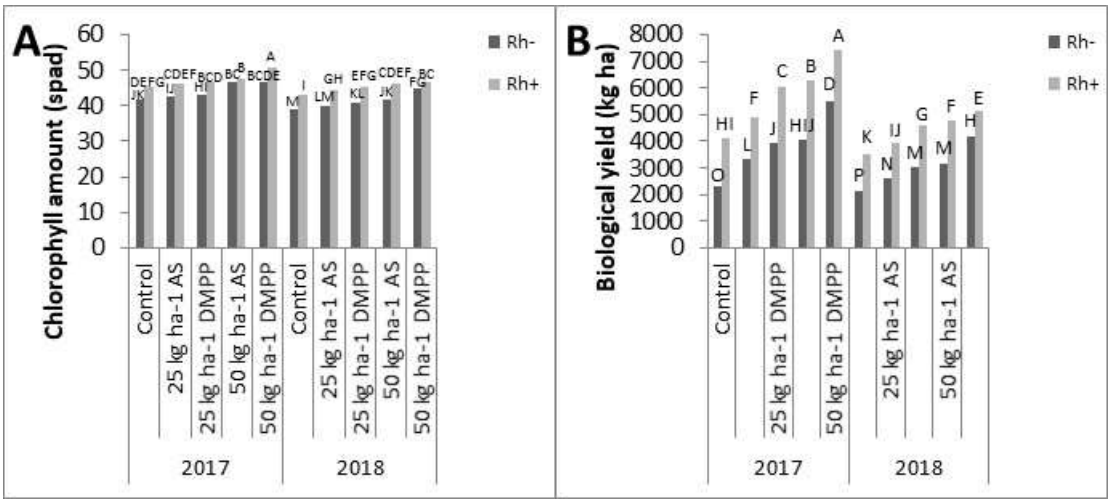

Figure 4. The interaction between rhizobia and different nitrogen fertilization on chlorophyll amount (A) and biological yield (B) of bean. Letters on each bar represent significance level at $P < 0.05$

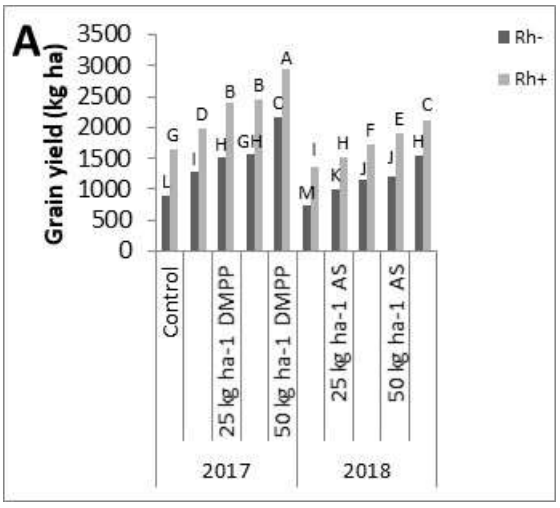

Figure 5. The interaction between rhizobia and different nitrogen fertilization on grain yield of bean. Letters on each bar represent significance level at $P < 0.05$

All of the investigated characters were higher in 50 kg ha^{-1} DMPP (Table 1). The use of DMPP makes that N is maintained in the soil for longer periods; therefore N losses are reduce (Rodrigues et al. 2019). Nitrogen fertilization promote vegetative development in plants (Tursun 2011). Hamodtov (2009) reported that leaf area index was incresing nitrogen doses. Leaf area index and NDVI might be higher in 50 kg ha^{-1} DMPP plots due to long-term nitrogen in soil (Table 1). Hokmalipour and Darbandi (2011) and Tursun (2011) were indicated that the chlorophyll content was increased with increasing nitrogen doses. The highest chlorophyll amount was achieved at 50 kg ha^{-1} DMPP in our research (Table 1). Tursun (2011) was reported that the canopy temperature was increased nitrogen fertilization. These results are similar to our results. Long-term nitrogen in soil was effected positively to physiologically characters. Many researchers have reported that there was significant increases in the biological and grain yield due to DMPP nitrogen fertilization (Diez-Lopez et al. 2008; Liu et al. 2013; Eyles et al. 2018; Hatano et al. 2019). The most likely reason for the increase of crop yield was that N loss through soil NH_3 volatilization was not increased by DMPP application. The effectiveness of nitrogen inhibitors may vary depending on climatic conditions (especially precipitation and temperature), soil moisture, pH, soil texture and mineral N content. May, June and July were very rainy in both years of our study. Excessive

precipitation causes nitrogen losses in soil and thus benefits of DMPP are more observed. Therefore, biological yield and grain yield may be higher in 50 kg/ha DMPP plots in our study.

CONCLUSIONS

All of the investigated characters were higher in the first year than the second year due to climatic conditions except for the canopy temperature. Rhizobia application and DMPP nitrogen fertilization were positively affected all investigated characters. The effectiveness of nitrogen inhibitors may vary depending on climatic conditions. Excessive precipitation causes nitrogen losses in soil and thus benefits of DMPP might be observed in our research. The most likely reason for the increase of crop yield was that N loss through soil NH_3 volatilization was not increased by DMPP application. Benefits for the nitrification inhibitors are increases in yields and reducing the fertilizer use.

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NEMATICIDAL ACTIVITY OF PINE ROSIN EXTRACTS AGAINST *MELOIDOGYNE INCOGNITA* AND *PRATYLENCHUS THORNEI*

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ABSTRACT

Plant parasitic nematodes cause significant yield losses in agricultural production. The main pests that cause this loss are *Meloidogyne* and *Pratylenchus* species. To control these pests, plant-based pesticides are an important role against synthetic pesticides. In this study, the effectiveness of the natural pine rosin against *Meloidogyne incognita* and *Pratylenchus thornei* was evaluated in laboratory conditions. The concentrations of 2%, 1% and 0.5% of the pine rosin were used in the experiments and their effectiveness at the end of 24, 48 and 72 hours were determined. The experiment was set up with 4 replications in a completely randomized design and repeated 2 times. According to the data obtained, the highest mortality was found at 64.88% in *Meloidogyne incognita* at 2% concentrations, and 66% in *Pratylenchus thornei* at the same concentration at end of 24 hours. The effect of the pine rosin has been determined to increase by dose concentration increase and time of impact. After 72 hours, 100% mortality was found in *Meloidogyne incognita* at 2% concentration, while 96.38% mortality was determined in *Pratylenchus thornei*.
According to the results, the pine rosin has been determined to have an effective nematicidal effect.

Keywords: Pine rosin, *Meloidogyne incognita, Pratylenchus thornei*, Biopesticide

INTRODUCTION

Rosin is a substance naturally secreted by herbaceous plants and trees. Especially the rosin produced by pine is called "*oleoresin*". When the tree is injured, the rosin covers the wound to protect it from diseases and pests, as well as to prevent water loss (Esen ve Dığrak, 2009). There is a limited publication on the effectiveness of pine rosin on some diseases and pests.

Plant parasitic nematodes are one of the important pests for agricultural production and cause a global yield loss estimated at $173 billion (Elling, 2013). Among plant parasitic nematodes, *Meloidogyne* spp. and *Pratylenchus* spp. species are important because they can cause damage to many important plants. Their control is not easy a few chemical nematicides are still authorized. Mainly, crop rotation, resistance cultivar or cultural practices are recommended. Farmer prefers chemical pesticides more. Due to the damage caused by chemical nematicides to the environment and non-targeted organisms, research of alternative methods of control has accelerated. Biopesticides are at the forefront of these studies. Extracts, essential oils, and products from plants are actively used in the control of nematodes. Some biopesticides have been licensed.

In this study, nematicidal activities of pine rosin was investigated on *Meloidogyne incognita* and *Pratylenchus thornei*.

MATERIAL AND METHOD

Preparation of pine rosin extracts

Pine rosin was collected from the garden of Atatürk Horticultural Central Research Institute in Yalova. They were kept in the refrigerator at 4°C until preparing extracts. 2 gr of pine rosin was dissolved in 10 ml ethanol and then 88 ml distilled water was added to the solutions. 2% solution was called stock solution and diluted 1% and 0.5% for the experiment from the stock solutions.

Nematodes Cultures

Meloidogyne incognita Cultures

Meloidogyne incognita was collected from tomato (Rio Grande cultivar) in the greenhouse at Atatürk Horticultural Central Research Institute in Yalova. Egg masses of *M. incognita* were picked off infected roots. They were placed on 0.525% NaOCl and shaken for 3-3.5 minutes. Egg was collected through 200 and 500 mesh sieves placed in series, rinsed with distilled water, and collected eggs inside the beakers (Hussey and Barker, 1973, Hussey and Boerma, 1981). Eggs were incubated for 48 hours by Modified Bearmann Funnnel, and freshly hatched were harvested. This newly hatched juvenile was used in the experiment.

Pratylenchus thornei Cultures

Pratylenchus thornei was produced on carrot cultures at Kırşehir Ahi Evran University Plant Protection Department. *P. thornei* was extracted from carrot discs by using modified Baermann funnel and kept at 4°C (Moody et al. 1973).

Bioassay Experiment

M.incognita and *P. thornei* experiments were conducted in the same way. The experiments were set up in a pedri dishes. *M. incognita* and *P. thornei* were counted under the light microscope at x10 magnifications. The experiments were prepared to contain 50 individuals in each Pedri dish. Nematodes were added to the pedri dishes containers in 100 µl distilled water. 2%, 1% and 0.5% solution of pine rosin were added to pedri dishes in 1 ml. The same amount of distilled water was used as a control. The experiment was set up with a completely randomised design, and 4 replicates and repeated 2 times. Pedri dishes were kept in incubator conditions at 28±2°C until 72 hours. After the solution was added, the experiments were counted at 24, 48 and 72 hours, and live or dead nematodes were recorded (Abbasi et al., 2008).

Statistical Analysis

The mortality was corrected using Abbot formula (Abbot, 1925). Data were analysed using SPSS Software (version 17.0, SPSS Inc., CA. USA). The data were analysed by one-way ANOVA and the comparison of means was done with Duncan's multiple range test ($P<0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

In this study, the nematicide activity of 2%,1% and 0.5% concentrations prepared from pine rosin against *Meloidogyne incognita* and *Pratylenhus thornei* were evaluated in vitro conditions. The experiment was conducted 72 hours after the solutions were added. According to the experiment results, highly effective nematicide activities were observed in both *M. incognita* and *P. thornei* at different doses and periods. Pine rosin was found highly toxic to *M. incognita* and *P. thornei* and mortality was observed after 24 hours of exposure. 24 hours after inoculation, the highest mortality was found at a 2% concentration applied in *P. thornei* (66 ±2.50%) This was followed by 2% concentrations applied in *M. incognita* (64.88±1.45%) and 1% concentration applied *M.incognita* (57.25±1.01%) respectively. 48 hours after inoculations, mortalities

were increased in both M. *incognita* and *P. thornei*. The lowest mortality was recorded at a 0.5% concentration applied in *P. thornei* (58.13±2.41%). The highest mortality was recorded at a 2% concentration applied in P. *thornei* (88.25±1.84%). This was followed by 2% concentrations applied in *M. incognita* (84.25±1.62%) and 1% concentration applied in *M. incognita* (75.13±0.79%) respectively. 72 hours after inoculations, the highest mortality was recorded at a 2% concentration applied in M. *incognita* (100%). This was followed by a 2% concentration applied in *P. thornei* (96.38±1.27%) and a 1% concentration applied in *P. thornei* (89.00±1.13%). (Figure 1.).

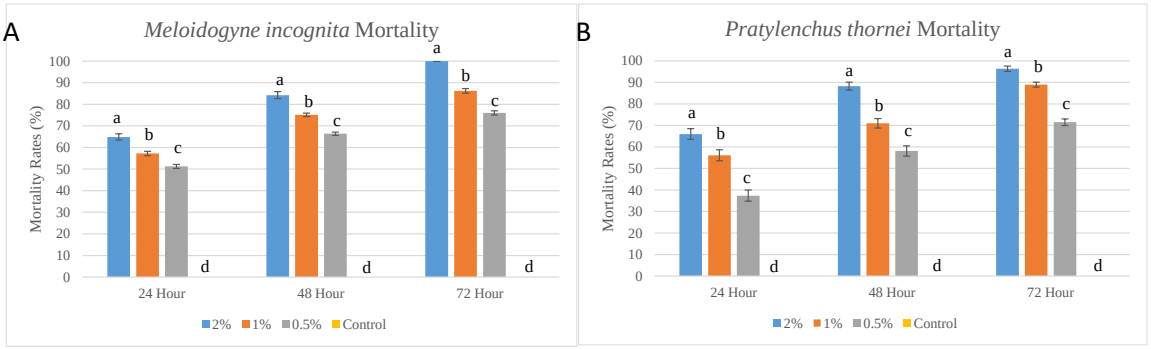

Figure 1.Effect of pine rosin extracts on mortality of *Meloidogyne incognita* (A) and *Pratylenchus thornei* (B) 24,48 and 72 hours after inoculations [*Meloidogyne incognita* (A) $F_{24\ hours}$: 866,54, df:31, P<0.05; $F_{48\ hours}$: 1521,54, df:31, P<0.05; $F_{72\ hours}$: 3961,15, df:31, P<0.05; *Pratylenchus thornei* (B) $F_{24\ hours}$: 171,86, df:31, P<0.05; $F_{48\ hours}$: 415,16, df:31, P<0.05; $F_{72\ hours}$: 1450,43, df:31, P<0.05].

The naturally obtained rosin has been used by different researchers for diseases. Li et al. (2014) in their study, tested chemicals synthesized from rosin against *Plutella xylostella* and *Mythimna separate*. It has been identified as an insecticide effective against *Plutella xylostella*. Tao et.al. (2020) in their study, pine rosin was tested in in-vitro conditions against *Valsa mali*, *Phytophthora capsici*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Fusarium oxysporum* and were found to more successful in suppressing the *Botrytis cinerea*. Mao et al., (2021) in their study, 4 substances synthesized from rosin were tested against *S. sclerotiorum*, *B. cinerea*, *F. oxysporum*, and *Magnaporthe oryzae*a and has determined that it is effective against *F. oxysporum*. Zhao et al. (2021) in their study tried the effectiveness of rosin synthesized against *P. xylostella* and found that it was effective.

Studies were also evaluated in terms of health. Alkan et al. (2016) investigated the antibacterial activity of pine, cherry and apricot rosins and determined that pine rosin has effective antibacterial activities.

CONCLUSIONS

When the studies were examined, information about the effectiveness of pine rosin on plant parasitic nematodes could not be reached. It is emphasized that pine rosin secretion has healing features in plants. Considering the results obtained, it is recommended to carry out pot and field experiments in future studies. The effect on the roots against injury and the effect on greenhouse and field runs will shed light on other work to be done.

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NEMATICIDAL POTENTIAL OF VERMICOMPOST TEA AGAINST *MELOIDOGYNE INCOGNITA* ON TOMATO

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ABSTRACT

Each year, pesticides and fertilizers are used extensively to ensure quality and high efficiency in plant production. Synthetic chemicals applied against soil pests and synthetic fertilizers applied to regulate plant development not only harm the environment and people health but also reduce soil fertility. Thus, beneficial organisms are reduced in the soil, plant resistance decreases, and the plant becomes sensitive to plant protection factors. In recent years, the sustainability of the soil has been ensured using vermicompost, while potential positive effects have been found against soil pests. With this study, the effect of vermicompost tea against *Meloidogyne incognita*, an important soil pest, was evaluated. In the experiment, doses of 100%, 75%, 50% and 25% were prepared from vermicompost tea. Abamax50 SC® was used as a positive control, and distilled water was used as a negative control. The experiment was set up with 5 replications in a completely randomized design and repeated 2 times. At the end of the experiment, some parameters were evaluated such as gall index scale, number of nematodes, plant height, number of plant leaves, and dry and fresh root weights. According to the results obtained, vermicompost tea has been determined to increase plant root development and the number of leaves. It has been determined that plant development is better at high doses of vermicompost tea. It was determined that the nematode gal index scale decreased as the dose of vermicompost tea increased. The lowest gall index scale was found to be 2.5 at %100 dose application; positive control application was determined as 3.30. In addition, the lowest nematode density was determined in the application of the highest vermicompost tea dose. According to the data obtained, vermicompost tea has been stated to improve soil quality and plant efficiency in its use as fertilizer, while also benefiting from biocontrol against *Meloidogyne incognita.*

Keywords: Vermicompost tea, *Meloidogyne incognita*, Biocontrol

INTRODUCTION

Inorganic fertilizer and pesticides are used in agricultural practice to increase the yield and quality of harvests. Both are linked to increased health risks for human and farm animals as well as substantial water and soil contaminations (Tikoria et.al.,2022). Vermiculture is a low-cost system that involves the process of using earthworms to convert organic waste into finer, more plant-available nutrient-rich, non-thermophilic compost (Buchannan et.al.,1988). Vermicompost

and vermicompost products, as a soil amendment, can be used to improve soil health, and control some soil-borne pathogens and pests.

Plant parasitic nematodes are one of the important pests for agricultural production and cause a global yield loss estimated at $173 billion (Elling, 2013). Especially *Meloidogyne* species is important in terms of causing damage to too many plants. *Meloidogyne* species affect the growth and development of plants. By causing damage to the roots of the plant, it prevents the intake of minerals and water necessary for the development of the plant. When plants are infected by nematodes, they show nutrient deficiency and signs of dehydration, and farmers can do intensive fertilization and give water. This causes the excessive use of fertilizer and water and damages the plant and the environment. In recent years, it is aimed at environmentally friendly methods in the control against *Meloidgoyne* spp. Recently, many studies have been done to control nematodes by using vermicompost and their products and found successful results (Awad-Allah et.al., 2019, Mondal et.al., 2021, Rostami et.al., 2021).

Vermicompost teas are the concentrated essence of vermicompost that are regularly prepared in the ratio of 5%-20% of solid vermicompost to water. Vermicompost tea can be applied to drench the soil surface or leaves by spraying directly. In this way, the beneficial nutrients from vermicompost tea can be forwarded effectively to target areas. Foliar spray or drenching the soil surface is more effective to protect against plant pests such as nematodes. Because of its liquid form is easier to reach the target areas in the soil where most nematodes in live (Yatoo et.al.2021). With this study, vermicompost tea was using to control *Meloidogyne incognita* on tomatoes plants in vivo conditions.

MATERIAL AND METHOD

Nematode Preparation

The culture of *Meloidogyne incognita* was collected from tomato (Rio Grande cultivar) in the greenhouse at Atatürk Horticultural Central Research Institute in Yalova. Egg masses of *M. incognita* were picked off infected roots. They were placed on 0.525% NaOCl and shaken for 3-3.5 minutes. Egg was collected through 200 and 500 mesh sieves placed in series, rinsed with distilled water, and collected eggs inside the beakers (Hussey and Barker, 1973, Hussey and Boerma, 1981). Eggs were incubated for 48 hours by Modified Bearmann Funnnel, and freshly hatched were harvested. This newly hatched juvenile was used in the experiment.

Preparation Vermicompost Tea

100% organic vermicompost is provided from Ecotarim Company. 5 kg vermicompost was placed in a cloth bag. It has been infused for 48 hours in 5 litres of chlorine-free water with the aid of an aquarium pump. To stimulate microorganism activity, approximately 100 ml of carob molasses have been added to the chlorine-free water. The obtained 5 lt solution was considered to be 100%. In the experiment, 100%, 75%, 50% and 25% vermicompost tea concentrations were tested against *Meloidogyne incognita* (IJ). Abamax 50 SC® pesticides was used as a positive control and distilled water was used as a negative control.

Greenhouse Experiment

Experiment was carried out in the unheated nylon greenhouse under controlled conditions from 20 August to 20 October 2021. Invictus seedlings were used as a tomato variety in the experiment. *Meloidogyne incognita* (IJ) inoculation was done at 1000 IJ per plant 4 days after planting. Concentrations [100%, 75%, 50%,25%, Abamax 50 SC® (IC(+), and distilled water (C(-)), only nematodes inoculation (C(+))] were applied at 1 ml per plants. During the study, the application concentrations were applied 15 days apart. The experiment was completed after 60 days. At the end of the experiment, some parameters were evaluated such as gall index scale,

number of nematodes, plant height, number of plant leaves, and dry and fresh root weights. The experiment was set up with 5 replications in a completely randomized design and repeated 2 times.

Statistical Analysis

Data were analysed using SPSS Software (version 17.0, SPSS Inc., CA. USA). The data were analysed by one-way ANOVA and the comparison of means was done with Duncan's multiple range test ($P<0.05$) (Duncan, 1955).

RESULTS AND DISCUSSION

Vermicompost tea was tested against *Meloidogyne incognita* in potted tomatoes in a greenhouse. All concentrations of vermicompost tea tested exhibited highly effective toxicity toward *Meloidogyne incognita* (Figure 1,2). After 60 days, tomatoes plants were carefully removed each pot and scored for *M. incognita* galls index. The lowest score was recorded from the 100% concentrations of vermicompost tea (2.50 ±0.17). This was followed by positive control (Abamax 50 SC®) (3.30 ±0.21) and 75% of vermicompost tea (3.40± 0.22) ($F=159,21$, $df=69$, $P<0.05$) (Figure 1).

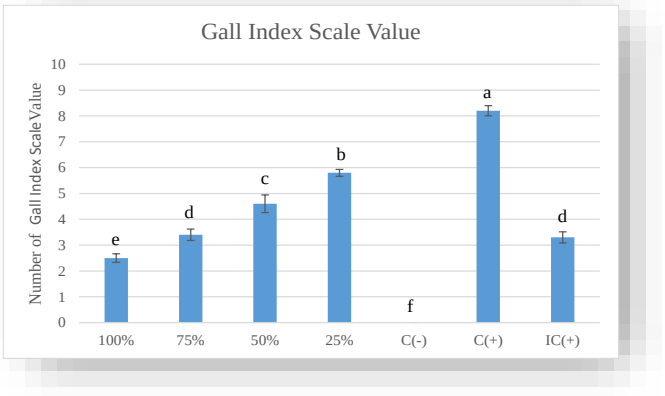

Figure 1. The effect of evaluated vermicompost tea on development of the number of galls on roots by *Meloidogyne incognita*.

The different concentrations of vermicompost tea recorded highly toxic effects on *Meloidogyne incognita*. 100% concentration was found the highest decrease effect on *Meloidogyne incognita* larvae. This was followed by 75% and 50% concentrations ($F=179,10$, $df=69$, $P<0.05$) (Figure 2). Mishra et.al., (2018) vermicompost tea was applied to cucumber and checked for *Meloidogyne incognita* development. They found that root penetration by *M. incognita* was lower in plants drenched with vermicompost tea as compared to control. You et.al., (2018) checked vermicompost tea (prepared from vermicompost with *Phyllostachys edulis*, *Pueraria lobata*, and vegetable food waste) against *Meloidogyne incognita* and *Rotylenchulus reniformis* and *Phyllostachys edulls* vermicompost tea was found more consistent than vegetable vermicompost tea for plant parasitic nematodes suppression. Mondal et.al., (2021) used vermicompost and biochar against *Meloidogyne graminicola*. Their result showed that vermicompost and biochar exudate was not effective against the hatching nematodes, however, after the experiment final population was found to reduce by using 1.2% biochar and 5% vermicompost. Zuhair et.al., (2022),

three different vermicompost's were applied and counted galls. The lowest number of galls was found in the vermicompost+saw dust applications compared to the control.

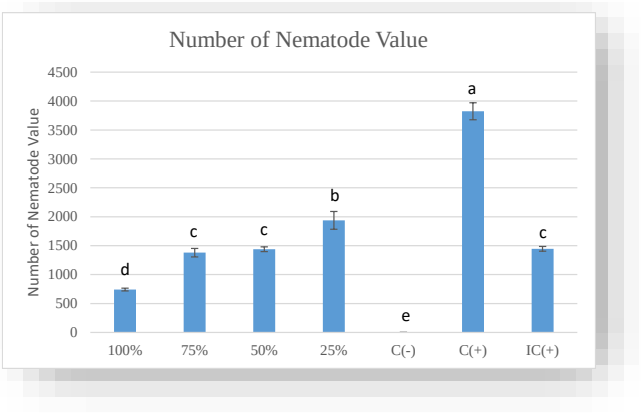

Figure 2. The effect of evaluated vermicompost tea on number of *Meloidogyne incognita* value in the soil and roots.

Fresh root weight was recorded and showed a vermicompost effect on tomato development. It has been determined that the 100% vermicompost application has increased the fresh root weight at the highest rate (31.25± 0.62)(F=18,16, df=69, P<0.05). This found value was higher than the plant without vermicompost [C(-)] and the difference was statistically significant (Figure 3).

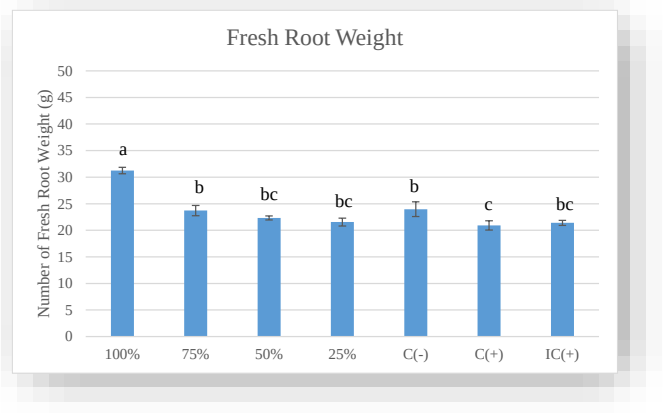

Figure 3. The effect of evaluated vermicompost tea on fresh root weights of tomato infected by *Meloidogyne incognita.*

Dry root weight was recorded. It has been determined that the 100% vermicompost application has increased the dry root weight at the highest rate (6.19± 0.28)(F=27.13, df=69, P<0.05). This found value was higher than the plant without vermicompost and the difference was statistically significant (Figure 4).

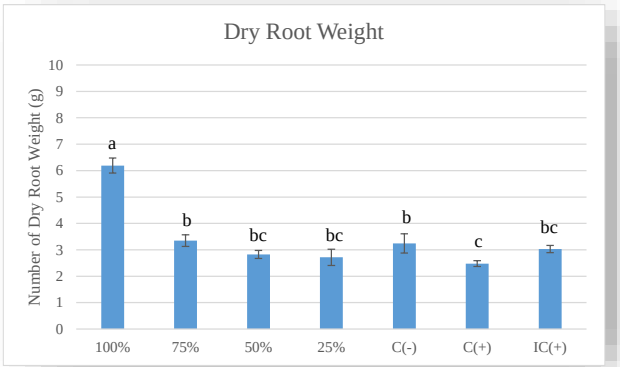

Figure 4. The effect of evaluated vermicompost tea on dry root weights of tomato infected by *Meloidogyne incognita.*

The plant height and number of leaves were measured every 15 days after the experiment was established. The last measurement showed the highest plant height compared to others. 100% vermicompost tea applied was found more effective than others (112.6± 2.20) (F=32.58, df=69, P<0.05) (Figure 5).

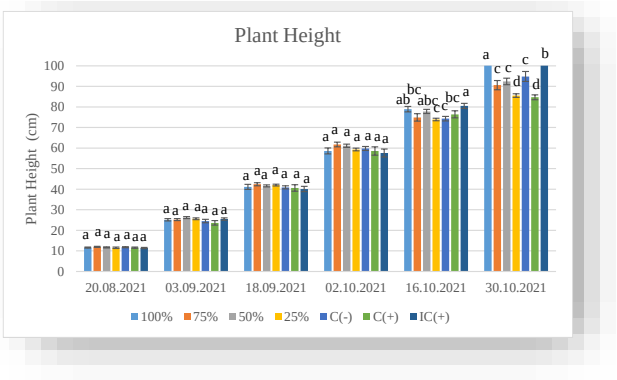

Figure 5. The effect of evaluated vermicompost tea on plant height of tomato infected by *Meloidogyne incognita*.

The plant's leaf development has been observed. 100% vermicompost tea applied was found the most effective concentration in the last measurement (21.9± 0.38) (F=3.09, df=69, P<0.05) (Figure 6). Zuhair et. al., (2022), three different vermicompost types inoculated to soil and recorded the highest number of leaves from the vermicompost +saw dust and vermicompost plain applications compared to controls.

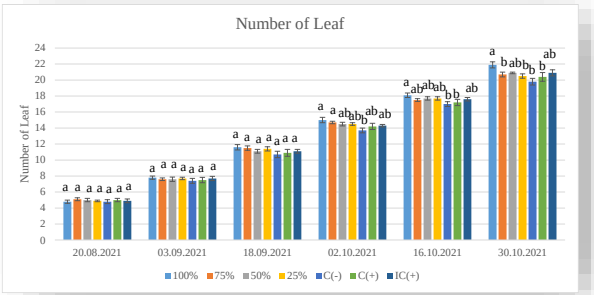

Figure 6. The effect of evaluated vermicompost tea on the number of leaves of tomato infected by *Meloidogyne incognita*.

CONCLUSIONS

Our main goal in plant production is to obtain high and quality yields, while leaving healthy agricultural areas for future generations. We must make correct practices by minimizing pesticides used for diseases, pests and weeds that affect production. In addition, we should apply correct and dosed fertilization techniques. Vermicompost and vermicompost products have shown positive effects on the growth and productivity of agricultural products. Also, vermicompost has been given cost-benefits where these organic materials can use by poor farmers (Yatoo et al., 2021). While vermicompost applications have observed productivity growth, beside that it has been revealed by different researchers that diseases, pests, and weeds are also suppressed.

In this study, it showed that the use of vermicompost tea in the control of *Meloidogyne incognita*, one of the major underground pests, has been given successful results. It is recommended that this study be carried out in field conditions and that the results of hope are shared with the farmers and that the use of vermicompost is increased.

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FRUIT QUALITY CHARACTERISTICS OF SOME APPLE VARIETIES CULTIVATED IN ORDU ECOLOGY

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ABSTRACT

This study carried out six apple cultivars grown in Ordu coastal ecology to determine some physical and biochemical properties. Within the scope of the study, fruit weight, fruit size, firmness, color characteristics, SSC, titratable acidity, and vitamin C values of Mondial Gala, Jeromine, Fuji, Granny Smith, Red Chief, and Scarlet Spur apple cultivars were investigated. In the study, fruit weight and fruit size were higher in Scarlet Spur (251.40 g) and Red Chief (240.70 g) cultivars, while the highest fruit firmness was determined in the Mondial Gala (73.05 N/mm) cultivar. In addition, the highest SSC content was found in Fuji (12.53 %), while the vitamin C content was found in Scarlet Spur (18.60 mg 100 g^{-1}). As a result of the research, it can be stated that Scarlet Spur and Red Chief varieties come to the fore in terms of features that directly affect the market value of products, such as fruit weight and fruit size.

Keywords: *Malus domestica*, Color Characteristics, Cultivars, Firmness, SSC, Vitamin C.

INTRODUCTION

Apple is a type of pome fruit grown in many places where temperate climate prevails the world. Turkey is among the homelands of apples (Özbek, 1978). It has a tremendous and rich genetic diversity and a suitable ecology for apple growing. In terms of apple production, Turkey ranks third in the world after America and China, according to 2020 data (Anonymous, 2022a). However, the number of standard varieties produced economically is limited. Although many new varieties have been developed, especially in the world, it takes time for our producers to adopt such (Özongun ve ark., 2014).

In recent years, with the development of dwarf and semi-dwarf rootstocks, intensive planting systems have increased the yield obtained per unit area in apples (Şensoy and Bostan, 2019). This has enabled producers to earn higher incomes. Again, earlier fruit laying in the gardens established with these systems and easier cultural processes brought an important advantage for the producers. Therefore, considering our production potential, cultivating popular varieties that meet consumer demands in national and international markets following modern techniques in our country's conditions may reveal a crucial economic input potential. Undoubtedly, adaptation studies have an essential place in the determination of these varieties. Many studies are carried out in different regions of our country to determine the vegetative development status, fruit quality characteristics, and yield amounts of different varieties (Soylu et al. 2033; Ceylan, 2008; Culha, 2010; Baytekin and Akca, 2011; Ozongun et al. 2014; Ozturk et al. 2016; Ozturk and Ozturk, 2016; Unuvar and Pırlak, 2016; Bayazit et al. 2019; Boyacı, 2019; Şensoy and Bostan, 2019).

This study aimed to determine the fruit quality characteristics of six apple cultivars grafted on M9 and MM106 apple rootstock under Ordu ecological conditions. The findings obtained from the study will facilitate the selection of varieties for the producers aiming to grow apples in the region.

MATERIAL AND METHOD

This study was carried out in the ecological conditions of Ordu province in 2019-2020. The plant material of the study consisted of Mondial Gala, Jeromine, Fuji, Granny Smith, Red Chief, and Scarlet Spur apple varieties grafted on M9 and MM106 clone rootstock grown in Ordu University Faculty of Agriculture Application and Research Field. According to the climate data for many years in Ordu (1959-2021), the average highest temperature is 18.4 °C, the lowest temperature is 11.3 °C, the annual average temperature is 14.5 °C, and the annual average precipitation is 1047.5 mm (Anonymous, 2022b). Plants were planted with 3.5 x 3.0 m between rows and pruned with the goble training system for grafted on MM106, and 3.0 x 1.2 m between rows and pruned with the central leader training system. Irrigation in the garden is done with a double-line drip irrigation system with 2 L/h drippers, and other cultural processes are also applied regularly.

During the harvest period, ten fruits were harvested separately from a total of 12 trees of each variety, and measurements and analyses were made. During the research, fruit weight (g), fruit size (mm), firmness (N/mm), skin color, soluble solids content (SSC), titratable acidity, and vitamin C characteristics of the cultivars were investigated. Fruit weight (g) was determined with a digital precision scale with an accuracy of 0.01 grams. Fruit size was determined by measuring with a digital caliper (Mitutoyo, Japon) with a sensitivity of 0.01 mm. Firmness was determined with a penetrometer using an 11.1 mm tip by measuring two different places where the skins were peeled from the equatorial region of the fruits. The skin color of the fruits was determined using a colorimeter (Minolta, model CR-400, Tokyo, Japan) in CIE L*, a*, and b*. SSC was determined by measuring with a digital refractometer (PAL-1, McCormick Fruit Tech. Yakima, ABD) from the juice sample obtained without sediment from the fruits. According to Öztürk et al. (2016), TA was calculated in terms of malic acid. Vitamin C content was determined using a reflectometer set (Merck RQflex plus 10, Germany).

The research was designed according to the random blocks trial design with three replications and four trees in each replication. The data obtained from the experiment were analyzed in the JMP 16.0 software, and the 'Tukey Multiple Comparison Test' ($p<0.05$) was used to compare the differences between the means.

RESULTS AND DISCUSSION

Apple consumers around the world generally prefer flamboyant, large fruits for table consumption in the first place. Therefore, for apple varieties, the weight and size of the fruit are among the main criteria affecting consumer preference. The fruit weight and size characteristics of the cultivars examined in the study are presented in Table 1. It was determined that the fruit weight of the cultivars varied between 193.04 g (Mondial Gala) and 251.40 g (Scarlet Spur). Scarlet Spur cultivar has the highest value in terms of commercially important fruit weight, followed by Red Chief, Fuji, Jeromine, and Granny Smith cultivars. All of these varieties attract attention with their fruit weights of over 200 g. However, statistically, the difference between Scarlet Spur and Red Chief cultivars was insignificant, while the difference between these cultivars and other cultivars was significant. In a study in which the quality characteristics of apple varieties grafted on M9 rootstock were determined in Ordu ecological conditions, it was stated that fruit weight ranged from 136.2 g (Red Chief) to 207.1 g (Granny Smith) (Ozturk et al., 2016). There are similar studies

in which fruit weight was determined in apple varieties grafted on MM106 rootstock in different regions of our country. It was reported as 122.8 g (Golden Delicious)-169.5 (Granny Smith) in Görükle (Bursa) conditions, 112.3 (Jersey Mac)-173.9 g (Starkrimson Delicious) in Samsun province ecological conditions, and 163.31 g (Braeburn)-215.00 g (Red Chief) in Kırşehir ecological conditions (Soylu et al., 2003; Oztürk and Ozturk, 2016; Boyacı, 2019). Accordingly, it can be said that the cultivars examined in this study generally have high fruit weight.

Fruit length was determined between 66.55 mm (Fuji) - 72.97 mm (Scarlet Spur), fruit width 74.43 mm (Mondial Gala) - 78.62 (Fuji), and fruit thickness between 76.23 mm (Mondial Gala) - 83.40 mm (Scarlet Spur) in cultivars. When the cultivars' length, width, and thickness values were examined, it was seen that the largest fruits belonged to the Scarlet Spur cultivar. In our country, there are studies in which dimensional measurements of different apple varieties are determined. In Turhal (Tokat) conditions, fruit width 70.33 mm (Granny Smith)-80.21 mm (Red Chief), and fruit length 60.72 mm (Granny Smith)-70.25 mm (S.S. Golden) (Baytekin and Akça, 2011); fruit width 69 mm (Early Red One)-84 mm (Novaja), fruit length 57 mm (Gala Selecta)-77 mm (Topred) (Özongun et al. 2014) in Eğirdir (Isparta) conditions; In Ordu ecological conditions, fruit width is 67.41 mm (Red Chief)- 77.60 (Granny Smith), fruit length is 58.65 mm (Red Chief)- 69.07 mm (Granny Smith) (Ozturk et al., 2016); fruit width was reported as 71.10 mm (Golden Delicious)-77.33 (Red Chief), fruit length as 63.20 mm (Granny Smith)- 71.26 mm (Red Chief) in Kırşehir province conditions (Boyacı, 2019). The dimensional characteristics of the cultivars examined in the study were generally compatible with the other studies.

Table 1. Fruit weight and fruit size characteristics of six apple cultivars grown in Ordu

#	CULTIVARS	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Thickness (mm)
1	MONDIAL GALA	193.04 d	67.95 bc	74.43 c	76.23 c
2	JEROMINE	206.60 c	69.14 b	75.81 bc	78.60 b
3	FUJI	229.70 b	66.55 c	78.62 a	81.90 ab
4	GRANNY SMITH	203.60 c	68.36 bc	76.20 b	79.39 b
5	RED CHIEF	240.70 a	72.26 a	76.60 b	82.28 a
6	SCARLET SPUR	251.40 a	72.97 a	78.37 a	83.40 a

Means in columns with the same letter do not differ according to Tukey's test at $P<0.05$.

The data on the fruit firmness and color characteristics of the cultivars are presented in Table 2. Accordingly, in cultivars, fruit firmness varied between 40 N/mm (Red Chief) and 73.05 N/mm (Mondial Gala). The Mondial Gala with the highest firmness was followed by Jeromine, Scarlet Spur, Granny Smith, and Fuji cultivars. In previous studies, the firmness was reported, 63.43 N/mm (Mondial Gala)-82.83 N/mm (Red Chief) in Ordu province (Ozturk et al., 2016), 48.5 N/mm (Jersey Mac)-78.3 N/mm (Granny Smith) in Samsun province (Oztürk and Ozturk, 2016), and 69.56 N/mm (Galaxy Gala)-86.11 N/mm (Granny Smith) in Çarşamba ecological conditions (Balta et al., 2020). Regarding firmness, Mondial Gala and Jeromine varieties have the highest values. In addition, the data of Fuji, Jeromine, Granny Smith, and Scarlet Spur cultivars were found to be compatible with the literature. In contrast, the Red Chief cultivar was lower than the literature.

When the color characteristics were examined, the L* value varied between 37.52 (Jeromine) and 61.77 (Granny Smith), a* value between 11.53 (Fuji) and 30.36 (Mondial Gala), and b* value between 15.78 (Jeromine) and 39.79 (Granny Smith). Granny Smith cultivar comes to the fore in this respect, as the L* value is a feature that affects the fruits' brightness and the consumer appeal of apples. Considering that a* value represents the red color and the b* value represents the green

color, it can be said that the Mondial Gala has the reddest fruits, and the Granny Smith has the greenest fruits. In different studies in which color characteristics were determined, Baytekin and Akça (2011) valued L* as 39.99 (Red Chief)- 73.13 (S.S. Golden), a* value -7.17 (S.S. Golden)-26.13 (Red Chief), b* value 16.79 (Red Chief)- 45.99 (S.S. Golden); Boyacı (2019) value L* 19.50 (Red Chief)- 78.86 (Golden Delicious), a* value -23.25 (Granny Smith)- 43.24 (Braeburn), b* value 10.10 (Red Chief)- 51.24 (Golden Delicious); Eskimez et al. (2020) L* is 29.47 (Arapkızı)-50.27 (Fuji Kiku), a* is 18.19 (Fuji Kiku)-20.11 (Jonagold), b* is 6.75 (Arapkızı)-22.76 (Fuji Kiku).

Table 2. Fruit firmness and color characteristics of six apple cultivars grown in Ordu

#	CULTIVARS	Firmness (N/mm)	Color characteristics		
			L*	a*	b*
1	MONDIAL GALA	73.05 a	50.36 c	30.36 a	26.04 b
2	JEROMINE	62.78 b	37.52 e	22.80 b	15.78 c
3	FUJI	53.30 c	54.32 b	11.53 c	27.50 b
4	GRANNY SMITH	56.14 c	61.77 a	21.61 b	39.79 a
5	RED CHIEF	40.00 d	37.71 e	21.64 b	19.37 c
6	SCARLET SPUR	56.73 c	41.41 d	22.64 b	19.99 c

Means in columns with the same letter do not differ according to Tukey's test at $P<0.05$.

SSC, TA and vitamin C data determined in cultivars are given in Table 3. Accordingly, the SSC content is 8.53% (Granny Smith) to 12.53% (Fuji), the TA content is 0.23% (Jeromine) to 0.86% (Granny Smith), and the vitamin C content is 11.60 mg 100 g-1 (Mondial Gala) to 18.60 mg 100 g-1 (Scarlet Spur). Granny Smith, Red Chief and Fuji cultivars followed the Scarlet Spur cultivar in terms of vitamin C, and the differences between these cultivars were found to be insignificant at the statistical level. In the study conducted in Ordu province, the SSC content was determined as 9.13% (Granny Smith)-11.25% (Mondial Gala), and the TA content as 0.27 (Mondial Gala)-0.67 (Granny Smith) (Ozturk et al., 2016). In cultivars grown under Çarşamba ecological conditions, the SSC content was 10.24% (Granny Smith)-12.07% (Royal Gala), and the TA content was 0.31 (Royal Gala, Galaxy Gala)-0.93 (Granny Smith) (Balta et al., 2020). Vitamin C content was determined as 9.9 mg/100 g in Granny Smith cultivar in a study conducted in Erzurum conditions (Karşı and Aslantas, 2016). In a different study conducted in Eskişehir conditions, the vitamin C content was reported as 3.78 9.9 mg/100 mL in Granny Smith variety and 2.89 mg/100 mL in Fuji variety (Mertoğlu and Evrenosoğlu, 2019). While the SSC content of the Granny Smith cultivar was found to be lower compared to the literature, it was determined that other cultivars had similar results. Vitamin C content, an essential feature for human health, was found at high levels in all varieties, especially Scarlet Spur and Granny Smith.

Table 3. Values of SSC, TA and vitamin C content of six apple cultivars grown in Ordu

#	CULTIVARS	Biochemical characteristics		
		SSC (%)	Titratable acidity (% malic acid)	Vitamin C (mg 100 g^{-1})
1	MONDIAL GALA	10.63 b	0.30 c	11.60 c
2	JEROMINE	9.10 c	0.23 d	15.40 b
3	FUJI	12.53 a	0.47 b	17.00 a
4	GRANNY SMITH	8.53 c	0.86 a	18.20 a
5	RED CHIEF	10.90 b	0.31 c	17.20 a
6	SCARLET SPUR	10.33 b	0.31 c	18.60 a

Means in columns with the same letter do not differ according to Tukey's test at $P<0.05$.

Generally, it can be said that the data on the fruit characteristics of the six cultivars examined during the research typically give results close to the values of the studies in the literature. It is thought that some of the differences may be caused by factors such as climatic conditions, soil structure, differences in cultural practices, and rootstock selection.

CONCLUSIONS

As a result, Scarlet Spur and Red Chief varieties grown in the ecological conditions of Ordu province came to the forefront with fruit weight and size, Fuji variety SSC content, Mondial Gala variety firmness, and Granny Smith variety vitamin C content. Generally, considering the literature findings, it can be stated that the examined cultivars perform well in terms of fruit characteristics.

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DETERMINATION OF SOME QUALITY PARAMETERS OF STANLEY AND PRESIDENT PLUM VARIETIES CULTIVATED IN ORDU ECOLOGY

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ABSTRACT

This study was carried out to determine some fruit quality characteristics of Stanley and President plum (*Prunus domestica*) cultivars grown in Ordu. Within the scope of the study, fruit weight, fruit dimensions, firmness, color, soluble solids content, titratable acidity, and vitamin C properties of fruits were determined. According to the research findings, the Stanley cultivar had significantly higher fruit weight and fruit size values than President cultivar. Similarly, while the titratable acidity content and vitamin C values of the Stanley variety were higher, the soluble solids content of the President variety was found to be higher. As a result of the research, it was found that the Stanley plum cultivar grown in Ordu province ecological conditions has superior characteristics in terms of important fruit quality parameters such as fruit firmness, fruit size, and biochemical characteristics.

Keywords: *Prunus domestica*, Color characteristics, Firmness, SSC, Vitamin C.

INTRODUCTION

Plum, included in the *Prunus* genus of the Rosaceae family, is a stone fruit species with high adaptability to climatic factors (Ertekin et al., 2006). It is reported that the history of plum culture dates to 2000 years ago, and there are more than 6000 plum varieties spread in Asia, Europe, and America (Karakaya, 2006; Topp et al., 2012). *Prunus domestica*, known as European plums, and *Prunus salicina*, known as Japanese plums, are among the plum species with high economic value and are grown intensively (Ertekin et al., 2006). European plums with large fruits can be used for canning, drying, and fresh consumption, while Japanese plums gained commercial importance due to their early maturity (Westwood, 1978).

World plum production was announced as 12 225 073 in 2020 data. China has the highest production rate, with 6 465 219 tons. On the other hand, Turkey ranked 5th after Romania, Serbia, and Iran, with a production of 329 056 tons (Anonymous, 2022a). Turkey is shown among the homelands of plum, which has a widespread area worldwide (Bolat et al., 2018). While this situation is effective in our country's genetic richness, it also reveals the suitability of ecological conditions for plum cultivation. Especially in recent years, with the widespread use of certified saplings and clone rootstocks in plum cultivation, there has been an increase in production amounts. However, the high quality of the products produced to meet the market demands is one of the most important points to be considered in cultivation. At this point, climate characteristics, variety and site selection, and cultural practices should be evaluated as a whole, and it is of great importance to determine the regional suitability of the varieties through adaptation studies (Crisosto ve ark., 1995).

In this context, this study aimed to determine the fruit quality characteristics of Stanley and President plum cultivars grown in Ordu province's ecological conditions.

MATERIAL AND METHOD

This study was carried out in the ecological conditions of Ordu province in 2019-2020. The plant material of the study consisted of Stanley and President cultivars in Ordu University Faculty of Agriculture Application and Research Field. According to the climate data for many years in Ordu (1959-2021), the average highest temperature is 18.4 °C, the lowest temperature is 11.3 °C, the annual average temperature is 14.5 °C, and the average yearly precipitation is 1047.5 mm (Anonymous, 2022b). Plants were planted with 3.5 x 3.0 m between rows and pruned with the Goble training system. Irrigation in the field is done with a double-line drip irrigation system with 2 L/h drippers, and other cultural processes are also applied regularly.

During the harvest period, ten fruits were harvested separately from a total of 9 trees of each variety, and measurements and analyses were made. During the research, fruit weight (g), fruit size characteristics (mm), firmness (N/mm), skin color, soluble solids content (SSC), titratable acidity, and vitamin C characteristics of the cultivars were investigated. Fruit weight (g) was determined with a digital precision scale with an accuracy of 0.01 grams. Fruit size characteristics were determined by measuring with a digital caliper (Mitutoyo, Japon) with a sensitivity of 0.01 mm. Firmness was determined with a penetrometer. The skin color of the fruits was determined using a colorimeter (Minolta, model CR-400, Tokyo, Japan) in CIE L*, a*, and b*. SSC was determined by measuring with a digital refractometer (PAL-1, McCormick Fruit Tech. Yakima, ABD) from the juice sample obtained without sediment from the fruits. TA was calculated in terms of malic acid. Vitamin C content was determined using a reflectometer set (Merck RQflex plus 10, Germany).

The research was designed according to the random blocks trial design with three replications and three trees in each replication. The data obtained from the experiment were analyzed in the JMP 16.0 software, and the 'Tukey Multiple Comparison Test' ($p<0.05$) was used to compare the differences between the means.

RESULTS AND DISCUSSION

The fruit weight and dimensional characteristics of the Stanley and President plum cultivars examined in the study are presented in Table 1. Accordingly, the fruit weight of the Stanley variety was 74.88 g, and the President variety was 55.71 g. The difference between cultivars was statistically significant ($P<0.05$). Fruit weight, 41.63 g in the Stanley variety and 68.34 g in the President variety in Çorlu conditions (Alkış, 2010); 24.45 g (Stanley)- 70.29 g (President) in Malatya conditions (Çöçen et al. 2019); 50.4 g for Stanley variety and 52.6 g for President variety in Georgia conditions (Maglakelidze et al. 2017) were reported. The values obtained from the research were higher for the Stanley variety compared to the literature. In President variety, fruit weight was lower than Alkış (2010) and Çöçen et al. (2019) and similar to the results of Maglakelidze et al. (2017).

Fruit width, fruit length, and fruit thickness of the examined cultivars were measured as 45.31 mm, 56.25 mm, 46.63 mm in the Stanley variety, 41.53 mm, 55.68 mm, and 42.52 mm in the President variety, respectively. In terms of fruit size characteristics, the difference between fruit length was insignificant between cultivars. In contrast, fruit width and thickness were significantly higher in the Stanley cultivar ($P<0.05$). In Çorlu conditions, the fruit width of Stanley variety is 37.47 mm, fruit length is 74.39 mm, in President variety fruit width is 67.93 mm, fruit length is 44.63 mm (Alkış, 2010); In Malatya conditions, fruit width for Stanley variety is 30.67 mm, fruit length is 44.85 mm, fruit thickness 32.82 mm, for President variety fruit width is 45.84 mm, fruit

length is 57.23 mm, fruit thickness is 48.49 mm (Çöçen et al. 2019); In Georgia conditions, fruit width was 30.70 mm, fruit length 40.60 mm, fruit thickness 30.50 mm for Stanley variety, fruit width 30.70 mm, fruit length 57.23 mm, fruit thickness 30.60 mm for President variety (Maglakelidze et al. 2017).

Table 1. Fruit weight and fruit size of Stanley and President plum cultivars

#	CULTIVARS	Fruit weight (g)	Fruit Length (mm)	Fruit Width (mm)	Fruit Thickness (mm)
1	STANLEY	74.88 a	56.25 a	45.31 a	46.63 a
2	PRESIDENT	55.71 b	55.68 a	41.53 b	42.52 b

Means in columns with the same letter do not differ according to Tukey's test at P<0.05.

Table 2. Fruit firmness and color characteristics of Stanley and President plum cultivars

#	CULTIVARS	Firmness (N/mm)	Color characteristics		
			L*	a*	b*
1	STANLEY	27.81 a	32.46 a	8.67 a	9.08 a
2	PRESIDENT	19.52 b	25.46 b	4.63 b	2.17 b

Means in columns with the same letter do not differ according to Tukey's test at P<0.05.

Table 3. SSC, TA, and Vitamin C characteristics of Stanley and President plum cultivars

#	CULTIVARS	SSC (%)	Titratable acidity (% malic acid)	Vitamin C (mg 100 g^{-1})
1	STANLEY	13.83 a	1.43 a	14.90 a
2	PRESIDENT	14.10 a	0.71 b	13.65 b

Means in columns with the same letter do not differ according to Tukey's test at P<0.05.

The firmness and color values of the Stanley and President varieties examined in the study are shown in Table 2. Firmness was determined as 27.81 N/mm in Stanley variety and 19.52 N/mm in President variety. The difference between the cultivars was statistically significant (P<0.05). Firmness was 12.75-26.02 N/mm for Stanley variety in a study conducted in Antalya (Korkuteli) (Ertekin ve ark., 2005); in the study conducted in Holovousy (Czech Republic), it was reported as 16.1 N/mm for President variety (Blažek and Pištěková, 2009). In terms of firmness, the data obtained from the study is compatible with the literature results.

The color characteristics of the examined cultivars were determined in terms of L*, a*, and b*. Accordingly, the L* value of the Stanley cultivar was 32.46, the a* value 8.67, and the b* value 9.08. In the President variety, the L* value was 25.46, the a* value was 4.63, and the b* value was 2.17. In the research, the findings obtained regarding color characteristics between varieties were statistically significant (P<0.05). Previously, L* value of Stanley variety was reported between 29.650- 40,830, a* value between 0.600-4.890, b* value between -10.250 and -2.670 in Antalya province (Ertekin ve ark., 2006). In President variety grown in Tokat province, L* value was reported between 49.59-61.39, a* value between -5.26 and -6.62, b* value between 26.51-39.03 (Altuntas et al. 2020).

In the study, SSC, TA, and Vitamin C values of Stanley and President cultivars are given in Table 3. In the measurements, the SSC content of the Stanley variety was determined as 13.83%, TA content 1.43, and vitamin C content as 14.90 mg 100 g^{-1}. In the President variety, the SSC content was 14.10%, the TA content was 0.71, and the vitamin C content was 13.65 mg 100 g^{-1}. No significant differences were found in SSC content between cultivars. On the contrary, the Stanley cultivar had significantly high values in TA and vitamin C ($P<0.05$). In previous studies, in Çorlu conditions, the content of SSC was 12.55% in Stanley, 18.60% in President (Alkış, 2010); In Malatya conditions, SSC content was 18.60% in Stanley and 17.60% in President (Çöçen et al. 2019); In Georgia conditions, the content of SSC was reported as 13.78% in Stanley and 12.64% in President (Maglakelidze et al. 2017). Altunas et al., (2020) reported the TA content of President variety between 1.15-1.28. Bozhkova (2014) reported the vitamin C content as 11.92 mg 100 g^{-1} for Stanley variety and 3.57 mg 100 g^{-1} for President variety in Plovdiv (Bulgaria) conditions. In addition, the vitamin C content of different plum varieties has been reported as 8.62-9.79 mg 100 g^{-1}, 9.04-12.05 mg 100 g^{-1} (Družić et al., 2007; Voća et al., 2009). According to the data obtained from the study, the SCKM content of the cultivars was found to be lower compared to the results of Alkış (2010) and Çöçen et al. (2019), but similar to Maglakelidze et al. (2017). Vitamin C content was found to be relatively high compared to the literature.

CONCLUSIONS

As a result, fruit characteristics of Stanley and President cultivars were determined in this study carried out in Ordu province's ecological conditions in 2019-2020. The findings obtained during the study showed that the Stanley variety has superior characteristics compared to the President variety in terms of fruit weight, size, firmness, coloration, and vitamin C.

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HEAD PROPERTIES OF SOME ARTICHOKE POPULATIONS

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ABSTRACT

Marmara is one of the regions that have the most artichoke production in Türkiye. Plant materials were collected from artichoke gardens in the provinces of Balıkesir, Bursa and Bilecik located in this region which were selected as a result of survey studies, in 2019-2020. Hopeful plants were marked through observations during plant vegetation and pre harvest assessments. Later on, the sucker-shoots were brought to Yalova Atatürk Central Horticultural Research Institute by going to the selected artichoke gardens in these provinces in order to be used in breeding studies. In this study, head weights (g), head diameter (cm), head heights (cm) and diameter/height ratios were determined with and without artichoke head bracts. Balıkesir population's average yield properties were determined as (with bracts) head weight 280,61g, head diameter 9,25 cm, head height 8.41 cm. Bursa population values were 232,67 g, 8,82 cm and 8,22cm and Bilecik ones 155,36 g, 7,57 cm, 7,50 cm, respectively. Artichoke breeding study, which includes these data, continues and it is aimed to determine artichoke clones with superior characteristics

Keywords: Artichoke, selection, breeding

INTRODUCTION

Artichoke is a vegetable from Astraceae family. Its origin has been reported as the Mediterranean basin and the island of Cyprus (Vural et al. 2000). Artichoke has been a known and consumed vegetable since ancient Greek and Roman times (Günay, 1993). The flower head and immature bractes of the artichoke are eaten. In Turkey, besides its fresh consumption, it is also consumed canned or frozen (Vural, 2000). 85-90% of the edible part of fresh artichoke consists of water. Artichokes are rich in minerals such as potassium, calcium and magnesium (Table 1). Table 1 shows the composition of 100 grams of artichokes (TURKOMP, 2021).

When peer in the Table 2, it is understood that, Italy is one of the countries that produce the most artichokes in the World. Italy is followed by Egypt and Spain, Turkey is in the 10th place with a production of approximately 39.000 tons (FAOSTAT, 2020).

While Italy produces over 370.000 tons, it varies by years, Egypt with about 300.000 tons and Spain follows with a production of over 200.000 tons (Table 2). Turkey lags behind with a production of 34,000 tons. In addition to the ecology of the country, consumption habits are also effective. When the production in Turkey is examined, it is understood that there has been an artichoke production of 30.000 – 35.000 tons for many years.

The underground stem of the artichoke plant is perennial. The dormant bud on the roots emerges after the first rains or watering in autumn and plant shoots are formed that will produce early next year. Artichoke has a wide and deep root system. Roots can go deeper than 120 cm in very good soil conditions. Artichoke plant is a type of vegetable that is generally grown by vegetative propagation. Therefore, production can be made with the bottom shoots that are driven from the eyes on the underground body (Abak, 1987). Although there are dormant bud in artichoke roots, production with bottom shoots is preferred because it is easier in the propagation stage (Günay, 1993; Vural vd., 2000). Ilbi et al. (2007), in a study they conducted, plant height (cm), plant width (cm), leaf color (L, a, b), leaf lobe (more or less), petiole thickness (cm), average head weight (g), height (cm), width (cm), the presence of anthocyanin in the bract, the harvest time and the number of harvested heads, including yield parameters such as 15 traits were assessed. In a study conducted

in Brazil, plant height, plant width, flower stalk length, main stem diameter, length and width of the largest leaf, number of side shoots formed after harvest, fresh weight, height and diameter of main head, and number of secondary heads were observed (da Costa et al. 2012).

Table 1. Consumption of artichoke (per 100 grams)

Component	Unit	Average	Component	Unit	Average
Water	g	89,22	Mg	mg	55
Ash	g	0,77	K	mg	425
Protein	g	2,46	Na	mg	65
Nitrogen	g	0,39	Zn	mg	0,53
Lipid, Total	g	0,2	Vit. C	mg	9,6
Carbohydrate	g	2,62	Thiamin	mg	0,042
Fiber, Total diet	g	4,74	Riboflavin	mg	0,04
Salt	mg	164	Niacin	mg	0,866
Fe	mg	0,88	Vit. B-6	mg	0,078
Protein	mg	51	Vit. A	RE	4
Ca	mg	99	Beta-caroten	µg	53
			Lutein	µg	73

Source: www.turkomp.gov.tr

Table 2. The main artichoke producing countries in the world and their production amounts (tons)

	Country	Production (ton)	
		2019	2020
1	Italy	378.820	367.080
2	Egypt	307.100	308.844
3	Spain	199.940	196.970
4	Peru	131.707	82.096
5	Algeria	119.636	126.762
6	Argentina	109.055	105.236
7	China Main Land	80.148	80.401
8	U.S.A	48.618	41.251
9	Morocco	44.774	45.012
10	Turkey	39.071	39.280

Source:www.fao.org/faostat

While “Sakız” type artichoke is grown in Aegean and Mediterranean regions, where the most artichoke cultivation is produced in our country, “Bayrampaşa” type artichokes are produced in ecologies such as the cooler Marmara region (TUIK, 2021).

Table 3. Artichoke production amounts by years in Turkey (ton)

Years	Production (ton)
2015	32.701
2016	36.368
2017	38.431
2018	39.477
2019	39.071
2020	39.280
2021	40.114

Source:www.tuik.gov.tr

Table 4. Provinces with the highest artichoke production in Turkey (2021).

Provinces	Production Amounts (Ton)
İzmir	11466
Aydın	8363
Bursa	6137
Sakarya	6100
Adana	1511
Bolu	1501
Antalya	1200
Konya	880
Balıkesir	799
Manisa	701

Source:www.tuik.gov.tr

Besides, foreign artichoke varieties produced with seeds have entered our country in recent years, it is seen that they are not preferred by breeders. These artichoke varieties are mostly grown by those who produce for industry. It is understood from the markets that those who consume fresh artichokes mostly prefer Sakız and Bayrampaşa type artichokes. Otherwise, farmers state that they do not prefer artichokes produced with seeds because they are hybrids and because of the necessity of seed renewal every year. Owing to the fact that, artichoke is propagated vegetatively, intraclonal selection methods are used more in breeding. Head features such as size, earliness and diameter were taken into consideration more as selection criteria (Şimşek, 1988 and Sürmeli, 1996). The study was conducted in order to determine some “Bayrampaşa” artichoke type heads features.

MATERIAL AND METHOD

The material of the study consisted of Bayrampaşa type artichokes to be collected from the provinces of Bursa, Bilecik and Balıkesir in the Marmara region. Artichoke plants collected from these provinces between 2019 and 2020 were planted on the Institute's trial realm. At least 20 plants of each artichoke type were examined and their head characteristics were determined. Assessed head properties were as head weights (g), head diameter (cm), head heights (cm) and diameter/height ratios.

The trial area is the artichoke plots in Yalova Atatürk Central Horticultural Research Institute. Artichokes were watered and fertilized by drip irrigation method.

RESULTS AND DISCUSSION

Evaluations were made by harvesting the artichoke plants that completed their development and formed the head. The data obtained as a result of the harvests are given in Table 5-7.

Table 5. Artichokes harvested in Balıkesir genotypes and some properties.

	Bractes Not Cleaned					Bractes Cleaned				
Artichoke Lines	Head Weights (g)		Average Head Diameter (cm)	Avg Head Height (cm)	Avg Diameter/ Height Ratios	Weight (g)		Avg Head Height (cm)	Number of Harvested Head	
	Mean	Total				Mean	Total		Mean	Total
Balıkesir 01	300,00	900	11,00	9,50	1,16	68,33	205,00	7,67	1,00	3,00
Balıkesir 02	308,33	925	10,00	9,50	1,05	126,67	380,00	8,33	1,00	3,00
Balıkesir 03	350,00	700	10,00	9,25	1,08	105,00	210,00	9,00	1,00	2,00
Balıkesir 04	302,50	1210	9,38	8,13	1,18	92,50	370,00	8,50	1,00	4,00
Balıkesir 05	421,67	1265	11,33	10,33	1,10	126,67	380,00	9,67	1,00	3,00
Balıkesir 06	221,67	665	8,33	7,33	1,15	76,67	230,00	7,50	1,00	3,00
Balıkesir 07	277,50	555	9,00	8,25	1,11	87,50	175,00	8,25	1,00	2,00
Balıkesir 08	272,50	545	8,75	8,50	1,02	85,00	170,00	8,25	1,00	2,00
Balıkesir 09	200,00	200	8,00	8,00	1,00	65,00	65,00	7,00	1,00	1,00
Balıkesir 10	287,50	575	9,00	6,75	1,32	100,00	200,00	8,25	1,00	2,00
Balıkesir 11	*145,00*	*145*	*7,00*	*7,00*	*1,00*	*50,00*	*50,00*	*6,50*	*1,00*	*1,00*
Mean	280,61	698,64	9,25	8,41	1,11	89,39	221,36	8,08	1,00	2,36
Min	130		7	5,5	0,875	40		6,5	1	
Max	510		12	11	1,42857143	180		10,5	4	

Line number 5 generally was shown the best performance in artichoke plants collected from Balıkesir province. Nearly all of the highest head characteristics were taken from Line 5. But Line 11 produced the worst results.

Table 6. Artichokes harvested in Bursa genotypes and some properties.

	Bractes Not Cleaned					Bractes Cleaned				
Artichoke Lines	Head Weight (g)		Avg Head Diameter (cm)	Avg Head Height (cm)	Avg Diameter Height Ratios	Weight (g)		Avg Head Height (cm)	Number of Harvested Head	
	Mean	Total				Mean	Total		Mean	Total
Bursa 01	277,50	555,00	9,75	8,50	1,15	82,50	165,00	8,25	1,00	2,00
Bursa 02	225,00	225,00	9,00	8,50	1,06	75,00	75,00	8,00	1,00	1,00
Bursa 03	127,00	381,00	8,00	7,00	1,13	60,00	120,00	7,25	1,00	3,00
Bursa 05	290,00	580,00	10,25	9,50	1,08	92,50	185,00	8,75	1,00	2,00
Bursa 06	210,00	210,00	8,00	8,00	1,00	70,00	70,00	7,00	1,00	1,00
Bursa 07	375,00	375,00	11,00	9,00	1,22	125,00	125,00	9,50	1,00	1,00
Bursa 08	210,00	210,00	9,50	9,00	1,06	45,00	45,00	6,50	1,00	1,00
Bursa 09	*50,00*	*50,00*	*4,00*	*5,00*	*0,80*	*20,00*	*20,00*	*3,50*	1,00	1,00
Bursa 10	276,67	830,00	9,83	9,33	1,05	75,00	225,00	8,33	1,00	3,00
Bursa 11	270,00	810,00	9,67	8,33	1,17	66,67	200,00	8,00	1,00	3,00
Bursa 12	283,67	851,00	9,83	9,17	1,07	70,00	210,00	8,33	1,00	3,00
Bursa 13	181,67	545,00	8,17	8,00	1,02	45,00	135,00	6,50	1,00	3,00
Bursa 14	181,25	725,00	7,88	7,75	1,02	45,00	180,00	6,38	1,00	4,00
Bursa 16	285,00	570,00	9,25	8,25	1,13	90,00	180,00	8,50	1,00	2,00
Bursa 17	188,33	565,00	8,83	8,67	1,02	51,67	155,00	7,33	1,00	3,00
Bursa 18	233,33	700,00	8,83	8,50	1,04	63,33	190,00	7,83	1,00	3,00
Bursa 19	288,33	865,00	10,17	8,67	1,18	75,83	227,50	8,50	1,00	3,00
Bursa 20	215,00	215,00	8,00	8,00	1,00	60,00	60,00	7,50	1,00	1,00
Bursa 21	285,00	570,00	10,00	9,00	1,11	80,00	160,00	8,00	1,00	2,00
Bursa 22	215,00	215,00	8,00	9,00	0,89	70,00	70,00	7,00	1,00	1,00
Bursa 23	200,00	400,00	8,25	8,25	1,00	47,50	95,00	6,75	1,00	2,00
Bursa 24	267,50	535,00	9,25	8,75	1,06	72,50	145,00	8,00	1,00	2,00
Bursa 25	95,00	95,00	5,50	5,50	1,00	30,00	30,00	5,00	1,00	1,00
Bursa 26	255,00	510,00	8,50	8,50	1,00	72,50	145,00	8,00	1,00	2,00
Bursa 27	155,00	155,00	7,00	6,50	1,08	45,00	45,00	6,50	1,00	1,00
Bursa 28	222,50	445,00	8,25	7,75	1,06	65,00	130,00	7,75	1,00	2,00
Bursa 29	322,50	645,00	10,50	9,50	1,11	80,00	160,00	8,50	1,00	2,00
Bursa 30	322,50	645,00	10,25	9,50	1,08	85,00	170,00	8,50	1,00	2,00
Bursa 31	205,00	410,00	9,00	7,50	1,20	52,50	105,00	7,25	1,00	2,00
Bursa 32	280,00	560,00	10,00	8,50	1,17	70,00	140,00	8,00	1,00	2,00
Bursa 33	220,00	440,00	9,00	7,50	1,19	62,50	125,00	7,50	1,00	2,00
Mean	232,67	480,23	8,82	8,22	1,07	65,97	131,85	7,51	1,00	2,03
Min	26,00		4,00	5,00	0,80	20,00		3,50	1,00	
Max	411,00		11,00	9,50	1,33	125,00		10,00	4,00	

Among the Bursa artichoke line, Line 7 was the best. The heights values in terms of mean of the not cleaned head weight (375 g), head diameter (11 cm) etc. were taken from Line 7. Line 9 gave the lowest values.

Table 7. Artichokes harvested in Bilecik genotypes and some properties.

	Bractes Not Cleaned					Bractes Cleaned				
Artichoke Lines	Head Weight (g)		Avg Head Diameter (cm)	Avg Head Height (cm)	Avg Diameter /Height Ratios	Weight (g)		Avg Head Height (cm)	Number of Harvested Head	
	Mean	Total				Mean	Total		Mean	Total
Bilecik 01	135,00	135,00	8,00	7,00	1,14	*25,00*	*25,00*	*4,50*	1,00	1,00
Bilecik 02	160,00	320,00	7,75	8,25	0,94	40,00	80,00	6,25	1,00	2,00
Bilecik 03	170,00	170,00	8,00	8,00	1,00	50,00	50,00	7,00	1,00	1,00
Bilecik 04	217,50	435,00	8,75	8,25	1,06	57,50	115,00	7,50	1,00	2,00
Bilecik 05	200,00	200,00	9,00	8,50	1,06	55,00	55,00	7,00	1,00	1,00
Bilecik 07	*80,00*	*80,00*	*5,00*	*5,00*	1,00	35,00	35,00	4,50	1,00	1,00
Bilecik 08	125,00	250,00	6,50	7,50	0,86	32,50	65,00	6,00	1,00	2,00
Mean	155,36	227,14	7,57	7,50	1,01	42,14	60,71	6,11	1,00	1,43
Min	60		5	5	0,83	20		4,5	1	
Max	245		9	9	1,14	75		8,5	2	

Although the highest results within Bilecik lines were taken from Line 4 and 5, the worst values were obtained from Line 7 and 1. The results of this study are similar to the research of Ilbi et al. (2007) and Sürmeli (1996).

CONCLUSIONS

According to this study, a few more years of studies are needed with artichoke plants collected from different provinces. Since artichoke is propagated vegetatively, it is very difficult to find a wide variation between lines. Although the variation found is not high, there are differences in artichoke plants between provinces.

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LIQUID STATE FERMENTATION USING *Lactobacillus spp.* AFFECT THE NUTRITIONAL COMPOSITION OF THE CAP PARTS OF *Agaricus bisporus*

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ABSTRACT

The effect of *Lactobacillus* spp. liquid state fermentation on the nutritional composition of *Agaricus bisporus* cap was investigated in this study. *Agaricus bisporus* was obtained fresh, and the cap part was separated from the stem part and cut into small pieces. Each 100 gram of cap was mixed with 400 ml of distilled water in the fermentation flasks. The mixture was enriched with 8.4 g urea (46% N) as a nitrogen source. The caps were separated into two groups, and the fermentation media was adjusted to two different pH values (6 and 7) by using 1 N HCl and 1 N NaOH. All mushrooms were sterilized at 121 °C for 15 min by autoclave and then inoculated with 1 ml *Lactobacillus* spp. (10^8 CFU/ml). One uninoculated group was also made for each pH level as a positive control. The inoculated mushrooms were incubated at 30°C for 48 hours. At the end of the fermentation, *Lactobacillus* spp. count, pH value, and crude protein and ash content were determined in raw, fermented, and uninoculated *Agaricus bisporus* caps. *Lactobacillus* spp. count was higher in the pH 6 group than in the ph 7 group. Similarly, liquid state fermentation increased the crude protein and decreased the ash content of *Agaricus bisporus* cap in the pH 6 group. However, no change was determined between uninoculated and fermented groups in the pH 7 group. Mushroom and liquid pH were lower ($P<0.05$) in the fermented caps compared with the uninoculated mushrooms in both pH groups. The obtained results showed that liquid state fermentation using *Lactobacillus* spp. improved the nutritional composition of *Agaricus bisporus* cap when the initial pH was six.

Keywords: Button mushroom, mushroom caps, Liquid state fermentation, *Agaricus bisporus, Lactobacillus* spp.

INTRODUCTION

Mushrooms have been considered important food sources since ancient times because of their nutritional values and therapeutic properties (Atila et al., 2021). The white button mushroom (*Agaricus bisporus*) is an edible Bacidiomycete fungus. *Agaricus bisporus* has important bioactive phenolic components such as cinnamic acid derivatives (cinnamic acid, p-coumaric acid, ferulic acid, and chlorogenic acid) and benzoic acid derivatives (p-hydroxybenzoic acid, protocatechuic acid, and gallic acid) (Ramos et al., 2019). Mushrooms also have strong antioxidant, immunostimulatory, anti-inflammatory, antibacterial, antiviral, and hypocholesterolemic effects on broiler chickens (Bederska-Łojewska et al., 2017).

Fermentation can be used to improve the nutritional composition of agricultural residues (Gungor et al., 2021). Fermentation is divided into liquid-state and solid-state fermentation. Solid-state fermentation refers a microbial growth in moistened solid substances without free water

(Gungor and Erener, 2020). However, liquid state (submerged) fermentation refers to microbial cultivation in the presence of plenty of free water (liquid medium) (Behera and Ray, 2019).

The objective of this study was to investigate the effect of the liquid state fermentation using *Lactobacillus* spp. on the nutritional composition of the cap parts of *Agaricus bisporus* at different pH levels

MATERIAL AND METHOD

Fresh *A. bisporus* was obtained from a local market at Samsun, Türkiye. The cap parts of *A. bisporus* were separated from the stalk parts. After cutting into small pieces, each 100 grams of mushroom cap was mixed with 400 ml of distilled water in the fermentation flasks. The mixture was enriched with 8.4 g urea (46% N) as a nitrogen source. The fermentation flasks were allocated to two pH groups (6 and 7), and the pH of the fermentation media was adjusted to 6 and 7 by using 1 N HCl and 1 N NaOH. Mushroom caps were sterilized at 121 °C for 15 min by autoclave.

Lactobacillus spp. were cultivated with MRS broth in a shaking incubator at 30 °C and 120 rpm for 48 hours (Kumoro and Hidayat, 2018). The sterilized mushroom caps were inoculated with 1 ml *Lactobacillus* spp. (10^8 CFU/ml). One uninoculated flask was also separated as a positive control for each inoculated flask. The inoculated mushrooms were incubated at 30 °C for 48 hours. At the end of the fermentation, raw, fermented, and uninoculated mushroom caps were analyzed to determine the *Lactobacillus* spp. count, pH value, crude protein, and ash content, according to AOAC (2000).

All data were analyzed by one-way ANOVA using SPSS 21.0 Statistics. The statistical differences between treatments were determined by Duncan's multiple range test. The level of statistical significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Lactobacillus spp. count was higher in the pH 6 group than in the ph 7 group. Similarly, the higher productivity of Lactobacillus spp. was observed at pH6 level compared with 6.5 and 6.75 pH levels (Aasen et al., 2000). Similar results were also reported from the study comparing the pH6 and pH8 levels (Tang et al., 2016).

Liquid state fermentation increased the crude protein content of *A. bisporus* cap in the pH 6 group. However, no change was determined between uninoculated and fermented groups in the pH 7 group. Similarly, *Lactobacillus* spp. was reported to increase the crude protein content of durian seed with submerged fermentation (Kumoro and Hidayat, 2018).

The ash content of the mushroom cap was also decreased ($P<0.001$) after liquid fermentation in the pH 6 group. Similarly, *Lactobacillus* spp. decreased the ash content of durian seed after submerged fermentation (Kumoro and Hidayat, 2018). The soluble minerals in the mushroom cap can be dissolved in the liquid medium during fermentation. This can be a reason of the decrease in the ash content of both uninoculated and inoculated cap.

Mushroom and liquid pH were lower ($P<0.05$) in the fermented caps compared with the uninoculated mushrooms in both pH groups. Lactobacillus spp. can produce lactic acid during submerged fermentation (Brinques et al., 2010). Decrease in the pH level can be due to the lactic acid production of *Lactobacillus* spp.

CONCLUSIONS

The obtained results showed that liquid state fermentation using *Lactobacillus* spp. improved the nutritional composition of *Agaricus bisporus* cap when the initial pH was six.

The fermentation conditions need to be studied to investigate the usability of the liquid state fermentation for improving the nutritional composition of mushroom cap.

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NUTRITIONAL COMPOSITION OF FERMENTED SOUR CHERRY KERNEL USING *Bacillus subtilis* IN SOLID-STATE FERMENTATION

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ABSTRACT

Sour cherry kernels are agricultural residues of fruit juice factories. Solid-state fermentation has been used to improve the nutritional values of agricultural by-products. The effect of *Bacillus subtilis* on the nutritional composition of sour cherry kernel in solid-state fermentation was investigated in this study. Sour cherry kernels were subjected to solid-state fermentation using *Bacillus subtilis* (ATCC 21556) for 48 hours. Sour cherry kernels were analyzed before and after fermentation to determine the crude protein (CP), ether extract (EE), ash, crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) content. *Bacillus subtilis* increased ($P<0.05$) the CP, EE, ash, CF, NDF, and ADF content of sour cherry kernel. However, the nitrogen-free extract was decreased ($P<0.001$) in sour cherry kernel after solid-state fermentation. *Bacillus subtilis* can be used to increase the crude protein, ether extract, and ash content of sour cherry kernel.

Keywords: Sour cherry kernel, Solid-state fermentation, Crude protein, *Bacillus subtilis*

INTRODUCTION

Sour cherry (*Prunus cerasus* L.) is a stone fruit from the Rosaceae family, which has reached 1.4 million tonnes of annual production worldwide (FAOSTAT, 2020). Cherry kernels are discarded after being separated from the fleshy parts in fruit juice factories (Yilmaz and Gokmen, 2013).

The fermentation method has received significant interest from researchers for detoxification and biotransformation of agricultural by-products. Fermentation can be divided into liquid-state (submerged) and solid-state fermentation. Solid-state fermentation (SSF) refers to the development of microorganisms within moistened solid substrates without free water (Gungor and Erener, 2020). The fermentation method can be used to improve the nutritional composition of agricultural products (Altop, 2019). *Bacillus subtilis* is used as probiotic in animal nutrition (Teng et al., 2012). *Bacillus subtilis* increased the crude protein and decreased the crude fiber content of sweet cherry kernel (Altop et al., 2020) and pomegranate seed (Güngör et al., 2020) in solid-state fermentation conditions. The effect of solid-state fermentation using *B. subtilis* on the nutritional composition of sour cherry kernel was investigated in this study.

MATERIAL AND METHOD

Sour cherry kernels were supplied from a fruit juice factory in Türkiye. Sour cherry kernels were fermented with *B. subtilis* in solid-state fermentation conditions, according to Altop et al. (2020). In brief, sour cherry kernels were milled to pass through a 2 mm sieve and enriched with the nutritional salt (glucose: urea: (NH4)2SO4: peptone: KH2PO4: MgSO4.7H2O = 4:2:6:1:4:1). Kernels were sterilized at 121°C for 15 min. *B. subtilis* were cultured in Tryptic Soy Broth and

inoculated to the sour cherry kernel at 10^{10} CFU per kg. Afterward, samples were incubated at 60 °C for 48 hours and dried at room temperature for six days till reaching %90 dry matter.

The dry matter, ash, crude protein, and ether extract of the raw and fermented sour cherry kernel were determined according to AOAC (2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were conducted according to Van Soest et al. (1991).

All experimental analyses were performed in triplicate. Data were analyzed with the Student t-test (SPSS 21.0 Statistics). Results were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Solid-state fermentation using *B. subtilis* increased ($P<0.001$) the crude protein content of sour cherry kernel. Similar to the results of the present study, *B. subtilis* increased the crude protein content of sweet cherry kernel (Altop et al., 2020), pomegranate seed (Güngör et al., 2020), and soybean meal (Dai et al., 2017). The microbial protein produced by *B. subtilis* during solid-state fermentation can cause an increase in the crude protein of substrates (Raimbault, 1998).

The ether extract content was increased ($P<0.001$) by *B. subtilis* solid-state fermentation. Similar to the results of the present study, ether extract was increased in pomegranate seed by *B. subtilis* solid-state fermentation (Güngör et al., 2020).

Solid-state fermentation increased ($P<0.001$) the ash content of sour cherry kernel. Similarly, *B. subtilis* increased the ash content of sweet cherry kernel (Altop et al., 2020) and pomegranate seed (Güngör et al., 2020). These results may be due to the relative increase of ash content because of the decrease in NFE content of sour cherry kernel by fermentation.

Bacillus subtilis decreased ($P<0.001$) the nitrogen-free extract of the sour cherry kernel. Soluble carbohydrates are the firstly-preferred carbon source compared to other nutrients for the microorganism (Papagianni, 2007). Similarly, *B. subtilis* decreased the nitrogen-free extract of the sweet cherry kernel (Altop et al., 2020) and pomegranate seed (Güngör et al., 2020).

The crude fiber, neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents of sour cherry Kernel were increased after solid state fermentation using *B. subtilis* in this study. *Bacillus subtilis* can produce cellulase in the conditions of solid state fermentation (Ritter et al., 2018). However, the structural carbohydrates were increased after solid-state fermentation in this study. Similar to the result of the present study, solid-state fermentation increased the crude fiber content of grape pomace (Gungor et al., 2021).

CONCLUSIONS

The results showed that solid-state fermentation using *Bacillus subtilis* can be used to improve the nutritional composition of the sour cherry kernel with increasing cruder protein, ether extract, and ash content. However, detailed studies need to be conducted to determine the reason for the increase in structural carbohydrates in the sour cherry kernel after solid-state fermentation.

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THE INVESTIGATION OF PHYLOGENETIC RELATIONSHIPS OF BACTERIAL NEUTRAL PAPAIN CYSTEINE PROTEASES

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ABSTRACT

Proteases break the peptide bonds of proteins to form amino acids and short peptides. Based on the difference of catalytic amino acids, these enzymes are divided into five categories as serine protease, cysteine protease, aspartate protease, metalloprotease and threonine protease. Among the proteases that make up 60% of the enzyme sales in the world, papain cysteine protease is one of the most widely used enzyme families for the meat tenderizing process in the food industry, and they generally work at a neutral pH (pH 5-8). Papain cysteine proteases are generally obtained from plants. However, recently, with the development of omics technologies, large number of bacterial whole genome sequences have been rapidly accumulated, thus it has been determined that these bacterial sequences include many papain cysteine protease enzyme sequences. The sequences from bacterial whole genome data automatically annotated in UniProt/TrEMBL database as unreviewed data. The aim of this study is to investigate the phylogenetic relationship of six bacterial papain cysteine protease enzymes having neutral theoritical pI derived from unreviewed amino acid sequences of UniProt/TrEMBL database using the MEGA11 program. Further analyses are planned to perform multiple sequence alignments, three-dimensional structure models and molecular docking analyzes of these enzymes.

Keywords: MEGA11, Papain, Cysteine protease, Meat tenderization

INTRODUCTION

Proteases, which are known to form amino acids and short peptides by breaking the peptide bonds of proteins, are an important class of enzymes. Considering the mechanisms of action of this class of enzymes, they are separated into four categories as serine protease (EC 3.4.21), cysteine/sulphhydryl protease (EC 3.4.22), aspartate protease (EC 3.4.23), metalloprotease (EC 3.4.24) and threonine protease (EC 3.4.2) (Hedstrom, 2002). Proteases, which make up 60% of the enzyme sales in the world, have a wide application area in many industrial processes such as food, detergent, medicine, leather, silk, chemicals and paper (Li et al., 2013; Theron and Divol, 2014).
Proteases are produced by various organisms such as animals, plants, yeast and bacteria (Jisha et al., 2013). Microorganisms (especially bacteria) are used as a source in industrial enzyme production due to the rapid growth and ease of gene manipulation (Ahmetoğlu et al., 2015; Annamalai et al., 2014). The majority of proteases are known to be neutral and alkaline. The pH range of bacterial proteases, frequently used in the food industry, is 5.0 to 8.0 (Rao et al., 1998).
The meat reconstruction technology has emerged for the evaluation and development of underutilized meat and by-products with low value (Gadekar et al., 2015). One of the biggest problems of reconstructed meat is that it retains its firmness even after the application of binding agents (e.g. salt and phosphate) and the heating of meat products (Istrati et al., 2008). To overcome this problem, it have been developed several meat tenderizing techniques including enzymatic treatment (Bhat et al., 2018). Among these, enzymatic meat tenderizing is more advantageous for reconstructed meat products since it can be easily fortified in this process. (Ha et al., 2012).

One of the most studied enzymes for meat tenderization is papain, a type of cysteine protease enzyme. Papain cysteine protease (EC 3.4.22.2) is derived from the latex of the papaya plant (*Carica papaya*), which is thought to play a physiological role in the protection of plants against insects (Konno et al., 2004). Papain cysteine protease is also produced by microorganisms including bacteria (Berti and Storer, 1995). Some studies have been reported on the application of meat tenderization process of the plant-derived papain (Botinestean et al., 2018; Naveena et al., 2004).

Rapid advances in biotechnology and protein purification capacities have encouraged the use of microbial (bacterial and fungal) sources for the production of proteases with greater specificity towards substrates, facilitating production in greater quantities and purity. Another obvious advantage is that, unlike plants, microorganisms are not seasonal and thus microbial enzymes can be produced at any time (Bekhit et al., 2014).

With the advances in omics technologies, whole-genome sequences of large quantities of bacteria and fungi have been rapidly revealed. Sequences derived from these data are automatically available as unreviewed data in the UniProt/TrEMBL database (The UniProt Consortium, 2021). Therefore, a wide variety of unreviewed protease sequence data can be obtained from the UniProt/TrEMBL database. This study aims to investigate the phylogenetic relationships and sequence analysis of six bacterial and neutral papain cysteine proteases. Phylogenetic relationship and sequence analysis were performed using amino acid sequences of six neutral papain cysteine protease enzymes from different bacterial species, which were unreviewed from the UniProt/TrEMBL database.

MATERIAL AND METHOD

Unreviewed 118 amino acid sequences of bacterial papain cysteine proteases derived from whole-genome shotgun (WGS) sequences were retrieved from the UniProt/TrEMBL database (The UniProt Consortium, 2021). The theoretical isoelectric point (pI) values of these bacterial papain cysteine proteases were determined by the ProtPram tool (Gasteiger et al., 2005) and six enzymes with neutral pI values from different bacteria were selected for the further analyses.

The analyses of phylogenetic relationships and sequence alignment of the six selected neutral papain cysteine proteases belonging to different bacterial species were performed via MEGA11 and Clustal Omega programs, respectively. For this purpose, the analysis of phylogenetic relationship of the six papain enzymes was analyzed via the maximum likelihood (ML) statistical technique involving 500 bootstrap replications and Jones-Taylor-Thornton (JTT) substitution model (Tamura et al., 2021). In addition, the comparison of the amino acid sequences of these enzymes was performed by the Clustal Omega program, which is a multiple sequence alignment method (Madeira et al., 2019).

RESULTS AND DISCUSSION

In this study, the six bacterial and neutral papain cysteine proteases were analyzed to detect the phylogenetic relationship and sequence resemblance using MEGA11 and Clustal Omega, respectively. For this purpose, the amino acid sequence of six neutral papain cysteine proteases in different bacterial species was selected among 118 UniProt/TrEMBL unreviewed data entries based on their pI values. The selected six neutral papain cysteine proteases were found to be *Pseudomonas viridiflava* protease (*Pv*Cys*)*, *Pseudomonas caricapapayae* protease (*Pc*Cys), *Pseudomonas coronafaciens* protease (*Pco*Cys), *Pseudomonas syringae* protease (*Ps*Cys), *Ethanoligenens harbinense* protease (*Eh*Cys) (The UniProt Consortium, 2021), and *Liberibacter crescens* proteaz (*Lc*Cys) (Leonard et al., 2012). Theoretical pI values of these enzymes were determined by the ProtParam tool, and they were shown to be in a range of 6.82-7.67.

The phylogenetic relationship of six neutral papain cysteine proteases was investigated with the maximum likelihood (ML) statistical method through the MEGA11 program (Lao et al., 1999). The amino acid sequence of the G protein from *Arabidopsis thaliana* was used as the output group. According to the results of molecular phylogenetic analysis, the amino acid sequences of enzymes from *Pseudomonas* were closer to each other, whereas papain enzymes from *Liberibacter crescens* and *Ethanoligenens harbinense* (*Lc*Cys and *Eh*Cys, respectively) had a more distant evolutionary relationship. Among the enzymes from *Pseudomonas*, the enzymes with the closest amino acid sequences were determined as *Pv*Cys and *Ps*Cys (Figure 1). The sequence similarity between *Pv*Cys and *Ps*Cys was found to be 96.47%.

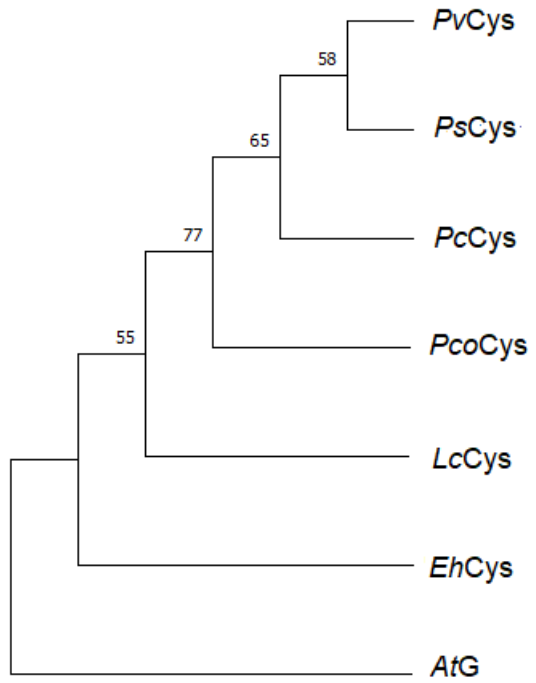

Figure 1. Phylogenetic tree of six bacterial and neutral papain cysteine protease enzymes. G protein from *Arabidopsis thaliana* (UniProt ID : Q9XIP7) was used as output group.

The amino acid sequences of six papain cysteine protease enzymes were compared through the Clustal Omega program, which is a multiple sequence alignment method (Madeira et al. 2019) and investigated in reference to the cysteine protease XCP2 (UniProt ID: UQ9LM66) from *Arabidopsis thaliana*. According to its automatically annotation via UniProtKB/TrEMBL, the active site amino acids of the cysteine protease XCP2 have been identified as C162, H298 and N318 (The UniProt Consortium, 2021). Multiple sequence alignment analysis showed that these active site amino acids were conserved in all the six bacterial and neutral papain cysteine protease enzymes (Figure 2).
A striking feature of papain cysteine proteases is the presence of the active site containing the catalytic triad amino acid (Cys-His-Asn) (Liu et al., 2018). Because of cysteine in the active site, these proteases have been included in the subclass 3.4.22 (Cysteine endopeptidases) (Amri and Mamboya, 2012). Moreover, active site residues including catalytic amino acids have been shown to be the most stable part of the three-dimensional structure of papain cysteine protease (Kamphuis et al., 1984).

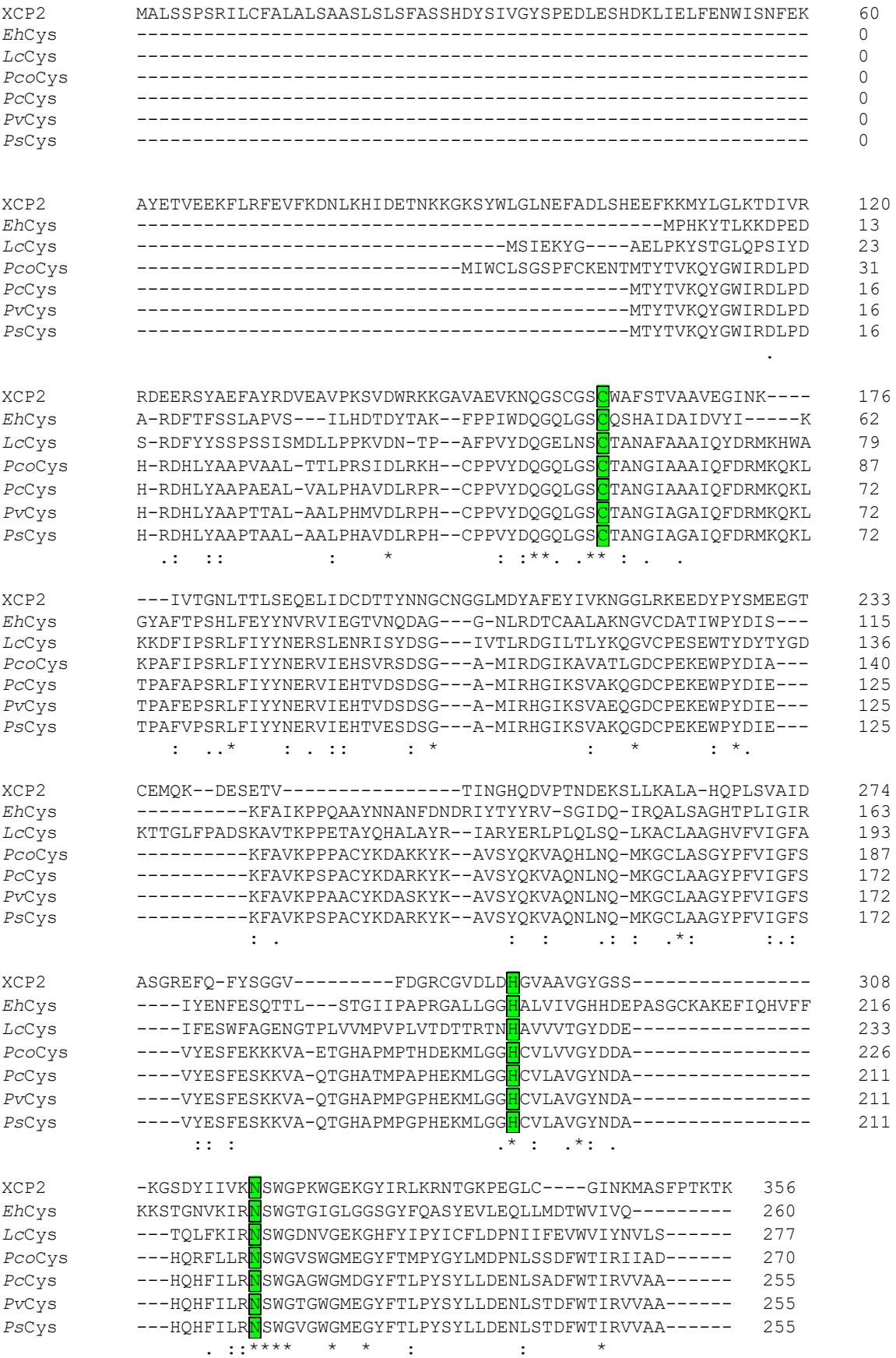

Figure 2. Multiple sequence alignment of six papain cysteine proteases. Amino acids marked in green indicate catalytic amino acids.

CONCLUSIONS

This study included the analyses of phylogenetic and multiple sequence alignment to determine the evolutionary relationship of six bacterial and neutral papain cysteine protease enzymes (*Pv*Cys, *Pc*Cys, *Pco*Cys, *Ps*Cys, *Eh*Cys and *Lc*Cys) and to compare their amino acid sequences, respectively. These enzymes were selected among 118 bacterial UniProt/TrEMBL unreviewed data entries and shown to have theoretical pI values in the range of 6.82-7.67. In addition, according to the results of molecular phylogenetic analysis, the enzymes with the closest amino acid sequences among the six papain cysteine proteases were determined as *Pv*Cys and *Ps*Cys, and their sequence similarity was found to be 96.47%. Multiple sequence alignment analysis results showed that the catalytic triad amino acid (Cys-His-Asn) responsible for catalysis in six papain enzymes was conserved in reference to the plant-derived papain enzyme sequence. Further analyses including three-dimensional homology models and molecular docking are planned for the six bacterial and neutral cysteine protease enzymes.

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A SOCIO-ECONOMIC STUDY ON THE INTERNATIONAL POTENTIAL AND COMPETITIVE CONDITIONS OF RICE PRODUCERS IN THE THRACE REGION: SAMPLE OF IPSALA DISTRICT

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ABSTRACT

Rice is an important product consumed as one of the main nutrients throughout the world. It is preferred as one of the basic nutrients also in Turkey, and rice consumption is increasing from year to year in Turkey. Along with the increasing demand for rice, there is an increase in rice imports. Turkey meets the need of rice with a high proportion of import. When the international potentials of domestic rice producers are examined, it is seen that their relations are oriented towards imports. Due to the high amount of imports, domestic producers have to compete with foreign enterprises in the domestic market as well as in international markets. The domestic rice producers in Turkey need to be aware of their international potential and develop competitive strategies in order to cope with tough competitive conditions. For this purpose, 100 Keşan Chamber of Commerce and Industry (represents İpsala, Enez and Keşan districts.) members who participated in the research were asked questions to learn their international potentials and competition strategies. Thrace region is the leader at the rice production in Turkey, and most of the rice producers are located in Edirne. Therefore, data obtained from the sample of the study provides current information about the positions of the rice producers in Turkey. With the study, it has been tried to analyse the international economic potential of the rice producers in Meriç Plain and bring out a socio-economic review about competition conditions in the sector. In this study we see that, even though rice producers in Meriç plain mostly do not think the competition in the sector is increasing tightly, they are aware of the trouble and getting necessary actions in order not to lose their positions in the sector.

Keywords: Rice, Production, Competition, Import, Economic Potential

INTRODUCTION

Rice is grown in all regions of Turkey but has the largest cultivation area and most production quantities in Marmara and Black Sea regions, respectively. In Turkey, paddy cultivation is basicallydone in Thrace region, South Marmara segment of Marmara region, in some parts of the Black Sea region and in South-eastern Anatolia region. On the other hand, the share of total production of paddy cultivation in other regions of Turkey do not exceed 10% (Özturk et al., 2010). Cultivation is very low in Aegean and Eastern Anatolia regions and Turkey's production is not enough for domestic consumption, therefore Turkey has been importing rice at increasingly levels. Rice imports have increased rapidly in recent years and have even exceeded domestic production.

According to the second estimation results of 2010, the paddy cultivation area in İpsala district is 19 500 ha and the total paddy production amount is 175 500 tons (Anonymous, 2011). 42,76% of the total paddy production (410 436 tons) in Edirne province is obtained from İpsala District (Bellitürk et al., 2012; Tokatlı and Varol., 2021).

İpsala District is a region located on the Meriç Plain and known by fertile agriculture lands and rich natural water resources. Due to the characteristics of the region, agriculture is the fundamental economic activity and thanks to wide natural water resources, the region is one of the most important agricultural areas of Turkey (Varol and Tokatlı.,2022).

There are many agricultural products produced in the region, but the most important is probably rice. Since land conditions and natural resources are so convenient for paddy cultivation, an important amount of rice production can be provided in the region, which is approximately equal to 35% of Turkey's total amount of rice production (Arda et al., 2015; Tokatlı, 2021; Varol and Tokatlı.,2021). The most popular agricultural activity in the region is paddy cultivation with a great contribution to the total rice production of Turkey. Rice producers in the region sell their products not only to domestic markets but also abroad. When considered that selling prices of rice is relatively better than other agricultural products, this economic opportunity, of course, brings up an increasing competition among producers. Paddy plant is a product with high yield compared to other cereal products and offers the opportunity to gain high profit in international markets (Taşlıgil and Şahin., 2011). In addition to these features, rice is a basic nutrient for more than half of the people living in the world, so it makes rice a very advantageous product to produce due to high demand and high international trade potential (Güngör and Şapaloğlu, 2016). These advantages increase the interest of world countries to rice. United States, one of the leading countries in the industrial area of the world, is located in the forefront of the countries that Turkey import rice. Turkey provides the large portion of rice needs from imports. Although having a suitable climate and soil conditions for rice production, Turkey's rice production level does not meet even the needs of the country (Gümüş, 2007) and this situation adversely affects the economy of Turkey.As it is known, since it is not possible to increase agricultural areas, it is necessary to use the existing resources more economically and effectively in order to meet the food demand of the increasing population. For this reason, it is necessary to increase the productivity and income per unit area. Productivity increase can be achieved with the correct execution of new technology and agricultural practices, and income increase can be achieved by reducing production costs (Şişman and Bilgin.,2016).

Rice produced domestically is more preferred by consumers living in Turkey, but there is no production at enough levels (Şapaloğlu, 2015). The reasons should be discussed why the production level of rice is inadequate in Turkey, while there is a high demand and suitable conditions for production. In order to solve the problem, the current situation should be analysed and appropriate solution suggestions should be developed. Turkey's international rice trade figures are given in Table 1. The data obtained for the year 2018 are from the first 9 months of 2018.

The data in Table 1 reveals the size of Turkey's import and export figures of rice. The increase in the total consumption of rice together with the decrease in the production, increases the dependence on foreign sources. This increase in rice imports adversely affects the balance of payments. In order to mitigate these problems, domestic producers should review their international potential and determine competitive strategies.

Table 1. Rice Import and Export Volumes of Turkey

Years	Amount of Import (ton)	Value (thousand $)	Amount of Export (ton)	Value (thousand $)
2014	191.067	136.414	19.467	19.261
2015	119.830	78.026	24.065	23.122
2016	73.046	36.826	45.812	35.956
2017	148.608	77.687	49.072	36.488
***2018**	186.963	109.547		

Source: www.zmo.org.tr; *First 9 months of 2018

When the data in Table 1 is analysed, a big difference between the exported and imported rice quantity of Turkey is observed. According to the table, which reveals Turkey 's international potential on rice product, Turkey has an increasing momentum in rice export, from year to year. However, when the figures of the last five years are analysed, it is seen that the amount of rice imports is much larger than the number of exports in each period. In the study, in order to analyse the international export potential of rice product, the producers were asked whether they have any activity in any country outside Turkey. Producers activating internationally were asked, with how many countries, how long they have been in operation and how many staff they have carried out for their international activities. In line with the questions asked, it was aimed to reveal the international potential of the rice producers in the Thrace region.

When Table 1 is examined, it is seen prominently that rice export companies in Turkey face with tough competitive conditions also in the domestic market. The surplus in the amount of imported rice also affects the competitive conditions in the sector. In order to survive in the national and international market, enterprises should take into account the conditions of competition. In the light of the data obtained from the research sample, the information received about the opinions of the companies about the existence of competition in the sector and which techniques they use in the struggle against competition.

MATERIALS AND METHODS

In this study, which conducted to investigate the perspectives of the members of the Agricultural Enterprises of Keşan Chamber of Commerce and Industry(represents İpsala, Enez and Keşan districts.) to competition conditions, the methods they apply to beat the competition and their potential in international trade; the data of 100 agricultural enterprises that are members of the Keşan Chamber of Commerce and Industry obtained byface-to-face survey method in the year 2019.A survey form has been created in order to obtain data about the period of working life of the paddy producer enterprises in İpsala district, the number of personnel in these enterprises, whether they are engaged in foreign activities, the duration of their international activities, and the number of personnel working in international activities. The research material is the data of the information obtained through this survey. These data were obtained by mutual interview method. In the research, the socio-economic competitive situation of paddy farming in the region was examined on the basis of producers. In the survey, in order to learn the perspectives of the enterprises on the competitive situation in their sectors and the methods of combating competition, Likert type questions have been directed to the participants. The data obtained has been analysed by frequency analysis method and presented using a pie chart. In line with the data obtained, the current potential of the enterprises in the international market and their strategies for competition have been revealed.

In the research, a socio-economic review was performed in terms of competition by using some systematic data collection techniques and some questions were asked to the rice producers to understand whether they have any activity outside Turkey. With the information's obtained, it has been tried to understand about the international marketing opportunities of the sector and determine the international economic power and potential of the producers. In this study, some questions were directed to the rice producers living in İpsala and Keşan and the results of the quantitative data collection technique was analysed

RESULTS AND DISCUSSION

Thrace Region is an important region where agriculture and agro-industrial sectors are common. Although the climate and soil conditions are suitable for the cultivation of many products; wheat, sunflower and paddy are among the products that are widely and economically produced in terms of cultivation area and production.

In this section, the findings collected from the paddy producers in İpsala district through the survey are evaluated under various topics. The reason for conducting a survey with the producers is to investigate whether they have any activity outside of Turkey for rice. With the information obtained, it has been tried to understand the international marketing possibilities of the sector and to determine the international economic power and potential of the producers.

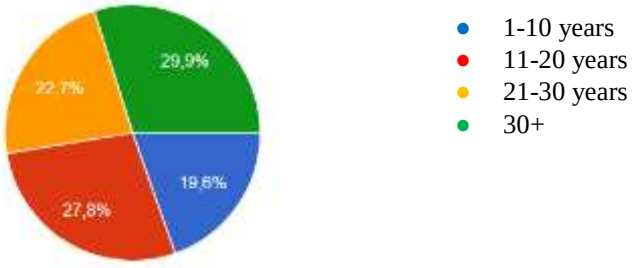

Figure1. How long your business is operating actively?

In Edirne province, the district with the highest rate of employees in the agricultural sector is Meriç district with 85,12%, followed by İpsala with 76,42% and Lalapaşa with 72,05% (Dinçer et al., 2003).

Paddy farming is carried out by 7925 producers in 7 districts and 91 villages in Edirne. Although the average paddy land per producer in the province is 31 decares; there are large paddy lands belonging to a single family or to several siblings from a family, especially in İpsala region (Oğuzhan and Özalp, 2005).

Meriç Plain, which has extensive land and water resources, is known as "Agricultural Land" and is considered one of the most important agricultural regions of Turkey. This region constitutes the largest rice cultivation area in Turkey and makes a great contribution to rice production (Helvacıoğlu et al., 2016).

The businesses operating in the region have generally been transferred from father to son and their existence has continued among the brothers. The closeness of the proportional distribution of the existence of the enterprises in terms of years is due to the brothers' desire to open new businesses for themselves.

Figure 2. How many personnel are working in your business?

It seems that most of the rice production firms in İpsala are small firms with less than 30 staff (with a big percentage of 75,8%). Moreover, nearly half of the firms are working with less than 10 staff.

The population in agricultural enterprises undertakes many tasks from the management of the enterprises to the labor force (Özüdoğru, 2010). The fact that the family structure in the regions where rice farming is carried out is suitable for this type of agriculture (usually there is no extended family structure, the families are in the form of nuclear families, the number of children is low and the number of people who can participate in the workforce in the family is low) provides significant advantages in terms of rice farming in those regions (Öztürk and Akçay, 2010). In this context, the fact that family members take part in agricultural enterprises in the region and undertake many tasks, also keeps the number of employees in the enterprises limited. Businesses generally use seasonal workers.

Figure 3. Is there any country that you are operating outside of Turkey?

As seen in Figure 3, 13% of the producers operate outside of Turkey. Since the paddy production of our country cannot meet the consumption, a certain amount of paddy or rice is imported almost every period. Turkey has been a paddy and rice importer since 1984 (Hekimoğlu and Altındeğer, 2019). Paddy import reached 409 thousand tons in 2010, the highest level of the last 16 years. In 2017, around 165 thousand tons of paddy was imported. Due to the record production in the 2019/20 market year, the rice competence level rose to 84,9%, while the share of imports in supply decreased to 28% (Anonymous,2021). Since paddy production in Turkey cannot meet rice consumption, a certain amount of import is actualised in every period to close the deficit (Yazıcı, 2020). Rice consumption per capita in Turkey has been increasing over the years. Although rice production in Turkey increases every year, the level to meet the consumption has not been reached yet. Therefore, imports are actualised every year to meet the needs of the domestic market. The ratio of production to consumption has increased over the years. While the ratio of production to meet consumption was 42,2% in 2000, this ratio reached 70,6% in 2016.The increase in the competence ratio during the period reached approximately 67,3%. Although rice production in Turkey has increased in the examined period, production cannot meet the consumption due to increase in both population and consumption per capita, so the imports still continue (Yılmaz and Avkıran, 2019). This need also affects the exports of the producers in the region.

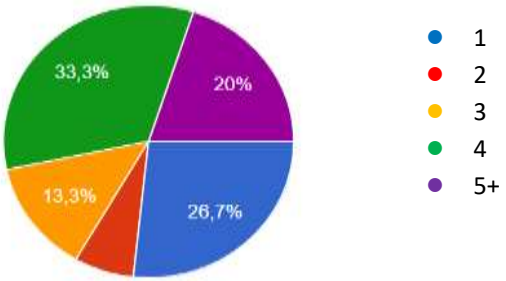

Figure 4. What is the number of countries that you are operating outside of Turkey?

In Figure 3 and Figure 4, there are graphics related with the questions trying to understand the competition conditions and expectations of the rice producers.

According to the answers given, it was understood that only a small ratio of producers have any activity outside of Turkey (13%) but from those having outside activity, 66,6% of them is working with 3 or more countries. Although Turkey is an importer country in rice, it is exported a little bit over the years (Yazıcı, 2020).

The main reason why paddy farming has become widespread in Edirne and has been cultivated for many years is that the Meriç and Ergene rivers are important water sources for paddy production. The production of the paddy plant, which has a higher monetary return per unit area than other products, is intensively made especially in the Meriç plain in İpsala, Meriç and Uzunköprü districts. Most of the 45 factories where the produced paddy product is processed into rice are gathered in Uzunköprü, Keşan, İpsala and Meriç districts, and create an important employment area and source of economic vitality in the region (Anonymous, 2005).

Although our country is an importer of rice, it has been started to export to Middle Eastern countries within the scope of Inward Processing Regime (DIR) in recent years. In this context, 14 thousand tons of rice was exported in 2009 and 52 thousand tons in 2010 (Anonymous, 2013). A significant part of the export is in the form of processing the paddy imported from the USA and Russia and selling it to countries such as Syria, Libya, Iraq and Egypt. In other words, it is an export in the form of re-export.

Except some most of the paddy factories operating in the Thrace region have started the production of new products by establishing additional facilities due to the decrease in competition and production. The majority of the factories in the Thrace region focus on the production of sunflower oil and compound feed. For this reason, there are only about 20 enterprises dealing with paddy processing literally, from 36 factories operating throughout Thrace. These enterprises are not satisfied with the paddy produced in their regions and they also process the paddy imported sometimes (Erdem, 2012). By this way, they export to various countries.

Figure 5. Since when do you have international activity?

According to the graph above, it is also seen that 76,9% of these international companies maintain their international activities more than 4 years. This shows that, the companies in international activity are quite experienced.

Figure 6. What is the number of staff working internationally in your business?

As seen in Figure 6, in 72,2% of the producers, just a manager is working internationally. This ratio is followed by 16,7% which indicates producers having 4 personnel working internationally.

Figure7. Competition in our industry is increasing day by day

It is shown in the graph that, only 45,3% of the producers agree with the opinion that competition in the sector is increasing tightly, others are either unstable or disagree.

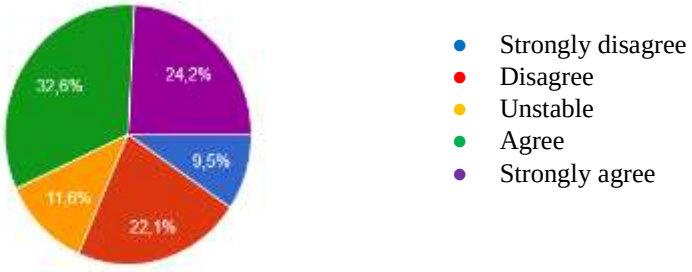

Figure 8. Due to increasing competition conditions, we are trying to gain competitive advantage by reducing our costs.

In Figure 8, it is seen that more than half of the producers try to gain competitive advantage by reducing their costs due to the increasing competitive conditions, whereas 31,6% of them do not make such an effort.

Figure 9. Due to increasing competition conditions, we are trying to maintain our relationships with our customers on a long-term basis by placing greater emphasis on customer relationships.

It is shown in the Figures 8-9 that, 56,8% of the producers are trying to reduce their costs and 58,7% of the procuders started to place greater emphasis on customer relationships in order to take precaution for increasing competition. The ratios are similar to each other, probably these are the same firms aware of the problem and trying to do something to avoid the trouble.

Figure10. Due to increasing competition conditions, we are trying to increase productivity by using new technological applications.

Turkey has the potential to become self-sufficient in terms of paddy production and even to become a net exporter country. In terms of both efficiency and the technology used, paddy producers are strong enough to compete with foreign producers (Sezer et al., 2012). As can be seen from Figure 10, producers generally care about productivity increase and follow new technological applications that will increase productivity.In a similar manner, in the research conducted by Kudal (2019) in Edirne's İpsala District, when the rice farmers were asked how often they meet with the experts on any subject related to their activities, 57% of the producers answered "at least once a month", 29% answered "once every three months" and 14% gave the answer “rarely”.These data show that more than half of the paddy producers give importance to expert knowledge about their activities.

It is seen that only 27% of the producers either do not have any effort to use new technological applications or unstable about the issuein order to avoid increasing competition conditions.

CONCLUSION

In the second graph, it is seen that most of the companies are operating with less than 10 persons which shows that most of these companies are small firms. Similarly, it is seen in the sixth graph that, in 72,2% of the international companies, just the manager works internationally, this is because these firms are quite small firms and do not have qualified staff at enough level. These datas actually show, although ratio of producers in international activity is quite less (just 13%), those entering international markets could be successful and spread over many different countries.

İpsala is just 4 kilometres away from Greece, in other words European Union. So, there is an important international potential for rice producers in İpsala District, but it is understood that, most of them cannot use this opportunity due to lack of qualified staff.

In the question number 7, it has been realised that more than half of the producers do not think the competition is increasing tightly. However, in next questions, it has been seen that most of the producers attempt to decrease costs in order to take an advantage in the competition.

In a similar manner, it has been understood in the next answers of producers that most of them are also giving more importance to customer relations and trying to increase productivity by using new technological methods in order to avoid increasing competition.

These results show us that, even though rice producers in Meriç plain mostly do not think the competition in the sector is increasing tightly, they are aware of the trouble and getting necessary actions in order not to lose their positions in the sector.

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FORECASTING OF DEMAND OF WATER FOR AGRICULTURE SECTOR IN LIBYA

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ABSTRACT

One of the main objectives of econometric modelling is to predict what is going to happen in the future forecasting means that the model will solve its endogenous variables beyond the originally utilised set of data. This forecasting exercise needs the predetermined variables of the model for the forecasting period. The major constraint on Libya's agriculture sector is water because of low rainfall, scarce renewable water resources, and increasing water demand because of increasing population. Most Libyan farms depend on bore-wells. The arid nature of much of the country's territory means irrigated farming systems are of decisive importance Non-renewable groundwater from desert aquifers in southern Libya provides the bulk of Libya's water needs. Libya is mostly arid and semi-arid and covers a total of 1,759,540 square kilometres. The cultivable area of the Libyan state is estimated to be about 2.2 million hectares, that is 1.2% of the total area Groundwater is the main source of fresh water in the country. More than 80% of agricultural production is from irrigated lands. Also, under conditions of the rapid growth in the population, water demand exceed 83% of the total annual consumption Rapidly increasing population in many parts of the world, Libya is mostly arid and semi-arid covers a total of 1,759,540 square Kilometres, The cultivable area of the Libyan state is estimated to be about 2.2 million hectares(1.2% of the total area Groundwater)

Keywords: Libya. Forecasting Agricultural Water

INTRODUCTION

A forecast is a conditional statement about the future. It is about what is expected to happen in the future. If various assumptions turn out to be valid, there are objective and subjective components involved in forecasting. The objective component consists of explaining past levels and patterns. While the subjective component is the application of the resultant knowledge the future (Bolan, 1984; Prasifka, 1988). There are several terms that are similar. However, they do not have exactly the same meaning as the words 'forecast' and 'prediction'. Prediction is used in a more general way than forecast. Predication is a statement about the future, whether or not conditional. Forecast may be regarded as predictions, but not all predictions are forecasts. Many forecasts rely on a set of assumptions, which include the continuation of at least some past trends and/or relationships. Such forecast is called projections (Lawgall, 2009) depending on the forecasting method employed. of Libya is covering a total of 1,759,540 square kilometres. Of with the arable area is estimated to be about 2.2 million hectares (1.2% of the area). Groundwater is the main source of fresh water in Libya. More than 80% of agricultural production depends on irrigation (Aman, 2011). The population in North Africa is growing rapidly and as we know the growing demand for water from the increasing population is of two types, direct and indirect. The direct demand arises from need of water for drinking and cooking. The other human needs for water arise from human activities such as agriculture and industry, (FAO, 2011) Water resources are very unevenly distributed among the countries of the world. While some countries have an abundance of water, many others must manage under the conditions of extreme scarcity. Also, some countries may appear to have abundance of water, but often that water is not accessible or not close to the cultivable lands or is very expensive to exploit.

Source: http://www.ewpnet.com/libya/river.htm

Figure (1) Map of Libya

Time series Definition

Econometrics is used to estimate the economic relations and is interested in testing their conformity to reality and predict its path in the future. This involves collecting, recording, and tabulating data and views (AL fakari, 2016). A time series definition is a set of data for a given variable over a given period. For example, the time series (Ẏt……1……2), the data for the variable (Y t) are shown during the period (t ....), considering that (Y t) is a value of (Y) when the value of t = 1. Time series is important in predicting the behaviour of the variables in the future. The study of the behaviour of economic variables such as production, consumption, investment, and others are useful in drawing up future policies. Most of the time series we deal with are non-static, and the reason is that one of the key elements of these strings is the arithmetic mean, the variance and the common variation may be unstable and change according to the time., A time series model for the observed data {Xt} is a specification of the joint distributions (or possibly only the means and covariances) of a sequence of random variables {Xt} of which {Xt} is postulated to be a realization. Stationarity and Unit Root Tests.

Classical econometric literature assumes and requires that all the variables should be stable over time and stationery. The desired properties of a stationary series are described as; i) constant mean; ii) constant variance; and iii) constant autocovariances for each of its given lags. Stationarity is considered a major test for considering the stability and degree of reliability of the results presented under various econometric methods (Brooks, 2008). The literature on the subject shows the use of non-stationary series

MATERIAL AND METHOD

Using time series for analysing the data from (1975-2014) and investigating the factors that have the maximum impact on demand for water demand such as the price, income, population, and the temperature, The main aim of this study is forecasting of demand of the water in Libya for (agriculture, water use) using the time series analyzing on Eviwes 10 software from 1975 -2014 and the forecasting until 2050. Using ARIAM model. **WITH Two Hypotheses we use for this paper:** Null hypothesis HO: variable is not stationary or got unit root, Alternative H1: variable is stationary the first method which can be used to check stationarity of the variables is to graph the series. Using the augmented Dickey-Fuller (ADF) test for unit roots all variables are difference,

the log A (**agricultural water use**) will be stationary at the level with intercept, T-statistic= -3. 88 more than -2.93 at 5% then **we can** reject the Null Hypothesis H0 and accept the alternative hypothesis.

ARDL Model OR Bound Test Autoregressive distributed log model

APPROACH: This research uses the Autoregressive Distributed Lag (ARDL) bounds test approach proposed by Pesaran et al. (2001) to estimate the long-term trade model in equations 33-34. The main advantage of this technique over the other cointegration methods is that it is robust enough to cope with the stochastic behaviour of the variables and has no underlying assumptions about the order of integration of the variables. This approach avoids the pre-testing problem associated with the order of integration of variables that standard cointegration techniques encounter. It also allows the researcher to distinguish between the short-term and long-term effects of the variables, which is important in economic analysis (Pesaran et al., 2001). An ARDL model includes the lags of the variables in the model. Lags are presented in economic models for different reasons. The economic lags consist of the recognition lag, the decision lag, and the effect lag. Models that include these lags into the analysis are very attractive for economic analysis (Brooks, 2008). One of the most applicable models of this kind is the error correction; the error correction model is a short-run dynamic model consisting of the first difference of the variables and an error correction term. It is a modified version of an ARDL model. In an ARDL model, the dependent variable y is a function of its own lag and other independent variables and their lags. A general ARDL (1,1) model can be written as follows:

$yt = \alpha 0 + \alpha 1 yt{-}1 + \alpha 2 xt + \alpha 3 xt{-}1 + ut$ (1)

This general model above can easily be transformed into an error correction model by subtracting yt-1 from both sides of the equation and using the first difference of x (Δxt): $\Delta yt = \alpha 0 + (\alpha 1 - 1)\, yt{-}1 + \alpha 2 \Delta xt + (\alpha 2 + \alpha 3) xt{-}1 + vt$ (2) Furthermore, in order to use the error correction (ECM) model, the following assumption is required:

$(\alpha 1 - 1) = -(\alpha 2 + \alpha 3)$ (37) Or $(\alpha 1 + \alpha 2 + \alpha 3) = 1$

LWA= +LPOP+LPRIC_AGR +LTEMP+L INCOME (1.1)

Where LWA = log of agricultural water use.

LPOP= log of population.

LPA = log of price of agricultural water use.

LTEMP = *log of temperature.*

L INCOME= log of income

Estimated Agricultural Water Use Using ARDL Model.

R-squared is Hight 0.99 % probe (f-statistic) is 0.0 less than 5 %, this model should be free form (serial correlation, the residual should be normal distrusted, and heteroskedasticity).

Table (1): The result of ARDL Model for Agriculture water use.

R^2	Serial correlation	Normal distribution test	Heteroskedasticity Test: Harvey	ARDL Long Run and Bounds Test	Selected Model ARDL
0.99	0.11	0.34	0.31	F- Statistic 6.129	(1.0.0.3.1)

EC=LWA-(3.5400*LTEMP+ 2.7080*LPOP-1.9282*LPA+0.4685*LINCOME

The first check in the model is serial correlation if the Probability Chi-Square is more than 5%; then we cannot reject the null hypothesis, the null hypothesis is the variable not serial correlation, in our model probability. Chi-Square = 0.11 or more than 0.005, so, we cannot reject the null hypothesis. So, we accept our null hypothesis; the model is free from serial correlation. The second step is to check the normal distribution using the Jarque-Bera test. If the probability more than 5% (Probability=0.34), that means the residuals series has normal distribution, and the last check is the heteroskedasticity

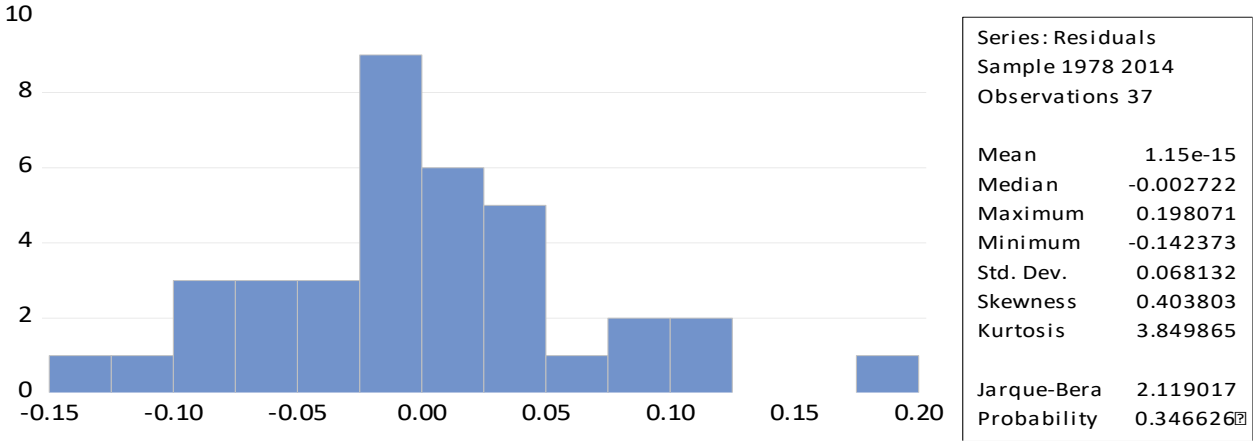

Figure (2): Residual Normal Distribution Test

For checking the long relationship among the variables, that is whether variables have a relationship, if the F statistics exceeds the upper bound, we can reject the null hypothesis and accept the alternative. The hypothesis here F – statistic = 6.12, that is, more than the bound test at 1%,5%, and 10%. Therefore, we can reject the null hypothesis and accept alternative hypothesis That means the variables have long relationship. Then using the cusum and cusum of squares test to check the stability by observing whether the blue line
goes out two red lines that mean the agriculture water consumption does not has relationship in the short time in 2003 the blue line goes out of two red line.
The first cusum test for stability shows the result that our model has not stability. The second test for check the stability is (cusum of squares test) The result is good because the blue line is between two red line.in the long time

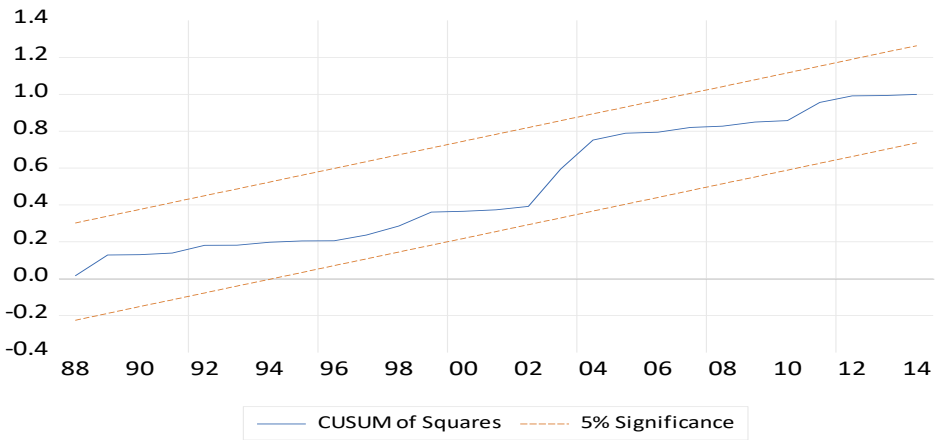

Figure (3): Stability Test (Cusum of Squares Test).

Estimation and Forecasting Using Box-Jenkins Modelling

One of the main objectives of econometric modelling is to predict what is going to happen in the future; forecasting means that the model will solve its endogenous variables beyond the originally utilised set of data. This forecasting exercise needs the predetermined variables of the model for the forecasting period.

ARIMA Forecasting Models

The Box-Jenkins approach to modelling Autoregressive Integrated Moving Average (ARIMA) processes was described in a highly influential book by statisticians George Box and Gwilym Jenkins in 1970. An ARIMA process is a mathematical model used for forecasting Box -Jenkins modelling involves identifying an appropriate ARIMA process Fitting it to the data, and then using the fitted model for forecasting. This section examines the stationary and nonstationary time series by formally testing for the presence of unit roots. Various Box-Jenkins Autoregressive Integrated Moving Average (ARIMA) models are estimated over the period 1975–2014 for demand for agricultural domestic and industrial use. The ARIMA models provide a useful framework understand how the water time series is generated. The ARAMA approach requires a water demand time series to be tested for no stationarity prior to undertaking estimation and forecasting if a series is not stationary (that series has a mean and variance that are not constant over time), the series has been to difference to transform it to stationary series, before generating forecasts.

Estimation of The Arima Model

Using the Best Fitting Model during the period 1975–2014. The best fitting ARIMA models are estimated separately for the water demand series from 1975 to 2014 and the test indicates the ARIMA for agricultural water are (2.1) (0.0) and the lowest AIC (12.39)

ARIMA Forecasting for Agricultural Water Use

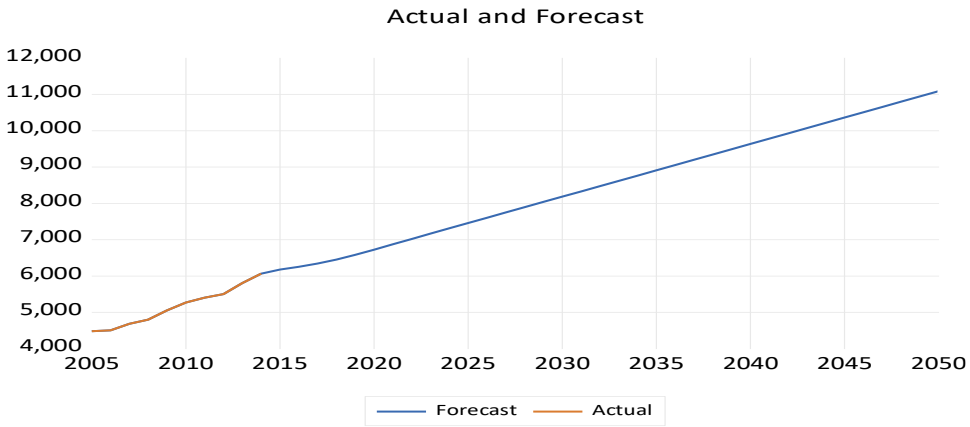

Figure (4): Actual and Forecast for Agricultural Water Use

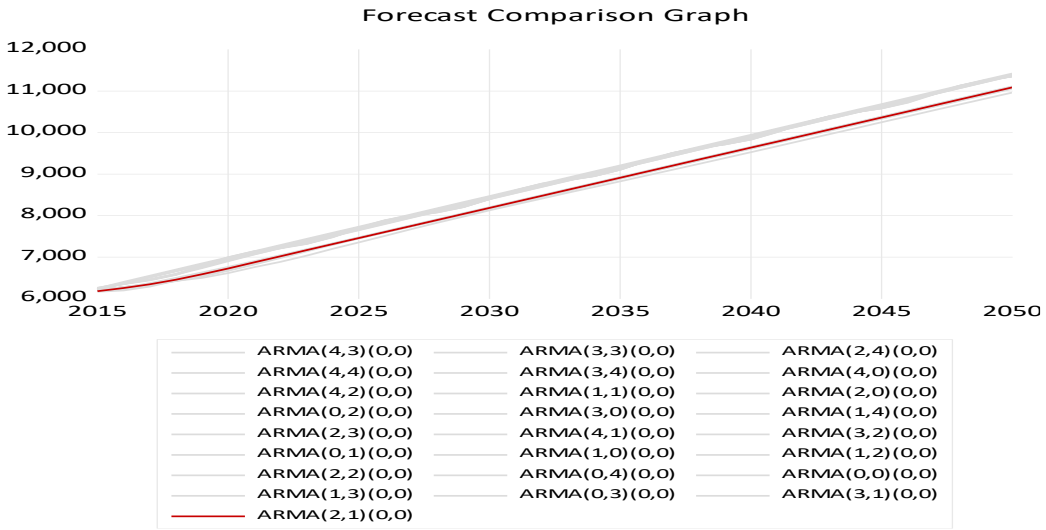

Figure (5): ARMA Forecast Graph

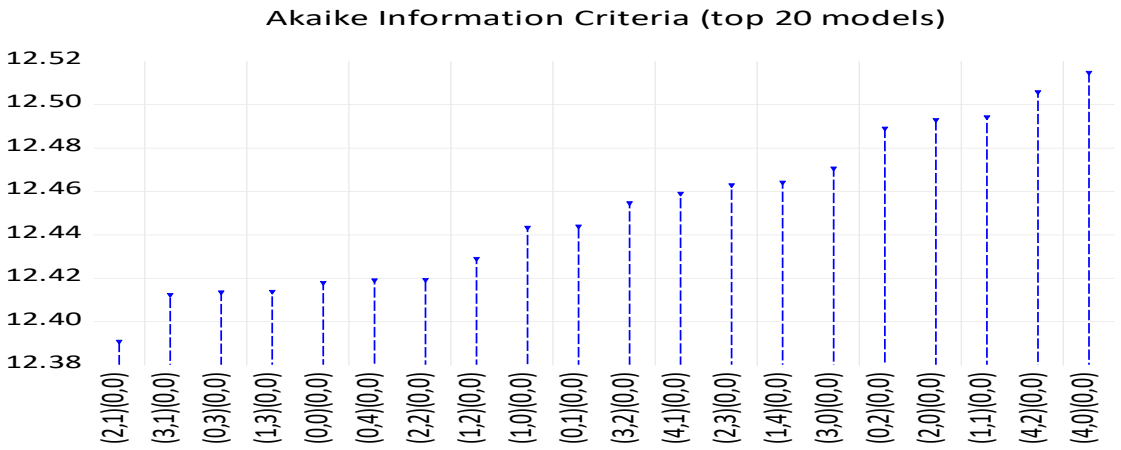

Figure (6): Akaike Criteria Graph

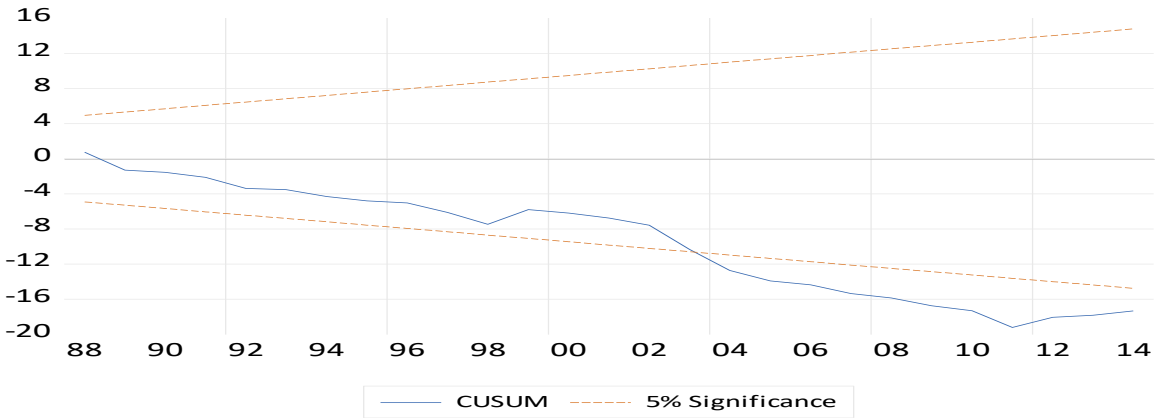

Figure (7): Stability (cusum) Test

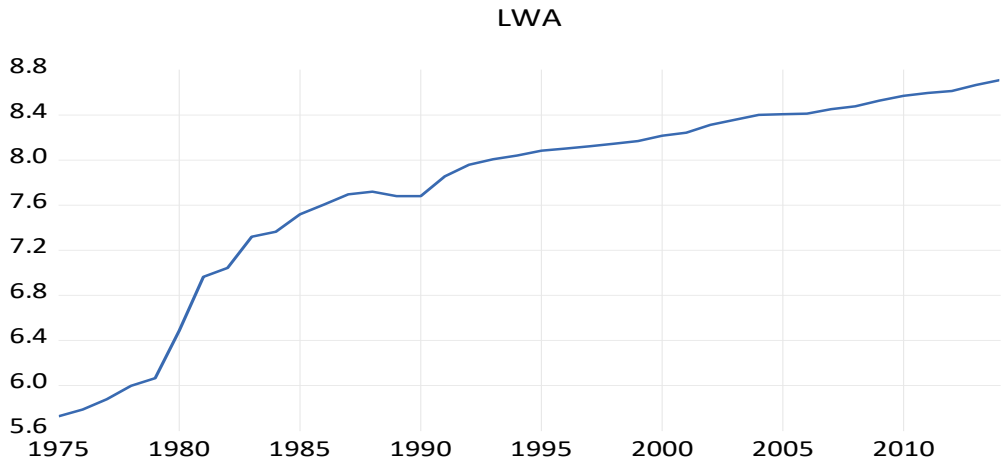

Figure (8): The original time series in logarithm for agricultural water use

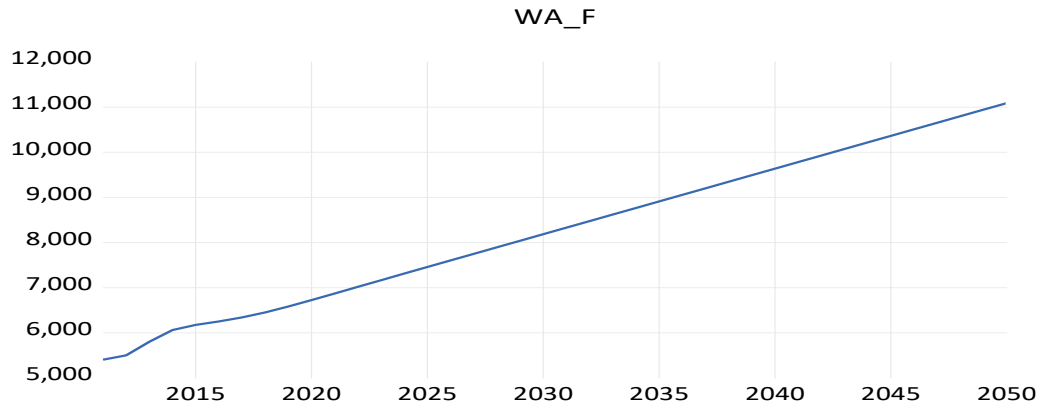

Figure (9): Agricultural Water Use Forecast from 2015 to 2050 Graph

RESULTS AND DISCUSSION

The demand for water is relatively high due to the warm climate prevailing during much of the year. Demand also keeps increasing with time because of the high growth rate of the population. Since the surface water resources of many arid countries are limited, and the management of these sources are still incomplete, there is a growing need to fulfil some part of the demand from groundwater resources. Experience has shown that groundwater abstraction. Libya experiences scarcity of water due to its arid location (Pastel et al., 1996). Population growth and rising income, coupled with rapid urbanization, assure the continued steep increase in the demand for water for irrigation, industrial and municipal uses, 90% of the land is desert and semi-desert area. The area features typical continental arid/semi-arid climate. There is no perennial river in Libya, and the country depends almost entirely on groundwater for its water supplies (Iglesias et al. 2007). The exploitation and utilization of water resources display spatial non-uniformity because almost 80% of the population inhabit cities in the northern coastal plain. Libya depends heavily on groundwater which accounts for more than 97% of water use (Omer, 2007). The demand for water has more than doubled between 1977 and 1994, thus intensifying pressure on this finite resource (Alghraiani, 2003). Libya has five principal regions with substantial water resources; they are as follows: Jifarah Plain and Jabal Nafusah region; the Middle Zone; Al Jabal Al Akhdar region; Fezzan region and Kufrah and Assarir region The main aim of this study is to forecast the demand for water in Libya using Eviews 10 software for the water demands, agricultural, This section has two aims. The first

aim is to estimate the model by (ARDL) Autoregressive distributed log model. Then to use the time series for the analysing the variables, income, temperature, price, and population. To achieve this aim, an econometric model of Libyan water demand is constructed for the period 1975-2014. Then using ARIMA forecasting approach, the forecast for water demand from 2014 to 2050 is made for the three uses. The best-fitting models are estimated separately for water demand series from 1975 to 2014.

Table (2) The results of forecast of the Agricultural water in Libya

Years	Agricultural Use
2015	6177.439
2016	6254.414
2017	6343.067
2018	6454.678
2019	6585.044
2020	6726.443
2021	6872.654
2022	7020.053
2023	7167.079
2024	7313.358
2025	7459.026
2026	7604.334
2027	7749.485
2028	7894.596
2029	8039.719
2030	8184.866
2031	8330.034
2032	8475.213
2033	8620.397
2034	8765.583
2035	8910.768
2036	9055.952
2037	9201.136
2038	9346.320
2039	9491.503
2040	9636.686
2041	9781.869
2042	9927.052
2043	10072.24
2044	10217.42
2045	10362.60
2046	10507.79
2047	10652.97
2048	10798.15
2049	10943.34
2050	11088.52

The tests indicate that the model performs well. The coefficients are all significant, and they satisfy the stationarity and invertibility conditions. Each model has the highest R^2 and the lowest AIC and

SIC. The second aim is forecasting of the water consumption Libya until 2050. The results of the study indicate that agriculture will continue to be the major water consumer. It continues to be the biggest consumer of water. It represents about 68% of the estimated water consumption in 2050 (11088.52 million m^3), up from 891.768 891.768in million m^3 in 2025. **Agriculture** will continue to be the major water consumer. It becomes the biggest consumer of water as shown in the table. It represents about 68% of the estimated water consumption in 2050.

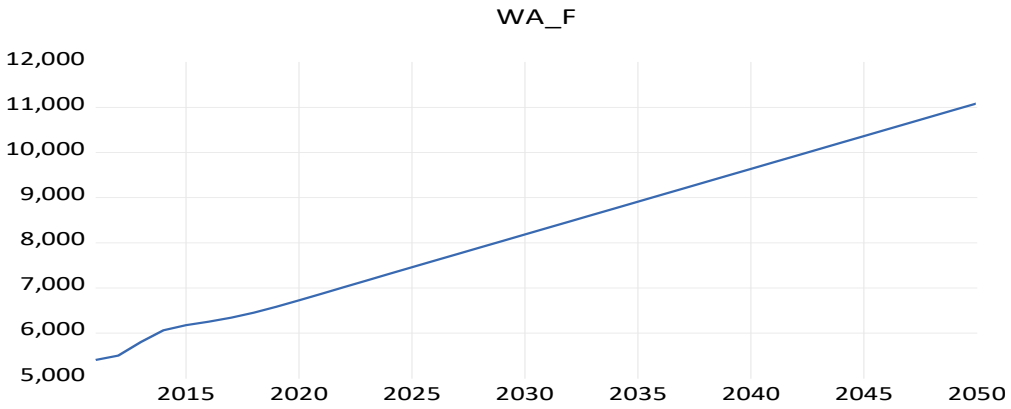

Figure (10): Agricultural Water Use Forecast from 2015 to 2050 Graph

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RECOVERY OF THORIUM FROM PURIFICATION PROCESS OF RARE EARTH ELEMENTS

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ABSTRACT

Rare earth elements (REE) will be able to bring today's world where we live to high technology, everything that may be possible is going through the miniaturization of electronics. Rare-earth (RE) industries generate a large amount of radioactive residue containing thorium concentrations at a high rate. Due to the fact that thorium is considered a non-economic element, large volume of these RE processed residues are commonly disposed of without any recovery methods. It is very important to study an appropriate recovery method that could reduce the volume of waste materials for final disposition. It will be able to cause technological developments that can change our world, including defense systems and telecommunications, from green energy to the pharmaceutical industry. Thorium that is a mamber of rare earth elements is electrochemical, optical, magnetic, nuclear, alloying, luminescent, catalytic, etc. due to their use in their processes, they are becoming increasingly important resources. Thorium elements are extremely important due to their use in components or equipment of many state-of-the-art products such as electric vehicles, green energy production, electronic devices and high-performance aircraft. There are no alternative materials that can replace these kind of elements yet. Radioactive elements of uranium and thorium are usually associated with rare earth deposits. The separation of thorium from rare earths is often a big concern in rare earth industry in order to reasonably manage the radioactive nuclides. It is not the main raw material of the products it is used for, but it is usually added as an additive in certain proportions and allows these materials to gain superior properties. This paper reviews the technologies used for separating thorium from rare earths in rare earth production.

Keywords: Rare earth elements, Radioactive residue, Thorium, Green energy production

INTRODUCTION

It has long been known that thorium-232 is a fertile radioactive material that after being irradiated with neutrons can produce energy in nuclear reactors for conversion to electricity. Thorium-232 performs this function by transmuting into uranium-233 permitting reactor energy by fission. As do other fertile materials, thorium-232 requires a source of neutrons for the transmutation to take place, from a fissile material (such as uranium-235 or plutonium 239) or from

an external source such as spallation neutrons. Thorium-232 appears in nature unmixed with isotopes, does not require enrichment for use as reactor fuel, and only needs relatively inexpensive chemical separation from ore impurities (Schaffer, 2013).

Thorium is not found in nature in a free state like uranium. It is included in the structure of about 60 minerals. Of these minarets, only monazir (Ce, La, Nd, Th) and Torite ((Th,U(SiO4) are used in the production of thorium. Thorium, which makes up 0.0007% of the earth's crust, is usually found together with Rare earth elements. Thorium, atomic number 90, atomic weight about 232 g/mol, density of 11.7 g/mL, melts at 1700 °C, lead-colored, does not deteriorate in air, is a radioactive element used as a source of atomic energy.

Thorium is known as a promising resource for its application as an alternative to nuclear fuel dating back to the emergence of the nuclear era. The predicted depletion of conventional uranium resource within the next 80 years has instigated the search and discovery of this new resource (Ismail and Yim, 2015) Thorium, which is the second in the actinide series, is found in many different minerals. They are not found in a free state in nature and are usually concentrated in certain regions together with NTES (Sahin et al..2002). In Figure 1, we can examine the ways in which the thorium mineral is found in nature.

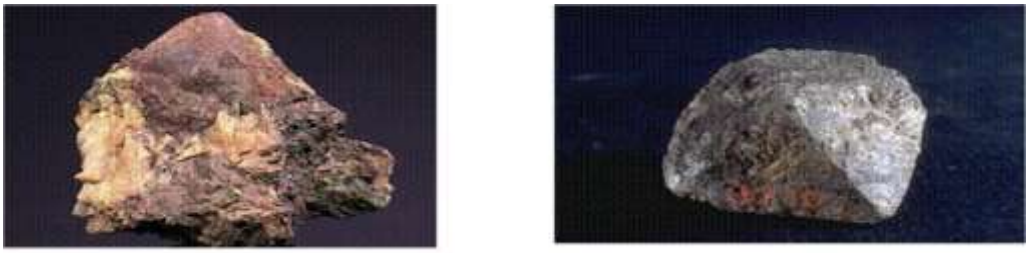

Figure 1. Occurrence of thorium mineral in nature

In order for thorium to be used as a nuclear fuel, an element capable of fission must be present next to it. 232TH, a fertile isotope, must be converted to 233U, a uranium isotope that can perform a fission reaction as a result of neutron capture.

There are initiatives for Rare earth element deposits that have been found in our country and have not yet been operated. In addition, the geology of our country is suitable for the formation of these elements. Thorium has the potential to become the nuclear fuel of the future as an alternative to uranium. Thorium is called the green nuclear. Instead of selling the found mines as they are, Turkey should manage to export them by processing them and increasing their added value.

In general, they are objects extracted by mining methods, mainly consisting of minerals, the processing of which provides a profit. From an economic point of view, the important thing in considering a substance as an ore is that it can be processed economically. This also depends on some factors. For example, a rich mineral deposit that is difficult to extract and transport, such as the way it is obtained, transportation and other costs, is not considered ore, but a low-grade deposit that can be processed economically can be considered ore.

The world's total thorium sources are reported to be about 1,400,000 (USGS, 2013) to 5,307,800 tonnes (OECD, 2015). The extracted ore is crushed and then ground with various grinders (jaw, ball, etc.). After grinding, screening is carried out according to the grain size. As a result of this screening process, it is checked whether there is enrichment in any grain size.

Physical pre-enrichment of ore

Most mines are usually compacted by chemical methods. But in some advantageous cases, physical pre-enrichment is applied. In general, physical pre-enrichment is carried out according to the optical property, according to the density difference, according to the magnetic property, taking advantage of the electrostatic property and by flotation (flotation).

The purpose of physical pre-enrichment is to increase the concentration of the desired minerals or minerals contained in the ore. Increasing the amount of minerals contained in a unit amount of ore allows you to get more minerals by spending less solvent.

Enrichment by size

Classification by grain size: Ores can be broken into different sizes and shapes, usually because they consist of or contain different minerals at the size reduction step. The resistance of different minerals to fracture allows them to break differently from each other, this property distinguishes large and small grains and provides significant enrichment.

Enrichment by extraction

In other words, triage is one of the first applications of mining and its application is still being carried out today. In addition to sorting by elimination, automatic debuggers are also used. By using the physical properties of minerals (color, shape, radioactivity, brightness, x-rays and phosphorescence properties), the desired mineral can be separated from the undesirable ones.

Enrichment by taking advantage of weight difference

The specific gravity of each mineral present in the ore is different. This difference is also exploited in this enrichment method. This method is more preferable due to its affordability. The scope of application is very large. The difference in the specific gravity of various minerals, such as coal, chromium, manganese, coastal sands, barite, makes them easy to enrich. This method is a physical separation method and has many varieties (rocking table, spiral groove, etc.) there are.

Enrichment using the magnetic separation method

It is an enrichment method applied based on the different magnetic susceptibility of minerals from each other. We can divide mineral varieties into two groups as minerals that can be enriched with magnetic separators and minerals that cannot be enriched. Minerals with strong magnetic properties are called ferromagnetic, while those that are not affected by the magnetic field are called diamagnetic, and substances that are affected by the magnetic field are also called paramagnetic.

Enrichment by electrostatic separation

It is a method based on the difference of the electrical conductivity of minerals. Enrichment is carried out using electrostatic separators after loading minerals of different densities and usually with different electrical charges.

Enrichment by flotation method

Flotation is one of the most widely used enrichment methods to Decouple the desired minerals from undesirable minerals by taking advantage of the surface and intermediate surface properties of the minerals. Minerals can be classified into 5 groups depending on their degree of polarization

- Pure metals and metal sulfides

- Sulphate minerals
- Carbonate minerals
- Oxide/Hydroxide minerals
- Silicate/alumina silicate minerals

Leaching

Leaching is mainly a diffusion process. The dissolution rate is proportional to the reactant concentration, temperature, the surface area of the solid, and the diffusion rate from the liquid to the solid surface. The diffusion coefficient decreases with increasing viscosity of the medium. In the leaching process carried out under pressure, the oxidation rate is proportional to the surface area of the reacting substance and the partial pressure of oxygen in the system. In the process of acid leaching, sulfuric acid is usually used due to its cheapness and low corrosive properties.

Leaching methods

The method to be chosen for the leaching process of an ore or a waste substance depends on the metal content of the substance, the grinding cost, the cost of bringing the ore to the surface and the processes that must be performed before various leaching processes such as roasting, and the simplicity of the dissolution process. The preferred method should contain low cost, dissolution should be fast and high efficiency should be realized.

On-site (insitu) leaching

This method is usually used for ores with a low tenor, since it will not be economical to cover the costs of mining and transfer. The ore has been leaching where it has been for a long time. For beds with very low tenor but large reserves, on-site or bulk type leaching systems are much more suitable. This is due to the fact that it is able to save the cost of operating a low-grade ore. The cost of on-site and bulk leaching methods compared to leaching and mixing leaching is very low. In addition, the applicability of bulk and in-situ leaching methods at large reserve sites is much higher. If there are acidic components in the mineralogical structure of the ore, it becomes more economical to use a basic solvent, since the use of reagents with acidic properties will be high due to the fact that the reagent consumption will be high due to the ability of the ore to consume acid.

Stack leaching

In bulk leaching, the region where the leaching process will be applied is prepared first. This area is generally prepared at or near mining sites and a floor covered with waterproof asphalt/plastic sheets is created. If it is permeable, the leaching solution moves away from the leaching area without contacting the ore. Ore is transferred from the mine or January to the area where it will be leached. The ore, usually reduced to a size of -15 cm, is deposited immediately on top of the impermeable layer, and the top of the pile is leveled.

Sewage drainage channels are opened on the piles to prevent leakage solutions and contact of the leaching solution with precious metals. Solvent pumps could be supplied to the upper part of the pile in different ways, such as irrigation or sprinkling. The solution collected through the drain is fed back to the pile again with the help of pumps in the same way. Figure 2 shows the in situ leaching system

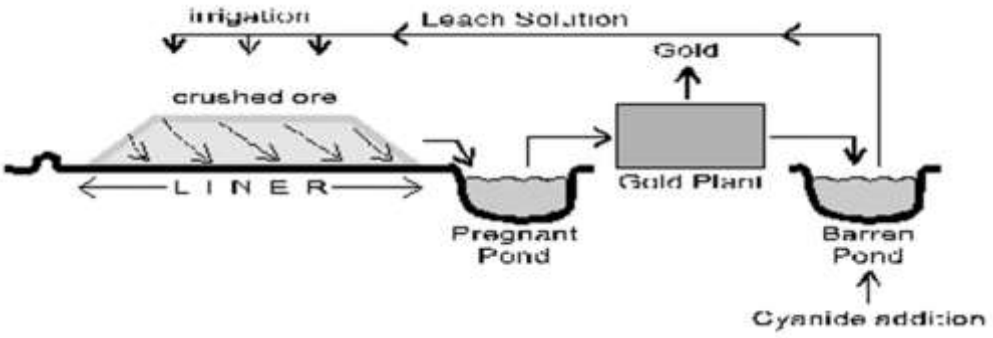

Figure 2. In-situ leach system

This method can only be applied if the grain size of the ore is large enough to not interfere with the circulation of the solution. In this study, the grain size is 9-12.5 mm. After the ore is filled into the tanks, it is brought into contact with the solvent. The solvent is either given from the bottom of the tank and the solution is taken from the top by overflow, or the solution is given from the top and taken from the bottom. It is applied in a serial circuit. It works according to the principle of reverse current. The first solvent is usually diluted or in the form of a metallic residual solution. The last solvent, on the other hand, is highly concentrated for the complete realization of leaching. the ore in the tanks located at the end of the thaw is washed with water and the solution between the grains is Decanted. No thickeners or filters are used. The pseudo-base already acts as a filter. The leaching time can reach 24 hours. It is mainly used for Fr, Cu, U, ZnO leaching. The reagent expenditure is low. The metal concentration of the solutions is high (Boyrazli,2008).

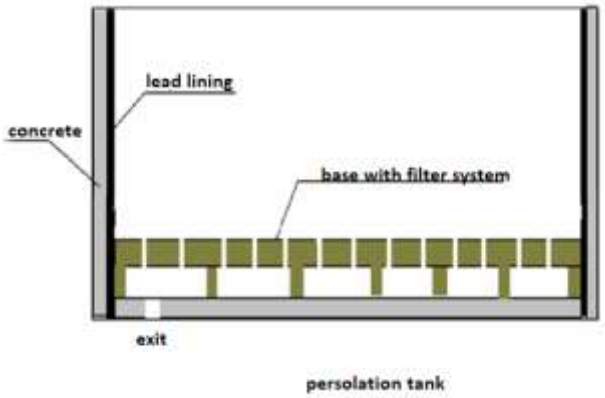

Figure 3. A classic percolation leaching scheme

Mixing (agitation) leaching

It is usually applied to ores or concentrates with a high tenor. The ore or concentrate should be reduced to- 0.5 mm before leaching. Mixing is carried out to prevent solid grains from collapsing, to increase the contact of the leaching solution with the metal and to facilitate the transfer of gas to the medium by ion transfer in the medium. Almost all of the metal is Decanted into the solution for about 1-10 hours. Due to this feature, it is a very preferred method. A classic mixing leaching unit is shown in figure 4.

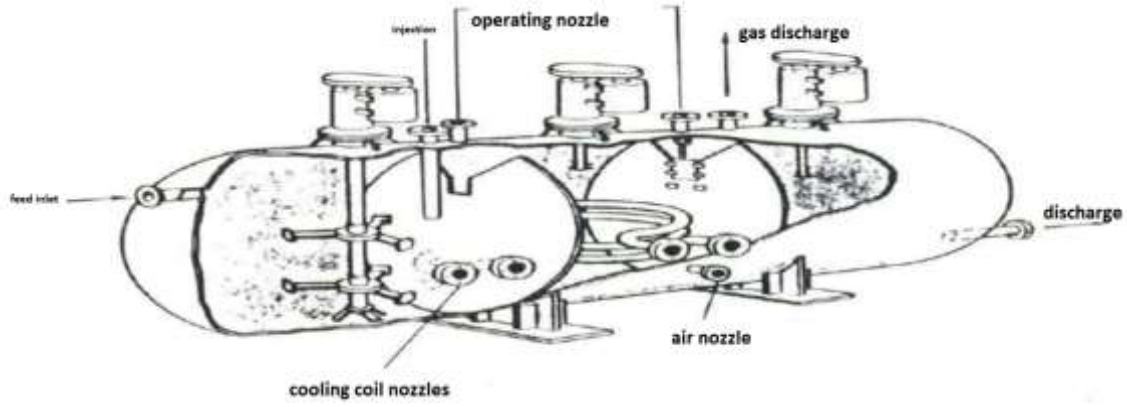

Figure 4. A classical mixing leaching operation scheme

RESULTS AND DISCUSSION

Thorium, which are members of the actinide group, are usually found together in rocks and minerals, but differ significantly in their properties under environmental conditions (Kabata-Pendias, 2011). Thorium was found in the terrestrial part and roots of meadow plants (Malikova et al., 2016).

Thorium is a fertile substance with quite good properties for the production of nuclear energy. Its use in nuclear power plants is superior, more advantageous and more sustainable than uranium in many respects. Thorium-containing fuels have high performance. In addition, their waste can be re-processed and evaluated. They are suitable for use in combination with less enriched fossil fuels. The waste of thorium fuels contain less heavy metals. Therefore, the rich thorium reserves are a great and important potential that is waiting to be evaluated in order to produce energy for Turkey. Unfortunately, Turkey is an outward dependent country in terms of energy. He cannot use his own resources in sufficient effectiveness. For this reason, he is forced to face various problems. Using the thorium reserves it has in energy production through nuclear power plants will undoubtedly help Turkey solve its problems. Although nuclear technology applications are high-cost, their long-term returns for Turkey will cover all kinds of costs paid.

In principle, the separation of thorium from its ores or solid waste streams consists of several processes, including leaching, solid-liquid separation, ion exchange, solvent extraction, and precipitation (Bahri et al., 2019). Apart from that, other conventional techniques could also be applied to separate thorium ion from aqueous phases, such as membrane filtration (Bisset et al., 2013), ion exchange (Heshmati et al., 2014), and adsorption means (Mahmoud et al., 2014). Cracking and leaching of solid streams are crucial processes at the initial stage of thorium separation, whereby these processes determine the solubility of thorium either in acid or alkali to form an aqueous solution. The solution concentration can be further modified by using various conventional methods (Meng et al., 2019).

Bekir (2007) studied, ammonium oxalate and ammonium hydroxide solvent with a mixture of oxalic acid for leaching of thorium, which may affect the conditions of grain size. His study was about solvent type and concentration, temperature, solid/liquid ratio and contact time parameters. He examined that, instead of thorium has determined the optimum conditions for defrosted. Positive results were obtained in the study and the variables were optimized. The method shows promise that thorium and Rare earth elements in the ore can be separated from each other only at the leaching stage. In the light of these data,it is envisaged that economic operation of the ore can be achieved by developing processes that will enable the recovery of rare earth elements that remain insoluble.

CONCLUSIONS

Bold decisions must be taken in order to move forward and advance in the production of thorium-containing nuclear power in Turkey. First of all, more detailed studies of rich thorium reserves and data on the amounts and ratios of thorium reserves should be able to be determined and updated with various studies. In addition, in order to effectively evaluate our strategy by using thorium fuel reserves, from which we can produce our own fuel, it is necessary to create products that will evaluate fuel and waste, to establish our own facilities with universities and research centers, and to work on this subject with our technology. Hence, separated thorium from rare-earth residue may provide a sustainable thorium resource in the future. To produce enough thorium at the national level, it is necessary to develop nuclear energy. In conclusion, the separation of thorium and the potential reduction volume of rare-earth residue could give positive sentiments and perceptions.

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EVALUATION OF THE DAILY VARIATION OF AERODYNAMIC RESISTANCE OF GRASS IN LOW-TECH MEDITERRANEAN-TYPE GREENHOUSES

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ABSTRACT

Aerodynamic resistance (r_a) is one of the parameters required to estimate the reference evapotranspiration (ET_o) with the Penman-Monteith equation. Various models were developed to determine r_a in field conditions depending on wind speed. Since the wind speed is very low in low-technology greenhouses, the r_a values calculated with these models cause an incorrect estimation of ET_o. This study, it was aimed to determine the changes in daily r_a values of the grass in low-tech Mediterranean-type greenhouses. For this purpose, first, the ET value of the grass was determined by weight type lysimeter, and then the r_a values were calculated by taking the inverse of the Penman-Monteith equation (I.P.M). Although there was a moderate relationship between the ET values measured by lysimeter and estimated by the P.M equation (R^2=0.60), it was not homogeneously distributed around the 1:1 reference line. The results showed that r_a values changed depending on the daily indoor climate. The obtained results showed that the ra values were very strongly dependent on the indoor climate.

Keywords: Referance evapotranspirtation, Penman-Monteith, greenhouse, lysimeter

INTRODUCTION

Given that agricultural production can be carried out under more controlled conditions, the efficiency of water use and yields per unit area are higher in greenhouse conditions than in field conditions (Rabbi et al., 2019). There is a positive relationship between the level of technology and indoor climate control in the greenhouse. The level of technology in the greenhouse is divided into 3 as low, medium, and high depending on the heating equipment, ventilation, and cooling system, thermal screens, carbon dioxide (CO_2) enrichment, supplementary lighting and, automation system (Ilahi, 2009). Most greenhouse cultivation areas in the world are in the countries in the Mediterranean basin. In countries like Turkey that are characterized by a Mediterranean climate with an annual average temperature of 10-20 ºC, greenhouse cultivation is carried out in low-tech greenhouses (Karaca, 2020).

Over the past 50 years, total water demand in all sectors of activity in the Mediterranean region has doubled to 280 km³ per year (Ferragina, 2010). Future estimates predict a 25 per cent increase in consumption by 2025 in the south and east of the Mediterranean, especially in Turkey, Syria and Egypt. In the Mediterranean basin, 72% of the water resources are used for irrigation, 10% for domestic, and 16% for industry. (Mandi, 2014). Therefore, accurate irrigation scheduling is important for the future of the region in terms of water resources.

One of the most widely used methods for estimating crop evapotranspiration (ET_c) and crop water requirement is the FAO methodology (Allen et al., 1998) based on reference evapotranspiration (ET_o) and crop coefficient (k_c) (ET_c=ET_o x k_c) (Campos et al., 2012; Karaca et al., 2018). The Penman-Monteith equation (Allen et al., 1998), recommended as the standard method for ET_o estimations, clearly underestimated the measured greenhouse ET_o (Fernández et

al., 2010). This method combines radiation and aerodynamics terms, and aerodynamic resistance (r_a) is determined based on wind speed, crop height, and vegetation type (Brutsaert and Stricker, 1979). However, the very low wind speed inside the greenhouse causes the r_a to oscillate between 20,800 and 2,080 s m^{-1} (Fernández et al., 2010). For this reason, some researchers calculated the r_a with the convection-based method (Qiu et al., 2013; Rouphael and Colla, 2004), while some (Fernández et al., 2010; Yan et al., 2018) fixed the r_a throughout the growing season. On the other hand, Yan et al. (2018) directly calculated the r_a value by taking the inverse of the energy balance equation (Inversing Bulk Transfer equation (IBTE). For this purpose, the researchers determined the ET values directly with the lysimeter.

In this study, the changes in the aerodynamic resistance values of grass in low-tech Mediterranean-type greenhouses were observed. For this purpose, aerodynamic resistance values, which is one of the important parameters to determine plant water consumption, were calculated by taking the inverse of the Penman-Monteith equation (I.P.M).

MATERIALS AND METHOD

This research was conducted in a low-tech Mediterranean-type greenhouse in the experimental area of Akdeniz University (30° 38' 30"N - 30° 39' 45"E), Antalya-Turkey. The greenhouse was a low-tech Mediterranean-type plastic greenhouse (180 μm polyethylene film with UV + IR + EVA + AD additives) without ventilation (passively ventilated) and heating systems.

The ET_o was measured directly by the soil water balance method (Equation 1) and was estimated with climate data by Penman-Monteith equation (P.M) (Monteith and Unsworth, 1990).

$$ET_o = I - D \pm \Delta S \qquad \text{(Eq-1)}$$

Where ET_o is the reference evapotranspiration; I is the total volumes of applied irrigation water; D is the total volumes of drainage water; ΔS is the change in volumetric soil water content between consequently two days.

For this purpose, the weight-type lysimeter consisting of a cylindrical pot (0.52 x 0.45) and a weighing instrument was used. Synthetic envelope materials were placed under the micro lysimeter to ensure adequate drainage conditions. Tifway Sport Bermudagrass (*Cynodon dactylon* L.) grass variety, which is a perennial plant that has adapted well to the region, was used as crop material. As recommended by Allen et al. (1998), the grass height was always kept at 0.12-0.15 m during the experiment. The change in soil water content and the volume of drainage water from the pot were measured every day at 9:00 a.m. In the study, temperature (PT100 1/3 Class B-Pessl Instruments), relative humidity (ROTRONIC IN1-Higrometre) and net radiation (CNR4-Kipp & Zonen) values were obtained from the meteorology station located (1.5 m) in the center of the greenhouse.

P.M equation used for ET_o estimation with climatic data was given in Equation 2.

$$ET_o = \frac{\Delta R_n + \rho C_p \left(\frac{e_s - e_a}{r_a}\right)}{\Delta + \gamma \left(1 + \frac{r_s}{r_a}\right)} \qquad \text{(Eq-2)}$$

Where ET_o is the reference evapotranspiration (mm day^{-1}); Δ is the slope of the saturation vapour pressure temperature curve (kPa $°C^{-1}$), R_n is the net radiation (MJ m^{-2} day^{-1}); ρ is the air density (kg m^{-3}); γ is the psychometric constant (kPa $°C^{-1}$); C_p is the specific heat of the air at

constant pressure (J kg^{-1} $°C^{-1}$); e_s is the saturation vapour pressure (kPa); e_a is the actual vapour pressure (kPa); r_a is the aerodynamic resistance (s m^{-1}); r_s is the stomatal resistance (70 s m^{-1}).

The change of daily aerodynamic resistance was calculated directly by taking the inverse of the Penman-Monteith equation (I.P.M). The relationship between measured and estimated ET_o values was determined by simple linear regression (Equation 3).

$$R^2 = \frac{\left[\sum_{i=1}^{n}\left(X_i - \overline{X_i}\right)\left(Y_i - \overline{Y_i}\right)\right]^2}{\sum_{i=1}^{n}\left(X_i - \overline{X_i}\right)^2 \sum_{i=1}^{n}\left(Y_i - \overline{Y_i}\right)^2} \quad \text{(Eq-3)}$$

Where n is number of observations, X_i is estimated ET_o, Y_i is measured ET_o and $\bar{X}_i$ is mean value of estimated ET_o, $\bar{Y}_i$ is mean value of measured ET_o.

Descriptive statistics and distributions of calculated daily aerodynamic resistance values were determined with Microsoft ® Excel®.

RESULTS AND DISCUSSION

Daily climatic data measured in the greenhouse were given in Figure 1.

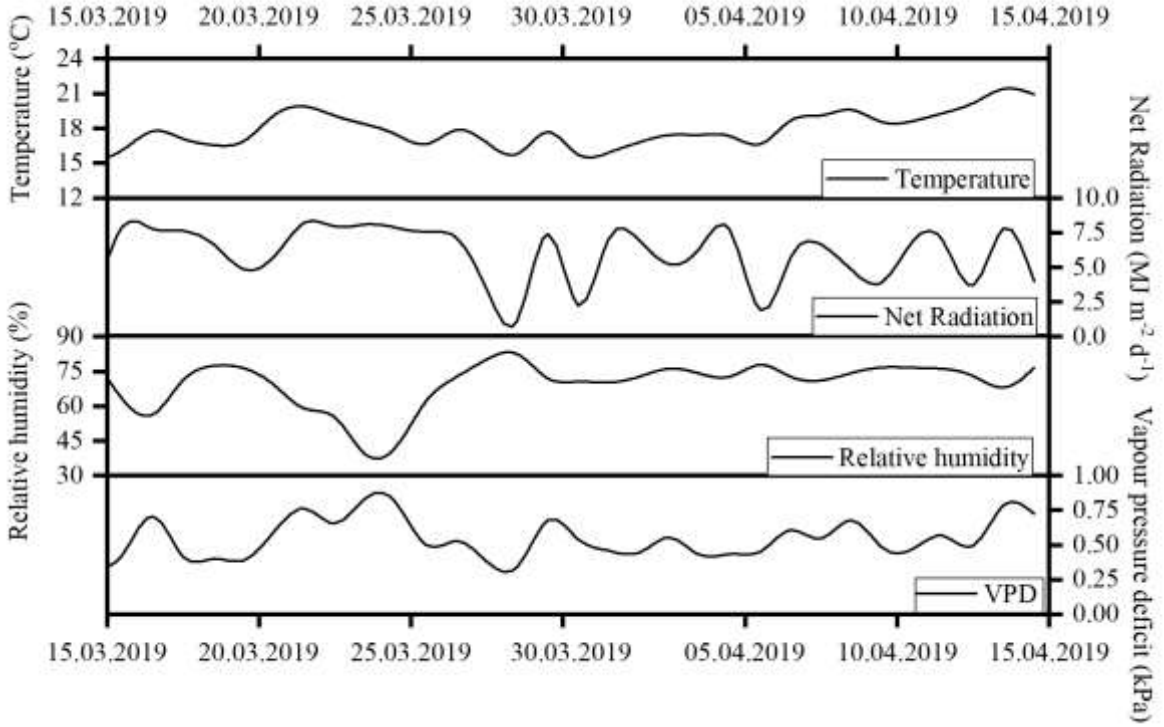

Figure 1. Daily climate data measured inside the greenhouse

The highest and lowest average daily temperature in the study is 14.69 and 21.85 (ºC), respectively. Net radiation values ranged between 1.18 - 8.23 MJ m^{-2} s^{-1} while a daily average of 5.90 MJ m^{-2} s^{-1} during the experiment (Figure 1). The similarity of the high and low peak values in the daily net radiation and temperature showed that the net radiation affected temperature. Throughout the research, the lowest relative humidity occurred on 24.03.2019 (34.28%) and the difference between high and low peak values decreased starting from 26.03.2021 (average 72.18%). The daily VPD values depending on the temperature and relative humidity ranged

between 0.33 and 0.84, while the average was 0.5. The change of measured and estimated ET (P.M) values was given in Figure 2.

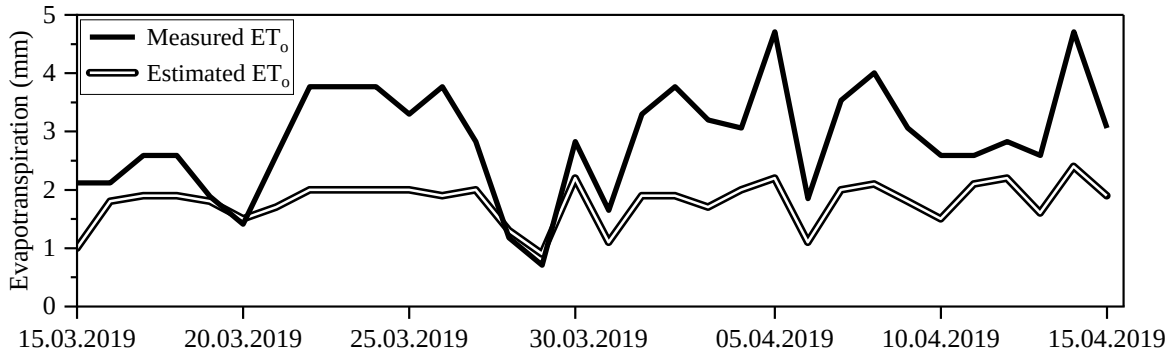

Figure 2. Change of measured and estimated (P.M) ET_o values

The measured ET_o values were higher than the estimated ET_o values except for a few days (Figure 2). At the end of March, when the net radiation value was lower than on other days, the estimated ET_o values were higher than the measured ET_o values (Figure 1-2). In addition, the decreased net radiation value caused a decrease in the difference between estimated and measured ET_o. Low relative humidity and high VPD on cloudless days when net radiation was measured at seasonal norms caused an increase in the difference between measured and estimated ET_o values. Measured and estimated ET_o values ranged from 0.71- 4.71 and 0.90-2.40, respectively, while the average was 2.74 and 1.79 mm, respectively. Figure 3-(a) showed the scatterplots of measured and estimated ET (P.M) values with 1:1 reference line.

Although there was a significant relationship between measured and estimated ET_o (R^2=0.60), its distribution around the 1:1 line showed that the estimation model had an insufficient performance (Figure 2-(a)). Although P.M and different versions of this model (FAO, FAO-56, ASCE) were the most commonly used model to estimate ET_o, numerous studies showed that this model underestimated the ET_o values in the greenhouse (Fernández et al., 2010). In this method, aerodynamic resistance is calculated as a function of wind speed. Since the wind speed in the greenhouse oscillates between 0 and 0.2 m s^{-1}, the r_a values calculated with this method range between 20,800 and 2,080 s m^{-1} (Fernández et al., 2010). This causes an erroneous estimation of the ET_o value in the greenhouse. For this reason, inaccurate calculation of the r_a value limits the use of the model in the greenhouse. The value of r_a was calculated from Equation 1 (I.P.M) by fixing the value of r_s to 70 s m^{-1}. Figures 3-(b) and Figure 3-(c) showed the daily variation of the r_a value during the experiment period and the relationship between the ET_o values estimated by the I.P.M equation and directly measured, respectively. Since r_a was calculated from the measured values, there was a perfect fit. During the experiment period, the r_a values varied between 350.2 s m^{-1} and 976.9 s m^{-1}, while the average was 585.5 s m^{-1}. The r_a value increased on cloudy days when net radiation was less than on other days. r_a values showed normal distribution and most of the data were between 450 and 650 s m^{-1}. The value of r_a oscillated throughout the study. This indicated that the climate inside the greenhouse affected the r_a value. Fernández et al. (2010) suggested using r_a as a fixed value of 295 s m^{-1} for FAO56-P.M in the Mediterranean-type greenhouse. The researchers stated that the shadow powder application affects the r_a value. Rahimikhoob et al. (2020) estimated ET_o at high performance by using r_a value as fixed 53 s m^{-1} in controlled greenhouse conditions. Because the researchers determined the r_a in more controlled conditions with a heating and ventilation system, they achieved a lower r_a than our study.

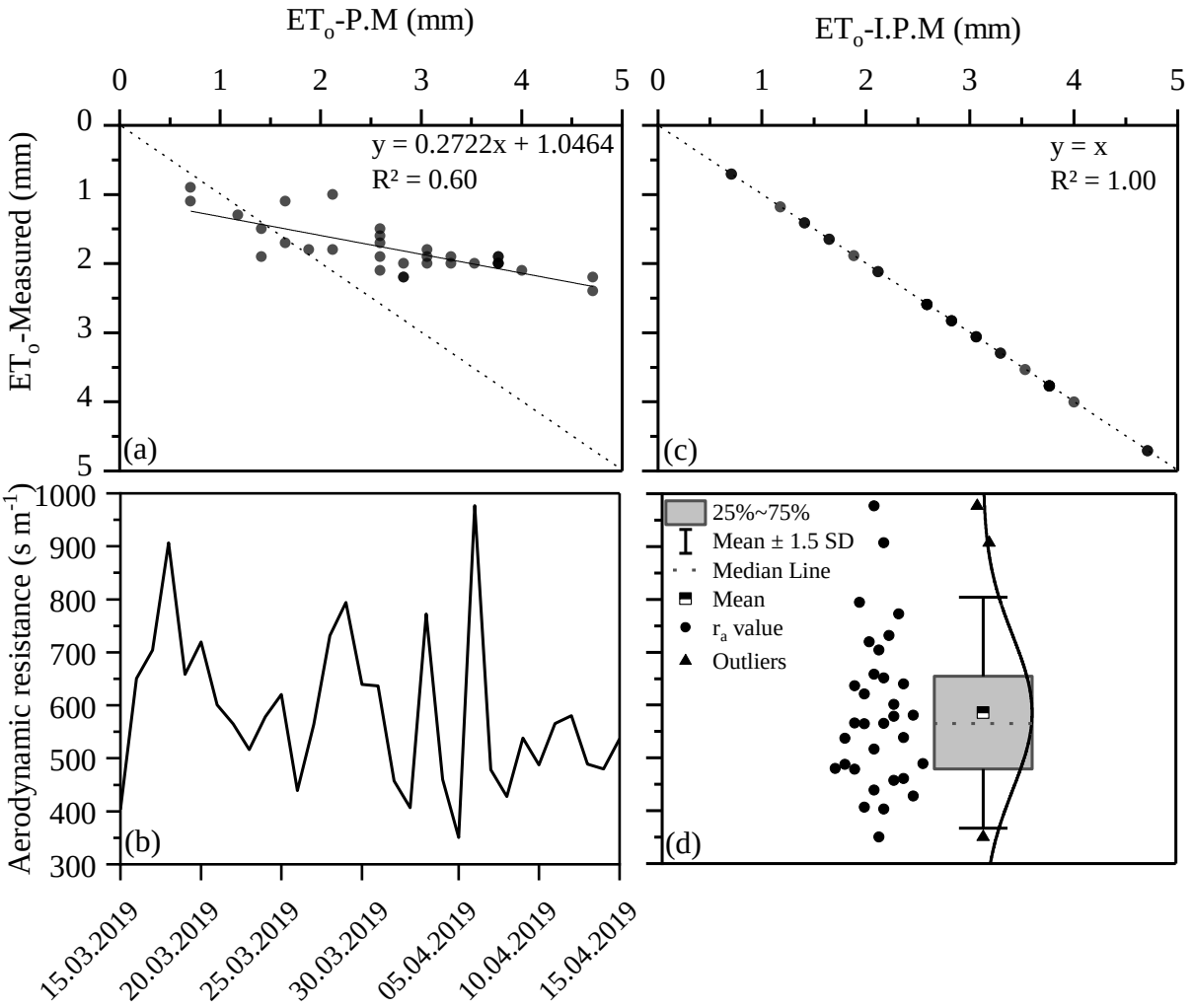

Figure 3. (a), Comparison between daily ET_o value estimated by P.M equation and measured directly; (b), the variation in the calculated daily ra value throughout the study; (c) comparison between daily ET_o value estimated by I.P.M equation and measured; (d), descriptive statistical information about the value of r_a.

CONCLUSION

This study aimed to calculate the r_a value of the grass using the Inverse Penman-Monteith equation in pot conditions in a low-tech Mediterranean-type greenhouse. For this purpose, climatic parameters such as temperature, net radiation and relative humidity were measured. ET_o values were determined both empirically with the help of these parameters and directly with the water budget equation. The daily value of r_a in the P.M equation was calculated using the directly measured ET values in the equation. The study indicated that the greenhouse climate effect the r_a value and therefore the daily changes of the r_a value oscillated. Long-term studies should be carried out in order to better evaluate the r_a value in low-tech Mediterranean-type PE greenhouse conditions.

DECLARATION OF COMPETING INTEREST

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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DETERMINATION OF AGRICULTURAL USES OF ORGANIC AND ORGANOMINERAL FERTILIZERS OF PRODUCED IN BIOGAS FACILITY: "A CASE STUDY IN SÜTAŞ I.C."

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ABSTRACT

This study, it was aimed to determine the effect of organic fertilizers formed as a result of fermentation in biogas plants fed with organic vegetable and animal wastes on the yield and soil properties of sunflower plants. Solid fermented organic fertilizer (S), liquid fermented organic fertilizer (L), organomineral fertilizer (6 10 6) (OM), and chemical fertilizer (C) applications were included in the research conducted under field conditions. Fertilizer applications were used in two doses. In the study, the effect on yield and nutrient uptake was determined. In addition, soil samples were taken from the parcels after harvest and the changes in some properties were determined. According to the data obtained; In fertilizer applications, the highest yield was obtained from the second doses of C, L, S, and OM applications. However, there are no statistically significant differences between the second doses of organic and chemical fertilizers. The applications positively affected the yield and the amount of nutrients uptake from the soil. According to the results of the soil analysis, significant effects were determined on the pH, EC, organic matter, and nutrient content of the soil.

Keywords: Sunflower, Organic fertilizer, organomineral fertilizer, solid fermented fertilizer, biogas plant, plant growth, soil properties,

INTRODUCTION

In recent years, it is important to increase production area and yield which is the raw material of products in the food sector such as wheat, corn, soybean, and sunflower in the world and our country. Especially sunflower production and consumption have become the most strategic agricultural activity. Sunflower is one of the most important raw materials in the oil industry since it contains a high amount of oil in its seeds (25-50%) and meets 74% of vegetable oil production in Turkey (Arıoğlu, 1999; Göktaş and Gül, 2018).

Sunflower is very important for health, as it contains oleic, linoleic, and linolenic fatty acids, which are highly unsaturated fatty acids, and reduces the risk of cardiovascular diseases and cholesterol, and contains vitamins A, D, E, K (Gürbüz et al., 2003). In addition to the use of sunflower oil in cooking and frying, the pulp and plant residues obtained after the oil is extracted are used as animal feed by making green fodder or silage (32.3% protein). It is used as the raw material for products such as paint, paper, plastic, soap, cosmetics, and insulation material in the industrial sector (Ghaffarzadeh, 2005).

To meet the food needs of the increasing world population, intensive tillage, fertilization, and pesticide use are increasing to achieve the goal of getting more efficiency from the unit area. For years, efforts have been made to increase productivity in agricultural areas with chemical fertilizer

applications. At this point, the increase in the use of chemical fertilizers and misapplications make it difficult to make economic production in line with increasing chemical fertilizer prices, while reducing soil quality and hindering agricultural sustainability.

As in every plant, using the right fertilizer in sunflower increases the yield and quality of the product positively (Gül et al., 2019). One of the conditions for making production by reducing the use of chemical fertilizers without disturbing the natural balance of the soil is to expand and increase the use of organic fertilizers to increase the soil's organic matter content. It has not been taken into account for a long time that there should be sufficient organic matter in the soil for the plant to benefit effectively from the chemical fertilizers used in production. However, recently, this situation has changed in the soil and the interest and use of organic or organomineral fertilizers have started to become widespread.

Organomineral fertilizers are defined as a type of fertilizer that is produced by blending organic materials with chemical fertilizers or raw materials by the technique. The organic matter it contains is obtained from leonardite, animal waste, domestic waste, and compost. Organomineral fertilizers are thought to reduce nutrient losses in soils and increase the uptake efficiency of plants. The use of organomineral fertilizers, by chelating nitrogen, phosphorus, and other plant nutrients of organic material with organic functional groups aimed to prevent nitrogen from mixing with the atmosphere in gaseous form and being washed away by precipitation, and to prevent the conversion of phosphorus into a form that cannot be taken by plants, and to increase the yield by minimizing it.

Today, while the protection of the natural environment and its sustainability are becoming increasingly important, it is of great importance to recycling the resulting wastes in an environmentally compatible manner. Therefore, the application of organic-based wastes to the soil for agricultural purposes is one of the accepted ideas. Biogas plants, whose activities are increasing in our country as well as all over the world, provide electricity as a result of the fermentation of plant and animal organic wastes while enabling the use of solid fermented and liquid fermented organic fertilizers, which are formed as final products, in agricultural areas.

This study, it is aimed to determine the effects of organic fertilizers and organomineral fertilizers obtained from biogas plants on soil fertility and quality by determining agricultural use efficiencies. In the study, it was tried to determine the effectiveness of organic and organomineral fertilizers in sunflower cultivation as a comparison with chemical fertilizers.

MATERIAL AND METHOD

This research was carried out in producer fields (3,1 hectares) in Bursa ecological conditions in the 2021 growing season, with a randomized complete block design with 3 replications. Statistical analysis was performed with JMP statistical program. Organic and organomineral fertilizers used in the research were obtained from which operates the biogas facilities, Enfaş I.C. Enfaş I.C.. is one of the group companies of SÜTAŞ I.C. It operates in Bursa-Karacabey district. The production flow process chart is given in Figure 1. The amounts of N, P, K, organic matter, and humic matter were determined in the solid fermented organic fertilizer (S), liquid fermented organic fertilizer (L), organomineral fertilizer (OM), and chemical fertilizer (C) samples used in the study are given in Table 1.

Solid Fermented Organic Fertilizer: It is a material with high organic content, which is formed as a result of anaerobic fermentation of plant and animal organic wastes in biogas plants, free from harmful pathogens and weed seeds, and contains plant nutrients at certain rates.

Liquid Fermented Organic Fertilizer: It is a liquid fertilizer obtained after the separator process of the products that are purified from harmful pathogens and weed seeds as a result of anaerobic fermentation of plant and animal organic wastes in biogas plants.

Organomineral Fertilizer: It is a fertilizer obtained by mixing or reacting organic content and/or organic fertilizer(s) with one or more primary, secondary or micro plant nutrients. The organomineral fertilizer used in this study is in the content of 6-10-6.

In the study, the compose fertilizers (15-15-15 and URE 46 %) were used as chemical fertilizers.

Before the applications, the soil samples were taken for analysis of soil fertility (Table2). The doses were determined by considering the organic matter, N, P, and K levels of the soils where the study was carried out, and plant nutrient demand (Table 3).

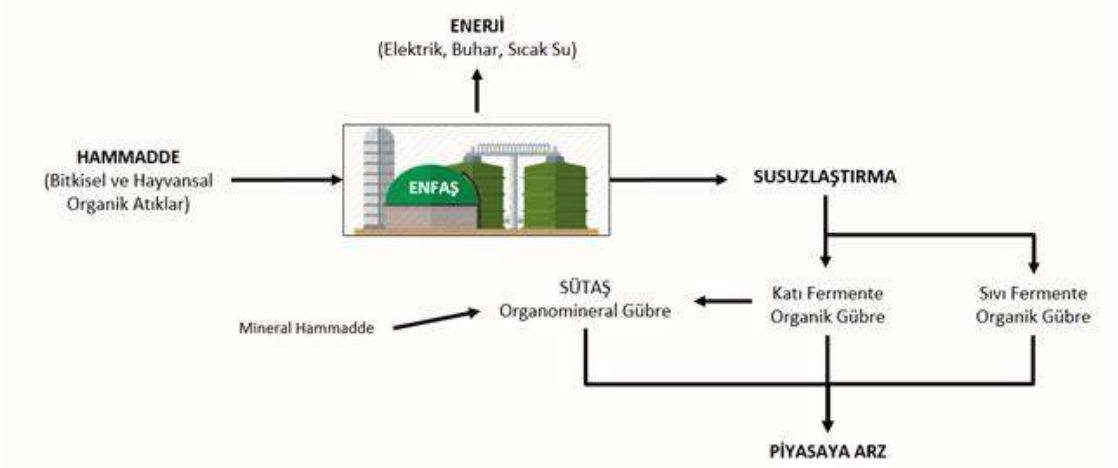

Figure 1. The production flow process chart in Biogas Facility

Table 1. Fertilizers contain used in the trial

Parameters	Solid Fermented Organic Fertilizer	Liquid Fermented Organic Fertilizer	Organomineral Fertilizer
pH	7,7	8,5	6,5
EC	3,2	3,4	38,3
Organic matter, %	77,09	5,1	46,8
Nitrogen(N), %	2,6	0,6	6,0
Total P_2O_5,%	2,4	0,2	10,0
Total K_2O, %	0,98	0,6	6,0
Humic + Fulvic,%	34,5	2,59	9,0
Cd	0,01	0,16	2,75
Cu	55,4	18,9	31,0
Ni	5,10	1,25	17,8
Pb	1,21	1,20	3,54
Zn	397,5	121,1	1,14
Hg	0,01	2,69	0,01
Cr	20,21	0,89	40,4

Table 2. The properties of the soil before applications

Parameters	Values
pH	7,54
EC, µS/cm	599
Organic matter %	0,86
N, %	0,072
NH_4-N	47,0
NO_3-N	13,5
$NaHCO_3$-P	25,2
NH_4OAc-K, g/kg	0,708
NH_4OAc-Ca, g/kg	5,10
NH_4OAc-Mg, g/kg	1,55
NH_4OAc-Na, g/kg	0,112
DTPA-Fe, mg/kg	16,1
DTPA-Cu, mg/kg	2,13
DTPA-Zn, mg/kg	0,57
DTPA-Mn, mg/kg	15,6
DTPA-Cd, mg/kg	trace
DTPA-Cr, mg/kg	trace
DTPA-Ni, mg/kg	2,08
DTPA-Pb, mg/kg	3,12

Table 3. Fertilizers and doses

Applications	tons ha^{-1}
Control	0
Solid Fermented Fertilizer (S1)	30
Solid Fermented Fertilizer (S2)	60
Liquid Fermented Fertilizer (L1)	30
Liquid Fermented Fertilizer (L2)	60
Organomineral Fertilizer 6-10-6 (OM1)	3,5
Organomineral Fertilizer 6-10-6 (OM2)	5,0
Chemical Fertilizer 15-15-15 (C1)	2,0
Chemical Fertilizer 15-15-15 (C2)	3,0

RESULTS AND DISCUSSION

The yield per hectare was calculated by harvesting the plants at a distance of 3 meters in each plot. Meanwhile, plant nutrients (N, P, K, Ca, Mg, Na, Fe, Cu, Zn, and Mn) were analyzed in samples for harvested (Kacar, 2014). Soil samples were taken from the parcels after harvest. The changes in soil properties were determined depending on the applications. The pH, EC, total N, NH_4-N, NO_3-N, available P, exchangeable K, Ca, Na and DTPA and extractable Fe, Cu, Zn, and Mn amounts were determined in the soils according to the Kacar (2012).

The Effect Of The Fertilizers Applications On The Plants

The results of the applications are determined important for statistical ($p<0.05$). The yield results were determined between 4,24-5,64 t ha^{-1}. The highest yield was obtained from second doses of C2 (5,64 t ha^{-1}), L2 (5,41 t ha^{-1}), S2 (5,35 t ha^{-1}), OM2 (5,28 t ha^{-1}) (Table 4).

Table 4. The yield results

Applications	**Yield, ton ha^{-1}**
Control	4,24 d*
Solid Fermented Fertilizer (S1)	4,73 cd
Solid Fermented Fertilizer (S2)	5,35 abc
Liquid Fermented Fertilizer (L1)	4,79 bcd
Liquid Fermented Fertilizer (L2)	5,41 ab
Organomineral Fertilizer 6-10-6 (OM1)	5,07 ab
Organomineral Fertilizer 6-10-6 (OM2)	5,28 abc
Chemical Fertilizer 15-15-15 (C1)	5,07 abc
Chemical Fertilizer 15-15-15 (C2)	5,64 a

The effects of fertilizer applications on the yield of the plant and the content of plant nutrients uptake from the soil are given in Table 5. The results were found to be statistically significant ($p<0.05$). The amount of nitrogen uptake from the soil by the sunflower plant is between 76,9 and 123,7 kg ha^{-1}. The lowest amount of nitrogen was determined from the control application, and the highest nitrogen amount was determined in L2>S1>C2>S2 applications. The amount of P uptake from the soil was determined between 24,7 and 31,9 kg ha^{-1}. According to the control application, the highest amount of P uptake from the soil was determined on this application OM2>L2>S2>C2. Depending on the applications, the amount of K uptake from the soil is between 21,6 and 40,0 kg ha^{-1}. The highest amount of K was determined as C2>S1>OM2=S2 respectively. With the sunflower yield, the amounts of Ca, Mg, and Na uptake from the soil were determined between 8,1-15,2 kg ha^{-1}, 5,8-7,5 kg ha^{-1}, and 1,5-2,6 kg ha^{-1}, respectively. Especially, the applications of OM and C obtained higher results. Meanwhile, the microelements were also analyzed from the soil samples which are taken after the harvest. The amounts of Cu, Zn, Fe, and Mn uptake from the soil with yield were obtained respectively between 92,0-135,0 kg ha^{-1}, 160,0-227,0 kg ha^{-1}, 190,0-342,0 kg ha^{-1} and 63,7-92,0 kg ha^{-1},. The highest results were obtained from C, S, and L applications.

Table 5. The amounts of nutrients uptake from the soil depend on the applications

	kg ha^{-1}						g ha^{-1}			
	N	P	K	Ca	Mg	Na	Cu	Zn	Fe	Mn
Control	76,9 e**	24,7 e*	21,6 f**	8,1 g**	5,8 c*	1,5 d**	92,0 h**	160,0 e**	190,0 e**	63,7 e**
S1	106,5 b	27,6 cde	39,4 a	13,4 bcd	6,5 abc	2,5 a	107,0 f	184,0 c	324,0 a	90,8 ab
S2	102,5 bc	31,1 ab	27,8 c	11,8 e	7,5 a	2,0 bc	135,0 a	198,0 b	282,0 b	87,3 abc
L1	97,8 cd	26,7 de	25,0 de	13,7 bc	6,1 c	1,9 c	116,0 e	227,0 a	234,0 d	72,1 d
L2	123,7 a	31,4 a	34,6 b	15,2 a	6,5 abc	2,3 ab	123,0 c	187,0 c	281,0 bc	86,4 bc
OM1	100,0 cd	27,5 de	25,5 d	12,1 de	6,6 abc	1,8 cd	116,0 e	166,0 e	261,0 c	63,7 e
OM2	98,5 cd	31,9 a	35,4 b	14,1 ab	7,4 a	2,6 a	129,0 b	187,0 c	290,0 b	82,6 c
C1	94,3 d	28,4 bcd	23,2 of	10,3 f	6,3 bc	1,9 c	104,0 g	187,0 c	342,0 a	92,0 a
C2	102,6 bc	30,5 abc	40,0 a	12,6 cde	7,2 ab	2,6 a	119,0 d	175,0 d	289,0 b	88,3 ab

Kaya (2008) was determined to obtain the desired yield and quality product in sunflower, 1,9-2,0 kg N, 1,5-1,6 kg P_2O_5, 2,3-2,4 kg K_2O, 0,3 Ca are uptake from the soil with each 100 kg grain yield. According to the results, sunflower uptakes the most K from the soil and ıt uses the K for stem and grain. The most of nitrogen is used till the sunflower head, the phosphor is used for flowering, and potassium is used for grain in sunflower production.

Patil and friends (2009) applied 80 kg ha^{-1} N, 40 kg ha^{-1} P_2O_5 ve 40 kg ha^{-1} K_2O and they obtained the best results. Yağmur and Okur (2017) obtained the highest yield, oil ratio, 1000-grain weight, and plant height from 120 kg K_2O ha^{-1} potassium fertilizer application. Kacar and Katkat (2007) determined that the most needed nutrients for sunflower production were K and also sunflower uses Fe of microelements. The amount of K uptake from the soil is 2.9 times more than N, 4,4 times more than P. According to the results of many studies, it has been reported that the amount of nutrients uptaken by the varieties varies depending on the environmental and variety characteristics. They reported that, the oil acids of oilseeds could be different according to genetic, ecological, morphological, physiological, and cultural factors and this result causes plant nutrient requirements to be different between plant species and varieties.

Atıcı (2000) determined that, organomineral fertilizer applications are more effective than chemical fertilizers in terms of yield and quality factors. Considering that the contents of nutrients in organomineral and chemical fertilizers are equal, the reason why organomineral fertilizers are more effective is due to the organic materials used as the additive. This situation is explainable that, the organic materials uses for organomineral fertilizer production connect the nutrients as organic. It means the mineralization on the soil. At the same time, the organic matter in OMG has a positive effect on soil properties and plant nutrient uptake, increasing yield. In a study conducted by Süzer and Çulhacı (2018), they reported that they obtained higher efficiency from OMG application than chemical fertilizer application.

The Effect of Organic Applications on Soil Properties

The soil samples which is taken from 0-30 cm after harvest were analyzed pH, EC, organic matter, and macro-micro elements. The effects of the applications on soil properties are given in Table 6. Differences in soil properties were found to be statistically significant.

Table 6. The effect of the application on soil properties after harvest

Parameters	Applications								
	Control	S1	S2	L1	L2	OM1	OM2	C1	C2
pH	7,23 b**	7,59 a	6,80 d	7,61 a	7,61 a	7,05 bc	6,85 cd	7,04 bc	7,12 b
EC, µS/cm	639 f**	1160 d	1421 b	852 e	852 e	1269 c	1519 a	663 f	1243 c
Org matter, %	1,63 ef	1,92 bc	2,02 b	1,89 bc	2,22 a	1,76 cde	1,83 cd	1,66 def	1,53 f
N, %	0,096 b	0,068 g	0,080 de	0,092 c*	0,076 f	0,077 ef	0,081 d	0,096 b	0,104 a
NH_4-N	47,00 d**	34,00 f	88,80 a	28,70 g	34,00 f	56,20 b	28,70 g	40,50 e	51,00 c
NO_3-N	13,50 bcd	13,00 bcd	14,60 b	9,10 f	10,20 ef	20,50 a	11,80 de	12,70 cd	13,80 bc
$NaHCO_3$-P	31,70 b	16,50 d	40,30a	21,00 c	29,40 b	32,70 b	20,10 cd	23,30 c	23,50 c
NH_4OAc-K, g kg^{-1}	0,324 bcd**	0,248 e	0,331 bc	0,343 bc	0,259 e	0,308 d	0,421 a	0,321 cd	0,344 b
NH_4OAc-Ca, g kg^{-1}	6,10 de**	7,03 a	6,80 ab	6,63 bc	7,07 a	6,24 cd	6,24 cd	6,24 cd	5,82 e
NH_4OAc-Mg, g kg^{-1}	1,10	1,01	1,05	0,72	0,77	0,96	1,12	1,13	1,10
NH_4OAc-Na, g kg^{-1}	0,113 b*	0,103 cd	0,110 bc	0,112 b	0,122 a	0,101 d	0,109 bc	0,11 b	0,113 b
DTPA-Fe, mg kg^{-1}	17,00 bc	16,30 cd	17,00 bc	12,10 e	17,80 b	10,20 f	15,50 d	21,10 a	20,50 a
DTPA-Cu, mg kg^{-1}	2,98 bc**	2,61 d	3,26 ab	2,83 cd	2,98 bc	2,22 e	2,72 cd	3,48 a	3,31 a
DTPA-Zn, mg kg^{-1}	0,87 d**	0,94 c	0,82 d	1,03 b	2,51 a	0,70 e	0,82 d	0,71 e	0,72 e
DTPA-Mn, mg kg^{-1}	16,60 c**	10,60 d	21,00 b	9,40 d	9,50 d	10,10 d	18,00 c	22,20 b	24,90 a

The pH value in the soil samples taken after harvest was determined between 6,80 and 7,61 values. More decreasing pH values were detected in S2 and OM2 applications. pH value, which expresses the hydrogen ion concentration in the soil solution, it is one of the most important parameters affecting the availability of nutrients in the soil. Soil pH value changes depending on organic matter, colloids, and environmental factors (Zincircioğlu, 2010). the effect of organic applications on soil ph value is related to the pH of the material and the specifications of organic acids (humic-fulvic) which is in the contain (Baran et al.,1998).

The change in EC values in the soil was determined statistically significant. The values were determined between 639 and 1519 µS cm^{-1}. Higher determination of EC values in organic and organomineral fertilizer applications is related to fertilizer properties and mineralization. It is an expected result that the amount of soil organic matter increases with organic fertilizer applications. Soil organic matter content varied between 1,63 % and 2,22 %.

Organic and organomineral fertilizers contain plant nutrients which are in the chemical fertilizers and organic matter together. These fertilizers contain nitrogen (N), phosphorus (P), potassium (K), sulfur (S), zinc (Zn) plant nutrients, and humic-fulvic acids and organic matter (Süzer and Çulhacı, 2018). The contents of organic and organomineral fertilizers affect the soil structure, physical, chemical, and biological structure ıf soil sustainable of soil positively. Therefore, organic matter increases the mineral holding capacity of soils (cation exchange), water and air retention, and mineral element levels. These positive properties of the organic matter contained in organomineral fertilizers, which improve the soil structure, positively affect plant growth and the yield harvested from the hectares (Kacar and Katkat 1999; Makinde et al., 2011; Olaniyi et al., 2010; Süzer 2010a; Süzer 2010b; Süzer and Çulhacı 2016).

The total amount of N, NH_4-N, and NO_3-N was analyzed in the soil samples taken after harvest. The total N was determined between 0,068 % and 0,104 %, NH_4-N 28,7-88,8 mg kg^{-1}, NO_3-N 9,1 mg kg^{-1} and 20,5 mg kg^{-1}. The change between nitrogen and its forms in the soil changes with organic fertilizer properties, soil properties, and plant nitrogen uptake. The results of P analysis in soil samples taken after harvest varied between 16,5 mg kg^{-1} and 40,5 mg kg^{-1}. The amount of available P in the soil varies depending on the P content of organic fertilizers, the degree of mineralization, and soil properties. The exchangeable K, Ca, Mg and Na contents of the soils varied between 0,248-0,421g kg^{-1}, 5,79-7,07 g kg^{-1}, 0,72-1,13 g kg^{-1}, 0,101-0,122 g kg^{-1}, respectively.

In general, the exchangeable cation contents of the soils differed due to the chemical properties of organic fertilizers, the emergence of nutrients with mineralization and the amount of

nutrients uptake from the soil during the development of the plant. The amount of N of organic fertilizers and composts must be converted (mineralized) into ammonium or nitrate by soil microorganisms before using by plants. The rate of mineralization is determined by the level of microbial activity, which varies with environmental factors (such as temperature and humidity), the characteristics of the organic material (such as the C:N ratio and lignin content).

Incompatibility of N uptake for plant and N released from organic material can lead to N deficiency and plant nutrient deficiencies or release of more N than needed during the growth process. If the form of N released from organic matter and the form usable for plants is different, it causes negative effect on the plant (IPNI, 2017). Most organic fertilizers do not have an N:P ratio compatible with plant needs. This type of fertilizer can be applied at 3-5 times the plant's need for P when added to the N requirement of the plants. If this imbalance is not taken care of, long-term organic fertilizer application may cause P accumulation (IPNI, 2017).

Extractable microelement contents were analyzed with DTPA in soil samples taken after harvest. The results of Fe, Cu, Zn and Mn extractable with DTPA of the soils varied between 10,2-21,1 mg kg^{-1}, 2,22-3,48 mg kg^{-1}, 0,70-2,51 mg kg^{-1} and 9,4-24,9 mg kg^{-1}, respectively. According to the control application, Fe, Mn, and Cu were determined at the highest C application after harvest, while Zn was determined in L application. It has been reported that organic compounds formed by the decomposition of organic materials in the soil contribute to the increase in the amount of plant nutrients that cannot be taken in the soil (Chen and Avnimelech,1986).

Animal manures show significant differences in terms of their physical and chemical properties according to certain feeding and management practices. N fertilizers exists in both inorganic and organic forms. The N content in fresh manure is variable because ammonia can be lost gaseously quite easily. In some conditions, the application of fertilizer to the soil surface can result in N losses. Application timing and location are important parameters to consider in reducing such losses. Accurate determination of the appropriate application dose for fertilizer requires accurate estimation of nutrient content analysis and possible N mineralization rate after application. Most of the P and all of the K in animal manures and composts are in inorganic form, which is available to the plant. The materials composted with the right methods will decompose slowly, they act as slow-release N sources for months or years. Composts can have significant differences in quality, maturity, and nutritional content, depending on the material used, composting processes, and processing style (IPNI, 2017).

CONCLUSIONS

In the study, the effects of different organic and organomineral fertilizers (S, L, OM, and C) were different from each other. In the study, organic applications increased the yield and effect positively soil properties.

According to the results, organic and organomineral fertilizers which are produced from biogas plant which is fed organic wastes are utilized for their effect on soil properties and yield. According to the results obtained, organic and organomineral fertilizers produced from the biogas plant fed with organic wastes are preferred because of their effects on soil properties and yields. For this reason, chemical fertilizer applications have decreased and more sustainable agriculture has been achieved. This is very important for determining fertilizer application doses in fertilization programs causing less chemical fertilizer to be applied to the soil.

In the literature studies, when the studies on organomineral fertilizers in the world are examined, the results obtained are summarized as follows (Cengiz and İrget, 2018);

1. Compared to conventional agriculture, the yield was found to be lower in some cases, mostly at the same level, and in some cases higher.
2. Results similar to yield were found in terms of quality.
3. It has been observed that there may be opportunities to benefit from lots of materials in the preparation (production) of organomineral fertilizers.
4. The effects of organomineral fertilizers on the physical, chemical, and biological properties of the soil are mostly positive.
5. According to the cost-benefit analysis, it was determined that the use of organic and organomineral fertilizers is more beneficial than chemical fertilizer applications.

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RESEARCH OF RESISTANCE OF WESTERN FLOWER THRIPS, Frankliniella occidentalis (PERGANDE) (THYSANOPTERA: THRIPIDAE) POPULATIONS TO DIFFERENT INSECTICIDE GROUPS

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ABSTRACT

Frankliniella occidentalis [(Pergande) (Thysanoptera: Thripidae)], also known as Western flower thrips, harms vegetables and ornamental plants in our country, is a difficult pest to control. Especially in confined areas such as greenhouses, producers apply insecticides intensively and frequently in order to have a short-term effect on this pest. It has been shown that *F. occidentalis* develops resistance to insecticide classes from different groups in many agricultural products. Due to the failure of the control as a result of resistance development, overdosing and frequent spraying is performed, which increases the product cost. This also causes other problems such as environmental pollution and the impact of pollinators. Resistance to pesticides in pests directly affects productivity in agricultural production. This study was carried out in greenhouses in different provinces in Aegean Region (İzmir and Manisa provinces) which is known to used plant protection products (PPP) intensively. The resistance status of *F. occidentalis* populations against synthetic pyrethroid, carbamates and spinosyn group insecticides (spinosad, spinoteram, acrinathrin, formetanate hydrochloride) was determined by capsule dipping bioassay.

Key words: *Frankliniella occidentalis*, insecticide resistance, bioassay studies.

INTRODUCTION:

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is a difficult to control pest that can cause significant yield losses by absorbing the sap of the leaves, flowers and buds of vegetables and ornamental plants in Turkiye. *F. occidentalis* have high reproductive power and reproduce rapidly in a short time. In addition, it can be protected from insecticide applications because the pest lays its eggs in the plant tissue, the adults and larvae are found in the inner parts of the flowers, and the pupae are located on the soil or plant (Robb and Parella, 1995).

Characteristic silvery spots appear on the tissues it feeds on, the leaves fade and fall in the process of time. In consequence of these suctions, the plant can dry out completely. The nymphs and adults of the pest are also harmful as virus vectors (Immaraju ve ark., 1992; Herron ve ark., 1996; Kontsedalov ve ark., 1998). Especially in closed areas such as greenhouses, producers apply insecticides intensively and frequently in order to have an effect in a short time to control against this pest. Studies have shown that *F. occidentalis* develops resistance to insecticide classes from different groups in many agricultural products. In Turkiye, *F. occidentalis* was first detected in the greenhouse areas of Antalya in 1993 (Tunç and Göçmen, 1995).

Studies have shown that *F. occidentalis* populations in many agricultural products develop resistance to insecticide classes from different groups and make the chemical control of the pest increasingly difficult (Immaraju et al., 1992; Brodsgaard, 1994; Zhao et al., 1995; Karadjova, 1998; Kontsedalov et al., 1998; Jensen, 2000; Espinosa et al., 2002; Herron et al., 2005; Bielza et al.2007).

This study was carried out in greenhouse vegetable cultivation areas in İzmir and Manisa provinces in Aegean Region, which were known to have intensive PPP (plant protection product) use. Resistance situation of *F. occidentalis* populations against synthetic pyrethroid, carbamate and spinosyn group insecticides was determined by bioassay studies. The high resistance rates determined in our study have emerged as an undeniable result of intensive and frequent insecticide application against thrips in greenhouses. With the failure of chemical control, vegetable producers increase the application dose and the number of applications. Therefore, as a result of the development of resistance, the cost of the product increases and it causes residues and threatens human health. It also causes many problems such as environmental pollution and affecting bees, birds and beneficial insects. In addition, incorrect insecticide selection and incorrect applications cause chemical control to fail. It is necessary for the producers to have information about the mechanisms of action of pesticides and to be supported in this regard.

MATERIAL AND METHOD

Material

The main materials of the study were *F. occidentalis* populations collected from greenhouse vegetable cultivation areas in İzmir and Manisa provinces in the Aegean Region, susceptible population, active substances of synthetic pyrethroid, carbamate and spinosyn group insecticides licensed and widely used against western flower thrips (Table 1).

Table 1. Information on insecticides used in bioassay studies

Active Ingredient Name	Active Ingredient Ratio	Formulation form	Insecticide Group
Spinosad	480 g/l	SC	Spinosyn
Spinoteram	120g/l	SC	Spinosyn
Acrinathrin	75 g/l	EW	Synthetic Pyrethroid
%50 Formetanate hydrochloride	%50	SP	Carbamate

In bioassay studies, materials such as green bean plants, stereomicroscope, bioassay cups, filter paper, Triton X-100 (Roche Diagnostics GmbH, Manheim), stopwatch, petroleum jelly, dip cups, glass measuring tapes, and forceps were used.

Cultivation of *Frankliniella occidentalis* Populations

The sensitive *F. occidentalis* population used in our project was obtained from Bornova Plant Protection Central Research Institute. *F. occidentalis* populations and the sensitive population were grown on green beans in insect growing rooms with 25 ± 1°C temperature, 40-50% humidity and 16 hours light and 8 hours dark conditions in plexiglass cabinets. Plastic containers covered with filter paper were used as production cages. Beans disinfected with bleacher were left for 3 hours to dry. After drying, the prepared honey and pollen mixture was applied to the bean capsules. Green bean fruits were replaced with new ones 3 times a week.

Bioassay Studies

The method recommended by IRAC (Insecticide Resistance Action Committee) was used in the bioassay studies applied on *F. occidentalis* populations in the laboratory (Anonymous 2022). First of all, a stock solution was prepared with bidistilled water containing 0.02% Triton X-100. Bidistilled water containing 0.02% Triton X-100 was used in all dilutions made from the stock solution and in the control. Green bean capsules were cut into pieces of 20 mm length and sealed the cut ends with vaseline. This prevented thrips from hiding in the cracks in the cut ends and drying out of the bean segments. The cut beans were immersed in the solution for 30 seconds. After dipping, the bean capsules soaked in the solutions were dried under the fume hood about 2 hours.

Petri dishes for bioassay were closed by placing 20 adult individuals in each petri dish. They were kept for 24 hours in a climate chamber with 25 ± 1 ℃ temperature and 16 hours light and 8 hours dark conditions. Mortality was assessed after 24 hours using a binocular microscope. Thrips were stimulated with a brush, and those that did not show any signs of movement were recorded as 'dead'. LC_{50} values obtained by bioassay experiments were determined by probit analysis (Finney 1964) using POLO-PC (LeOra Software 1994) program.

RESULTS AND DISCUSSION

11 populations were collected from greenhouses in İzmir and Manisa provinces and were cultured in insect breeding rooms. Information about *F. occidentalis* populations is given in Table 2.

Table 2. Information about the collected *F. occidentalis* populations

Population Name	District	Product
Manisa/Akhisar	Selçikli	Green pepper
İzmir/Menemen	Türkeli	Cucumber
İzmir/Menemen	Değirmendere	Green pepper
İzmir/Menemen	Emiralem	Cucumber
İzmir/Menemen	İnönü	Cucumber
İzmir/Menderes	Çamönü	Cucumber
İzmir/Menderes	Altıntepe	Green pepper
İzmir/Menderes	Akçaköy	Cucumber
İzmir/Menderes	Merkez	Cucumber
İzmir/Bayındır	Merkez	Cucumber
İzmir/Bayındır	Karaveliler	Vegetable marrow

At first screening for resistance, LC_{50} values of each active substance were calculated for the sensitive population in order to determine the diagnostic dose. This LC_{50} value (diagnostic dose), which was also determined, was applied to the populations collected from the greenhouse vegetable areas. As a result of bioassay studies, populations with low mortality rates were mass produced. LC_{50} values were calculated by performing a bioassay again on these populations produced and estimated to be resistant. In this way, it was easier to find the resistant population in *F. occidentalis* populations (Halliday and Burnham, 1990). LC_{50} values obtained from susceptible populations were performed are given in Table 3.

Table 3. LC_{50} values and confidence intervals obtained from susceptible populations in bioassay studies

Insecticide	Populations	LC_{50} (ppm)	0.95 Confidence interval	Heterogeneity
Spinosad	Susceptible population	0.745	0.441-1.041	0.83
Spinoteram	Susceptible population	0.623	0.296-0.851	0.62
Acrinathrin	Susceptible population	0.202	0.005-0.545	0.55
%50 Formetanate hydrochloride	Susceptible population	0.356	0.047-0.690	0.76

Our studies of differential dose application to populations collected from greenhouses have been completed. Bioassays of populations of Bayındır/Merkez, Bayındır/Karaveliler, Menderes/Çamönü, Menderes/Türkeli Menderes/Akçaköy, Menemen/Emiralem populations were eliminated due to the high mortality rate as a result of differential dosing. The resistance ratios to the insecticides used in the tests were calculated by dividing the LC_{50} values determined for all populations to the LC_{50} values of the susceptible population. The LC_{50} and LC_{90} values of our populations are given in Table 4 below.

When all data are evaluated, it was seen that *F. occidentalis* populations collected as a result of bioassay studies have different levels of LC_{50} values against insecticides from different groups. According to the results of the studies conducted on the populations collected from the greenhouse vegetable areas of İzmir and Manisa provinces, it has been determined that there is a significant level of resistance against insecticides.

The Menderes/Altıntepe population was found to have the highest resistance ratio and LC_{50} values for synthetic pyrethroids, carbamates and spinosyn group insecticides (spinosad, spinoteram, acrinatrin, formetanate hydrochloride). It was determined that LC_{50} value and resistance ratio of Bayındır/ Karaveliler population were lower than the other populations. Nevertheless, it was observed that there was a more specific resistance against to spinosyn and carbamate groups of insecticides compared to others. Dong-Gang et al. (2016) performed bioassay studies on *F. occidentalis* populations in vegetables, in China and found a high resistance to spinosad in all populations when compared to the susceptible population. It was emphasized that the efficiency of spinosad used to control against *F. occidentalis* populations decreased and resistance management strategies should be applied as soon as possible to reduce the development of resistance. On the other hand Dağlı (2018) suggested that spinosad resistance was permanent in the Antalya-2015 population. They emphasized that the application of insecticide rotation in the struggle was absolutely necessary in terms of resistance management strategies.

Insecticide resistance has been detected in many *F. occidentalis* populations, which is an economically important pest found in crops grown around the world. Wan et al., (2021), conducted to determine the main thrips species in Mexico, they found that *F. occidentalis* species were more resistant than other species among 13 populations. Gholam and Sadeghi (2016) stated that the use of all Integrated Pest Management Strategies would be more effective in the control of *F. occidentalis* that they collected from greenhouse vegetable areas in Iran. It has been indicated that a new approach to the insecticide resistance management of *F. occidentalis* was required due to the resistance to most of the conventional insecticides. Strategies need to be developed to reduce selection pressure, minimize the use of insecticides, and prevent cross-resistance.

Table 4. LC_{50} and LC_{90} values obtained as a result of diagnostic doses

Insecticides	Populations	LC_{50} (ppm)	LC_{90} (ppm)	Resistance Ratio
Acrinathrin	Menderes Merkez	8.514	33.110	42.148
	Menemen Değirmendere	6.189	28.704	30.638
	Akhisar/Selçikli	10.362	53.941	51.297
	Menderes/Altıntepe	14.978	62.463	74.148
	Menemen/İnönü	5.038	22.971	24.940
	Bayındır/Karaveliler	4.136	20.253	20.475
Formetanate hydrochloride %50	Menderes Merkez	9.209	24.985	25.867
	Menemen Değirmendere	7.102	20.917	19.949
	Akhisar/Selçikli	12.051	56.884	33.851
	Menderes/Altıntepe	17.618	68.204	49.488
	Menemen/İnönü	7.129	21.235	20.025
	Bayındır/ Karaveliler	4.927	21.269	13.839
Spinosad	Menderes Merkez	19.830	86.136	26.617
	Menemen Değirmendere	12.485	67.148	16.758
	Akhisar/Selçikli	28.901	92.037	38.793
	Menderes/Altıntepe	34.603	101.240	46.446
	Menemen/İnönü	10.469	51.703	14.052
	Bayındır/ Karaveliler	6.038	20.412	8.104
Spinoteram	Menderes Merkez	14.067	56.459	22.579
	Menemen Değirmendere	7.553	44.690	12.123
	Akhisar/Selçikli	19.085	146.74	30.634
	Menderes/Altıntepe	26.032	77.621	41.784
	Menemen/İnönü	8.675	23.814	13.924
	Bayındır/ Karaveliler	5.131	19.872	8.235

CONCLUSIONS

The situation of preventing and delaying the adaptation of non-target pests to plant protection techniques, which includes their control, is called resistance management. Resistance management is also an important part of Integrated Pest Management and must be put into practice. Detection of resistance in the early period increases the success of the struggle studies.

Providing that chemical control from Integrated Pest Methods is required, unnecessary spraying should be avoided by taking into account the economic damage threshold. Besides to these, attention should be taken to apply insecticide applications to other pests, including *F. occidentalis*, during periods when populations are high. However, spraying with broad-spectrum drugs should be avoided when beneficial insects are active. In the use of pesticides, attention should be paid to the application dose, application interval and spraying time. It should not be forgotten that the

calibration of the spraying tools increases the expected advantage from the pesticides. In addition to all of these, monitoring studies should be realized regularly.

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THE EFFECT OF IODINE PREPARATIONS APPLIED BY DIFFERENT METHODS ON YIELD CHARACTERISTICS OF OATS

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ABSTRACT

Iodine is an essential nutrient for all living organisms to maintain their vital functions. Fertilization of oat with iodine may be increasing its yields. This study, it is aimed to investigate whether the yield of oats, whose production and consumption has increased in recent years, can be increased by applying to the seed before planting, to the soil after planting, and to the foliar during the early maturation period of the plants. Two different iodine forms [biologically active iodine (BAI) and potassium iodine (KI)] and three different application methods (seed, soil and leaf) were applied to oats grown in field conditions. In the study, panicle length (cm), panicle weight (g), number of seeds per panicle (number), grain weight per panicle (g), and grain yield (kg/da) were investigated. According to the research findings, the length of the panicle increased with the application of iodine. Properties that directly affect yield such as panicle weight, number of seeds per panicle and grain weight per panicle increased with the application of BAI from the soil. The highest yield was obtained from the application of BAI (366.78 kg/da) from the soil. As a result of the research, it was determined that the application of BAI from the soil was effective in increasing the yield and improving the yield characteristics of the oat plant. In addition to basic fertilization in cereal cultivation, iodized fertilization from the soil can contribute to obtaining high-yield products. Since the product containing biologically active iodine will limit the use of chemicals, it can be recommended as an environmentally friendly application.

Keywords: Biologically active iodine, Potassium iodide, Oat, Yield components.

INTRODUCTION

Iodine is one of the important nutrient elements required for the normal growth and development of living organisms. Its most important function is to control the functioning of the thyroid glands in the human body. It is thought that iodine deficiency can be controlled with less cost by increasing the biological availability and assimilation of the amount of iodine in foods (Cakmak et al., 2017).

The low iodine concentration in crops may be related to the low iodine content in the soil. Because the iodine content of the plants depends on the iodine content in the soil where they grow, and the plants take the iodine into their roots and stomata in their leaves. In most cases the elements are transported to the upper parts of the plants through the root system. The main source of iodine in soils is atmospheric iodine, and the main source of iodine in the atmosphere is the sea and oceans

(Tsukada et al. 2008). Because of the evaporation of water from the oceans and falling to the soil as rain, iodine joins the terrestrial food chain (Caffagni et al. 2011). Due to the calcareous and clayey soils of our country, the level of microelements, especially iodine, is gradually decreasing in the crops grown. It should be ensured that essential nutrients for humans and animals such as iodine are present in also plants by fertilizations.

In recent years, both in the world and in our country, depending on the increase in the income and welfare level of people, the understanding of healthy and balanced nutrition has increased, and this understanding brings oats to the forefront alongside other alternative products. Studies have shown that oats contain important antioxidant substances in terms of human and animal health (Dokuyucu et al., 2003; Peterson et al., 2005), high in fiber content, which has a cholesterol-lowering effect, and rich in Fe (Wood, 2001). While the oat plant is rich in P, Fe and Mn, its husk contains a large amount of Si. There is very little amount of vitamins A, D and E in the grain and oil. In addition, it is reported that oat flour containing soluble fiber beta-glucan reduces the risk of heart disease when consumed 5-10 g per day (Peterson et al., 2005). In addition to being animal feed and human food, oat has gained importance especially in recent years due to the increase in its use in the pharmaceutical and cosmetic industry.

This study has been investigated whether the yield of oats, whose production and consumption has increased in our country in recent years, can be increased by the applications made to the seed before planting, to the soil during planting and to the foliar during the early maturation period of the plants.

MATERIAL AND METHOD

A field trial was conducted with Albatros oat cultivar to examine the response of oat yield to iodine application. The plant and iodine fertilizer materials, environment characteristics, field trial procedure and set-up of the experiments, are fully described by Kutlu et al. (2022).

The plants were harvested in the second half of July, when the panicles were fully mature. Important yield parameters such as panicle length, panicle weight, number of grains per panicle and weight were determined in oats. The parcel yield was found by weighing the grains obtained from the parcel and converting them to kg/da.

The data obtained from the experiment were evaluated according to the factorial trial design in the IBM-SPSS 20.0 statistical package program.

RESULTS AND DISCUSSION

This project aimed to contribute to the effective meeting of the iodine needs of people who have to be fed with foods with low iodine content, especially in our country, and to determine the effective use of seed application, which is an alternative method to iodine fertilization applications. In our previous report, which examined the protein, beta glucan, ADF, NDF, iodine and potassium contents of oats, which are indicators of grain nutrition quality, the effectiveness of BAI was emphasized (Kutlu et al., 2022). As it is known, agro-strategies that can be applied to obtain bio-fortified crops have been the focus of attention in recent years. Among the nutritional elements mentioned as important for biofortification, iodine has come to the fore especially in recent years (Smoleń et al. 2016). Biologically active iodine, which is the iodine form used in this study, was tried for the first time as a plant fertilizer. "Iodis-Concentrate" was used as a biologically active iodine source. "Iodis-Concentrate" is an advanced technology product with international patents

and certificates used in 21 countries. It is mainly used as a human food supplement through the iodine enrichment of drinking water or juice (Orozbaeva et al. 2017). In addition, due to its antifungal, antiviral and antibacterial properties, it is used to obtain both food preservatives and iodine-enriched foodstuffs by adding to the content of dairy products and processed meat products. By adding it to the rations of poultry, it is used both to protect animal health and to obtain iodine-rich animal food (Indyukhova et al. 2019; Dalievska and Pokotylo, 2021).

"Iodis-Concentrate" is used in plant production with the bio-fertilizer called "Biojodis", which biologically active iodine is included in. In some studies related with Biyojodis, it has been argued that the superiority of "Biojodis" over other bio-fertilizers is due to the biologically active iodine in its content (Gaurilčikienė et al. 2008; Piotrowski et al. 2016; Sosnowski et al. 2020). For this reason, in this study, we tried for the first time whether iodine could be used for the development and iodine enrichment of the oat plant by applying "Iodis-Concentrate" to plants in three different ways (seed, soil and foliar). Thus, we aimed to introduce an alternative source of iodine to researchers interested in iodine fertilization. We hope that this study will be the first step towards understanding the mechanism of biologically active iodine in plants, unlike mineral and organic iodine compounds.

It was found that the iodine forms used in the study were statistically effective at the level of 1% on all features except the number of grains per panicle. While the application methods did not make a difference on the panicle length, the interaction of "iodine form × application method" was found to be significant in all properties except panicle length (Table 1). This shows that the effect of the iodine form may vary according to the application method.

Table 1. The F values obtained from the analysis of variance of the examined features

	Iodine form (I)	Application Method (A)	I × A
Panicle length	10.20**	0.36ns	0.64ns
Panicle weight	7.30**	6.15**	11.35**
Grain number per panicle	1.24ns	5.26**	5.30**
Grain weight per panicle	6.16**	27.92**	53.70**
Grain yield	231.05**	65.66**	25.93**

** P < 0.01, ns: nonsignificiant

The effect of iodine applications on the panicle characteristics of oats is shown in Figure 1. The panicle length increased with the application of iodine. While the highest panicle length was obtained from foliar BAI application, KI application from soil presented the second highest value. Properties that directly affect yield such as panicle weight, number of seeds per panicle and grain weight per panicle increased with the application of BAI from the soil.

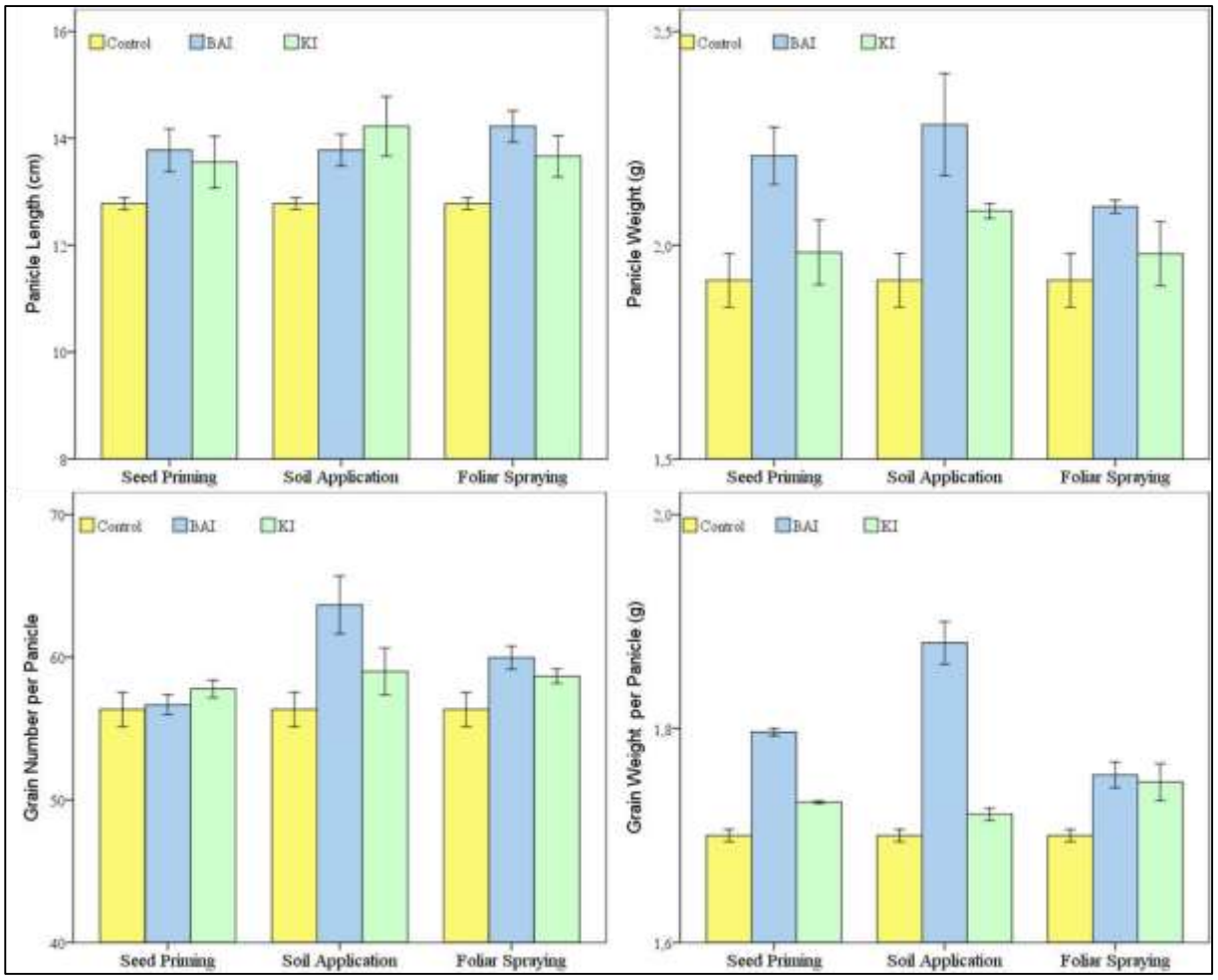

Figure 1. The effect of iodine applications on the panicle characteristics of oats

The positive effects of iodine applications on the growth and development of various plants have been found after relatively low doses of iodine applications (Medrano-Macias et al. 2016; Gonzali et al. 2017). Cakmak et al. (2017) reported that the application of high doses of iodine in the form of KI from the soil or foliar affects plant growth negatively and reduces yield. The BAI used in our study is a product that can be used in organic agriculture and its negative effects in overdose, such as KI, have not been recorded. Gaurilčikienė et al. (2008) reported that this organic product has positive effects on seed health and can increase the yield and quality of grains when applied from seed or foliar.

In the study, the grain yield of oats increased with iodine applications. While the highest yield was obtained from soil application of BAI (366.78 kg/da), it was followed by foliar application of BAI (317.91 kg/da). Similarly, the application of KI from the soil and foliar provided an increase in yield compared to the control, while the least increase in yield was obtained from the application of BAI from the seed (241.12) (Figure 2).

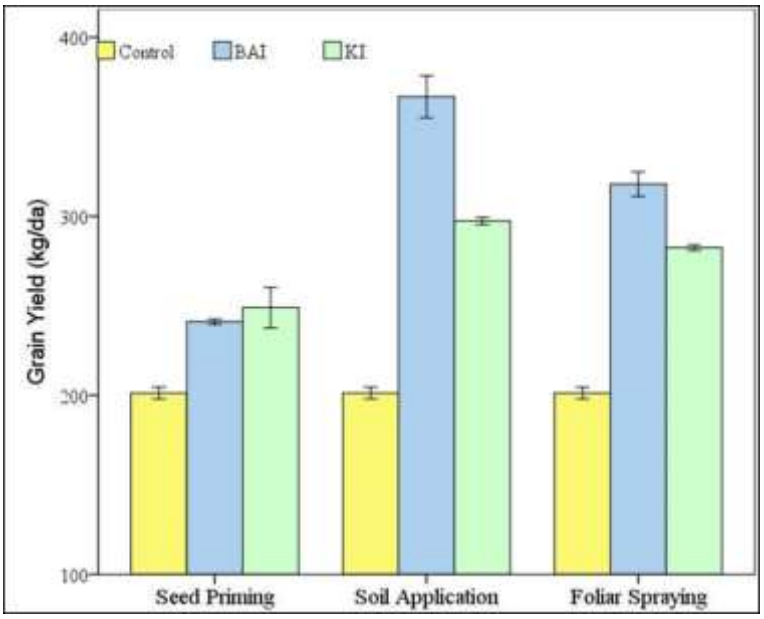

Figure 2. The effect of iodine applications on the yield of oats

CONCLUSIONS

As a result, it was found that the increase in yield and its components reached very high values with the application of BAI from the foliar and soil. In order to determine the optimum dose for high yield should be continued the studies. It can be said that especially seed applications with BAI are effective by ensuring that the first development of plants is healthy and strong. However, the most effective application for both forms of iodine is the foliar application. Since BAI is an organic product, it can be used safely as an environmentally friendly method to increase the yield and enhancement morphological traits of oats.

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DETERMINATION OF BUD FERTILITY AND PRUNING TYPE OF SOME TABLE GRAPES

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ABSTRACT

The aim of this research is to determine bud fertility and pruning type of some table grapes, which are Osmanca, Alphonse Lavalle, Superior Seedless, Cardinal, Samancı seedless and Red Globe cvs. (*Vitis vinifera* L.) planted in faculty application vineyards. During the winter pruning, ten one-years-old cane above to 10 bud were pruned for each cultivar. The concerning bud position, flower clusters per shoot were counted on developing summer shoots. Average bud fertility (number of flower clusters /shoots) were ranged between 0.46 and 2.40 depending on cvs. While the highest bud fertility was obtained from Alfonse Lavalle and Cardinal cvs, the lowest bud fertility was taken from Superior seedless. When bud fertility and cluster size considered, it is advised that spur pruning for Alfonse Lavallee, Cardinal and Osmanca cvs, and semi long cane pruning for Samancı seedless and long cane pruning for Superior Seedless and semi long cane or spur pruning for Red Globe cvs.

Key words: bud fertility, table grapes, *Vitis vinifera* L., pruning type

INTRODUCTION

Turkey is one of the gene centers of grapes (*Vitis vinifera* L.). Turkey total fresh grape production is about 4.2 million tons (FAO, 2020) (Fig.1). In terms of grape production, Turkey is ranged in 6th place among the World countries. Grape and wine have been produced since ancient times in Turkey.

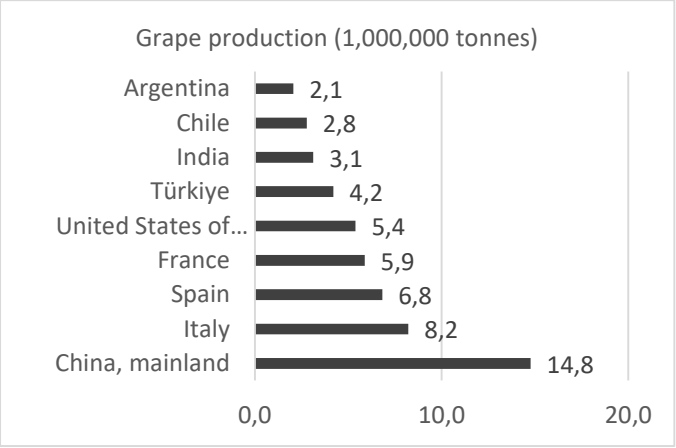

Fig.1. Main grape productions of countries (1,000,000 tons)

Grape production depends on many factors such as maintenance, ecological conditions and bud fertility. Bud fertility means that number of cluster primordia development during preceding years of bud break. Bud fertility has been determined by mainly cluster numbers and cluster sizes in bud or in shoot.

At Thompson seedless cultivar, bud differentiation occurs during second half of May during the flowering time for 4 to 9 nodes from basal on the shoots in California in USA. Bud fertility are affected by genetic structure, light, temperature, irrigation and mineral nutrition during the bud development in preceding years of bud break(Williams, 2000).

One of the research results show that foliar application of 2.5 kg 15 N ha−1 in pre-bloom and in post-bloom 15N-enriched-ammonium nitrate increased bud fertility of Trebbiano Romagnolo vines (*Vitis vinifera* L.) (Baldi et.al, 2017).

Early defoliation method, before flowering, removing six leaves from basal has been used for decreased cluster compactness and enhanced the berry composition of wine grapes and for alternative method of cluster thinning. Early defoliation before flowering results in decreasing bud fertility because of decreasing carbohydrates (Gatti et al. 2012).

Bud fertility can be determined by different methods. One of the methods is to open the buds under stereomicroscope. The others are; microtome method by taking very thin cuttings, according to bud position on the cane, using one year old cuttings grown in the pots, and leaving long canes on vine to follow the developing shoots and clusters according to buds on cane positions. (Ağaoğlu, Y.S. 2002).

Uyak and Doğan, (2018) carried out the experiment with local grape cultivars of Seedless, Süleymani, Hurıst, Mılaki, Reşık, Keçimemesi, Veşifır and Sipiyaşin grown on their own roots in Şemdinli (Hakkari). Their objective was to determine the bud fertility and optimum pruning levels of the grape cultivars. Bud fertility (cluster/bud) of the cultivars was determined by counting the number of clusters on developing shoots from the single-bud scions taken from the 1-10th nodes. It was obtained that Sipiyaşin cultivar should be pruned above the above the 1-3rd buds; Süleymani, Hurıst, Mılaki, Reşık, Keçimemesi and Veşifır cultivars should be short-pruned above the 4-5th buds and Seedless cultivar should be long-pruned above the 7th bud.

The aim of these research is, according to bud positions on cane, to determine bud fertility and pruning type of Osmanca, Alfonse Lavelle, Superior seedless, Cardinal, Samancı seedless and Red Globe (*Vitis vinifera* L.) grape cvs.

MATERIALS AND METHODS

Materials: Six grape cultivars; Osmanca, Alfonse Lavelle, Superior seedless, Cardinal, Samancı Seedless and Red Globe (*Vitis vinifera* L.) were used in the application vineyards of Horticulture Department in Agriculture Faculty in Adnan Menderes University in Aydın province of Turkey.

Methods:

For each one of the cultivar, 10 dormant canes with 10 buds were chosen before winter pruning, and signed with colorful ropes and tighten by lobes written replication number. Canes which had medium size and medium internode length were chosen (Figure 2).

Figure 2. Choosing one years old canes and pruning at ten bud level

According to bud position, developing clusters between first and sixth node on young shoots were counted and recorded (Figure 3).

Figure 3. Developing shoots and clusters at the early spring

Results

As seen in Figure 4, among to cultivars, bud fertility ranged between 0.46 and 2.40 flower cluster/shoot or bud. Statistically, while Alfonse Lavalle and Cardinal had the highest bud fertility, Superior seedless had the lowest bud fertility. The others have the medium bud fertility.

According to bud position on canes, fertility of buds Superior Seedless and Samancı seedless cultivars were examined. For two cultivars, first, second and third buds on canes from basal side had the lowest bud fertility, but bud fertility between fifth and tenth buds increased. In addition that, Samancı seedless had the higher bud fertility than that of Superior seedless for all buds on canes (Figure 5). It can be advised that cane pruning for Superior seedless and spur pruning for Samancı seedless.

Average bud fertility of Alfonse Lavalle and Cardinal cultivars are the higher than the other cultivars. In all cultivars, first, second and third bud fertility is lower than that of bud fertility of between 4 and 10 buds. However, In Alfonse Lavalle and Cardinal cultivars, first three bud fertility ranged between 1.75 and 2.00 and is still high (Figure 6). These cultivars are proper to spur pruning. Çelik (2006) also advised that spur pruning for these cultivars. Dardeniz and Kısmalı (2005) also advised that spur pruning for Cardinal cv. In a similar way, because of basal bud fertility is high,

spur pruning is advised for Atak 77 cultivar (Şen and Atak, 2020). In this research, basal buds of Osmanca cv. had average 1.75 cluster / bud or shoot (figure 6), so it can be advised spur pruning for Osmanca cv. Çelik (2006) suggestions are also compatible with this research results for Osmanca cv. In the other hand, basal buds of Red Globe cv had average 1.0 cluster /bud or shoot (Figure 6) and half cane pruning can be advised. Yet, Çelik (2006) suggest spur pruning for Red Globe. The reason of the suggestion of spur pruning could be heavy clusters such as 1000g.

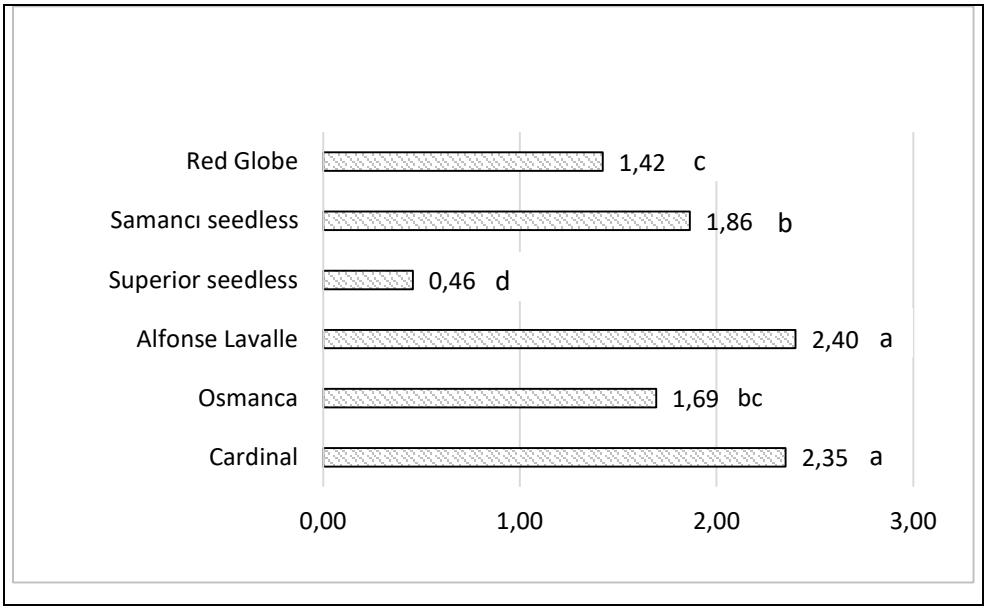

Figure 4. Average bud fertility, according to cultivars (clusters/shoot or bud)

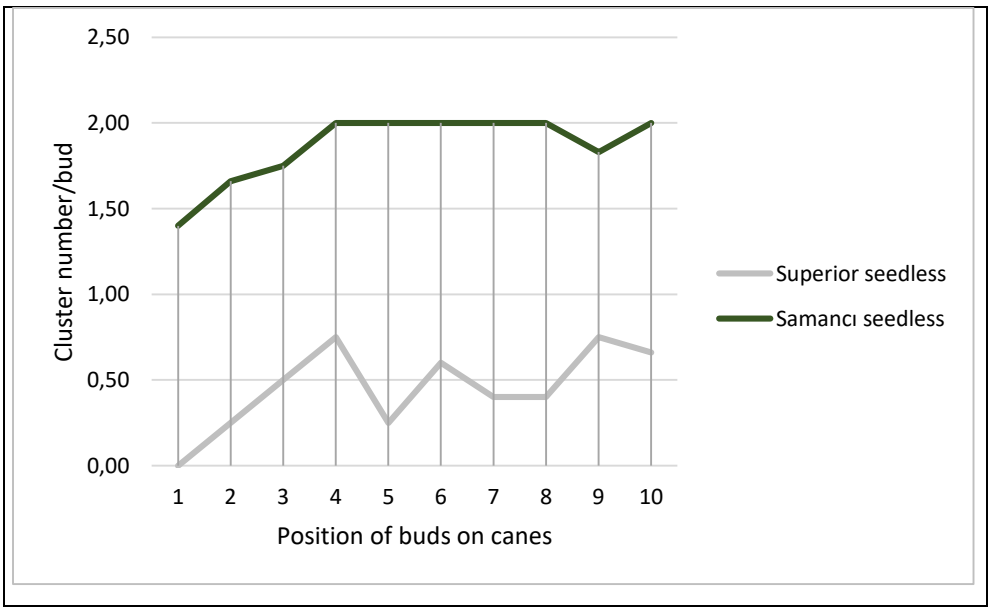

Figure 5. According to bud position on canes, bud fertility (cluster number /shoot) of Superior seedless and Samancı seedless cultivars

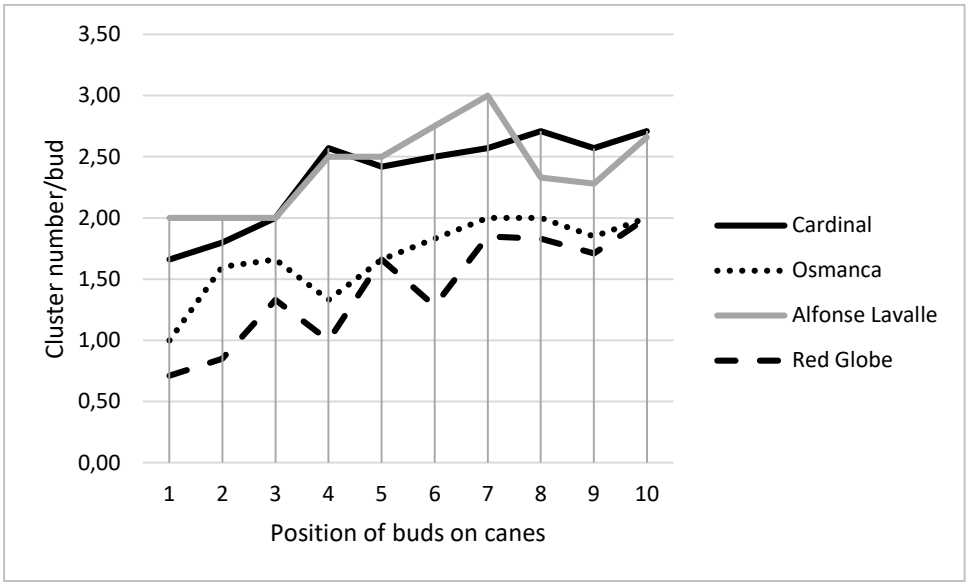

Figure 6. According to bud position on canes, bud fertility (cluster number /shoot) of some seeded cultivars

CONCLUSION

Bud fertility was between 0.46 and 2.40 cluster /bud for examining cultivars. While pruning levels of canes determined, basal buds fertility, especially first, second, third buds fertility have been considered When bud fertility and cluster size considered, it is advised that spur pruning for Alfonse Lavallee, Cardinal and Osmanca cvs, and, spur or semi long cane pruning for Samancı seedless and Red Globe cvs and, long cane pruning for Superior Seedless cv.

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MECHANICAL AND BEARING CONDITION EVALUATION BY VIBRATION ANALYSIS OF THRESHING UNIT OF COMBINE HARVESTER

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ABSTRACT

Due to hard to access areas of the threshing unit for sensor mounting the reliability testing of the threshing unit is hard and often neglected before the harvesting season resulting in breakdown maintenance in peak harvesting time. In this paper, threshing condition of combine harvester using vibration analysis is performed by introducing the bracket. The Adash A4900 Vibrio M analyzer (Adash spol. s.r.o., Ostrava, Czech Republic) is used for vibration signal measurement and DDS Adash software is used for signal processing. The FFT (Fast Fourier Transform) (500Hz-16kHz) demodulation method is used to evaluate the bearing condition and phase analysis along with DDS Adash FASIT (Fault Source Identification Tool) technology is used to evaluate other mechanical condition like looseness, misalignment, and unbalance of the threshing unit of the threshing unit of the NIVA SK5. The effective method for determining the bearing quality condition is identified as demodulated FFT spectrum analysis, which offers the necessary vibration information. The FASIT tool technology provided by Adash DDS is effective and easy to identify the machine conditions like misalignment, looseness, and unbalance.

Keywords: Combine Harvester, Threshing, Bearing, Demodulated FFT, Adash, FASIT,

INTRODUCTION

When combine harvester operates in the field, its components vibrate significantly under multi-source excitation forces which can cause complex vibration and structural deformation. Unexpected dynamic vibrations from different sources of rotating elements of combine harvester can cause a sudden breakdown which cause the delay in harvesting process and cause a huge financial loss and degrades grain quality. The combined harvester can achieve its goal when all the parts are in an expected condition so, the monitoring is necessary before any malfunction. Threshing is also one of the major parameters to complete the harvesting process which is advancing with the time (Špokas et al., 2016). Observing advancements and innovation on threshing technology, it is discovered that grain damage and grain loss are consistently the significant parameters of the threshing mechanism which can be monitored by vibration analysis for optimal performances(Zhao et al., 2011). Discharge beater is one of the essential tools provided at threshing mechanism located adjoining to the outlet and selectively rotated in response to feed rate. It is arranged to transfer crops remains from the threshing drum and conveyor arrangement and basically responsible for net production of grains outlet (Brian Hollaz; Porta Alegra (BR), 2012).

The Fast Fourier Transform (FFT) with Decimation-In-Time (DIT) and XGBoost algorithm was developed to identify the fault type of bearing quickly and accurately. Firstly, the original vibration signal of rolling bearing was transformed by DIT-FFT and divided into the training set and test set and the training set was used to train the fault diagnosis XGBoost model, and the test set was used to validate the well-trained XGBoost model. Finally, the proposed approach was compared with some common methods. It is demonstrated that the proposed approach is able to diagnose and identify the fault type of bearing quickly with almost 99% accuracy which is more accurate than Machine Learning (89.88%), Ensemble Learning (93.25%), and Deep Learning (95%) (Xiang et al., 2021).

Vibration data was recorded and processed for using signal collective processing techniques including self-adaptive noise cancellation (SANC), kurtogram and envelope analysis. Processing results indicate that the seeded planetary bearing defect was successfully detected on helicopters to monitor the health state of their transmission systems and predict remaining useful life of key helicopter components(Zhou et al., 2019).

Vibration analysis provides pertinent information regarding the progressive deterioration of bearings and gives a baseline signature for future monitoring. The wide vibration analysis techniques and its application are very popular in automobile industries (Senthil Kumar et al., 2013)(Liu et al., 2017)(Yu, 2021)(Qi et al., 2019) but few implementations in combine harvester specifying in threshing components. Most of the vibration analyses are conducted in cabin area of the harvester focusing on driver safety and comfort (Jahanbakhshi et al., 2020) (Xu et al., 2019), simulated vibration analysis(Chen et al., 2020) and in laboratory condition (J. Wang et al., 2021). However, the vibration analysis techniques of threshing unit's health monitoring are mostly in laboratory condition. The vibration analysis performed for threshing unit of combine harvester includes the complexity of instrumentation due to its complex structure. The components need to be dismantled to obtain the raw vibration signal for processing.

If bearing defects exist, the measured vibration signal would be amplitude modulated at its characteristic defect frequency. The modulating wave is a pass vibration signal corresponding to local defects, and various demodulation techniques have been developed for the separation. In this work, an envelope demodulation method based on Hilbert transform is employed(H. Q. Wang et al., 2014).

For a continuous time, signal x(t), the Hilbert transform $\widehat{x(t)}$ is defined as

$$\widehat{x(t)} = \frac{1}{\pi}\int_{-\infty}^{\widehat{+\infty}} \frac{x(\tau)}{(t-\tau)}d\tau$$

Then, combine x(t) and $\widehat{x(t)}$ to form a new analytic signal as

$$g(t) = x(t) + j\widehat{x(t)}$$

The envelope x(t) of is defined as the amplitude of g(t) as

$$A(t) = \sqrt{\{x(t)\}^2 + \left\{\widehat{x(t)}\right\}^2}$$

To extract characteristics defect frequencies, By FFT after Hilbert transform is obtained for envelop spectrum.

MATERIAL AND METHOD

The NIVA SK-5 were investigated for vibration analysis as shown in figure 1.

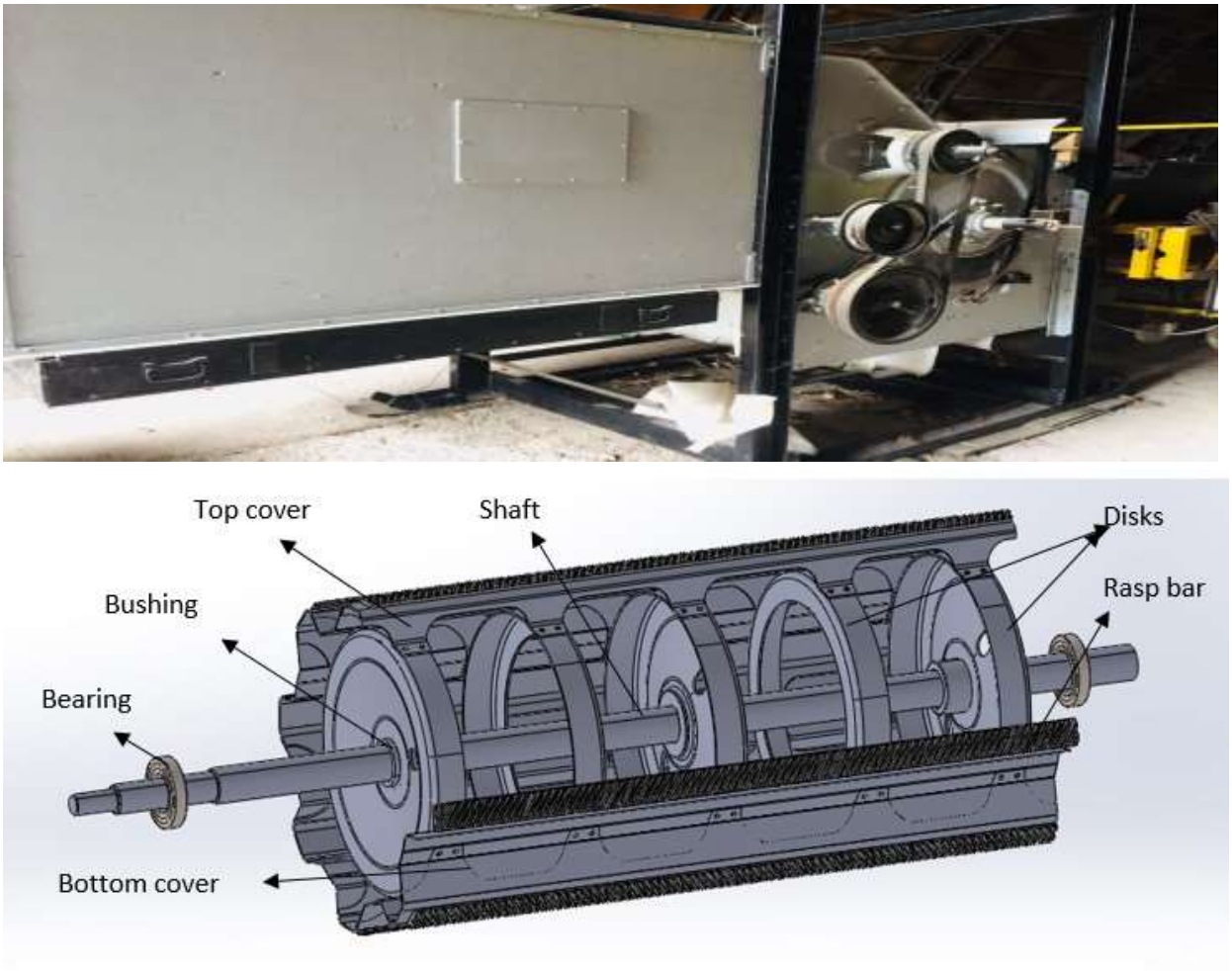


Figure 1. NIVA SK-5 Thresher stand.

The threshing drum bearings consist of KBC 6313 BS: 65× 140 × 33 (KBC Industrial CO. LTD, Zhejiang, China) The vibration measurement was conducted three times for each location. The detail of the technical condition of the threshing, platform and bearing are shown in table 1.

Table1.

Part	Parameters	Value
Platform	Length	*3700 mm*
	Width	*2060 mm*
	Height	*1540 mm*
Thresher	Length	1940 mm
	Diameter	597.88 mm
	No. of rasp bars	8
	Fillet Parameter of rasp bar (symmetric)	3 mm
	Rasp bar Length	1176 mm
	Wide	45 mm
	Distance between rasp bar	178 mm
Bearing (KBC 6313 BS)	Bore Diameter	65 mm
	Outer Diameter	140mm
	Raceway Width	33mm
	Number of Rollers	16
	Static load rating	59.5 KN
	Dynamic load rating	92.5 KN

The measurement was taken in bracket and the housing. The variable measuring threshing speed were same for both the driving and non-driving sides of the threshing. The measuring speed were 220, 210, 350, 420 threshing drum RPMs respectively.

The threshing cylinder rotation frequency was changed by a voltage frequency converter DeltaVFD-C2000 SERIES and a cylinder gear variator. The test bench was driven by a 30-kW electric motor. The conveying mechanism composed of a rotating belt with dimensions of 6 × 0.5 m. Manufactured S235 Steel bracket and its properties are shown in table 2 and figure 2. The measurement was taken in 45° with the horizontal radial axis of the threshing shaft.

Table 2. Material Properties of S235 steel.

Material Property	Value
Density ρ	≈ 7850 kg/m^3
Unit weight γ	≈ 78.5 kN/m^3
Modulus of elasticity E (Young's modulus)	210000 MPa
Shear modulus G	$G = E / [2 \cdot (1 + \nu)] \approx 81000$ MPa
Yield strength f_y	235 Mpa
Ultimate strength f_u	360 Mpa
Poisson's ratio in elastic range ν	0.3
Coefficient of linear thermal expansion α	12×10^{-6} °K^{-1}

Manufactured S235 Steel bracket and its mounting position. The sensors are mounted as shown in the figure 2. Vibration measuring device used is Adash A4900 Vibrio M device with AC150 piezoelectric sensor as shown in figure 3.

The Adash A4900 Vibrio M (Adash spol. s.r.o., Ostrava, Czech Republic) vibration accelerometer with a magnetic base and the Adash A4900 Vibrio M vibration analyzer were used for vibration measurement and data analysis. This type of equipment is useful in detecting early warnings related to bearing conditions for industrial applications, such as high-speed turbines, rotors, gearboxes, etc. This instrument satisfies the ISO 10816-3standard (ISO Standard. 10816-3: Mechanical Vibration. Evaluation of Machine Vibration by Measurements on Non-Rotating Parts.Part 3, 2009)and the vibration testing requirements specified in ISO 10816-3, 2009 ISO10816-3:2009 mechanical vibration—evaluation of machine vibration by measurements on non-rotating parts, Part 3: industrial machines, with nominal power above 15 kW and nominal speeds between 120 min−1and 15,000 min−1when measured in situ.

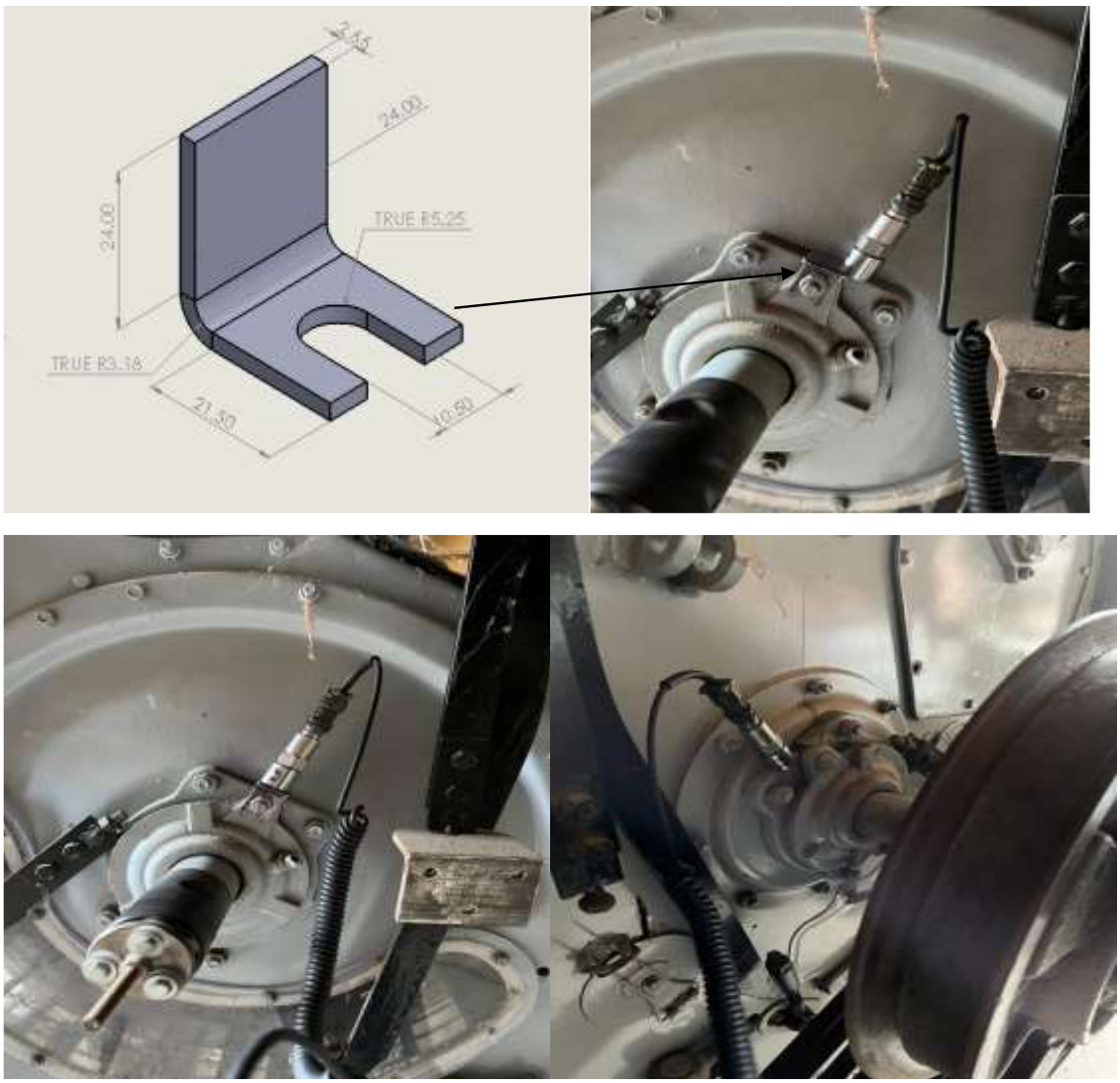


Figure 2: Manufactured S235 Steel bracket and its mounting position on bracket and housing.

Figure 3. Adash A4900 Vibrio M device with AC150 piezoelectric sensor.

For the bracket and housing bearing, modal analysis and harmonic response analysis were carried out using ANSYS software to locate and eliminate the typical natural frequencies that could lead to resonance. It is required to undertake adequate design, material selection, and simulation of that material to avoid the resonance problem before taking the true vibration measurement of the bearing from the made added materials. Before measuring vibration from the added bracket, the choice of material is examined and recommended from the standpoint of material qualities. The bracket is designed to prevent vibrational lapse, and modal analysis is carried out to prevent resonance of identical natural frequencies in related parts. SolidWorks was used to create the bearing, bracket, and housing designs in Figure 1&2.

RESULTS AND DISCUSSION

Modal Analysis

S235 steel was selected as the material of the bracket due to its high stiffness. Its physical properties were: Young's modulus = 210 GPa, Poisson's ratio = 0.3, shear modulus = 76.9 GPa, and density = 7850 kg/m^3 (*Material specification sheet Saarstahl - C45*, 2019). The design of the bracket and housing is designed on the solid works and bearing design is imported form the design library. The design is imported to the Ansys for modal analysis as shown on the figure 4.

Figure 4. CAD assembly of bearing, housing, bolt and manufacture barcket

Table 3. Mesh Statstics

	Bracket	Bearing and Housing
Nodes	4345	57,289
Element	2293	21,305

The eigenvalues for the modal analysis of the bracket in ANSYS were 2495 Hz, 4106 Hz, 8526.3 Hz, 4156 Hz, 9621.9 Hz, 22,433 Hz and 22,8433 Hz (Figure 5). These are the bracket's initial five-

mode shapes. The bracket is fastened to the base and the area of the contact surface for modal analysis using a bolt. Furthermore, the bearing, housing, bolt, and bracket used in the system were subjected to a modal analysis. The bearing races and housing (0.4), rollers and bearing races (0.21), bolts and bracket (0.21), and their frictional connections were described (0.23).

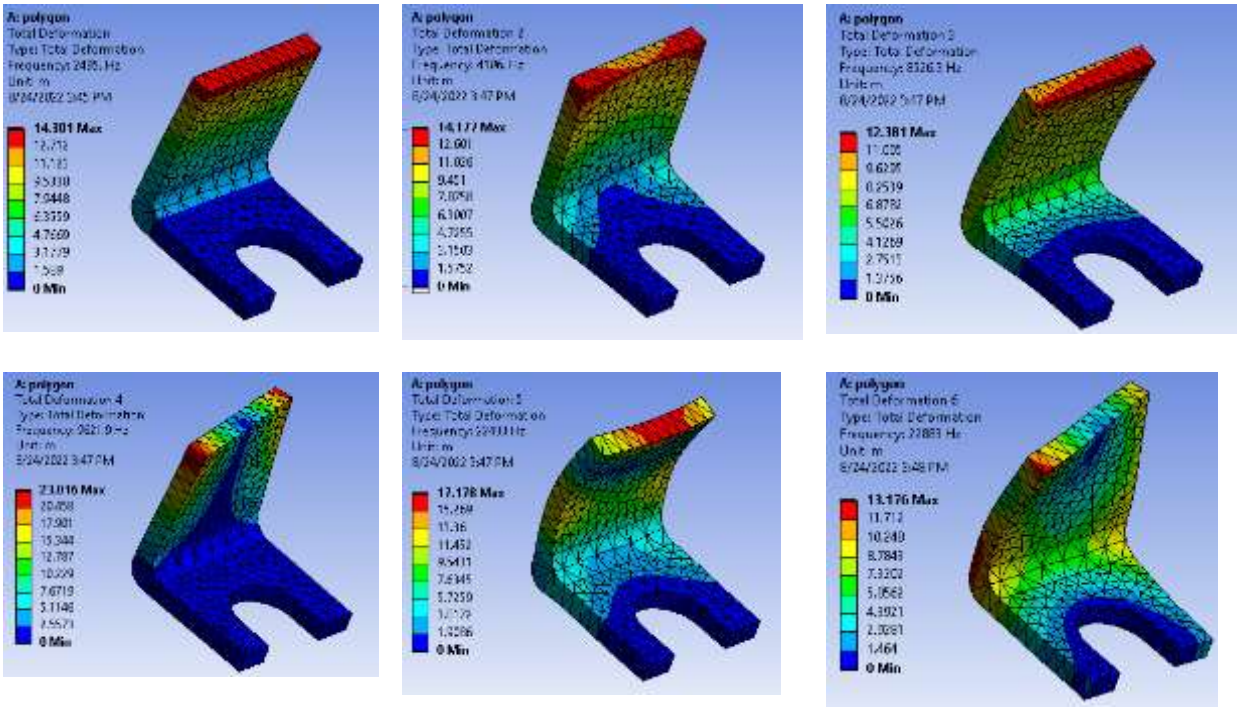


Figure 5. Eigen values and eigen vectors of S235 manufactured bracket.

From the modal analysis the eigen values and eigen vectors are obtained for bolt, bracket and housing as shown in table 4.

Tale 4. Mode shapes of components

	Eigen Values and Eigen Vectors					
	Mode 1 (Hz)	Mode 2 (Hz)	Mode 3 (Hz)	Mode 4 (Hz)	Mode 5 (Hz)	Mode 6 (Hz)
Bracket	2495	4106	8526.3	9621.9	22,433	22,8433
Housing	5027.4	5078.4	5086.	5153.3	5203.4	5748.7
Bolt	43893	44422	47372	85087	94161	94580

The natural frequencies of the components (bearing, bolt and housing) are dissimilar to the natural frequency of the the bracket. Therefore, it willl not create the resonance phenomenon and the bracket is manufactured as per the design and material selected.

Vibration Analysis

The comparative vibration data of the driving and non-driving end for 350 RPM and 420 RPM respectively are presented on figure 4&5 with the major vibration parameters like peak, RMS,

Demodulated peak, and Demodulated RMS. The amplitude of the vibration is greater in driving ends for all the presented parameters due to the pull force experienced by the shaft driven by the belt and pulley system.

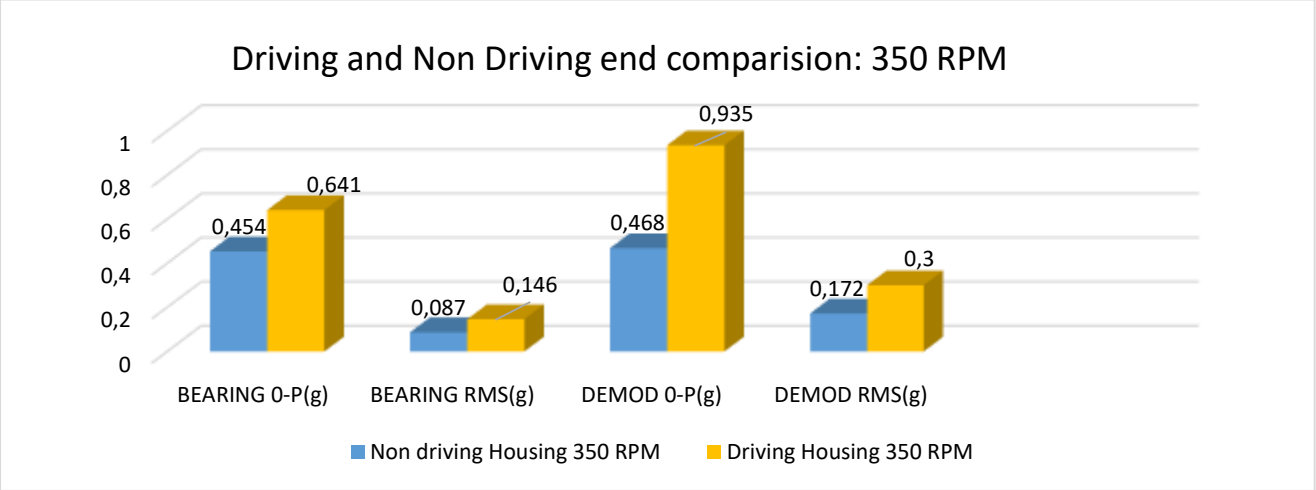


Figure 4. Driving and non-driving ends vibration comparison of NIVA SK-5 at 350 RPM.

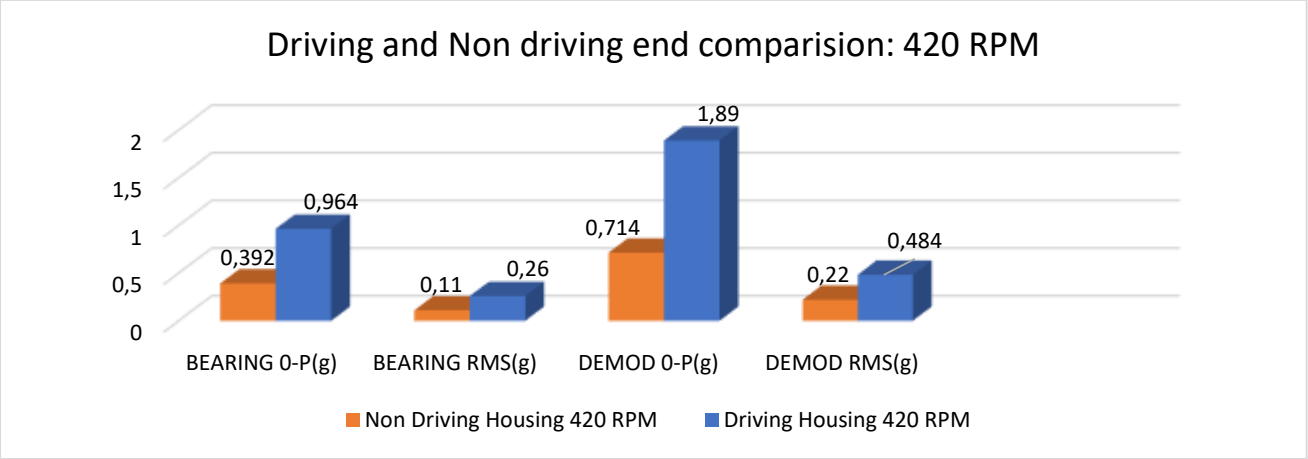


Figure 5. Driving and non-driving ends vibration comparison of NIVA SK-5 at 420 RPM.

The combine harvester's conveniently accessible bearing housing was used to compare bearing vibrations with and without a bracket. Comparing the data, measurements were made. The major goal of these measures was to create a database that would allow researchers to compare cases with and without the constructed bracket. RMS data are mostly employed for bearing vibration analysis. From the results, the measurement difference was determined, and the provided coefficient was applied to the examination of the bearings on the threshing drum. The information gathered indicated that using the bracket increased vibration by 17.72%. (Figure 5) for bearing analysis (DEMOD RMS).

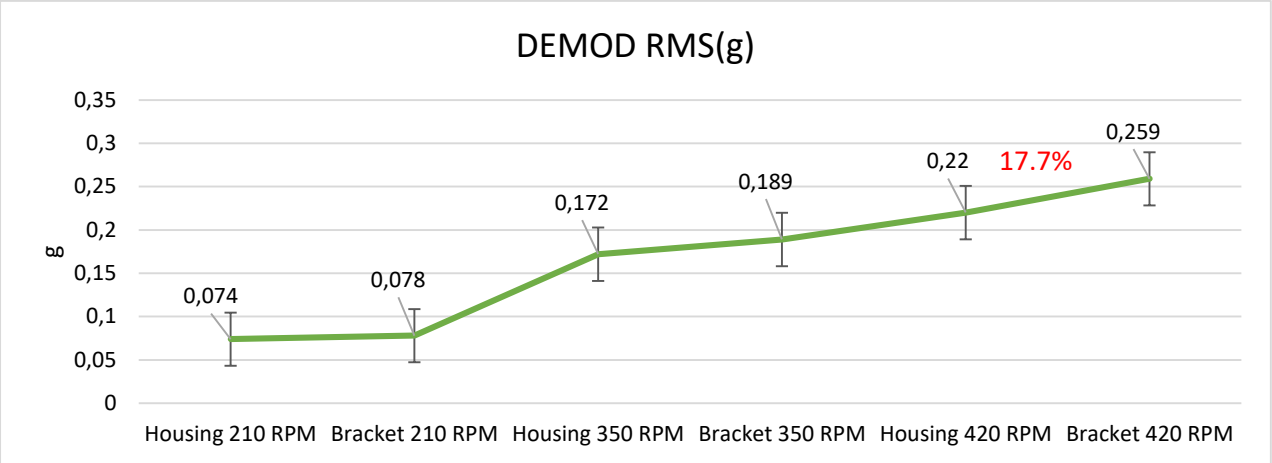


Figure 6: Demodulated RMS comparison for variable threshing shaft rotating speed.

To study about the bearing used in the stand, demodulated FFT is considered when the measurement is from accelerometer mounted on bracket and housing. The fundamental frequency is obtained DDS Adash bearing library and verified from KBC catalogue for bearing KBC (HANWHA, 2001) as shown in Table 3. Multiplying the rotational speed of the threshing drum with the fundamental frequencies of the bearing gives the fault frequencies for different operating speed.

Table 4. Fundamental Frequencies: 6310 SKF

Fundamental Frequencies: 6310 SKF	
FTF (Fundamental Train Frequency)	0.381Hz
BSF (Ball Spin Frequency)	2.04Hz
BPFO (Ball Passing Frequency of Outer Race)	3.07HZ
BPFI (Ball Passing Frequency of Inner Race)	4.93HZ

The demodulated FFT spectrum with amplitude RMS(g) with the threshing speed of 420 RPM when the sensor is mounted on housing for bearing failure frequencies FTF (2.69Hz, 0.004g), BPFO (21.5Hz, 0.005g), BSF (28.5Hz, 0.006g), BPFI (34.5Hz, 0.004Hz) were measured (Figure 7). The RMS higher values should be as near to zero as possible when assessing them. Peak amplitude is under noise threshold for Envelop Analysis.

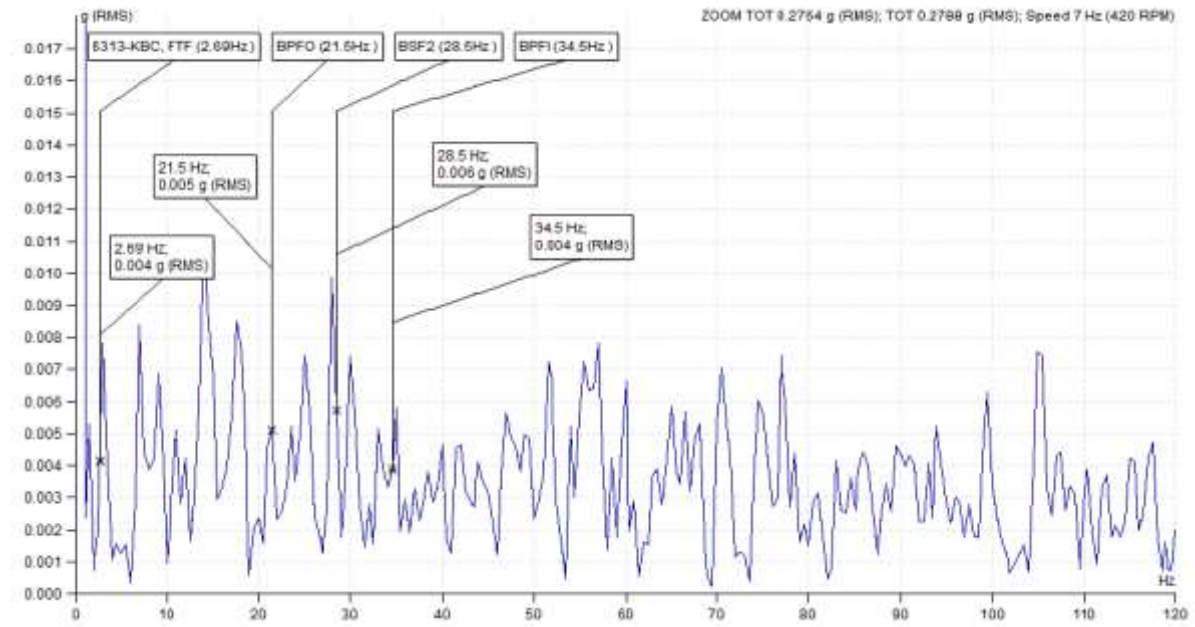


Figure 7: Envelop demodulation of the 6313 KBC of the non-driving end of the threshing drum of NIVA SK-5, sensor mounted on bearing housing.

Similarly, the demodulated FFT spectrum RMS analysis was performed when the sensor is mounted on manufactured bracket. The demodulated FFT spectrum with amplitude RMS(g) with the threshing speed of 420 RPM for bearing failure frequencies FTF (2.69Hz, 0.005g), BPFO (21.5Hz, 0.009g), BSF (28.5Hz, 0.004g), BPFI (34.5Hz, 0.001Hz) were measured when the data recorded from the accelerometer mounting on the bracket (Figure 8).

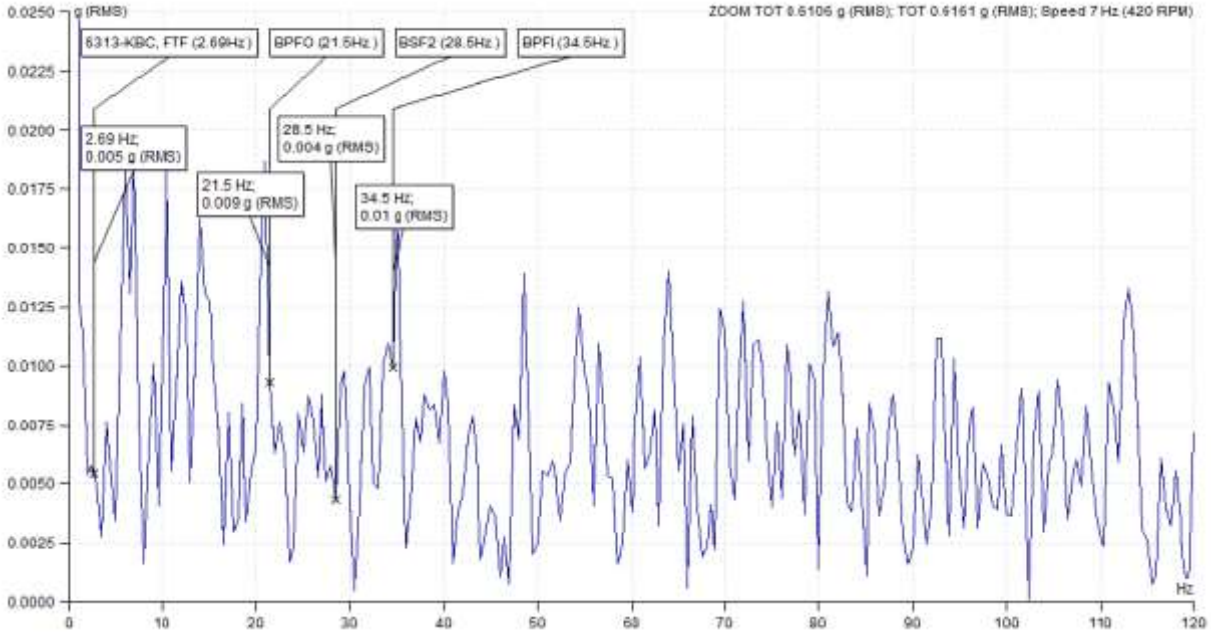


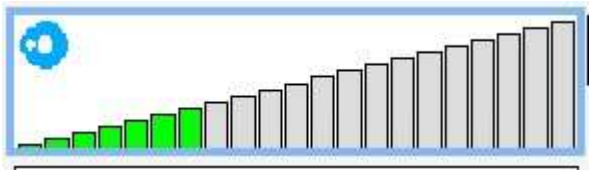
Figure 8: Envelop demodulation of the 6313 KBC of the non-driving end of the threshing drum of NIVA SK-5, sensor mounted on manufactured.

No harmonics and sidebands were recorded for the fundamental frequencies from envelop graphs. The top peaks are observed from the overall spectrum of the variable speed vibration measurement and unusual peaks were also not recorded. The bearing is working in a good condition and need to be examined in a frequent interval after consecutive long threshing work or when the unexpected noise is experienced.

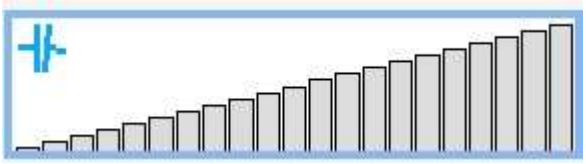
Alarm limit is set according to ISO 10816 with the group 2 specification. Green alarm level is found for bearing analysis. (0.194 g) Red alarm level is found for ISO RMS with velocity amplitude (8.28 mm/s). So, its need to be distinguished either its Unbalance, Misalignment or Looseness. The FASIT tool provided by the Adash is used to analyze the machine condition (misalignment, looseness and unbalance).

a. When the sensor is mounted on Housing directly:

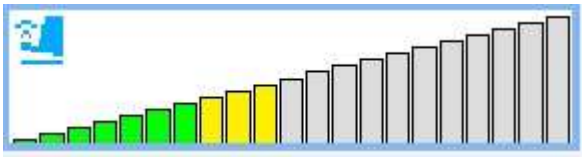
30% unbalance (Phase analysis- 90° phase difference in vertical measurement in radial direction. Amplitude due to unbalance will increase by the square of speed below first rotor critical. 1X RPM)



0% misalignment (Amplitude due to unbalance will increase by the square of speed below first rotor critical. 1X RPM-5X RPM)

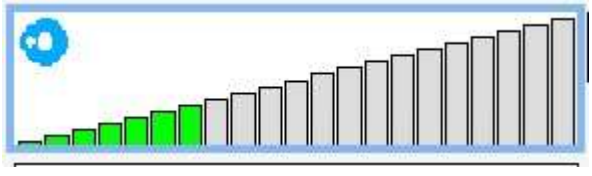


40% looseness (Time waveform- phase analysis- 180° phase difference in vertical measurement in radial direction)

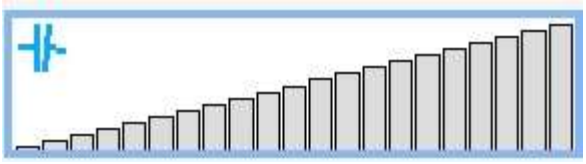


b. When the sensor is mounted on the bracket:

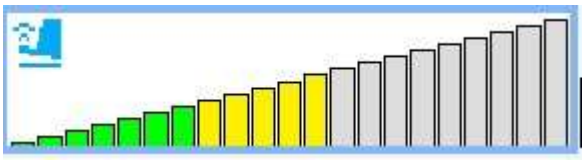
30% unbalance



0% misalignment



60% looseness



As the machine has 30% unbalanced and 40% looseness, machine need to be worked on correcting unbalance and looseness immediately to avoid the failure of the threshing machine. The looseness is 20% higher from the FASIT tool display taken from the manufactured bracket than bearing housing. It is recommended to tight the bolt as specified by the manufacturer in ctatlagoue to overcome theses sort of undesired results.

CONCLUSIONS

The most suitable vibration signals were selected to assess the threshing drum technical characteristics of a combine and to spot flaws when the combine is in use in the field. The most effective method for determining the bearing quality condition is demodulated FFT spectrum analysis, which offers the necessary vibration information. The FASIT tool technology provided by Adash DDS is effective and easy to identify the machine conditions like misalignment, looseness, unbalance.

Peak amplitude was under noise threshold for Envelop Analysis. So, peaks are not on specified frequency for separating the frequency family for bearing, motor, and pulley drive. The bearing is working in a good condition and need to be examined in a frequent interval after consecutive long threshing work or when the unexpected noise is experienced. As the machine has 30% unbalanced and 40% looseness, machine need to be worked on correcting unbalance and looseness immediately to avoid the failure of the threshing machine.

The RMS Bearing static amplitude is 0.121g, which indicates the lower number than the alarm limit specified on ISO 10800: Noise and Vibration standard alarm set on the DDS software. Therefore, the manufactured bracket can be used to conduct the reliability testing of the bearing and mechanical analysis like looseness, misalignment, and unbalance on the threshing unit of combine harvester where accelerometer mounting is not suitable.

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MAPPING GREENHOUSE AREA CHANGES USING SENTINEL-2 IMAGERIES AND DIFFERENT CLASSIFICATION TECHNIQUES: PILOT AREA IN AKSU, ANTALYA, TURKEY

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ABSTRACT

Present paper focused on the determination of greenhouse covered land changes (ha, %) around pilot area in Aksu district of Antalya Province, Turkey between 2016 and 2021. The study area was selected depending on the fact that, various types of land cover land use (LCLU) can be found together with the dense greenhouse areas. High resolution satellite images from Sentinel-2 sensor were freely downloaded and used to achieve the aim. Since thebands of the images have different spatial resolutions, 20 m resolution bands were resampled to 10 m prior to the classification process. Moreover, performances of different pixel-based supervised classification techniques were evaluated whereas same training samples fromdifferent classes were utilized in the classification procedure. The considered classes were included greenhouse (G), agriculture (A), dense vegetation (D), sparse vegetation (S), baresoil (B), residential (R), transportation network (T), and water surface (W) LULC types. Subsequent to the classification process, accuracy assessments were conducted for identification the reliability of obtained LCLU maps via 240 randomized control points using Google Earth. Results indicated that there were remarkable increases in greenhouse coverage in study area whereby random forest classification gave more accurate results among the tested algorithms. The study believed to serve as a baseline for further research that will be conducted in wider area within the Mediterranean region.

Keywords: Classification, Greenhouse, Mapping, Sentinel-2, Short-term changes.

INTRODUCTION

Greenhouse production plays an important role in economic activities by being a vital agricultural income source in Turkey, as well as many other countries especially in Mediterranean region, since such production activities provide fresh vegetables and fruits in all seasons (Ceylan et. al., 2020), and assist to guaranty food security. The country stands within the first five countries in regarding to the world greenhouse covered area (Briassoulois et. al., 2016), while standing in the 2nd order within in the Europe, following Spain. The reports of Republic of Turkey Ministry of Agriculture and Forestry (RTMAF) denoted that the greenhouse coverage is reached to almost eighty thousand hectares (ha) by 2020 (RTMAF, 2020). Moreover, according to the same report, the quarter part of the total vegetable production was announced to be obtained from greenhouses in 2019. Meanwhile, Turkish Statistical

Institute (TUIK) data indicated the rapid changes occurred between 2018 and 2019 were 2% and 4.5% increase in coverage area and production amounts, respectively(TUIK, 2020).

Even though greenhouse production is preferred in many areas of our country for growing of various products, Antalya Province is known to have widest greenhouse coverage with an approximately 30 thousand ha survey area, and producing almost 50 percent of total greenhouse production (TUIK, 2020). Therefore, the area has great importance in terms of agricultural production, and thus, in local, regional and even territorial economy.

The greenhouse production has several positive effects such as controlled, continuous and off-season production, independent from soil conditions by usage of hydroponic systems in some cases, and saving time, space and labor costs. On the other hand, there are some negative consequences of increased especially plastic greenhouse production. These are related with degradation, acidification, heavy-metal uptake increase of cultivated plants, and rural landscape metrics (Jiao et. al., 2003; Picuno et. al., 2011; Deng et. al., 2015; Sun et. al., 2021). Thence, identification and tracking of the current status and increase in greenhouse coverage can be helpful for evaluating the balance of such effects. Thereby, inventory records on greenhouse locations and areas have great importance at different scales. In Turkey,agricultural statistics are publicly available in TUIK databases. However, mentioned data are usually served after a definite period of each year. On this account, mapping and monitoringof the greenhouse area change using contemporary remote sensing techniques provide valuable, rapid, and up-to-date information.

The study was carried out within a part of Aksu district of Antalya Province, whereby different types of land cover land use (LCLU) are located in the area. In present study, the short-term changes between 2016 and 2021 years in greenhouse covered area were identified while the discrimination performances of different classification techniques such as maximum likelyhood and random forest were tested using selected bands of Sentinel-2 imageries.

MATERIAL AND METHOD

The study area is located Eastern part of the central residential zones and 16 kmdistance from of Antalya province center (Figure 1). The area is chosen due to the fact that impervious surfaces relating to settlements or industrial purposes can be found nearby the greenhouses, where results of the performance testing are may be highly significant (Figure2).

The satellite images of Sentinel-2 were freely downloaded from United States Geological Survey (USGS) website (USGS, 2021). The imageries were acquired in dryseason to avoid from the cloudy conditions and to guarantee similar illumination/shadow conditions. Acquisition dates of the used imageries were 31 July, 2016 and 15 July, 2021. Thespatial resolutions of Sentinel-2 bands are known to range between ten to sixty meters (m). Prior to the image processing, the 20 m bands were resampled to 10 m spatial resolution. Subsequently, study area was subset from both imageries prior to training sample collection for supervised image classification procedures. All image pre-processing, classification and steps were conducted using freely available SNAP software of European Space Agency (ESA).

The changes were identified through a post-classification technique. The greenhouse area differences that occurred from 2016 to 2021 were calculated and given in and percentages (%).

The performances of different classifier algorithms for discrimination of greenhouse areas were evaluated by validation process. Validation refers the determination of reliability levels for overall classification as well as the reliability levels for classification of individual classes. Accuracy assessments were conducted to validate the LCLU maps using stratified randomized points, according to Congalton and Green (2009), whereby the same control points were used for evaluation of all classifications to provide unity in assessments. Overall accuracy (%),

overall kappa statistics, G class accuracy and G class kappa statistics were interpreted among the data obtained from produced error matrices.

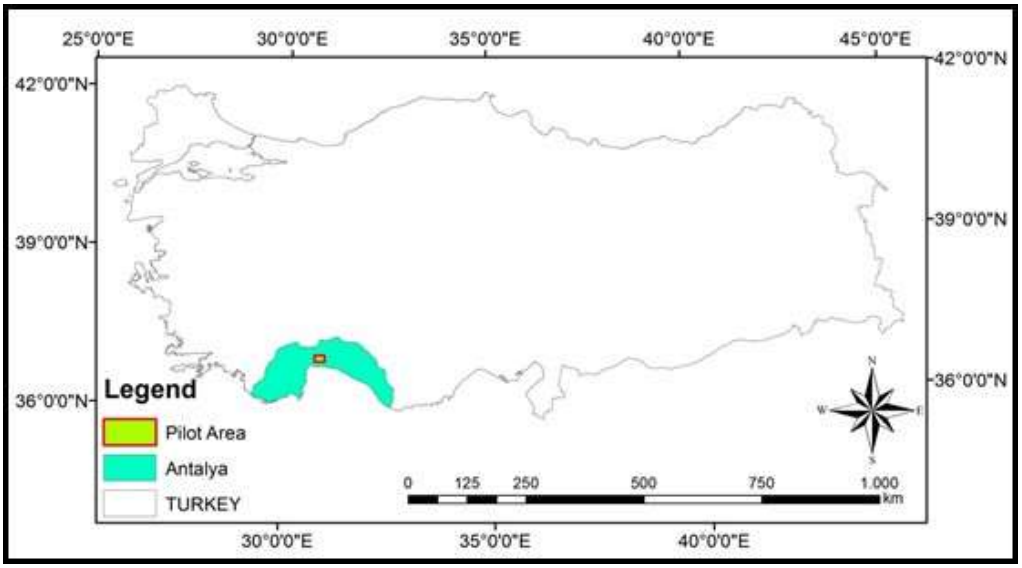

Figure 1. Location of the pilot area within Antalya, Turkey

Figure 2. Complex LCLU situations in the study area (Turkish Republic Aksu district governor's office, 2021).

The training samples for classification process were randomly collected from eight different LCLU types. These are namely, greenhouse (G), agriculture (A), forest (F), sparse vegetation (S), bare soil (B), residential (R), transportation network (T), and water surface (W). The images of both dates were classified using pixel based random forest (RF), maximum likelyhood (ML), minimum distance (MD), and k-nearest neighbor (KNN) algorithms to produce LCLU maps of the area. The same training samples were used in all classifiers.

RESULTS AND DISCUSSION

The LCLU maps that generated through different classifier algorithms can be seen on Figure 3 a-d for 2016 and 4 a-d for 2021 years. Due to the fact that the T and B classes were confused with R class, these areas are combined to R class latter, for further analysis. In addition, the calculated areas of $LCLU_{2016}$ and $LULC_{2021}$ classes, which are obtained from different classification algorithms, are given in Figure 5 and Figure 6, respectively. Moreover, the change amounts and directions are given in Figure 7. According to visual interpretation of figures 3 and 4, there are great differences between LULC maps obtained from different algorithms although the training samples were the same. Therefore, the coverages (%) of class areas differed obviously in figures 5, 6, and 7, especially in MD, which was followed by KNN. On the other hand, the results of accuracy assessments have pointed out that the classification accuracies of MD-derived LULC maps of both years were considerably low. The highest accuracies were obtained from RF and it was followed by ML in both years, showing that RF is quite more reliable than ML algorithym. On this account, the change analyses of RF classifications have revealed that G class area increased over 7% in the area within a short time period of 5 years. Similar results have found in the study conducted in another district of Antalya province (Inalpulat and Genc, 2021), which may be an evidence of the increment trend even in a short-term period.

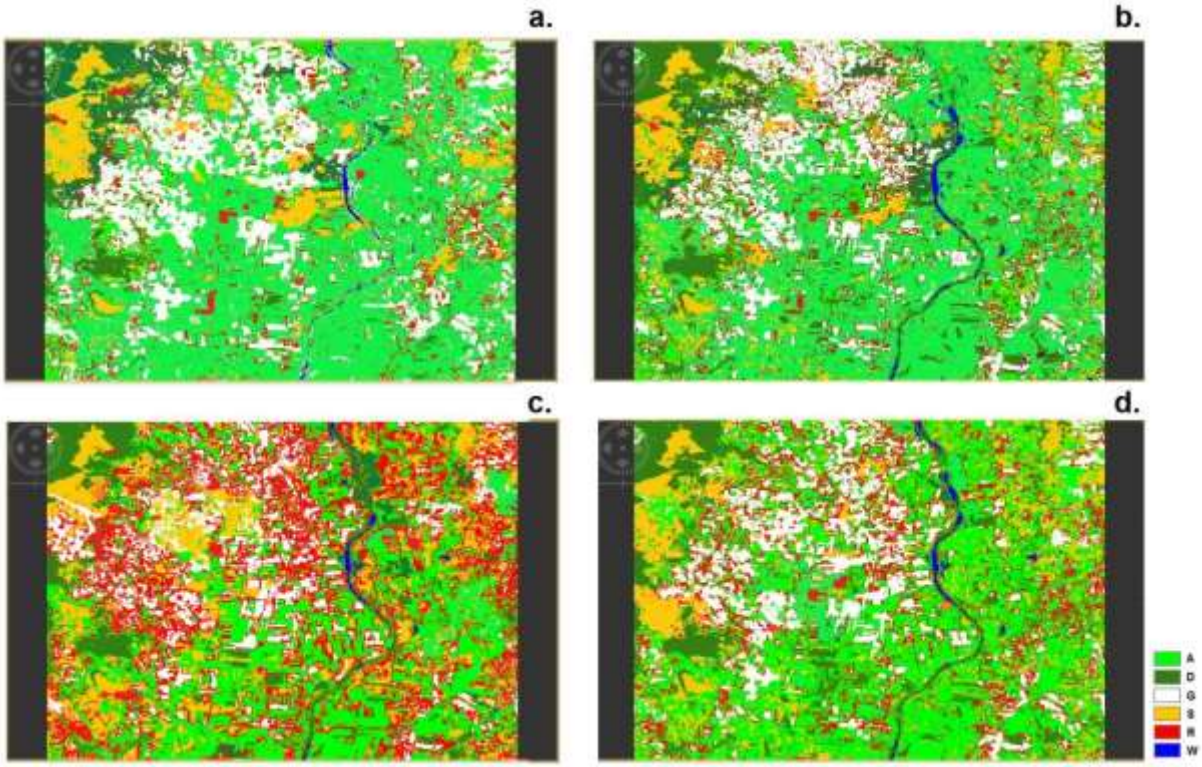

Figure 3. $LULC_{2016}$ from different classifiers a. RF, b. ML, c. MD, and d. KNN

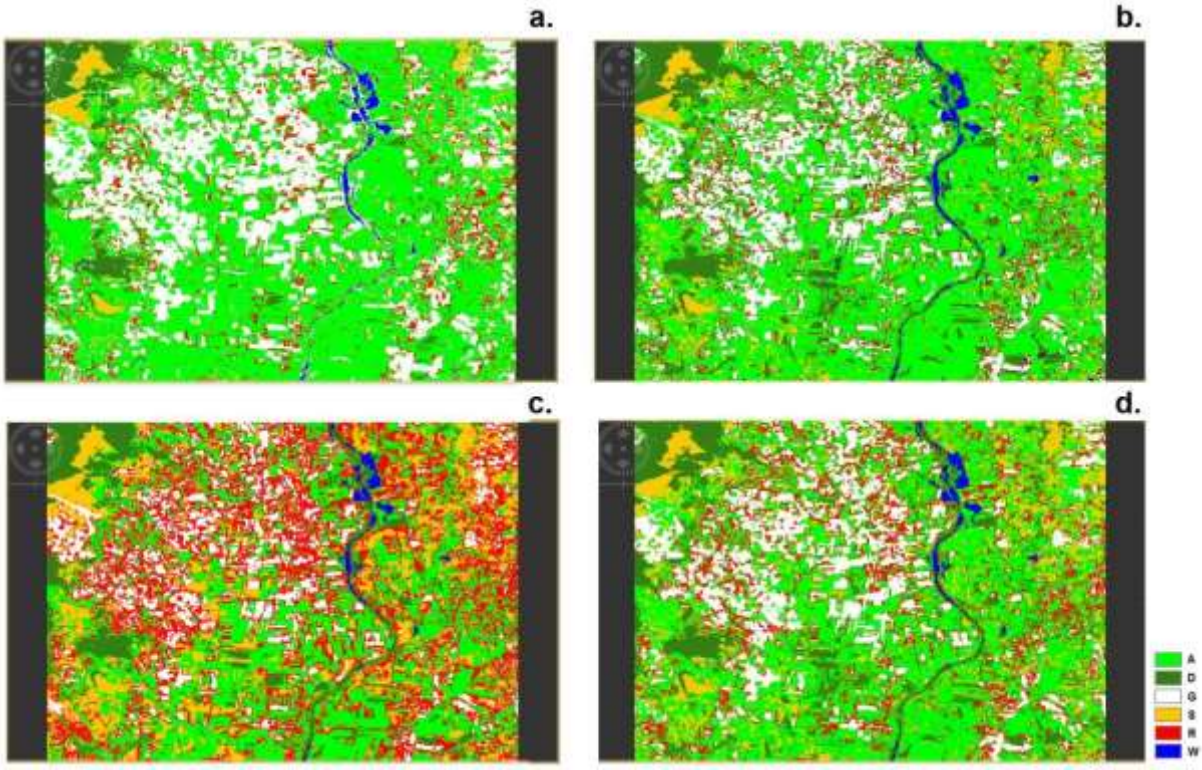

Figure 4. LULC$_{2021}$ from different classifiers : a. RF, b. ML, c. MD, and d. KNN

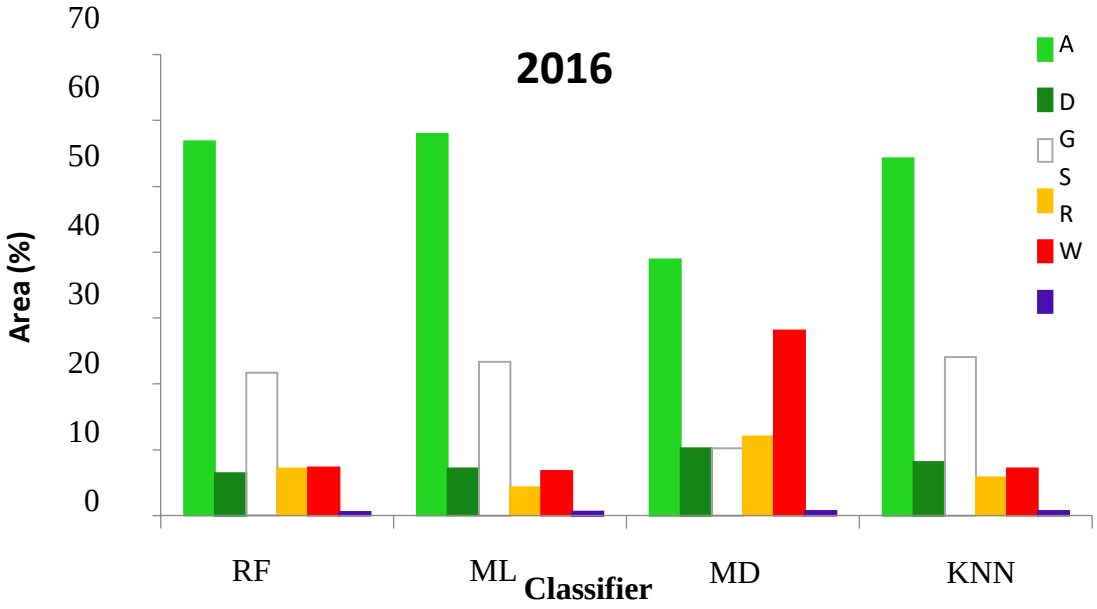

Figure 5. Areas of $LULC_{2016}$ from different classifiers

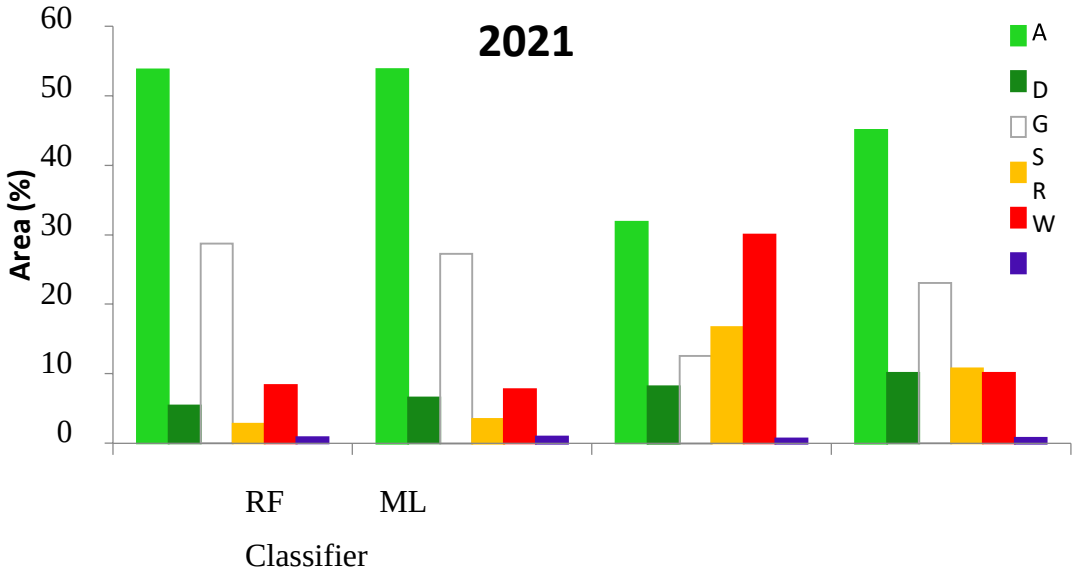

Figure 6. Areas of $LULC_{2021}$ from different classifiers

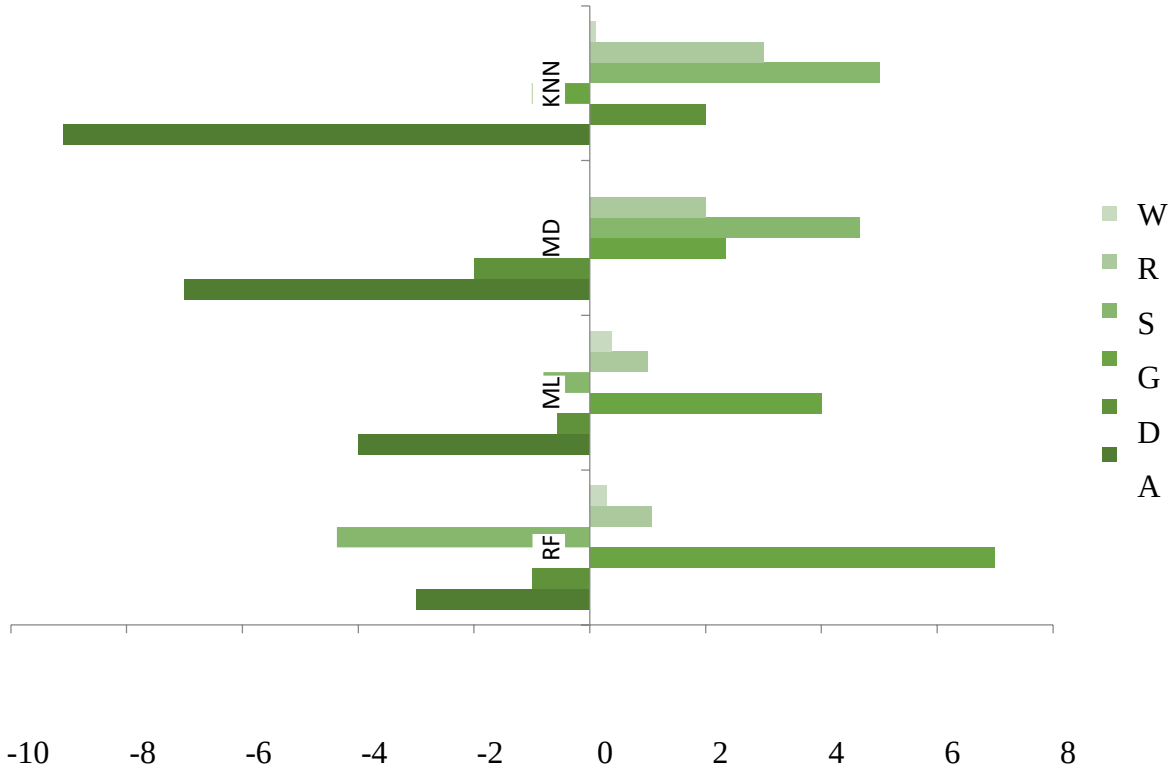

Figure 7. Change in areas of LCLU classes according to different algorithms

Table 1. Overall accuracies and overall kappa values for different classifiers in 2016 and 2021

Years	Overall Accuracy (%)				Overall Kappa			
	RF	ML	MD	KNN	RF	ML	MD	KNN
2016	83.2	81.4	68.7	78.8	0.8264	0.8088	0.6371	0.7698
2021	86.5	82.4	70.1	77.3	0.8556	0.8176	0.6436	0.7771

CONCLUSIONS

The study was conducted to monitor short-term changes in greenhouse coverage meanwhile performances of different supervised classification algorithms were evaluated within in pilot area located in Aksu district of Antalya province. Among with the considered algorithms, random forest gave the most satisfactory validation results with higher overall and individual accuracies together with overall and individual kappa values. On the other hand, findings of the study have revealed that greenhouse areas are increased by %7 between 2016 and 2021 according to the most accurate classification obtained through RF classifier algorithym. The study seemed to compose satisfactory results in terms of greenhouse classification. However, another important topic comes into the frame in remote sensing studies for greenhouse detection, which is namely, greenhouse type and coverage material discrimination. On this account, ongoing study includes testing of the capabilities of different band combinations and indices for discrimination between types of greenhouse coverage materials that may improve the findings of present study.

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MICRO-SCALE DRINKING WATER FOOTPRINT MAPPING OF BOVINE, OVINE AND POULTRY FARMING IN CANAKKALE, TURKEY USING GIS

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ABSTRACT

Agricultural activities are known to be one of the great water consumptive sectors whereas animal production holds approximately one third of the total agricultural water consumption. Identification of the annual consumed amount may provide better understanding for foreseen future situations for planners in the local authorities for maintenance of present resources and planning of future water collection units in the perspective of climate change. The main purpose of the study was to determine and map the drinking water footprint of different livestock types including bovine, ovine and poultry at town and village scale in Canakkale Province, Turkey. The inventory data representing the number of animals for each type were obtained from local inventory records of Canakkale Directorate of Provincial Agriculture and Forestry. Water footprints were calculated depending on standard water consumption assumptions accordingly to livestock type. The study presents the first attempt for subscale mapping in the area, and has potential to serve as a basis for further studies.

Keywords: Animal farming, Canakkale, Drinking water footprint, GIS, Micro-scale mapping

INTRODUCTION

Animal farming takes the first place in terms of agricultural water consumption since water footprint of animals greatly larger than any of agricultural plants. Water is the primary component in the body of poultry and livestock, and 50-80% of the animals' body weight is water. Water plays an important role in regulating the body temperature of animals, secretion of milk, regulation of digestion and metabolism, removal of waste materials, reproduction, sound and appearance (Cemek et. al.,2011). Daily water requirements of animals depend on many factors such as air temperature, quality and quantity of feed consumed, physiological condition of the animal. As it's well known, population increase has a trigger impact on demand of animal products, namely meat, milk, and egg due to their valuable nutrition ranks. Future projections denoted that the needs for such products are likely to increase in the next decades, and water resources are probable to face with more pressure as a result of thisprocess (Mekonnen and Hoekstra, 2012). In our country, different types of farming animals reported to be increased considerably even in a short period between December 2020 and June 2021 (TUIK, 2022). Therefore, determining the amount of water consumption may allow

controlling the use of water and provides effective water management. In this context, concept of water footprint came into the frame (Hoekstra et. al., 2011).

Present study focused on determination and mapping of ovine, bovine and poultry farming drinking water footprint in Canakkale province of Turkey. The paper presents the first micro-scale study in the area.

MATERIAL AND METHOD

The study was conducted in Canakkale Province, Turkey. There are 594 towns-villages within the city boundaries except the Imbros and Tenedos islands. Figure 1 represents the location and coverage of the province with sub-levels.

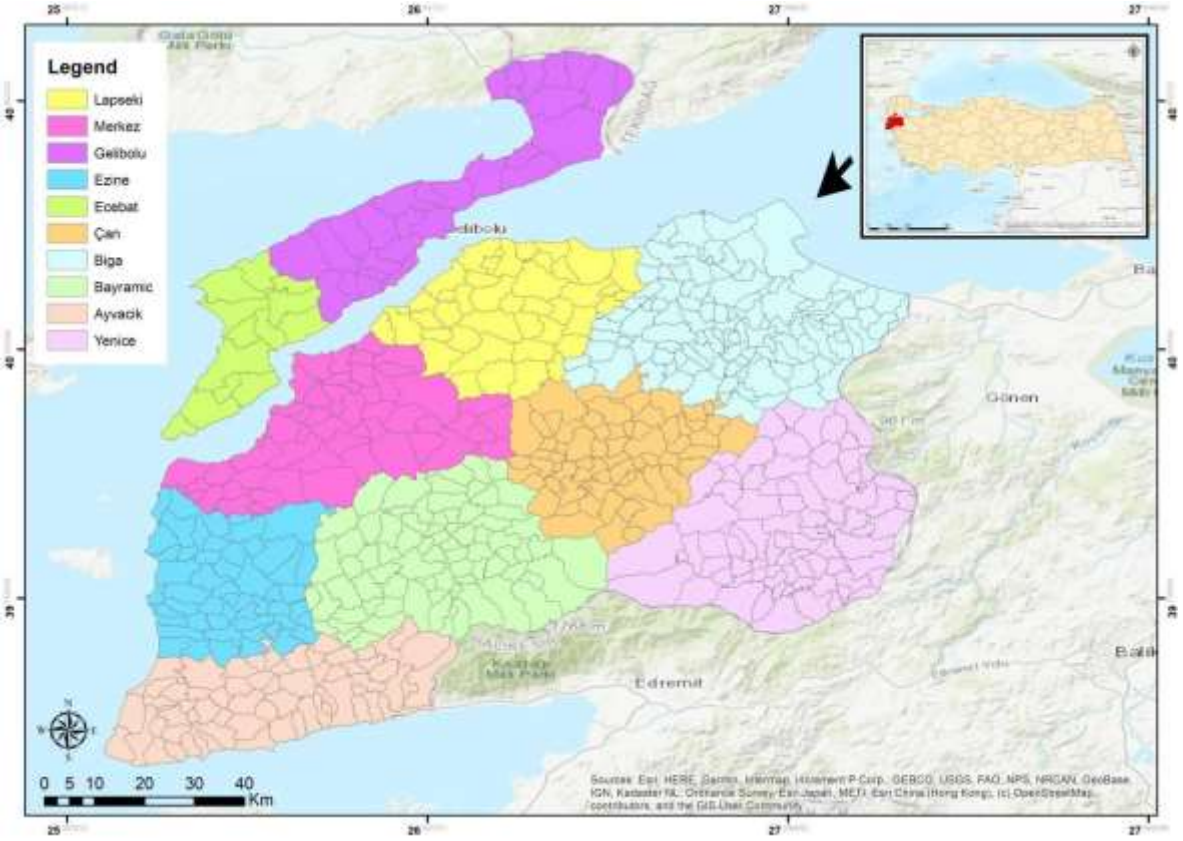

Figure 1. Study area of Canakkale Province

The number of different animal types presents the main data in the study. The data was obtained from village- and town-based inventory of Turkish Republic of Ministry of Agriculture and Forestry, Canakkale Provincial Directorate. Accordingly, the total and district based distribution different kinds of animals can be seen on Figure 2 a-d. When the figure is examined, it can be seen that number of poultry type is considerably high in comparison with ovine and bovine, whereas it is followed by ovine. The highest number of bovine reported to be found in Biga and Yenice districts respectively, while the lowest is in Eceabat district. When ovine type is considered, it was figured out that highest number of animals is living in Ezine district while the lowest found in Eceabat, as well as bovine type. Lastly, investigation of poultry type revealed that, highest number of animals are found in Biga and lowest number of animals are in Ayvacik districts.

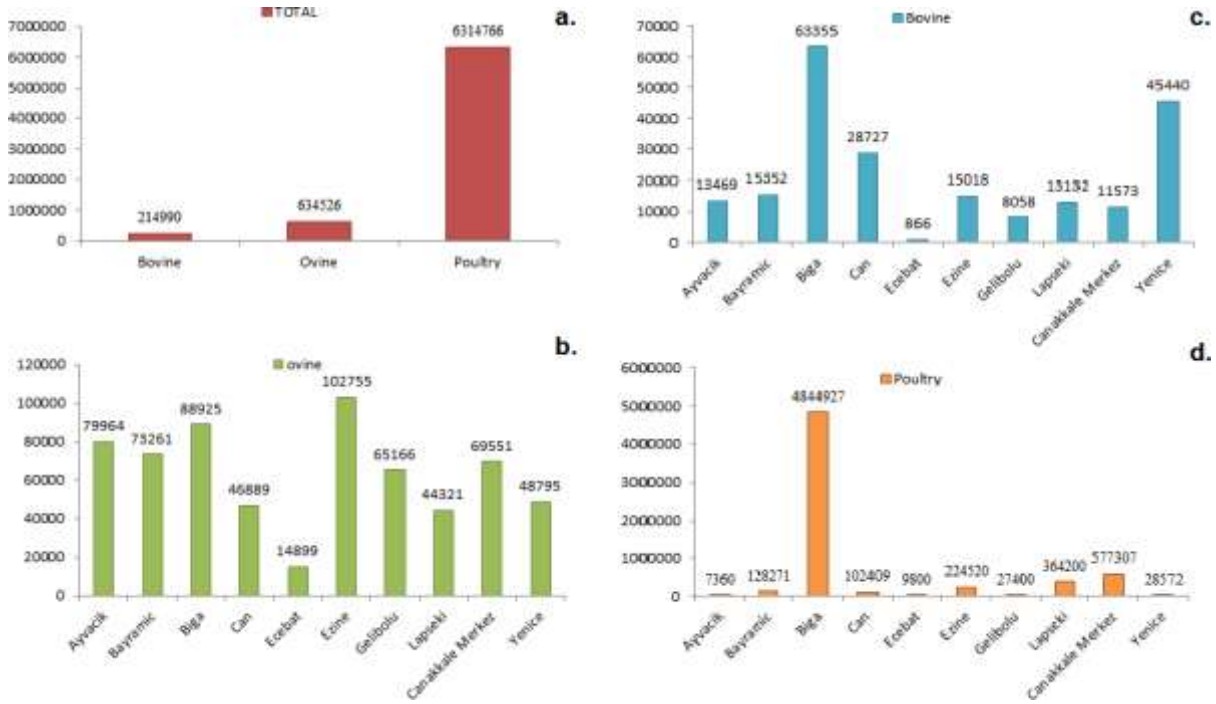

Figure 2. Number of animals in different types a. total number, b. district-based number of bovine, c. district-based number of ovine, and d. district-based number of poultry

Daily water consumption of animals determined via personal communications with local farmers. The average of daily water consumption values of 21 local farmer's animals used in the study in the further calculations (Table 1). Drinking water footprint determined asa function of consumption amounts and animal numbers for each category. The total and animal-type based drinking water footprints of animals are mapped using ArcGIS (10.3).

Table 1. Daily water consumption of different animal types

Animal Type	Water Consumption (l/day)
Male calf	6
Female calf	6
1 - 2 Years Old Male	35
Heifer	40
Cow	50
Ox	50
Bull	50
Sheep	5
Goat	5
Laying hen	0.2
Broiler chickens	0.12
Turkey	0.85
Goose	0.6
Duck	0.6

RESULTS AND DISCUSSION

The drinking water footprints bovine, ovine and poultry are represented in Figure 3, Figure 4, and Figure 5, respectively. The highest value for bovine calculated as 135920 l/day for Uvecik village of Ezine district, and it's followed by a few villages located in Biga district (Figure 3). Investigation of ovine consumptions has revealed that the highest value is obtainedfrom Adatepe village of Lapseki district with 143880 l/day, which is above the maximum consumption of bovine type. Moreover, all of the villages covered within given thresholds of highest consumption class, which covers the value over 120000 l/day to 135920 l/day, locatedin Lapseki district (Figure 4). Finally, the highest consumption of poultry is calculated for Yukaridemirci village of Biga district with a value of 391641 l/day. Accordingly, investigation of maximum rates of daily consumptions pointed out that highest daily drinking water footprint sourced from poultry type even though their individual daily consumption is significantly low in comparison with especially bovine type. Considerably high number of poultry resulted in higher footprint in the specified area.

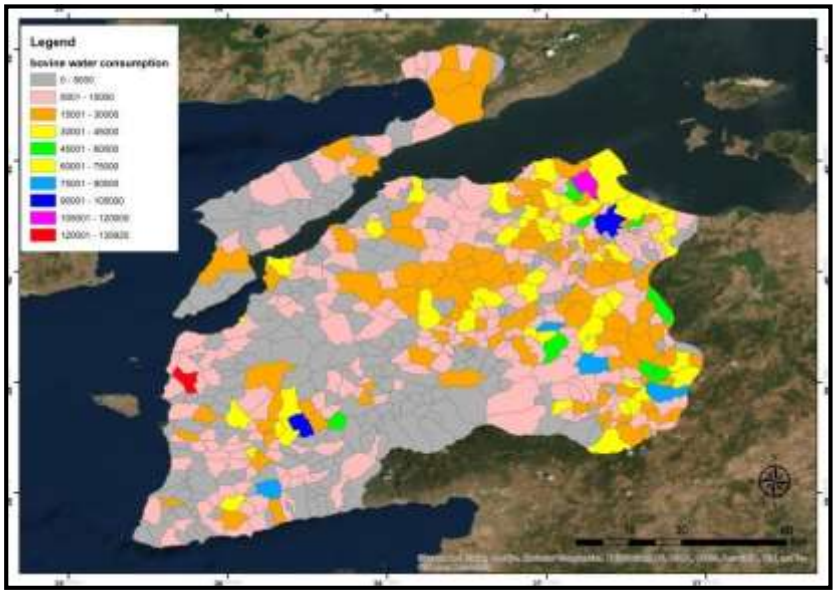

Figure 3. Drinking water footprint distribution map of bovine

Total drinking water footprint of bovine, ovine and poultry are given in Figure 6. In addition, the figure shows locations of stream networks and water collection units. Dependently, Yukaridemirci village of Biga district seemed to have highest drinking water footprint with 402636 l/day. Furthermore, visual interpretation designated that all of the villages or towns with high water footprint is located nearby a water collection unit or stream network, which is an important finding of the study since water supply to farms would be a vital problem, otherwise.

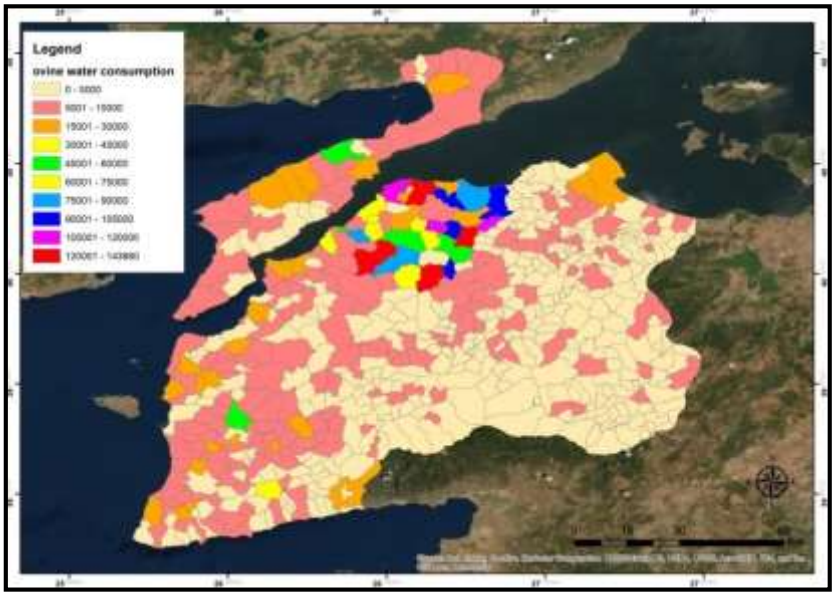

Figure 4. Drinking water footprint distribution map of ovine

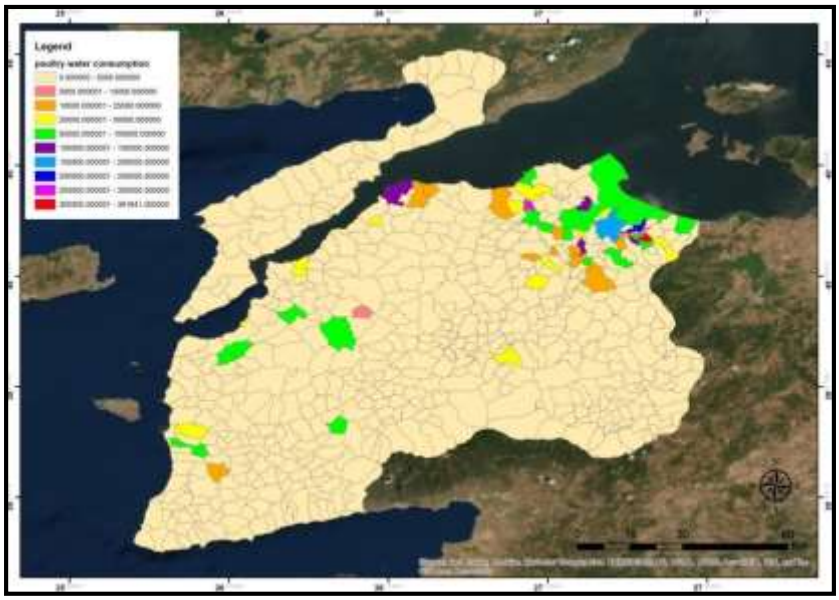

Figure 5. Drinking water footprint distribution map of poultry

Figure 6. Total drinking water footprint, stream network, and water collection units

CONCLUSIONS

Depending on the study findings, it can be concluded that highest drinking water footprint is sourced from poultry farming in the area, and it is followed by bovine and ovine farming, respectively. The highest total water footprint of all types of animals is calculated forYukaridemirci village of Biga district. The locations of villages with higher total drinking water footprint are concluded to be located around water resources in present day. Increments in number of animals would result in higher drinking water footprint in the future. Ongoing study is focused on assessment of sufficiency of current water resources using future estimations on number of animals for 2030 and 2050 years.

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IMPACT OF LATE SPRING FROSTS ON SOME PEAR CULTIVARS

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ABSTRACT

This research paper presents the results of late spring frosts impact to pear cultivars 'Abate Fetel', 'Conference' and 'William', on the rootstock MA. The spring of 2017 in Kosovo has been characterized by low unusual critical temperatures below 0°C degree. In the experimental as a randomized block system included 45 pear trees (15 for each cultivar). To each plant monitoring and examined some parameters as dynamic of flowering, inflorescence, fruits setting and fruits growing. At the same time was monitoring and examined some meteorological parameters through "Spectrum Weather" station. In the March – April period, temperature below the 0°C degree has dropped 13 time, with the lowest being - 4.72 °C degrees at the beginning of flowering and - 4.22 °C degrees at the settings and start growth of fruits time. The most resistant cultivar has been shown Conference which has managed to set and grow 40.87 fruits or 2.64%, per pear tree, then the William cultivar has created 36.07 fruits or 2.16% and the susceptible cultivar is shown Abate Fetel witch has managed to create only 3.47 fruits or 0.24% per pear tree. The damages by the frosts was to highs but with differences between the pear cultivars.

Keywords: pear, late spring frosts, damages, flowering

INTRODUCTION

European conditions where late spring frost can be expected, as it has a significant negative impact on architectural (and thus wood quality) traits (Fady et al., 2003). In many countries of continental Europe, in recent years there has been an increase in the rate of damage to fruit crops from late spring frosts. As NFO (Nederlandse Fruittelers Organisatie) in April 2016, in several European countries – such as Austria, Switzerland, Italy, Croatia, Germany, Slovenia, France and Belgium – apples, pears, cherries and grapes were frozen and very great damage has been caused. The freezing events affected farmers, local economy and cause the greatest economic losses in agriculture (Snyder et al., 2005).

According (Vitasse et al., 2018), during analyzed long-term temperature data from the period 1975–2016 in 50 locations in Switzerland and used different phenological models calibrated with long-term series of the flowering and leaf-out timing of two fruit trees (apple and cherry) and two forest trees (Norway spruce and European beech) to test whether the risk of frost damage has increased during this period.

The extent of frost damage depends essentially on ecophysiological factors. Main factors by which different environmental conditions (e.g., temperature, light, flood, and drought), and management practices (pruning, thinning, girdling, sheltering, water aspersion, irrigation, and fertilization) influence frost sensitivity and frost exposure of trees (Charrier et al., 2015). The level of damage is directly related to cold intensity and duration as well as bud developmental stage, fruit crops, that have different bud structure (Larsen, 2010). Cell walls structure and their composition play an important role against frost (Bartolo et al., 1987).

Genetic basis has an impact on the resistance of fruit trees to frost which determines their biomorphological properties. Cold resistant cultivars with acceptable commercial value, such as Flemish Beauty, Clapp's Favorite, Miney, Parker and Guyot. The most cold hardy cultivars tested in Quebec were Gaspar and Moe (Granger and Rousselle, 1984 cit. Palonen & Buszard, 2017). Flowering time is a conditional genetic property, which can to some extent be influenced by environmental factors. According to the time of flowering, fruit crops cultivars may have differences between them. Early - blooming fruit crops and cultivars are more vulnerable to late spring frosts (Efendija, 2000). Early-blooming pear cultivars tend to be more severely injured by spring frost, although wide differences between cultivars within date-of full-bloom classes occur (Lamb 1982 cit. Palonen & Buszard, 2017).

In terms of stages of fruit crops development, the highest risk of late spring frosts is posed by bud sprouting, flowering time and set of fruits (Ferraj & Thomaj, 2014; Štampar, 2005; Çakalli, 2005; Efendija, 2000; Bulatovič & Martič, 1996).

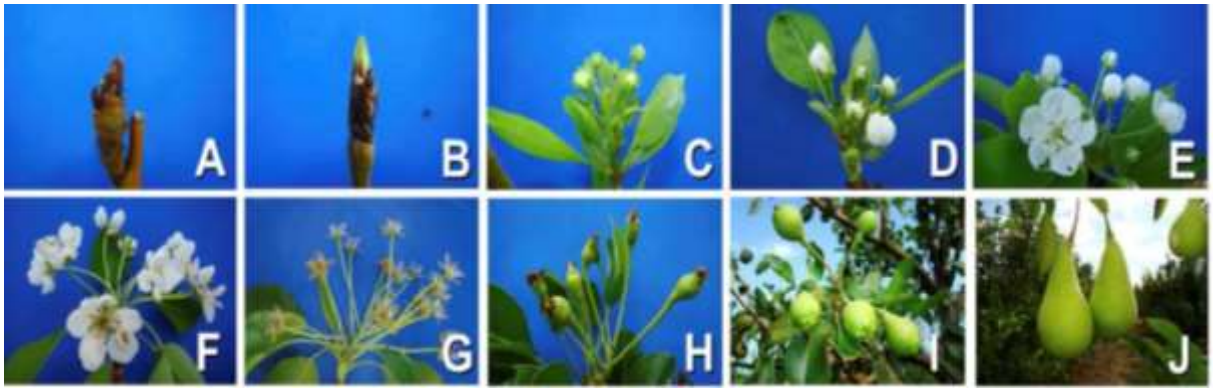

Figure 1. Some development stages of pear (Oliveira et al., 2016)

MATERIAL AND METHOD

The study was conducted during 2017, in a 4-year-old pear orchard, in the village of Vërboc, municipality of Drenas, Kosovo. Planting distances were 4 x 1.2 m, the altitude of the orchard 670 m. The research included three cultivars of pear, Abate fetel, Conference and William.

The experiment established in 3 repetitions at the random way. One repetition included 5 plants which means that for each cultivar 15 plants were researched. (Troni, 2001).

Meteorological data (the minimum, maximum and average temperatures and Growing Degree Days - GDD) were provided from the "Spectrum Weather" station of the IADK organization.

GDD - equivalent formulation uses the average of the daily maximum and minimum temperatures compared to a T_{base}. As an equation:

$$GDD = \frac{T_{max} + T_{min}}{2} - T_{base}$$

If the minimum temperature T_{min} is below the T_{base} one, if $(T_{max} + T_{min})/2 < T_{base}$, set $(T_{max} + T_{min})/2 = T_{base}$. The resulting GDD is 0. (https://mrcc.illinois.edu)

T_{base} = 5 ^{o}C *(Vitasse et al., 2018)*

From the beginning of vegetation in all plants included in the experiment were carefully monitored and examined some biomorphological parameters.

Dynamic of flowering

- Beginning of bloom (5% of flowers bloomed)
- Beginning of full bloom (25% flowers bloomed)
- Full bloom (75% of flowers bloomed)
- End of bloom (95% of flowers bloomed)

Every two days exanimated 100 clusters of flowers to calculate percentage of bloom

Inflorescence

- Number of flowers per cluster (average of 120 clusters of 15 plants)
- Number of flowers per plant (all flowers in all plant examined)

Fruits

- Number of fruits settings (all fruits in all plant examined)
- Number of fruits maturated (all fruits in all plant examined)
- % of fruits developed in relation to the number of flowers formed

Assessment of resistance to low temperatures (late spring frosts) –was realized through statistical analysis, JMP 2010 program.

RESULTS AND DISCUSSION

The year 2017 has been characterized by unusually low temperatures both during the winter period and in early spring. These low temperatures have been accompanied by frosts which have caused very great damage to fruit trees. In this case the most pronounced damage from late spring frosts.

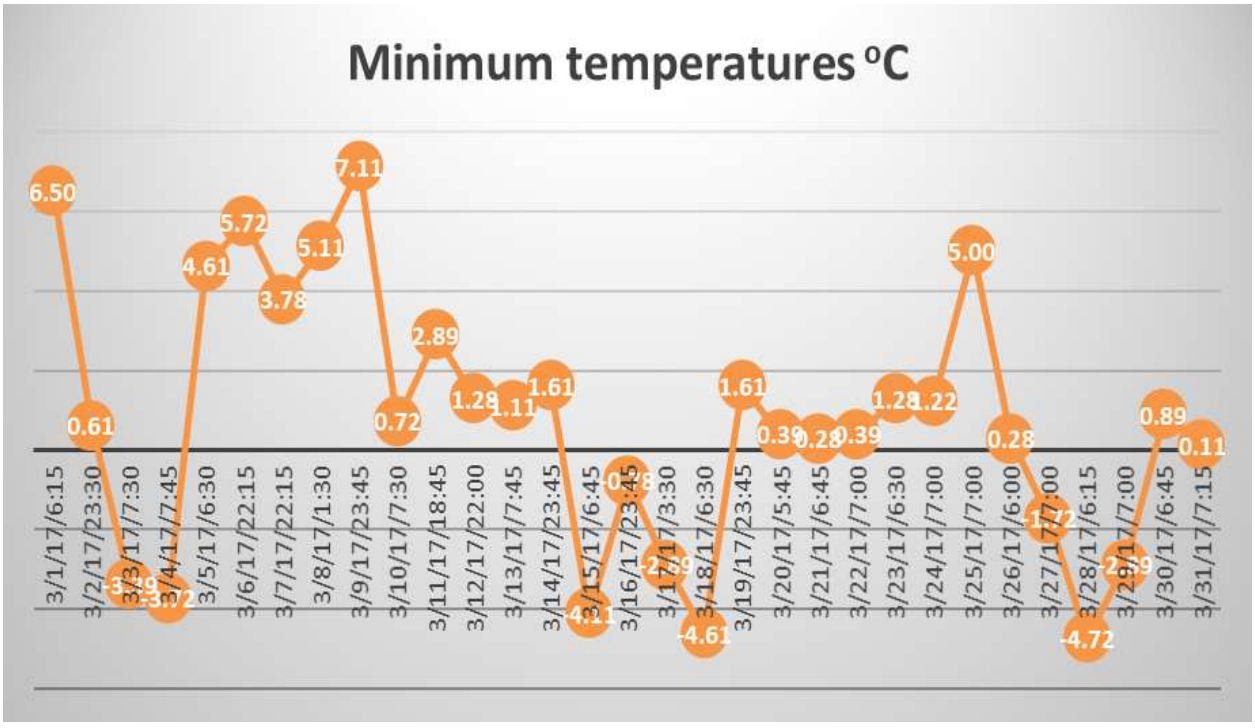

Figure 2. Minimum temperatures during March 2017

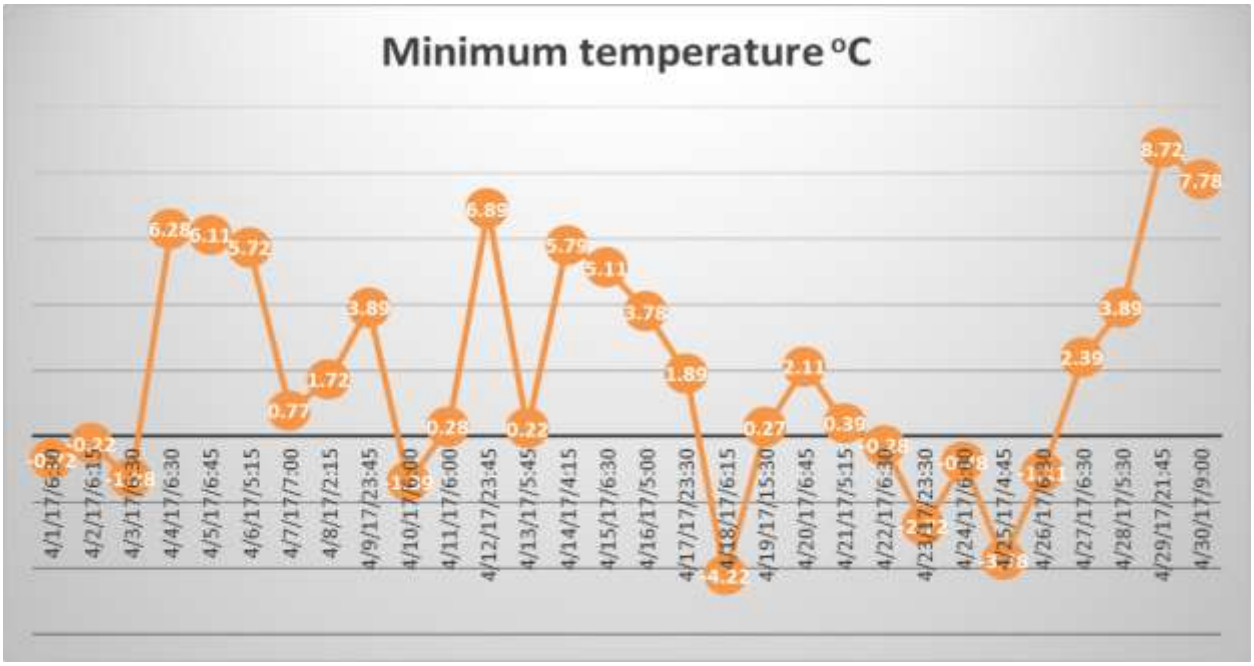

Figure 3. Minimum temperatures during April 2017

Based on the data presented in Figure 2. it is clear that in March it was constantly associated with temperatures below 0 ^{o}C. During these period 9 days the temperatures were below 0 ^{o}C. The lowest were -5.39 ^{o}C on 03.03.2017 and - 4.72 ^{o}C on 28.03.2017. A similar weather with low temperatures has also accompanied the month of April (Figure 3.). So this month throughout its period there have been 11 days of temperatures at 0 ^{o}C, where the lowest temperature was - 4.22 ^{o}C on 18.03.2017. All this, shows very clearly how endangered the fruit trees were, given the biological activity in this period.

Regarding this meteorological phenomenon and in our case study, a more complete explanation is given in Figure 4. From the flowering dynamics given for the three varieties of pear, Williams, Conference and Abate fetel, it can be seen that the three varieties have been exposed to frost throughout the entire period of flowering and fruit setting. Most of these frosts were exposed to the Williams cultivar which had an earlier flowering and the least to the Conference cultivar which had a later flowering. An oscillation of GDD is also observed in this period. At the stage of fruit formation, respectively at the end of April, GDD values were below the minimum parameters of plant development (below 1).

Critical temperatures in some stages of pear development are: bud sprouting stage, A - C (-1.5 to -3.9), flowering time stage D - F (-1.6 to -2.2), set of fruits stage G – I (-1.0 to -2.1). (Ferraj & Thomaj, 2014; Štampar qt al., 2005; Çakalli, 2005; Efendija, 2000). Temperatures - 4.4 ^{o}C in the flowering stage of pear, cause damage at the level of 90% (Bulatovič & Martič, 1996).

Always referring to the data in figure 4. we notice that the three pear varieties, in the stage after fruit setting have repeatedly been subjected to low temperatures above the critical limits of this stage. This happened on April 18, 23, 25 and 26 with temperatures (-4.2, -2.7, -3.8, and -1.1 ^{o}C).

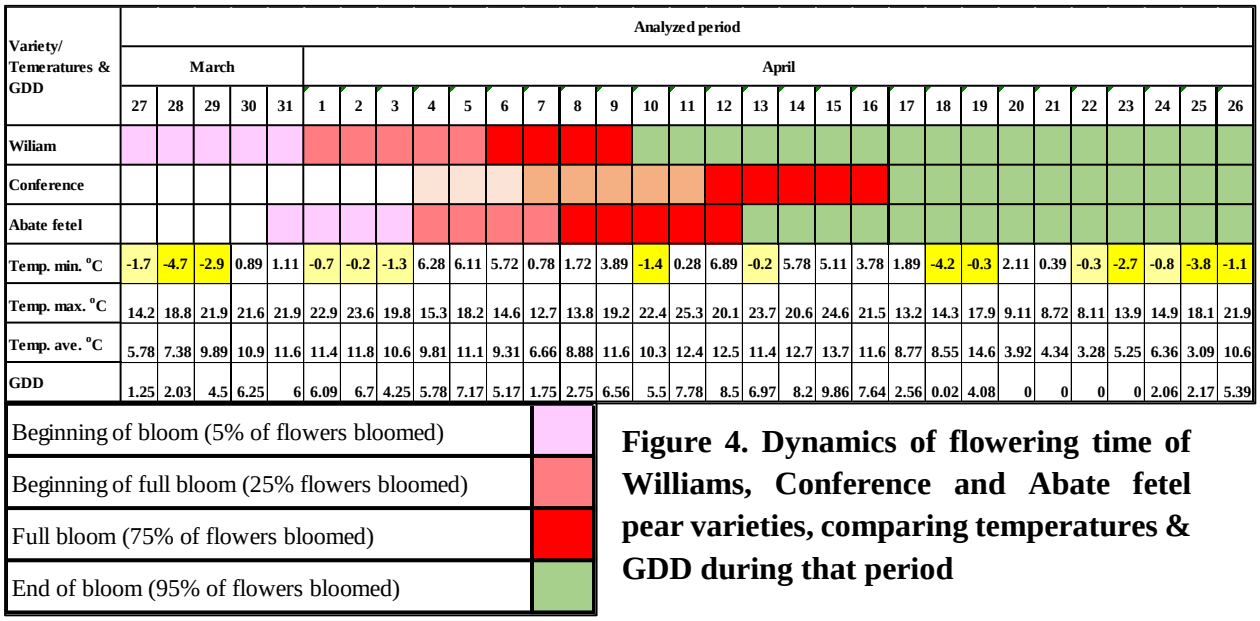

Figure 4. Dynamics of flowering time of Williams, Conference and Abate fetel pear varieties, comparing temperatures & GDD during that period

Analyzing the results from the data obtained between the three pear cultivars, it is seen that the flowers load at the beginning was approximately the same (Table 1, number of flowers clusters /plant). This fact is very important to have a correct conclusion on the resistance of the three varieties to late spring frosts.

Table 1. Comparison of the impact of late spring frosts on fruiting parameters in three pear varieties

Varieties	Parameters				
	No. of flowers clusters / plant	No. of flowers	No. of flowers	No. of fruits	% of fruits
William	203.93 a	8.17 a	1665.94 a	40.86 a	2.66 a
Conference	195.67 a	7.88 a	1542.54 b	36.07 a	2.23 a
Abate Fetel	195.27 a	7.53 a	1472.30 c	3.46 b	0.23 b
Comparisons for all pairs	q*		Alpha		
Tukey-Kramer HSD	3.07939		0.01		

Analyzing the number of fruits formed in relation to the total number of flowers per plant, a significant difference between varieties is clearly observed. Thus, the Conference and Wiliam pear varieties have achieved to create the bigger number of fruits per plant 40.87 fruits with (2.66%) and 36.07 fruits with (2.33%), in relation to the Abate Fetel variety which has achieved to create only 3. 47 fruits for plant with (0.29%) (Table 1.)

CONCLUSIONS

Occurrence of late spring frosts during period of 27 March and 26 April 2017 in Kosovo, has been very extreme both in terms of minimum temperature (-4.72 ^{o}C) and their duration (15 days below 0 ^{o}C). This has caused the three varieties of pears to suffer very large losses in yield. However, not all varieties have suffered the same damage, the Abate Fetel variety has suffered the most damage compared to the other two varieties of Conference and Williams pears. Although the Abate Fetel variety started flowering 4 days after the Williams variety, it has suffered much greater damage. This indicates that late flowering is not the only factor in reducing damage from late spring frosts, but also genetic resistance may be crucial for survival against these frosts. To reduce to some extent, the impact of extreme climatic conditions on fruit production, testing and selection of resistant varieties for specific regions is a must.

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EFFECTS OF FOUR EXTENDERS ON THE QUALITY OF FRESH SEMEN IN BULGARIAN SPORT HORSE BREED

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ABSTRACT

Ninety-five ejaculates were obtained from five Bulgarian sport horse to study the impact of four extenders (Tris, INRA 82, EquiPlus and Modified Kenny) on the quality of stallions' semen. The aim was to find a link between motility, velocity parameters, morphology and vitality with the enzyme activity of Alkaline Phosphatase (ALP), Lactat Dehydrogenase (LDH), g-Glutamyl Transferase (GGT) and Creatine Kinase (CK) in semen plasma, water and triton X100 extracts from spermatozoa. Results revealed that velocity (VCL, VSL, and VAP) and trajectory parameters (STR and ALH), percentages of live sperm and abnormalities were better in the modified Kenny ($P<0.05$). Concerning activity of ALP, CK, GGT in seminal plasma diluted with modified Kenny extender were significantly higher ($P<0.05$). Enzyme activity of ALP and LDH in seminal plasma and water extract were significant higher in samples with Tris ($P<0.05$). In conclusion, Tris and modified Kenny extenders give better protection to the sperm. Activity of ALP, LDH, CK and GGT enzymes could be used as quality markers of fresh stallion semen from Bulgarian sport horse.

Keywords: semen, extenders, enzyme activity, Bulgarian sport horse

INTRODUCTION

Artificial insemination with fresh, cooled or frozen semen is most common used technique in global equine industry for assisted reproduction. In this connection, semen quality is the major determinant for successful horse breeding programs. Many breed organizations now allow the use of liquid semen. Interest among breeders in this technology is increasing because unfortunately, not all stallions produce sperm that survive in the cooling process as cryoconservation (Varner et al., 2010).

In addition, when compared with those from natural mating, pregnancy rates have been shown to be equal, or even higher, after artificial insemination with fresh or cooled-stored semen (Janett et al., 2003; Melo et al. 2007).

Efforts to improve the preservation of semen are focused on the choose and modification of extenders (Coutinho da Silva et al. 2012) to maintain motility, viability and sperm membrane integrity (Kubovicova et al. 2010; Sarlos et al. 2002). Additionally, the routine studies such as measuring the ejaculate volume and concentration, or percentage of live and normal spermatozoa is not sufficient assessment to determine the possible causes of reduced fertility. Various enzymes in the semen have an effect on sperm fertility (Pesch et al. 2006), but in the literature, data on their activity are very scarce. It is not so clear higher or lower enzyme activity could be used as indicators on good semen quality (Diaconescu et al. 2014). In this connection, the aim was to find a link between motility, velocity parameters, morphology and vitality with the enzyme activity as a result of different extenders, used in fresh semen from Bulgarian sport horse.

MATERIALS AND METHODS

This study was exempted from approval from the Institution Animal Ethics because the semen collection does not affect the normal physiology of the animals.

1.1.Experimental design

95 ejaculates of stallions, Bulgarian sport horse breed, aged 3 to 23 years, coming from five different stud farms in Bulgaria were evaluated. All stallions were clinically healthy before and during the study. Animals were fed hay, oat and pellets supplemented with minerals and were exercised for at least 1 h daily.

Semen collection was done with the help of a phantom (Shrewsbury, Great Britain) or with a mare with an artificial vagina (Minitube, Germany). Each ejaculate was subjected to conventional pre breeding examination (volume, motility, progressive motility, concentration, live: dead-ratio). The gel fraction was removed after semen collection and then semen was filtered with sterile gauze. The volume was measured in a graduated cylinder. pH value was determined using pH indicator paper.

1.2. Extenders

Each semen sample was centrifuged at 800 g for 10 min to remove seminal plasma, divided into four part and re suspended (1:1) with the following extenders: Tris, INRA 82, EquiPlus and modified Kenny (Table 1). Samples with a minimum of 200×10^6 sperm/ml and motility >60% were used for this study.

Table 1. Semen extenders used on each group.

Tris Tris - 2.42 g Citric acid - 1.34 g Fructose - 1 g Streptomycin - 0.08 g Glycerol - 7 ml Deionized water (made up to 100 ml)	INRA 82 glucose monohydrate - 25 g\L lactose monohydrate - 1.5 g\L raffinose pentahydrate - 1.5 g\L potassium citrate monohydrate - 0.4 g\L sodium citrate dihydrate - 0.3 g\L HEPES -4.76 g Gentamycin -500 mg\L, SDS - 0.035% skimmed milk - 0.15%	EquiPlus Medium - 95 ml Minitube - Animal Reproduction Technologies (Germany)	modified Kenny Glucose - 4.9g/100ml skimmed milk- 2.4g/100ml sodium penicillin - 150.000 IU dihydrostreptomycin - 150mg Deionized water (made up to 100 ml) + 35% Tyroye's medium (Ijaz and Ducharme, 1995).

1.3.Computer-assisted sperm analysis (CASA)

The CASA system (Valencia, Spain) was used to assess the motility pattern immediately after the dilution of the semen. A sample (3 μl) from each tube was placed on a slide and semen motility assessment was performed based on five digital images from different fields, via a × 10 negative-phase contrast objective and warm stage at 37ºC. The motility pattern was measured according to total motile sperms, sperm viability, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity index (LIN) and straightness index (STR), amplitude of lateral head displacement (ALH). At least 300 sperm were analyzed from each sample, with the images turning read within 1s.

Sperm smears were stained with Microptic kits for vitality BrightVit and with Sperm Blue for morphological analysis, using a commercially available kit (Microptic, Spain). The

morphological characteristics included abnormal heads (%), abnormal midpieces and tails, loose heads as well as proximal and distal cytoplasmic droplets. The sperm cells were evaluated with an Olympus BX50 microscope. The measurements were performed on 100 randomly selected sperm in each ejaculate.

1.4. Enzyme assay

To obtain seminal plasma, the ejaculate was centrifuged at 3000 rpm for 10 min (Hermile Labor Technik, Z326 K). The procedure was repeated with the supernatant of the first centrifugation. The won seminal plasma was decanted and proceed for next analysis.

Pellet, received after first centrifugation was resuspended with saline and centrifuged at 3000 rpm/10 min. The procedure was repeated 3 times. After last centrifugation, pellet was resuspended in distilled water and freeze at -20ºC overnight. Next day, after thawing, pellet was sonicated 3 times x 10 sec and centrifuged at 12000 rpm for 30 min. Received supernatant contains water soluble proteins, extracted from sperm cells. The pellet was resuspended in Triton X100, centrifuged at 12000 rpm for 30 min and supernatant was used for analysis of membrane connected proteins in spermatozoa.

Both extracts and seminal plasma were used for analysed on the activity of enzymes alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH) and γ-glutamyl-transferase (GGT) by Biochemical analyzer (Mindray BA-88A). All steps were done following the manufacturer instructions, using the next reagents: for GGT- Tris buffer 100 mM pH 8.25,glycil-glycine 100 mM, L-Glutamyl-4-nitroanilide 4mM, wavelength 405 nm; for ALP- DEA buffer pH 9.8 1M,MgCl2 0.5 mM , 4-Nitrophenilphosphate 10 mM, wavelength 405 nm; for CK- Imidazole buffer 29 mM pH 6.50, creatine phosphate 30 mM, glucose 20 mM, N-Acetyl-L-cysteine 20 mM, magnesium acetate 10 mM, EDTA 2 mM, ADP 2mM, NADP 2mM, AMP 5mM, Di(adenosine-5)pentaphosphate 12 MikroM, glucose-6-phosphate-dehydrogenase >3kU/l, hexokinase>3kU/l, wavelength 340 nm; for LDH-phosphate buffer pH7.50 50 mM, sodium pyruvate 0.60 mM, NADH 0.18 mM, wave length340 nm. The standard sample was distilled water, which was analyzed in the system before introducing the samples. The enzymes were measured in U/L.

1.5.Statistical analysis

The one-way ANOVA test for comparison was used to analyze the data. Pearson correlation coefficient was used to evaluate the relationship of different sperm parameters with enzyme activities. All data are presented as mean ± SD (standard deviation) of the mean. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The present study provides a detailed description of fresh semen characteristics in the Bulgarian sport horse. The data of the effects of different extenders on sperm quality are presented in Table 2. The results showed significantly higher ($P<0.05$) total motility in samples diluted with Tris and modified Kenny compared with extender INRA 82. Sperm samples extended with modified Kenny had significantly higher values for the velocity (VCL, VSL, and VAP) and trajectory parameters (STR and ALH), ($P<0.05$) compared with other extenders. Therefore, sperm cells, diluted with this extender cover longer distance most rapidly. Sperm samples extended with Tris, had low values for the VSL and VAP and significantly high values for straightness index ($P<0.05$) in comparison with EquiPlus. These sperm cells cover shorter distances. Lower TMS in samples extended with EquiPlus were found compared to Tris and modified Kenny extenders, which was according with results in Icelandic stallions (Janett et al.

2012). Šichtař et al. (2017) claim that extender EquiPlus gives better results in sire with long-term storage of sperm, such as cryopreservation.

Spermatozoa vitality with the use of modified Kenny extender was greater compared with INRA 82 ($P<0.05$), but not significantly higher ($P>0.05$) compared with EquiPlus and Tris (Figure 1). According to Pugliesi et al. (2012), Kenney's extenter is effective in maintaining the motility, vitality and fertility of stallion sperm after 24 hours of refrigerated storage, which is according with results obtained. As yet, there is no available literature reporting on the quality and preservation of semen of Bulgarian sport horse breed and it is difficult to compare the results of different studies as it is known that breed, age, season of semen collection may influence semen quality (Dowsett et al. 1996; Janett et al. 2003).

Table 2. Effect of different extenders in fresh stallion semen on the total motility and velocity parameters.

Parameters	Tris	INRA 82	EquiPlus	modified Kenny
TMS (%)	87,25±18,02^{a}	54,65±27,24^{ab}	65,56±24,90	73,15±26,82^{b}
VCL (µm/s)	37,93±16,46^{a}	37,21±11,71^{b}	52,13±30,43	59,66±29,29^{ab}
VSL (µm/s)	15,19±6,22^{a}	16,98±8,09	18,20±11,0	24,11±11,22^{a}
VAP (µm/s)	24,08±11,16^{a}	24,54±9,14^{a}	30,83±19,36	36,76±16,51^{a}
LIN (%)	46,99±13,37	45,66±16,43	36,16±8,15	43,58±10,57
STR (%)	67,49±9,25^{a}	63,45±13,10	57,93±7,13^{ab}	66,24±8,18^{b}
ALH (µm)	2,15±0,78	1,84±0,48^{a}	2,63±0,98	2,84±0,94^{a}
Vitality (%)	36,35±5,48	18,38±10,21^{a}	31,05±7,85	48,66±5,72^{a}

∗ ^{ab} Values with different superscripts across rows indicate significant differences at $P < 0.05$.

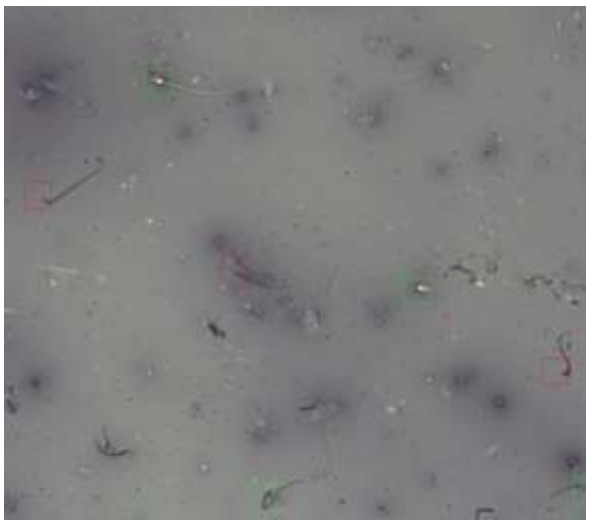

Fig. 1. Vitality of fresh stallion semen: live sperms appear white (green square), dead appear dark (red square)

The morphologic features of stallion sperm are shown in Figure 2. The results revealed no significant variation among all extenders, except percent normal cells (P<0.05) compared to defects in head, midpiece or tail. This emphasizes the performance of all used extenders targeting to preserve the sperm morphology. It is agreeing with Vlasiu et al. (2008) who report no significant difference in sperm morphology between different extenders.

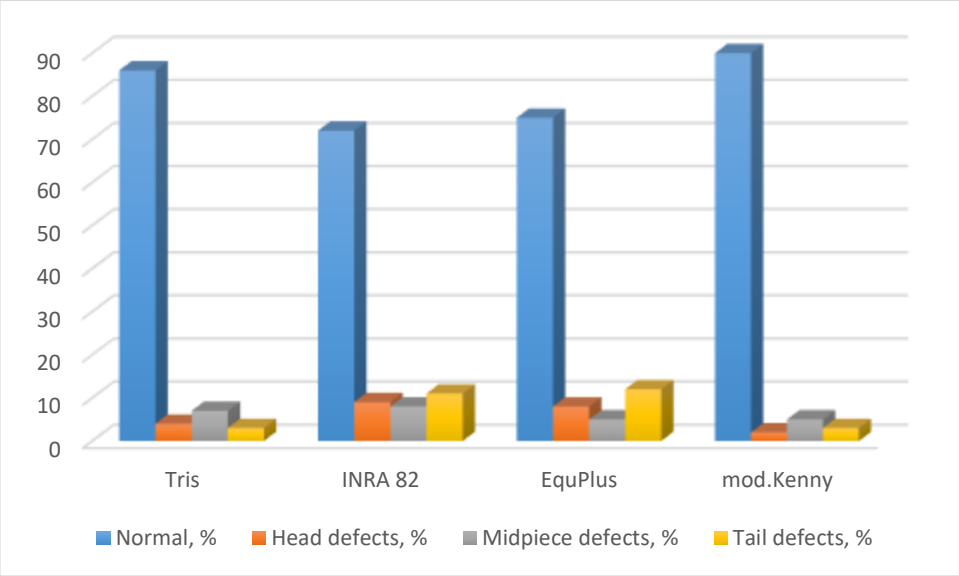

Fig. 2. Morphology of fresh stallion sperm

Analysis of the semen quality requires an accurate morphological assessment of the defects of individual sperm because some stallions may own good semen motility, but high abnormal morphology is not excluded (Varner, 2016).

An important role on semen quality plays some enzymes that were estimated in semen plasma, water and triton extracts from sperm cells (Table 3). Concerning activity of ALP in seminal plasma and water extract, the samples with Tris and modified Kenny were significantly higher (P<0.05) compared with INRA 82 and EquiPlus extenders. In Triton X100 extract, enzyme activity of ALP and LDH were significantly higher in samples with EquiPlus (P<0.05). Enzyme activity of LDH in seminal plasma and water extract were significant higher in samples with Tris (P<0.05) compared to INRA 82. The significant higher level of CK and GGT were found in seminal plasma diluted with modified Kenny (P<0.05) extender in comparison with INRA 82 and EquiPlus.

The composition of each extender significantly influences the lifespan and fertilization ability of the sperm in the insemination dose according Siddique et al. (2006), also affects the level of enzymes activity in the different fractions.

Table 3. Enzyme activity in seminal plasma (SP), water (W) and triton X100 extracts (Tr) of stallion semen

Enzymes / Extender		ALP (U/L)	CK (U/L)	LDH (U/L)	GGT (U/L)
	SP	545,14±119,22[a]	196,08±16,21	386,56±47,40[a]	256,80±26,63
Tris	W	377,23±74,70[b]	63,0±39,0	256,17±47,55[b]	60,46±14,91
	Tr	224,66±38,47[c]	61,60±34,21	56,45±16,98[c]	39,18±13,23
	SP	334,71±88,14[a]	104,43±22,18[a]	51,19±29,16[a]	208,50±25,76[a]
INRA	W	140,86±69,16[b]	60,21±17,92	95,43±15,07[b]	59,18±15,23
	Tr	261,0±67,04[c]	66,89±25,31	192,31±74,20[c]	38,50±17,19
	SP	60,0±11,0[a]	30,25±16,50[a]	276,0±55,20	100,0±20,21[a]
EquiPlus	W	112,20±29,30[b]	63,04±35,81	147,40±53,84	62,73±18,0
	Tr	830,50±163,78[c]	35,25±13,08[b]	639,80±181,22[c]	33,75±18,85
	SP	920,0±179,0 [a]	205,80±82,93[a]	221,40±96,85	668,60±66,27[a]
Modif. Kenny	W	372,53±24,25[b]	66,20±20,93	188,20±17,56	78,18±18,17
	Tr	138,62±33,32[c]	115,40±61,32[b]	171,0±80,60[c]	40,0±14,08

∗ [abc] Values with different superscripts across columns indicate significant differences (P< 0.05) between extenders

Table 4 summarizes relationship between sperm parameters and activity of enzymes in seminal plasma, water and TritonX100 extracts of corresponding samples. The extender Tris express positive correlation (P<0.05) between TMS, LIN and STR with activities of ALP, LDH, GGT in seminal plasma and water extract. About extender INRA 82, the positive correlation (P<0.05) was observed only between trajectory parameters LIN and level of LDH in seminal plasma and water extract. In the samples diluted with EquiPlus, correlation values were positively (P< 0.05) between velocity parameters VCL, VSL, VAP and enzyme activity of ALP, CK, LDH, but only in TritonX100 extract, extractive membrane-bound proteins. It was found positive correlation (P< 0.05) between ALP and CK in seminal plasma with TMS, velocity (VCL, VSL, and VAP) and trajectory parameters (STR and ALH), (P<0.05), as well as between also sperm parameters and activity of LDH and GGT in water extract of semen samples with modified Kenny extender.

Table 4. Correlation between sperm parameters and activity of enzymes in seminal plasma (SP), water (W) and triton X100 extracts (Tr)

		ALP			CK			LDH			GGT		
		SP	W	Tr	SP	W	Tr	SP	W	Tr	SP	W	Tr
Tris	TMS	0.797 *	0.699 *	0.407	0.304	0.099	0.168	0.988 *	0.706 *	0.205	0.933 *	0.133	0.088
	VCL	0.444	0.102	0.082	0.228	0.198	0.245	0.302	0.285	0.234	0.264	0.088	0.179
	VSL	0.094	0.372	0.188	0.467	0.164	0.096	0.228	0.199	0.23	0.381	0.154	0.418
	VAP	0.351	0.145	0.254	0.399	0.21	0.444	0.12	0.08	0.354	0.347	0.375	0.099
	LIN	0.904 *	0.822 *	0.337	0.395	0.272	0.492	0.923 *	0.601 *	0.33	0.621 *	0.092	0.212
	STR	0.774 *	0.685 *	0.415	0.333	0.424	0.252	0.598 *	0.903 *	0.40	0.701 *	0.421	0.500
	ALH	0.228	0.125	0.382	0.145	0.198	0.361	0.088	0.127	0.433	0.341	0.271	0.250
INRA	TMS	0.233	0.366	0.398	0.401	0.48	0.499	0.368	0.23	0.388	0.356	0.405	0.499
	VCL	0456	0.451	0.501	0.288	0.378	0.396	0.354	0.257	0.236	0.500	0.491	0.257
	VSL	0.258	0.401	0.444	0.388	0.339	0.448	0.502	0.236	0.380	0.401	0.258	0.238
	VAP	0.336	0.299	0.221	0.455	0.503	0.471	0.224	0.337	0.214	0.36	0.301	0.489
	LIN	0.385	0.369	0.223	0.339	0.496	0.448	0.705 *	0.69 *	0.099	0.122	0.23	0.418
	STR	0.501	0.462	0.485	0.392	0.273	0.42	0.361	0.491	0.231	0.502	0.484	0.441
	ALH	0.361	0.485	0.392	0.475	0.281	0.421	0.451	0.472	0.492	0.381	0.288	0.288
Equi Plus	TMS	0.264	0.389	0.50	0.444	0.365	0.12	0.288	0.277	0.33	0.46	0.51	0.333
	VCL	0.155	0.188	0.599 *	0.414	0.391	0.820 *	0.219	0.412	0.808 *	0.440	0.36	0.089
	VSL	0.399	0.227	0.666 *	0.344	0.225	0.723 *	0.303	0.399	0.900 *	0.272	0.028	0.255
	VAP	0.419	0.398	0.781 *	0.226	0.122	0.662 *	0.395	0.484	0.766 *	0.491	0.319	0.144
	LIN	0.233	0.366	0.233	0.288	0.500	0.335	0.345	0.252	0.095	0.103	0.351	0.336
	STR	0.381	0.212	0.24	0.366	0.488	0.328	0.351	0.501	0.201	0.499	0.423	0.322
	ALH	0.444	0.079	0.212	0.387	0.371	0.460	0.282	0.097	0.288	0.356	0.44	0.329
modif. Kenny	TMS	0.666 *	0.522	0.112	0.811 *	0.274	0.309	0.474	0.845 *	0.455	0.228	0.692 *	0.126
	VCL	0.740 *	0.499	0.451	0.995 *	0.125	0.377	0.202	0.727 *	0.235	0.098	0.609 *	0.253
	VSL	0.685 *	0.332	0.247	0.612 *	0.097	0.122	0.091	0.334	0.414	0.088	0.936 *	0.323
	VAP	0.712 *	0.501	0.338	0.833 *	0.088	0.440	0.399	0.777 *	0.333	0.182	0.909 *	0.212
	LIN	0.333	0.392	0.450	0.12	0.388	0.290	0.441	0.332	0.384	0.512	0.509	0.225
	STR	0.881 *	0.440	0.093	0.814 *	0.104	0.168	0.427	0.909 *	0.381	0.418	0.618 *	0.238
	ALH	0.790 *	0.099	0.088	0.906 *	0.097	0.102	0.713 *	0.807 *	0.067	0.292	0.712 *	0.319

* - indicate significant differences (P< 0.05);

Alkaline phosphatase (ALP) is an enzyme, which was reported to be able to prevent the premature spermatozoa capacitation that improves the sperm fertility (Bucci et al., 2014). Data about increase ALP levels and correlated with the better sperm motility in the samples with extenders Tris and modified Kenny is in agreement with other findings in the semen plasma of fresh semen of horses (Turner and McDonnell, 2003). Kareskoski et al. (2010), in turn, reported high correlation between the sperm concentration and ALP level in the stallion's seminal plasma. According to Zaja, (2016) the level of alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and g-glutamyl transpeptidase (GGT) enzymes is positively correlated with sperm function and responsible for semen quality.

On the other hand, the better sperm parameters in samples diluted with Tris and modified Kenny extenders, are explained by their positive correlation with the LDH, which generates the energy required to enhance the sperm motility feature. It has also been reported that LDH is an essential enzyme for sperm acrosome reaction (O'Flaherty et al. 2005), therefore, the higher LDH enzyme level corresponded with better semen quality.

In current study, the CK enzyme showed better result in seminal plasma with Tris and modified Kenny extenders, while EquiPlus extender leads to the release of this enzyme in the triton extract. It is considered that CK working along with other enzymes, associated with various processes during maturation of cells (Bucci et al. 2014). The author's team of Dogan et al. (2009) and Pesch et al. (2006) claimed that lactate dehydrogenase (LDH) and g-glutamyl-transferase (GGT) enzymes are the accurate parameters for determination of fresh stallion semen quality. These findings are consistent with Pero et al. (2017) who reported a positive relationship of GGT with higher sperm motility in bovine semen. Similar positive correlation was observed in our study in samples with Tris and modified Kenny, respectively in seminal plasma and water extract. This gives a reason to assume that these two extenders are more suitable for fresh stallion semen compared to others used in the present study. Also, it could be possible, that enzymes fractions in semen plasma, water and triton X100 extracts are different enzymes forms, present in stallion semen with specific function in sperm biology.

2. Conclusions

In conclusion, it can be said that Tris and modified Kenny extenders give better protection to the sperm, compared to other used extenders in current research. The using of alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK) and g-glutamyl-transferase (GGT) enzymes as markers of sperm quality is a positive sign in predicting the characteristics of fresh stallion semen from Bulgarian sport horse. In general, the fertility of stallion sperm can be estimated by a set of characteristics, where the motility and enzyme activity have the high prognostic value.

Disclosure statement

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Statement of animal rights

The manuscript does not contain clinical studies and patient data

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EFFECTS OF MEDETOMIDINE/PROPOFOL ANAESTHESIA AND ATIPAMEZOLE ON OCULAR PARAMETERS IN DOGS

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ABSTRACT

This study was aimed at investigating the effects of the combined use of medetomidine with propofol, and their reversal by atipamezole, on anesthesia monitoring (AM) and ocular parameters including intraocular pressure (IOP), tear production (TP), and horizontal pupil diameter (HPD) in dogs. Ten sexually intact female dogs (weighing 8.5-27 kg and aged 1.0-4.5 years), which were referred for ovariohysterectomy (OVH), were included in this study. General anesthesia was induced, 15 min after medetomidine 100 µg/kg IM premedication, with propofol (Anestofol 10 mg/kg, VIC, Russia) 4 mg/kg IV in group. The animals were monitored for electrocardiography, heart rate, respiratory rate, non-invasive blood pressure, pulse oximetry and rectal temperature throughout anaesthesia. At the end of the OVH procedure, atipamezole was administered at a dose of 500 µg/kg by IM route. Ocular measurements were performed in laterally recumbent dogs, such that the head was raised and care was taken to avoid the occlusion of the jugular veins and the placing of pressure on the eyeball while retracting the eyelids. While TP was significantly lower at T5 ($p=0.01$), no statistically significant difference was observed between the groups at T1, T2, T3 and T4 ($p=0.40$, $p=0.43$, $p=0.44$, and $p=0.50$ respectively). In conclusion, while MED-PRO was found to effective in reducing TP and the HPD, and altering the anesthesia monitoring response to general anaesthesia, atipamezole could be used in dogs for a quick and safe recovery to baseline values after abdominal surgery, such as OVH.

Keywords: Atipamezole, Dog, Intraocular pressure, Pupil diameter

INTRODUCTION

Increase in intraocular pressure (IOP) is a major problem in ophthalmic surgery such that avoiding and controlling the rise of IOP before, during and after surgical interventions is a basic requirement (Banga et al., 2015; Miller, 2019). Anaesthesizing a patient with an ocular penetrating injury on a full stomach is challenging for the anaesthesiologist. In such cases, anaesthesia should be induced rapidly without increasing the IOP. It is imperative for the anaesthesiologist to weigh the risk of aspiration for the injured eye against the risk of blindness that could be caused by an increased IOP and ocular extrusion (Banga et al., 2015). Anaesthesia induction techniques impact IOP in humans (Badrinath et al., 1986; Lev and Rosen, 1994). Sudden increases in the IOP in cases of glaucoma or near-perforating corneal trauma may trigger dramatic effects. Ocular content prolapse not only complicates the surgical intervention, but also results in poor prognosis (Chmielevski et al., 1997; Smith et al., 2019). In cases of glaucoma, a slight increase in IOP would suffice to reduce the axoplasmic flow in the optic nerve, and thereby, cause predisposition to further injury (Hofmesiter et al., 2009; Smith et al., 2019; Muench et al., 2021). Thus, procedures to prevent the increase of IOP during the

induction of anaesthesia would be of benefit in cases of ocular trauma and glaucoma and in patients anaesthesized for intraocular surgery.

Anaesthesia monitoring (AM) is used for ensuring sedation, comfort, amnesia, and anxiety relief during diagnostic and therapeutic procedures involving sedation and analgesia (Gelatt et al., 2007; Karabağlı et al., 2014; Senthil et al., 2021). Given that respiratory depression causes grave injuries (Karabağlı et al., 2014; Park et al., 2019), optimal AM refers to maintaining anaesthesia and normal cardiovascular functions without airway obstruction and severe respiratory depression. Another basic requirement of AM is the ability to rapidly regulate the depth of general anaesthesia if needed. Various analgesics, sedativesand narcotics are used for these purposes with minimum adverse effects (Karabağlı et al., 2014).

Anaesthesia induced with medetomidine/propofol (MED-PRO) combination, which can be antagonized by atipamezole, has been proven to be useful in animals (Badrinath et al., 1986; Lev and Rosen, 1994; Keating et al., 2020). The primary benefit of these combination is the competitive reversibility of medetomidine by atipamezole, which enables an improved control of anaesthetic depth, a shorter recovery period and reduced risk of hypothermia (Badrinath et al., 1986; Lev and Rosen, 1994; Keating et al., 2020).

While the pharmacokinetics and pharmacodynamics of various premedicants and general anaesthetics have been studied in detail, only scarce and controversial information is available on their effects on AM and functional ocular parameters, including IOP and pupil diameter in animals and particularly dogs (Karabağlı et al., 2014; Bruniges et al., 2016). To our knowledge, there is no previous study on the effects of MED-PRO anaesthesia on AM and ocular parameters in dogs.

This study was aimed at investigating the effects of the combined use of medetomidine with propofol, and their reversal by atipamezole, on AM and ocular parameters including IOP, tear production (TP), and horizontal pupil diameter (HPD) in dogs.

MATERIAL AND METHOD

This study was randomized and single-blinded. Ten sexually intact female dogs (weighing 8.5-27 kg and aged 1.0-4.5 years), which were referred for ovariohysterectomy (OVH), were included in this study. The study design was approved by the Local Ethics Committee (Approval Number: 2016-03/2).

The animals were randomly included to group (T0, baseline). Carprofen was administered intravenously at a dose of 4 mg/kg (Rimadyl, Pfizer, USA) to all dogs for analgesia. General anesthesia was induced, 15 min after medetomidine (Tomidine, Provet, Turkey; T1: after 10 min premedication) 100 µg/kg IM premedication, with propofol (Anestofol 10 mg/kg, VIC, Russia) 4 mg/kg IV in group. Physiological saline solution (Eczacıbaşı, Istanbul, Turkey) was administered intravenously at a dose rate of 10 ml/kg/h during the operation. The animals were monitored for electrocardiography, heart rate, respiratory rate, non-invasive blood pressure, pulse oximetry and rectal temperature (G9000, Guoteng Co Ltd, China) throughout anaesthesia (T3: 15 min after the start of the operation; T4: after the last suture). No other anaesthetic or analgesic drug was administered during the operation. At the end of the OVH procedure, atipamezole (Reversal 5 mg/kg, Provet, Turkey; T5: 10 min after reversal) was administered at a dose of 500 µg/kg by IM route.

The animals breathed spontaneously during anaesthesia. The dogs were monitored for respiratory rate (RR), heart rate (HR), mean arterial pressure (MAP), hemoglobin oxygen saturation (SpO_2), and body temperature (BT) with a multiparameter monitor (G9000, Guoteng Co Ltd, China). Clinical data, including the HR, RR, SpO_2, MAP, and BT were recorded together with ultrasonographic findings at six time points (T0, T1, T2, T3, T4, T5), excluding the time point T3. Arterial blood pressure was measured by oscillometry (Rap100, Riester,

Germany).

Ocular parameters were evaluated at the time points T0, T1, T2, T3, T4, and T5. Ocular measurements were performed in laterally recumbent dogs (except T3), such that the head was raised and care was taken to avoid the occlusion of the jugular veins and the placing of pressure on the eyeball while retracting the eyelids. One ophthalmologist, blinded to the anaesthetic technique, performed all of the IOP measurements and used a rebound tonometer (TonoVet, Tiolat, Finland). IOP was measured at the center of the cornea, in accordance with the instruction manual of the tonometer. The factory-calibrated tonometer was recalibrated before each measurement. Three readings were recorded for the right eye at each time point. The mean value of the three readings was calculated. No local anaesthetic was used prior to the IOP measurements. HPD measurements were performed with a caliper at the same time points. The Schirmer tear test I was conducted by placing commercial Schirmer strips (Schirmer Tear Test, Eickmeyer, Germany) in the lower fornix for 1 min.

The dogs underwent OVH after the T2 time point. All surgical operations were performed by a single veterinarian with assistance from veterinary students. All dogs were discharged 24 h after surgery.

Mean±SD values were used for data estimation. Given the small sample size and abnormal distribution of data, statistical analyses were performed with nonparametric tests. Differences in the effects of the drug from the median values were determined by comparatively analyzing the variables of both groups with the Friedman test. The study group was compared with the Mann-Whitney U test, and differences between the measurements over time were evaluated with the Wilcoxon test. Based on an assumption of equal variance and a two-sided confidence interval, statistical significance was set at $P<0.05$. Statistical analyses were performed using the SPSS software (22.0, IBM Company, USA).

RESULTS AND DISCUSSION

The treatment groups did not show any statistically significant differences for the body condition score (BCS), body weight (BW), age and baseline HR, RR, MAP, SpO_2, and RT values. The monitored baseline values of the anaesthetized dogs fell within the normal reference ranges (Table 1).

Table 1. Animal data and baseline (T0) physiological variables for both groups, Mean (±SE)

Patient variable	**MED-PRO**
Body weight (kg)	14.07±1.88
Body condition score	2.80±0.20
Age (years)	1.7±0.22
Baseline heart rate (beats/min)	132.80±6.54
Baseline respiratory rate (breaths/min)	12.50±0.72
Baseline blood pressure (mmHg)	131.00±3.70
Baseline SpO_2 (mmHg)	91.30±0.42
Baseline rectal temperature (°C)	39.16±0.14

SPO_2: Hemoglobin oxygen saturation, MED-PRO: medetomidine/propofol, NS ($p>0.05$).

A comparison of the serial data changes in group MED-PRO are presented in Table 2. In group MED-PRO, HR values showed a significant decrease after the administration of medetomidine and propofol. Statistically significant differences were determined between the time points (p=0.001). Significantly lower values were measured at T1, T2, T3, T4, and T5 compared to T0 (T1, T2, T3, T4, T5 vs. T0, p = 0.005, p=0.005, p=0.028, p=0.005 and p=0.013, respectively). Moreover, significantly higher values were measured at T3, T4 and T5, compared to T1 (T3, T4, T5 vs. T1, p=0.018, p=0.009 and p=0.037, respectively). While the HR values showed a temporary decrease, the quartile values were not within the normal range at the time points T1, T2, and T3 [T1:53.30 (12.24-89.89), T2:50.90 (14.46-86.86), T3:66.43 (4.84-117.15)]. SpO_2 values decreased after the administration of medetomidine and propofol. Statistically significant differences were determined between the time points (p=0.005). Significantly higher values were measured at T0, T1, and T2, compared to T4 (T0, T1, T2 vs. T4, p=0.012, p=0.016, p=0.007, respectively). Moreover, values were significantly higher at T1 and T2 compared to T5 (T1, T2 vs. T5, p=0.038, p=0.018, respectively). RR values displayed alterations after the administration of medetomidine and propofol. Statistically significant differences were determined between the time points (p=0.009). Values were significantly lower at T3 compared to T2 (T3 vs. T2, p=0.017). While the alterations in the RR values were temporary, the quartile values fell within the normal range at the time points T1, T2, T3, T4 and T5 [T1:12.20 (8.87-16.46), T2:13.90 (12.52-17.48), T3:11.14 (7.70-16.30), T4:11.40 (6.50-16.84), T5:12.90 (4.61-22.06)].

A comparison of the serial changes in the intraocular parameters of group MED-PRO is presented in Table 3. In group MED-PRO, the HPD values significantly decreased after medetomidine and propofol administration. Statistically significant differences were determined between the time points (p<0.001). Significantly lower values were measured at T1, T2, T3, T4 and T5 compared to T0 (T1, T2, T3, T4, T5 vs. T0, p=0.011, p=0.005, p=0.005, p=0.005 and p=0.007, respectively). Values were significantly lower at T2 versus T1 (T3 vs. T1, p=0.042). While the decrease in the HPD values was temporary, the quartile values fell within the normal range [T1:5.70 (0.40-11.07), T2:4.30 (3.08-4.92), T3:4.70 (4.22-5.50), T4:4.33 (2.90-5.77), T5:4.90 (4.31-7.69)]. TP showed a significant decrease after the administration of medetomidine and propofol. Statistically significant differences were detected between the time points (p=0.000). Significantly lower values were measured at T1, T2, T3, T4, and T5 compared to T0 (T1, T2, T3, T4, T5 vs. T0, p=0.005, p=0.005, p=0.005, p=0.007 and p=0.005, respectively). Values were significantly lower at T3 versus T1 (T3 vs. T1, p=0.018). While the decrease in TP was temporary, the quartile values fell within the normal range [T1:7.80 (3.30-8.99), T2:5.10 (3.28-5.86), T3:4.40 (3.31-6.41), T4:5.60(2.91-7.37), T5:10.85 (4.51-17.21)].

Table 2. The serial monitorized data changes in MED-PRO group

	Group	T0	T1	T2	T3	T4	T5	P within group
HR	MED-PRO	132.80±6.54	53.30±7.02*	50.90±4.86*	66.43±10.07$^{*\pm¥}$	76.80±6.45$^{*\pm¥}$	90.30±11.46$^{*\pm¥}$	**0.041**
O_2	MED-PRO	91.30±0.42$^{\perp}$	88.90±1.35$^{\perp+}$	85.80±3.87$^{\perp+}$	91.70±0.94	94.70±0.93	93.10±1.59	0.45
RR	MED-PRO	13.30±0.72	12.20±0.63	13.90±0.94	11.14±0.67$^{¥}$	11.40±0.81	12.90±0.77	0.36
BP	MED-PRO	126.30±3.70	119.20±3.34	109.43±5.92	121.14±4.81	119.40±2.65	110.88±5.14	0.55

*p<0.05: vs. T0 within the group, $^{\pm}$ p<0.05: vs. T1 within the group, $^{¥}$ p<0.05: vs. T2 within the group, $^{\perp}$ p<0.05: vs. T4 within the group, $^{+}$ p<0.05: vs. T5 within the group. HR: heart rate, O_2: oxygen saturation, RR: respiration rate, BP: blood pressure.

Table 3. The serial intraocular parameter changes in MED-PRO grous

	Group	T0	T1	T2	T3	T4	T5	P within group
IOP	MED-PRO	20.50±1.42	20.60±1.53	20.90±1.35	21.10±1.91	19.80±0.96	23.90±2.12	0.79
HPD	MED-PRO	8.30±0.63	5.70±0.86*	4.30±0.40*±	4.70±0.40*	4.90±0.59*	5.30±0.63*	0.94
ETP	MED-PRO	16.90±1.33	7.80±1.54*	5.10±0.69*	4.40±0.27*±	5.60±1.06*	5.60±0.90*	0.62

*p<0.05: vs. T0 within the group, ± p<0.05: vs. T1 within the group, ¥ p<0.05: vs. T2 within the group, ⊥ p<0.05: vs. T4 within the group, + p<0.05: vs. T5 within the group. IOP: intraocular pressure, HPD: horizontal pupil diameter, ETP: ear tear production.

CONCLUSIONS

In conclusion, while MED-PRO was found to be effective in reducing TP and the HPD, and altering the AM response to general anaesthesia, atipamezole could be used in dogs for a quick and safe recovery to baseline values after abdominal surgery, such as OVH. It is considered that a dilated HPD could aid in avoiding anterior sinechia during corneal surgery. Thus, the anesthetic protocol tested may not be suitable for corneal surgery. Future research on the effects of other premedicants and induction agents on the HPD, IOP, TP, and AM would either confirm or disprove our results for metetomidine, ketamine and propofol, and contribute to the achievement of a better understanding.

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SCREENING OF SOME WILD TOMATO ACCESSIONS FOR RESISTANCE TO TWO-SPOTTED SPIDER MITE (TETRANYCHUS URTICAE KOCH)

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ABSTRACT

The most widely used method of tomato pest management is chemical insecticides which are known to have a strong influence on agricultural production and also people health. The use of resistant plant to control pests is a beneficial method. Among to pests, the two-spotted spider mite (*Tetranychus urticae* Koch) is a significant tomato pest and all commercial varieties of tomato, *Solanum lycopersicum* L., are susceptible this pest. The objective of this study was to screen wild tomato genotypes against *T. urticae*. The experiment was conducted at the Akdeniz University and 17 tomato genotypes which were consisted of 15 wild tomatoes and 2 local varieties were used in experiment. According to no choice method, LA0716, LA1940 (*S. pennelli*) and LA0462 (*S. peruvianum*) were the most resistant and local variety Ayaş (*S. lycopersicum*) was determined as susceptible.

Key words: Tomato, insecticides, resistance, wild genotype

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important crops worldwide. It is produced 181 million tons in 2020 (FAO, 2020). But diseases, pests and management practices can be restricted of tomato production. More than 60 pathogens, including bacteria, fungi, oomycytes, viruses, and nematodes, have been reported to cause disease in tomatoes, producing serious problems in worldwide tomato production (Jones et al., 2014). Tomatoes also serve as a host for a variety of insects which can cause unsustainable growth or mortality, as well as fruit damage (Lange and Bronson, 1981).

Among to pests, the two-spotted spider mite is a significant tomato pest and all commercial varieties of tomato, *Solanum lycopersicum* L., are susceptible this pest (Salinas et al., 2013). Mites feed on tomato plants, causing significant damage to the leaves and fruits, as well as production loss at high infestation levels (Meck et al., 2013). *T. urticae* can cause direct damage to tomato fruit in the form of gold fleck, which can reduce the fruit's marketability (Meck et al., 2012). Under optimal conditions, the two-spotted spider mite has a fast generation time and can complete its life cycle from egg to adult in approximately seven days (Fig 1) (Namin, 2017).

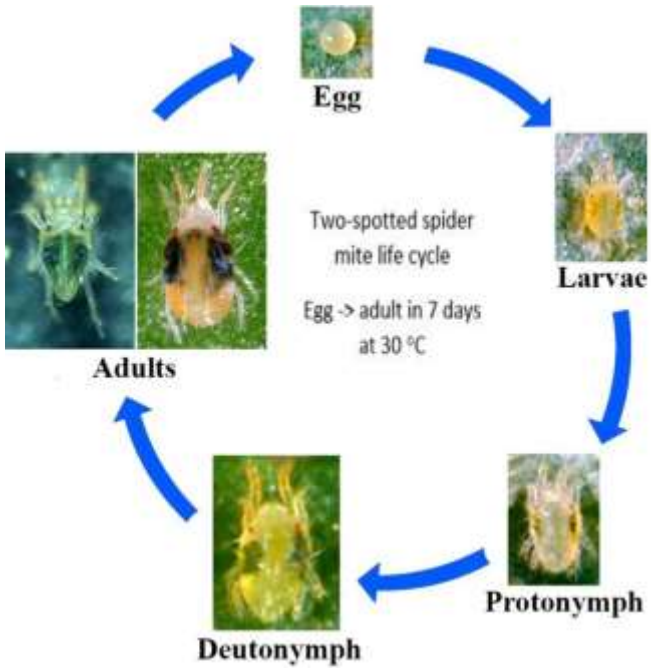

Fig 1. The life cycle of two-spotted spider mites five developmental stages of egg, larva, protonymph, deutonymph and adult (Namin, 2017).

T. urticae control mostly depends on the application of synthetic insecticides (Bolland et al., 1998). The use of synthetic pesticides for pest management negative affect both production costs and environmental impact (Salinas et al., 2013).

Cultivated tomato plants are susceptible to the some diseases and pests so breeding for resistant cultivars are used as an attractive alternative technique for tomato production. All tomato species are diploids (2n = 24) and belongs to the *Solanaceae* family (Rick, 1988). Plant breeders use the wild genotype to develop diseases and pests resistance that can be transferred to cultivated species (Lawson et al., 1997). Wild genotypes have a large genetic diversity (Rick, 1988) and are found growing in the western South American Andes, from central Ecuador to northern Chile, as well as in the Galapagos Islands (Peralta and Spooner, 2000).

Some wild tomato genotypes which are *S. pennellii*, *S. habrochaites*, *S. habrochaites* f. *glabratum* and *S. pimpinellifolium* linked to the spider mite have been determined as potential sources of *T. urticae* resistance (Saeidi et al., 2007; Snyder et al., 2005; Chatzivasileiadis and Sabelis 1997; Salinas et al., 2013).

Further research is necessary to identify for tolerance to resistance to two spotted spider mites which may be useful for developing tomato plants. The aims of this study, to evaluate some wild tomato accessions of tomato for resistance to two spotted spider mites.

MATERIAL AND METHOD

The study was carried out at Akdeniz University, Manavgat Vocational School and a total of 17 tomato genotypes which were consisted of 15 wild tomatoes and 2 local varieties were used in experiment (Table 1). Tomato seeds were germinated into 150-cell plastic trays filled with growing medium and they were grown in nursery until reached the 2-3 leaf stage. Then they were transplanted in to 10 cm diameter plastic pots filled with peat and vermiculite mix in the laboratory at the University. Plants were grown 25/20 ^{0}C day/ night temperatures in the laboratory.

Table 1. Genotype numbers, name of species and origin of tomato materials.

Genotype	Species	Origin

LA1940	*S. pennelli*	TGRC
LA0716	*S. pennelli*	TGRC
LA0407	*S. habrochaites*	TGRC
LA1778	*S. habrochaites*	TGRC
LA1393	*S. habrochaites*	TGRC
LA1777	*S. habrochaites*	TGRC
LA1579	*S. pimpinellifolium*	TGRC
LA2093	*S. pimpinellifolium*	TGRC
LA1651	*S. pimpinellifolium*	TGRC
LA0462	*S. peruvianum*	TGRC
LA1337	*S. peruvianum*	TGRC
LA1278	*S. peruvianum*	TGRC
LA1318	*S. chmielewskii*	TGRC
LA1982	*S. huaylasense*	TGRC
LA2157	*S. arcanum*	TGRC
Lice	*S. lycopersicum*	Local variety (Turkey)
Ayaş	*S. lycopersicum*	Local variety (Turkey)

TGRC= Tomato Genetic Resource Center

Mites: Adult females of *T. urticae* which were maintained for 3 years without any pesticide exposure were obtained from Plant Protection Laboratory of BATEM. The colony of was maintained on green cowpea seedlings (*Vigna unguiculata* L. Walp.) at 26 ± 1 °C, 65% ± 5% RH and 16 L: 8D photoperiod in Akdeniz University Manavgat Vocational School. Adult females (1–2 days old) of mite were used in the experiments.

Resistance tests

Free-choice' method that the *T. urticae* prefers and dislikes was used to determine the resistance of tomato wild genotypes and local varieties (Alba et al. 2009). The seedling of tomato plants was placed on a platform (64x64 cm) and no leaves overlapping and infested cowbean leaves were placed in the center of platform (Fig 2). 200 female adultmites (1–2 days old) were transfered on to center of tray with a soft brush in order to subject all the genotype to the attack of *T. urticae* freely. The experiment was repeated three dupication and all of the duplicates were carried out at the same time. The experiment was kept in a growth chamber with 26/14 °C (day/night temperatures, respectively); 65 ± 5% RH and 16 L:8D photo period during the test period. The *T. urticae* were allowed to make a choice of tomato cultivars in each replicate and after 3, 6 and 9 days, *T. urticae* were counted (Gong et al., 2018; Kirisik et al. 2021).

Fig 2. Tomato plants were placed on a platform.

Data Analysis Statistical analysis of the data was performed by using JUMP 10 statistical software.

RESULT AND DISCUSSION

Results of analysis showed that there was a significant effect among the evaluated genotypes at 3, 6 and 9 days which were infested by spider mites (Table 2).

Table 2. Results of analysis showed at 3, 6 and 9 days

Genotype	At 3 day	At 6 day	At 9 day
Ayaş	5.3 a	5.0 a	7.8 a
LA2093	5.0 ab	2.0 b-e	2.3 c-f
Lice	4.0 abc	3.0 bc	2.8 c-e
LA1579	3.8 a-d	3.8 ab	3.5 b-d
LA1651	2.8 b-e	2.5 b-d	3.8 bc
LA1393	2.0 c-e	2.0 b-e	2.0 c-f
LA1777	2.0 c-e	2.0 b-e	2.3 c-f
LA1778	1.8 c-e	1.0 de	1.0 d-f
LA1982	1.5 de	5.0 a	5.5 ab
LA1278	1.3 e	1.3 c-e	1.8 c-f
LA0407	1.0 e	2.0 b-e	0.8 ef
LA1318	0.8 e	1.3 c-e	0.5 ef
LA1940	0.8 e	0.8 de	0.3 ef
LA0462	0.8 e	0.3 e	0 f
LA1337	0.8 e	2.0 b-e	2.3 c-f
LA2157	0.5 e	1.0 de	1.8 c-f
LA0716	0.5 e	1.0 de	0.5 ef

The local genotype of Ayaş were determined very sensitive against *T. urticae* and the counts of *T. urticae* were observed 5.3, 5.0 and 7.8 respectively at 3, 6 and 9 days. On the other hand, LA0716

showed lowest values for the presence of *T. urticae* at 6 and 9 days. This results shown that the wild genotype LA0716 was the most resistant. Some studies have reported that LA0716 (*S. pennellii*) was resistant to spider mites *T. urticae* (Resende et al., 2002; Maciel et al., 2017; Peixoto et al., 2020). We obtained similar results our study. Another resistant genotype LA 1940 (*S. pennelli*) had number of 0.8, 0.8 and 0.3 *T. urticae* respectively. Statistically significant differences were observed in the experiment (Table 2). LA1940 had useful horticultural characteristics including biotic stress tolerance (Geethanjali et al., 2010). This genotype has arthropod resistance (http://tgrc.ucdavis.edu/). Previous study found that LA1940 (*S. pennelli*) was relatively resistant to *T. absoluta* (Sridhar et al., 2019). According to our results, LA1940 (*S. pennelli*) was tolerant to *T. urticae*.

Some other wild species particularly *S. galapagense* (VI057400, VI045262, VI037869 and VI037239) and *S. cheesmaniae* (VI037240) were determined as resistance based on choice and no-choice bioassays methods in AVRDC (Rakha et al., 2017). The genetic diversity present in wild relatives of the *Solanum lycopersicum* complex (*S. lycopersicum* var. *cerasiforme, S. pimpinellifolium, S. habrochaites, S. pennellii, S. chmeilewskii, S. neorickii,* and *S. cheesmaniae*) and the *Solanum peruvianum* complex (*S. chilense, S. peruvianum*) has been exploited extensively in genetic improvement of cultivated tomato (Rick et al., 1987)

CONCLUSION

Wild genotypes is the source of resistance genes in tomato. And disease resistance in most commercial cultivars has been derived from the related wild species. To determine disease resistance condition can be used in breeding studies. Thus genetic variation will be increased in obtaining new tomato varieties.

Cultivated tomato plants are susceptible to some diseases and pests so breeding for resistant cultivars are used as an attractive alternative technique for tomato production.

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DETERMINATION OF DECISION CRITERIA FOR CHEMICAL USE IN AGRICULTURAL ENTERPRISES

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ABSTRACT

Within the scope of the study, the process for the importance of the decision in the supply of foodstuffs of agricultural enterprises is aimed. All of the accounting records of agricultural enterprises in agriculture were made for their procurement within the scope of the study. Information on all of the chemical inputs and comprehensive comprehensive inspection information according to the research results. In the products examined, 32.38% from the payments of the material fee; total business controls were 15.59%. Materials not delivered per decare in the examined facilities were examined and it was determined that they were not grown for use. The AHP method was used in the course of the decision in the procurement of the relevant package materials. Unstable involved in the study; It can be quality, price, continuity, popularity, availability, substance, maturity. It has been determined that 3 criteria from the target from the call from AHP are quality, popularity and price. It can be explained by the fact that it is next to the first, that the enterprises are in the products that have quality products in growing better quality products, and that the expenses in the purchasing process are paid. Detailed suggestions about the supply of chemical inputs or the problems in agricultural enterprises in enterprises.

Keywords: Chemical Input, Input Supply, AHP Method, Decision Criteria

INTRODUCTION

Production is defined as all the activities carried out to meet the needs of people. At the same time, production, which is one of the three basic activities of an agricultural entrepreneurship that produces goods or services, is very important for agricultural entrepreneurship. The benefits of goods and services, which are a large part of production, need to be increased. Agricultural production, which is a sub-branch of production, is expressed as the production of plant, animal, aquatic products, microorganisms, and energy using soil, water and biological resources and agricultural inputs. It has a strategic importance in terms of the country's economy due to reasons such as contributing to the national income.

Chemical input, which is among the factors necessary for the realization of agricultural production, is important for yield increase. To ensure the sustainability of agricultural enterprises, enterprises must make a certain level of profit. Factors determining the gross profit of enterprises after production; used inputs and income from agricultural products. Input is an important factor affecting the income of agricultural enterprises as it affects the productivity and quality of agricultural products as well as production costs. It is known that because of the increase in the importance of agricultural inputs, the amount of chemical input usage per unit area in the world has increased over the years. When the amount of fertilizer and pesticide use between 1970 and 2019 in the 20 countries that use the most chemical inputs per hectare in the world, fertilizer is 359,09%; it is stated that there is an increase of 229.37% in pesticides (FAO, 2021).

There are many parameters that affect the behavior of agricultural enterprises in the supply of chemical inputs. These parameters can be classified as quality, popularity, price, recommendation, reliability, continuity, brand, maturity, accessibility, and active ingredient

(Peker, 2012). These parameters are called the decision criteria of enterprises in the supply of chemical inputs. Within the scope of the study, it is aimed to determine the important decision criteria in the supply of chemical inputs in agricultural enterprises.

MATERIAL AND METHOD

Material

The data used in the study consist of primary and secondary sources. Primary data were obtained by conducting a face-to-face survey with the managers of 277 agricultural enterprises operating in Konya province Ereğli, Karapınar, Çumra, Hadim, Bozkır, Seydişehir, Beyşehir, Yunak, Akşehir, Ilgın, Altınekin, Kulu, Cihanbeyli and Karatay. The questions in the survey application were obtained from similar studies. The secondary data used within the scope of the study were obtained from the statistical data, reports and related scientific studies of various institutions/organizations related to the subject.

Method

Accounting records are not kept by the agricultural enterprises in Turkey in the majority of the enterprises. For this reason, the necessity of conducting a survey within the scope of the study arises. The primary data to be used within the scope of the study, determined as the study area; Data were obtained by conducting a face-to-face survey with the enterprises engaged in agricultural production in Ereğli, Karapınar, Çumra, Hadim, Bozkır, Seydişehir, Beyşehir, Yunak, Akşehir, Ilgın, Altınekin, Kulu, Cihanbeyli and Karatay districts of Konya province. There are various factors in choosing the province of Konya as the study area. Konya province alone meets 4.8% of Turkey's agricultural production value with 12.90 billion TL. In addition, the fact that it ranks first in wheat, barley, sugar beet, carrot, dry beans and tulip production in Turkey reveals the importance of the agricultural sector in Konya. Due to the richness of the production factors of the province of Konya, it was chosen as the study area and a survey was conducted for agricultural enterprises. Stratified random sampling method was used to find the number of sample agricultural entrepreneurship belonging to the groups.

In the selection of the districts, the agricultural product pattern, agricultural entrepreneurship structures and representation of the ecology of the regions were taken into consideration. These districts were chosen because they constitute 61.94% of the total lands and agricultural entrepreneurship. Neyman's Stratified Random Sampling Method was used in sample selection and the following formula was used.

$$n = \frac{\sum(Nh * Sh)^2}{N^2 * D^2 + \sum(Nh * Sh^2)}$$

$$D^2 = \frac{d^2}{z^2}$$

In the formula;

n: Number of samples,

N: Number of agricultural entrepreneurship in the main audience,

Nh: the number of enterprises in the hth layer,

Sh: variance of the layer,

d: Allowable margin of error from the population mean,

z: Expresses the z value in the standard normal distribution table according to the error rate (Yamane, 1967).

In the determination of the number of samples drawn from the main mass, it was studied within the limits of 5% error and 99% confidence, and the following formula was used to distribute the sample numbers to the strata (Yamane, 1967).

$$n = (N_h S_h * n) / \sum N_h * S_h$$

Table 1. Number of Sample Agricultural Entrepreneurship by Agricultural Entrepreneurship Size Groups (Units)

Agricultural Entrepreneurship Size	Nh	Sh	Average	CV	Nh*Sh	Nh*(Sh)2	N
15-50	23.264	10,20	31,63	32,23	237.209,95	2.418.697	16
51-150	34.077	28,38	91,90	30,88	967.173,66	27.450.330	65
151-500	24.464	88,32	255,63	32,55	2.160.664,55	190.830.252	146
501-+	3.110	236,42	724,43	32,64	735.273,59	173.835.128	50
Total	84.915				4.100.321,75	394.534.407	277

Within the scope of the study, enterprises with 1500 decares or more and enterprises with less than 15 decares of land were not included in the population. The reason for this is that the data used belongs to the Farm Registration System (ÇKS) and there can be more than one record of the same enterprise in the ÇKS. In addition, 15 decares and below were not included in the population because of the thought that the land presence would not be an agricultural entrepreneurship scale in the conditions of the research region.

Method to be Used in Data Analysis

Capital structures of enterprises will be examined on the basis of their classification according to the liquidity of capital. (Açıl ve Demirci, 1984; Karagölge, 1987; Erkuş ve ark., 1995; İnan, 1998; Kıral ve ark., 1999; Özçelik ve ark., 2011; Oğuz ve Bayramoğlu, 2018).

Within the scope of the study, the Analytical Hierarchy Process (AHP) method was used to determine the decision criteria in the use of inputs by agricultural managers. Determination of decision criteria in the use of inputs of enterprises and the degree of impact of these decision criteria have been determined. The Analytical Hierarchy Process (AHP) is a method that helps to rank the probabilities of the realization of alternative decisions based on the decision maker's (producer) judgments about the importance of the criteria. For this reason, when the AHP method is used, it can be shown which option outweighs the producer, and therefore it constitutes a good alternative that can be used for producer behavior models (Günden ve Miran, 2008). The hierarchical organization of criteria is widely used in large decision problems (Prakash, 2003). AHP; It is based on three basic principles: parsing, paired comparison and hierarchical arrangement. A parsing problem is the process of structuring into various hierarchies. Paired comparison is the process of constructing a paired comparison matrix for options or criteria. Hierarchical arrangement is the process of combining comparisons on the hierarchy (Miran, 2008). The first step of AHP is to decompose the decision problem into its main components and create a hierarchical structure. It helps the decision maker to focus on the smaller parts of the relevant Decision (Braunschweig ve Becker, 2004). In paired comparison, how important

criterion A is compared to criterion B is determined by the preference scale with 1-9 points shown in Table 2. Values such as 2, 4, 6, 8 that are not in the degree of importance are intermediate (mean) values.

Table 2. Preference Scale Used in Analytical Hierarchy Process

Significant Level	Definitions	Explanation
1	Equal importance of the two criteria	Two criteria contribute equally to the goal.
3	Moderate importance of one over the other	Experience and judgment moderately favor one criterion over another.
5	Strongly important	Experience and judgment strongly favor one criterion over another.
7	Very strongly important	A criterion is strongly favored, and its dominance is evident in practice.
9	Absolutely important	Evidence for preferring one criterion over another is very reliable.
2-4-6-8	Average Value	Values falling between the judgments listed above to be used when reconciliation is required.

RESULTS AND DISCUSSION

Variable costs in agricultural enterprises; costs that vary depending on production. These costs, which vary depending on the production area, continue throughout the production process and end with the end of production. The varying costs of the enterprises examined in the study are given in plant production. Irrigation (29.80%), fertilizer (26.57%) and harvesting (12.36%) expenses constitute most plant variable costs in the examined enterprises, respectively. Within the scope of the study, the share of fertilizers and pesticides, which are classified as chemical inputs, in total vegetable variable costs was determined as 32.38%. In addition, the total operating costs were also examined within the scope of the study. The share of chemical input costs in the total operating costs in the examined enterprises was determined as 15.59%. In agricultural enterprises, it is seen that the share of chemical inputs in plant production changing, and total operating costs is high. Especially in recent years, with the increasing chemical input prices, the production costs of agricultural enterprises have increased. This situation affects the chemical input supply behavior of agricultural enterprises.

In agricultural enterprises, the varying costs of crops differ according to the size of the enterprises. Variable costs per decare in the enterprises examined within the scope of the study are examined in Table 3. In agricultural enterprises, it is assumed that the efficiency of resource use will increase as the scale of the enterprise increases. For this reason, it is expected that unit costs will decrease as the scale of the farm increases. However, it is seen that this is not the case in the examined enterprises. It is seen that the cost of fertilizers and pesticides per decare is similar in terms of agricultural entrepreneurship groups.

Table 3. Variable Herbal Costs per Decare in the Investigated Enterprises

	Entrepreneurship groups (TL)									
	15-50		51-150		151-500		501-+		Entrepreneurship Average	
	TL	%	TL	%	TL	%	TL	%	TL	%
Seed	57,00	11,46	60,95	12,08	64,38	10,95	67,29	11,63	65,48	11,34
Fertilizer	**136,12**	**27,36**	**169,66**	**33,63**	**155,98**	**26,52**	**148,86**	**25,73**	**153,38**	**26,57**
Pesticide	**34,38**	**6,91**	**22,38**	**4,44**	**30,96**	**5,27**	**37,51**	**6,48**	**33,52**	**5,81**
Irrigation	97,50	19,60	112,64	22,33	174,49	29,67	179,18	30,97	172,06	29,80
Workforce	19,99	4,02	18,54	3,67	19,36	3,29	18,46	3,19	18,88	3,27
Fuel	62,36	12,53	57,84	11,46	60,42	10,27	57,60	9,96	58,92	10,21
Harvest	83,06	16,70	55,48	11,00	78,21	13,30	66,92	11,57	71,35	12,36
Transport	7,10	1,43	7,00	1,39	4,26	0,72	2,78	0,48	3,76	0,65
Total	497,50	100,00	504,48	100,00	588,05	100,00	1.524,48	100,00	577,35	100,00

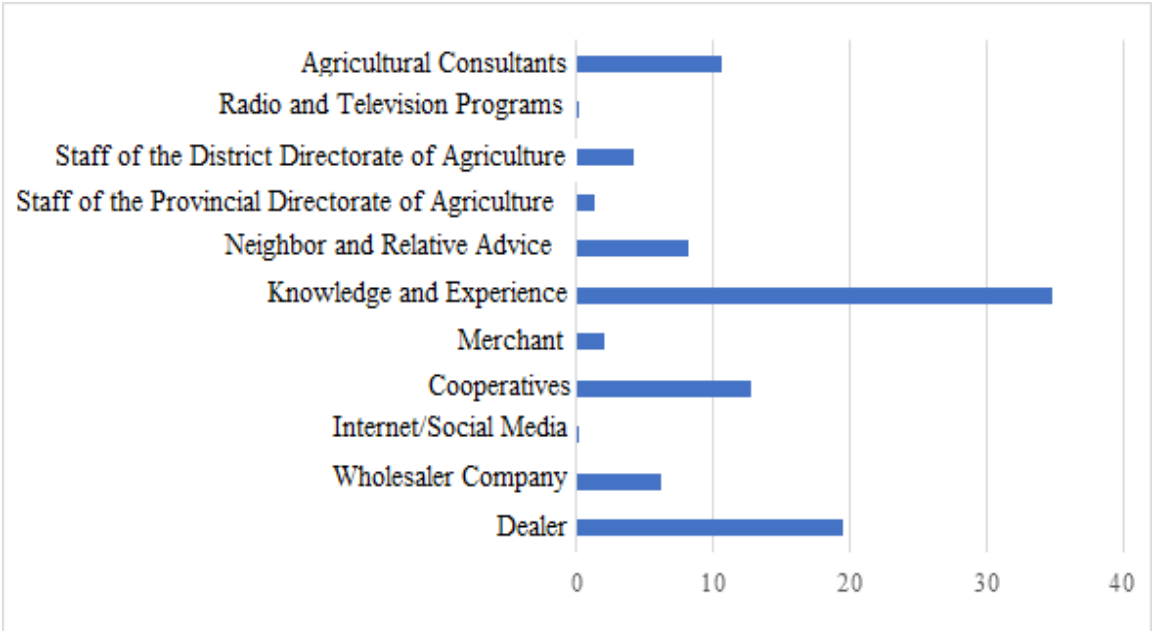

Figure 1. Sources of Chemical Input Supply Location in the Inspected Enterprises

There are many enterprises that provide the supply of chemical inputs to agricultural enterprises. In terms of agricultural enterprises, many factors affect the choice of enterprises that supply chemical inputs. These; Factors such as the prices determined by the companies providing chemical inputs for the chemical inputs and the maturity opportunity provided, the reliability of the enterprise, and the recommendation can be listed as factors. In addition, agricultural enterprises have information sources of chemical input supply location. These resources are given in Figure 1. In the scope of the study, the information sources of the chemical input supply location of the examined enterprises were examined. When the information sources of the fertilizer supply places in the examined enterprises are examined, the highest share is knowledge and experience (34.85%), followed by dealers (19.53%), cooperatives (12.77%), agricultural consultants (10.58%), neighbors, respectively. and relatives' suggestions (8.21%), fertilizer companies (6.20%), technical staff of the district directorate of agriculture (4.20%), technical staff of the provincial directorate of agriculture (1.28%), internet/social media (0%) ,18) and radio and television programs (0.18%). There are various factors that place knowledge and experience in

the first place among the sources of information. As mentioned in the text, the decision maker of the majority of the factors affecting the choice of the enterprise supplying chemical inputs for agricultural enterprises is the enterprise manager. For this reason, the rate of knowledge and experience among the chemical input source information sources may be higher than other sources.

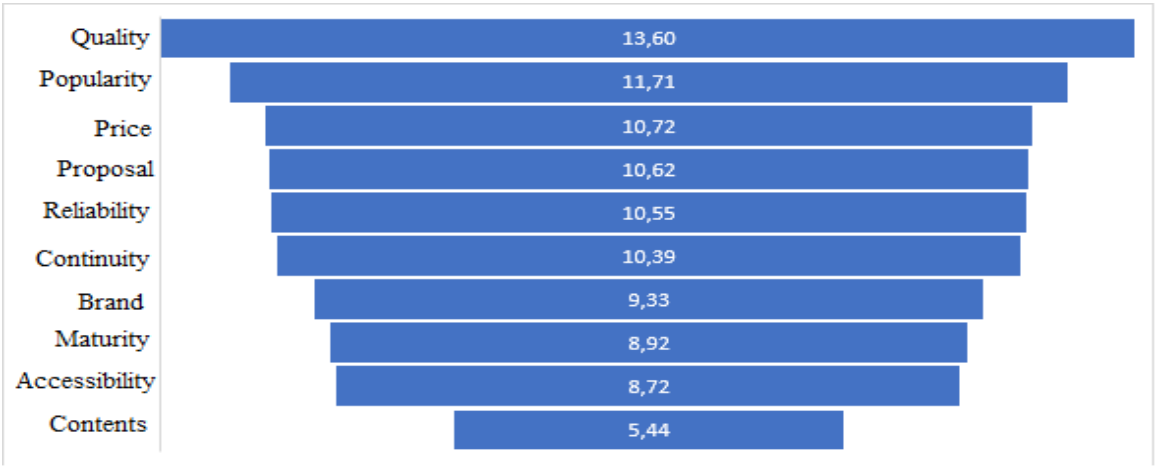

Figure 3. The Degree of Importance for Decision Criteria for Input Supply in the Enterprises

Since the use of chemical inputs affects operational efficiency, agricultural managers consider various decision criteria in the selection of these inputs. Decision making is defined as the process of choosing someone in line with goals and purposes. Various methods are used in the studies in the literature to examine the decision criteria, and one of these methods is AHP management. AHP method; It is a method that enables the selection and ranking of alternatives among decision criteria according to multiple criteria. Within the scope of the study, the AHP method was used to determine the decision criteria in the supply of inputs. 10 scales were used in the implementation of AHP management, and these scales are popularity, quality, reliability, active ingredient, brand, continuity, maturity, availability, advice and price. In the examined enterprises, these decision criteria were prepared according to the question techniques of the AHP method, and it was aimed to determine the most important criteria for the enterprises by comparing each criterion. According to the results of the research, quality has the highest share with 13.60% among the decision criteria in the selection of chemical inputs in the enterprises examined. Popularity (11.71%), price (10.72%), recommendation (10.62%), reliability (10.55%), continuity (10.39%), brand (9.33%), quality respectively. maturity (8.92%), accessibility (8.72%) and active substance (5.44%). There are various reasons why quality has the highest share in the decision criteria. Poor quality chemical inputs used in agricultural enterprises in order to obtain more products from the unit area can cause various damages to the grown products. In addition, the expected yield increase may not be achieved as a result of the use of poor-quality chemical inputs. For these reasons, input quality is important, and manufacturers pay attention. The results obtained in this study support that manufacturers attach importance to quality. The use of quality chemical inputs allows not only to obtain more products per unit area, but also to grow higher quality products. As a result, the prices of quality products can be high in market conditions. There are various reasons why the ratio of the price criterion among the decision criteria is higher than the other criteria. It is mentioned in the study that the share of fertilizers and pesticides is 32.38% in the herbal variable costs examined within the scope of the study. The fact that this share is high can bring the price to the fore in the selection of chemical inputs in agricultural enterprises. The increase in the share of chemical inputs in enterprises reduces the profitability of production. According to the results of the research, the criterion of the active ingredient should

be carefully examined. There is information about active substances on the packages of chemical inputs. As mentioned in the quality criterion, the active ingredient criterion also affects the productivity of the grown products. However, the low importance of the active ingredient criterion can be associated with the education level of agricultural managers. Within the scope of the study, the education levels of agricultural managers were examined, and it was determined that the primary school rate was 53.43%, the secondary school rate was 18.40%, the high school rate was 23.83%, and the university rate was 4.34%. When the education levels of agricultural managers are examined, it is seen that most of them are primary and secondary school graduates. As a result of the low education levels, it is thought that the importance of the active substance criterion is replaced by the selection of the inputs that are constantly used and recommended by the region.

CONCLUSION

Chemical inputs are examined under two headings as fertilizer and pesticide. Since the correct use of these input sources in the enterprise will change the amount of product obtained per unit area, it affects the efficiency of the enterprise. For this reason, it is necessary to examine all issues from the allocation of chemical inputs used in agricultural enterprises to the environmental effects after use. For this purpose, this study was carried out in Konya.

Within the scope of the study, first of all, the plant variable costs, and total operating costs of the enterprises were examined. The ratio of the cost incurred because of the use of chemical inputs in the total variable costs has been determined as 32.38%. In addition, it has been determined that the chemical input constitutes the highest share in the total variable costs. In addition to this, the chemical input cost among the total operating costs was also examined and its share in the total operating costs was determined as 15.59%. The fact that these rates are high is due to the high amount of chemical input usage, as well as the high price of the chemical inputs used. Especially in recent years, it is known that the share of agricultural enterprises in the changing and total operating costs has increased with the excessive price increases in fertilizers and pesticides. This situation reduces the profit of agricultural enterprises. As a result, it limits the sustainability of agricultural enterprises.

Within the scope of the study, the input procurement decision criteria of the agricultural managers were determined and the criteria with the highest degree of importance by the enterprises were determined as quality, popularity, and price. The fact that the quality criteria have the highest degree of importance by the enterprises is due to the risks of the use of chemical inputs. Since these inputs are chemical substances, it is thought that non-quality inputs will damage the products grown. The fact that the price factor is among the most important criteria is due to the high share of chemical inputs in production costs.

According to the results of the research, it has been determined that the chemical input costs in agricultural enterprises have a significant share in the total operating costs. It should be known that the sustainability of agricultural enterprises is under threat, assuming that this share is high, and this share will increase with the increase in chemical input prices. In this regard, it is necessary to minimize the price increases experienced by controlling agricultural input prices or to increase fertilizer supports.

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INFLUENCE OF THE APPLICATION OF ULTRASOUND ON THE SEED QUALITY AND THE DEVELOPMENT OF SEEDLINGS *OF LAGERSTROEMIA INDICA* L.

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ABSTRACT

The use of various physical methods aimed at increasing the germination and vitality of seeds in hard-to-reproduce ornamental species is increasingly applied in nursery practice. One of these methods is pre-sowing seed treatment with ultrasound. The present study was carried out in order to determine the influence of ultrasound on germination and seed viability of the ornamental Crape myrtle (*Lagerstroemia indica* L.) The experiments were carried out in the laboratory of the Department of Horticulture, Agricultural University - Plovdiv. Variants with exposure 5; 10; 15 and 20 minutes were studied. Untreated seeds were used as a control. Characteristics related to plant growth and phenological behaviour were studied. Ultrasonic seed treatment was found to have a positive effect on seed germination and subsequent development of Lagerstremia seedlings. Treatment with ultrasound for 5 and 15 minutes resulted in an acceleration of the rate of stem growth, especially in the initial stages of plant development. Stem height and diameter, as well as the number of internodes, were again highest at 5 and 15 min exposure. The number of stem branches is the parameter that is negatively affected by the ultrasound treatment. The 20-minute exposure treatment is not recommended because it negatively affects both the phenological and biometric parameters of the seeds and seedlings.

Key words: *Lagerstroemia indica*, ornamental plant, propagation by seeds, ultrasound treatment, germination.

INTRODUCTION

The genus Lagerstroemia L. is related to the family Lythraceae, includes about 53 species (Furtado and Montien, 1969; Tobe and Raven, 1990). The center of origin for lagerstroemia is Southeast Asia and Australia (Egolf and Andrick, 1978). The plant is endemic to Southeastern Asia, but is cultivated and naturalized in temperate and tropical regions worldwide (Raulston J.C, Tripp K.E., 1995). The majority of the species in the genus are tropical, and only a few species can grow well in temperate regions (Babele G.S, Kandya A.K., 1986). The crape myrtle has been cultivated in Southeast Asia for many centuries, and was introduced in Europe in the mid 1600s (Pooler, M.,2007). It was planted in Kew Gardens in England in the mid 1700s, and was introduced to the southeastern U.S. soon after ward (Egolf and Andrick, 1978).

Lagerstroemia, or crape myrtle, is best known for its value as a landscape tree or shrub, and as a container or bedding plant. The crape myrtle has become a mainstay in mild climate landscapes because of its ease of production and cultivation, long lasting mid- to late summer bloom, range of plant habit from miniature potted plants to large trees, and diversity of landscape uses (Chudnoff M., 1980; 1984). In addition to its place in the landscape, the introduction of dwarf and miniature cultivars has helped to establish crape myrtle as a potential floriculture, bedding or container plant, that can be grown in a range of hardness zones (Hartmann H.T. et all., 2002).

They can be used as specimen plants; mass planted in parks, estates, or along the streets and highways; as screens; as bedding or foundations plants; or as outdoor container plants. This diversity of form and function, along with often outstanding fall color and stricking exfoliating bark, make it little wonder that crape myrtle has been nicknamed the "autumn lilac". Although some species are grown commercially for timber, wood, or tannins (Egolf and Andrick, 1978), or for medicinal purposes (Hayashi et al., 2002).

Propagation of crape myrtle can be done using a variety of methods. Dirr and Heuser (1987) reported that seeds germinate readily without pre-treatment, although stratification for 1 month at 4 ^{0}C is sometimes advised to synchronize germination. As a stratification method they apply moist pre-chilling. The same find and Raulston and Tripp (1995). According Egolf and Andrick (1978) seeds sown at 15^{0}C germinated within 10 days, without stratification. They recommended seedlings to be transplanted into individual pots shortly after emergence and then fertilized lightly. In a warm greenhouse, such seedlings will make rapid growth, and often bloom the first summer from a December or January sowing. Dirr (1998) reported that germination occurs in 2 to 3 weeks for seeds sown immediately after seed collection in January. However, plants produced from seeds (with hybrid origin or not) will vary in habitus, flower color and shape, plant height and cold hardiness. At present, commercial propagation of crape myrtles is primarily by stem cuttings. For the most easily propagated cultivars, terminal or subterminal softwood cuttings, preferably taken before flowering, with or without rooting hormone, will root within three weeks (Byers, 1977; Einert, 1974). Hardwood cuttings, taken in the fall after defoliation, and stored in a cool place during winter, can be stuck directly in the field in spring (Byers, 1997; Salle, 1988). Hardwood cuttings taken in the spring may not perform as well, but achieve the highest rooting percentage using an IBA/ NAA rooting (Einert, 1974). Root cuttings can be made during any season, and will result in numerous shoots, which can then be rooted as softwood cuttings (Egolf and Andrick, 1978). Micro propagation trough tissue culture, using axillary buds on modified woody plant medium with 4-10 MBA, is also possible (Zhang and Davies, 1986; Shim and Mi, 1994). Propagation by seeds, although not desirable for production of cultivar material, is obviously required for breeding. Seeds can be sown without stratification, directly into flats, and will germinate within two weeks. With proper fertilizer, light, and temperature, many seedlings will flower during first growing season.

MATERIALS AND METHODS

The study was conducted in the period 2020-2021. The experiments were set in the laboratory of the Department of Horticulture, Agricultural University - Plovdiv. The seeds were collected from well-developed, healthy plants *Lagerstremia indica (L.) Pers.*, located in the area of the town of Hisar, Plovdiv region. The collection took place in late November 2020.

The seeds were separated by hand. Then lightly dried at room temperature. The ultrasonic treatment was carried out in an ultrasonic bath Ultrasonic water bath NAHITA, model 620/1, manufactured by AUXILAB, S.L., Spain with ultrasonic wave frequency 220-240 v - 50 Hz and ultrasonic power 35 W. The experiment was set on April 29, 2021 at the Agricultural University of Plovdiv. Lagerstroemia seeds were immersed in water, wrapped in gauze and treated with ultrasound for different periods of time. 160 seeds were used, which were divided into 5 groups of 32 each - one non-treated control and 4 groups treated for 5 minutes, 10 minutes, 15 minutes and 20 minutes respectively. Immediately after sonication, the seeds were sown in a peat-perlite mixture, four per cell in a tray with 40 cells. The seeds are then left outdoors and grown under natural conditions. Germination began in the middle of May 2021.

The following indicators were studied: Growth rate of the stem; Phenological observations: Seed germination (%); Plant height (cm); Diameter of the stem at the base (mm); Number of internodes (pcs.).

The experiment was reported every week after the emergence of the first plant. The experiment ended 4 months after sowing (in the end of August). All plants of each variant were analyzed. Seed germination was reported one month after sowing. The height of the stem was measured from the soil surface to the top of the plant in cm. The thickness of the stem was recorded at the base, above the root collar using a caliper in mm.

Figure 1. Seeds of *Lagerstroemia indica (L.) Pers.*

The obtained data were statistically processed by analysis of variance.

RESULTS AND DISCUSSIONS

The seed qualities of *Lagerstroemia* are presented in Table 1. The absolute mass per 1000 seeds is 0.978 g. Vitality percent is 75.07. The average germination is 5 %. The germination in the different variants is presented later in Table 7. The germination energy of the seeds is 23.96%. The length of the embryonic root is 0.67 cm.

Table 1. *Lagerstroemia* seed quality

Absolute mass per 1000 seeds (g)	Vitality,%	Average germination %	Germination energy,%	Embryonic root length (cm)
0.978	75.07	5.0	23.96	0.67

The rate of stem growth of *Lagerstroemia indica* is shown in Figure 2.

The most intensive during the entire period is the growth of the Crepe myrtles treated for 5 and 15 minutes. The plants from these groups are the most vigorous, the tallest and are almost at the same height all the time, and at the end of the experiment they outgrow the control group by more

than 3 cm, and the plant treated for 10 min by more than 2.5 cm. The difference between the highest (in the 15 min treatment group) and the shortest plant (in the control group) at the end is 4.2 cm.

This is more than half the total height of the control plants, which is 6.8 cm. The 10 min treatment group is in middle position - the plant is taller than the control group, but it is the only one alive, while in the control group 3 sprouted and 2 survived.

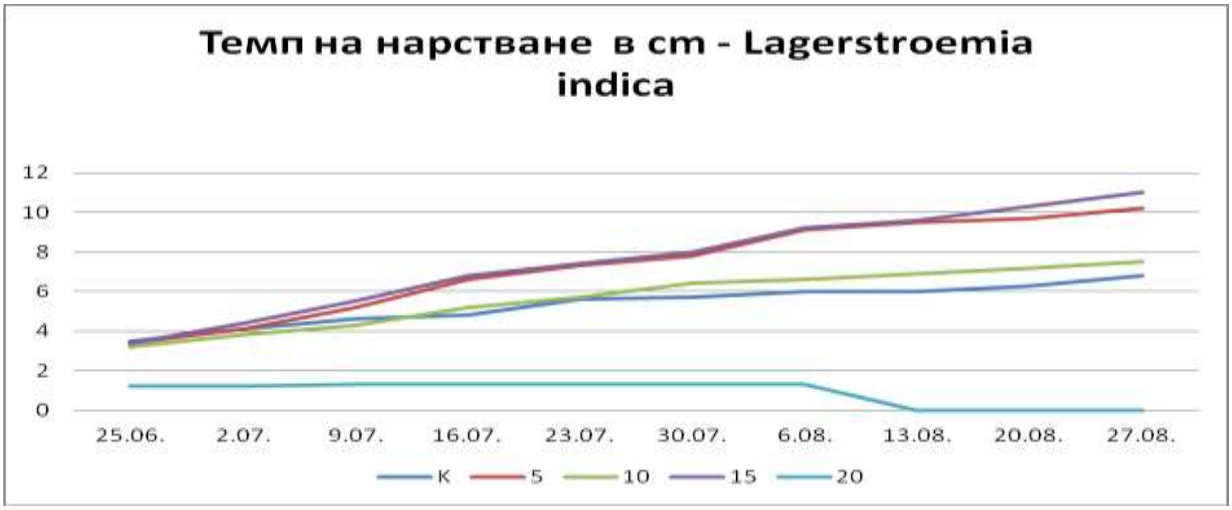

Figure 2. The rate of stem growth of *Lagerstroemia indica*

Compared to the 5 and 15-minute treatment groups, these plants are weaker. In the 20-minute treatment group there is no significant change from sprouting to early July, and the only plant is in the cotyledon stage and with height of 1.2 cm. The presence of a third germ leaf and the lack of a vegetation peak is very unusal. In the second week of July, however, there is a minimal increase in height of 0.1 cm. The third, fourth and fifth weeks of July show absolutely no change and the plant remains in the cotyledon stage at 1.3 cm. In the first week of August, most likely due to the high temperatures that occurred, the plant dies.

Table 2. Phenological observations in *Lagerstroemia indica*

Characteristics / Variants	germination			
	started		mass	
	date	No. days after sowing	date	No. days after sowing
f	21.05	22	28.05	29
5	21.05	22	21.05	22
10	14.05	15	14.05	15
15	21.05	22	21.05	22
20	21.05	22	21.05	22

The first initial and mass germination was observed in the lagerstroemia, which was treated for 10 min - on May 14. That is 15 days after the sowing.

Table 3. Phenological observations in *Lagerstroemia indica*

Characteristics / Variants	third pair of true leaves			
	started		mass	
	date	No. days after sowing	date	No. days after sowing
к	25.06	57	25.06	57
5	25.06	57	25.06	57
10	25.06	57	25.06	57
15	25.06	57	25.06	57
20	-	-	-	-

This happened last in the control group - initial emergence on May 21 and mass emergence on May 28 - 29 days after sowing. The groups treated for 5, 15 and 20 min. with ultrasound had initial and mass germination 22 days after sowing on May 21.
All plants, except for the 20-min treatment group, which remained only with cotyledons until the end, simultaneously entered the stage with 3rd, 4th and 5th pair of true leaves – respectively on the 57th, 64th and 71st day of sowing. The control, however, entered in the 5th pair of true leaves one week after the others - on the 78th day of sowing.

Table 4. Phenological observations in *Lagerstroemia indica*

Characteristics / Variants	fifth pair of true leaves			
	started		mass	
	date	No. days after sowing	date	No. days after sowing
к	09.07	71	16.07	78
5	09.07	71	09.07	71
10	09.07	71	09.07	71
15	09.07	71	09.07	71
20	-	-	-	-

The control and the 5 min treatment group first entered initial forming of the 8th pair of true leaves, but the control group was the last to enter the stage - 120 days after sowing. All other groups entered it 92 days after sowing.
The initial forming of the 11th pair of true leaves occurs simultaneously in the control and in the groups treated for 5 and 15 min. This was on day 113 of the sowing. Mass forming of 11th pair of true leaves occurred on the same day for the groups treated for 5 and 15 min. One week later, the group treated for 10 min. was the first to enter in stage 11- the pair of true leaves - 120 days after sowing. Control group never reached this phase en masse.

Table 5. Phenological observations in *Lagerstroemia indica*

Characteristics / Variants	eighth pair of true leaves			
	started		mass	
	date	No. days after sowing	date	No. days after sowing
к	23.07	85	27.08	120
5	23.07	85	30.07	92

10	30.07	92	30.07	92
15	30.07	92	30.07	92
20	-	-	-	-

Table 6. Phenological observations in *Lagerstroemia indica*

Characteristics / Variants	eleventh pair of true leaves			
	started		mass	
	date	No. days after sowing	date	No. days after sowing
к	20.08	113	-	-
5	20.08	113	20.08	113
10	27.08	120	27.08	120
15	20.08	113	20.08	113
20	-	-	-	-

In conclusion, it can be said that the best development had the Crepe myrtles treated for 5 and 15 minutes with ultrasound. The worst development had those treated for 20 minutes.
At the end of the experiment, the lagerstroemia treated for 15 minutes with ultrasound had the greatest height - 11 cm. The second best was the one treated for 5 minutes - 10.2 cm. The shortest Crepe myrtle was the one treated for 20 minutes - only 1.3 cm. Stem branches develop only in the control group and in the group of 5-minute treatment – an average of 3.5 in the control group and 1 in the 5-minute treatment group. The largest diameter of the stem at the base has the lagerstroemia, treated for 15 min. - 2.1 mm, followed by the one treated for 5 min. - 1.9 mm. The smallest diameter has the Crepe myrtle treated for 20 min. - 1 mm, which is almost 2 times less than the plants in 1st and 2nd place according to this indicator.

Table 7. Characteristics of the stem and germination of *Lagerstroemia indica*

Characteristics / Variants	Height cm	Number of stem branches, average	Diameter of the stem at the base, mm	Number of internodes, average	Germination %
к	6,8	3,5	1,75	10,5	9,37
5	10,2	1	1,9	12,5	6,25
10	7,5	0	1,7	12	3,12
15	11,0	0	2,1	13	3,12
20	1,3	0	1,0	1	3,12

The lagerstroemia, treated for 15 min. developed the most internodes - 13, followed by the one treated for 5 min. - 12.5. These two groups had at least 2 internodes more than the control group, which had an average of 10.5. The lagerstroemia treated for 20 min developed only one internode. Germination was highest in the control - 9.37%, followed by the group of 5 min. treatment - 6.25%, and was the lowest in the group of 10, 15 and 20 min. treatment -3.12%. Out of 160 seeds sown, only 8 germinated. The average germination rate is 5%, which is extremely low.

CONCLUSIONS

Plants growing from 5 and 15 minutes' seeds sonication are characterized by the most intensive and uniform growth rate. The seeds with 10 minutes' ultrasound treatment had the highest germination rate. Ultrasound treatment did not affect the phenological manifestations of

Lagerstroemia in the initial phases of plant development. All plants - both treated and control - formed a third pair of leaves at the same time. All treated plants formed fifth and eighth pairs of leaves at the same time, 7 and 28 days earlier than untreated plants, respectively. The eleventh pair of leaves did not form in the control group and in the plants treated with 20 minutes of ultrasound. Plants treated with 5 and 15 minutes of ultrasound were the first to form eleventh pair of leaves. Plants obtained from 10 and 15 minutes' ultrasound treatment have the tallest stems. The number of stem branches is negatively affected by ultrasonic seed treatment. Stem diameter and internode number were greatest at 15 min seed sonication.

In conclusion we can recommend 5 minutes' sonication, because germination does not decrease much, but the vitality of the plants increases significantly.

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STUDY OF THE POSSIBILITIES FOR IMPROVING THE SOWING QUALITIES OF SEEDS AND THE VITALITY OF SEEDLINGS FROM *CRYPTOMERIA JAPONICA* DON. THROUGH PRE-SOWING TREATMENT WITH ULTRASOUND

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ABSTRACT

Recently, in the production of planting material from ornamental species of trees and shrubs from seeds, more and more attention is paid to various physical methods aimed at increasing the germination and viability of seeds in difficult to propagate species. One of these methods is pre-sowing treatment of seeds with ultrasound. The present study was conducted to determine the effect of ultrasound on the germination and viability of seeds of ornamental species of Cryptomeria (Cryptomeria japonica D. Don.). The experiments were set in the laboratory of the Department of Horticulture, Agricultural University Plovdiv. The experiment with Cryptomeria was conducted from the end of February to the beginning of August. Variants with 5, 10, 15 and 20 minutes exposure were studied. Untreated seeds were used for control. Indicators related to the growth and phenological manifestations of plants were studied. It was found that the treatment of seeds with ultrasound affects the sowing qualities of the seeds of the studied species. Ultrasound treatment has a beneficial effect on germination in Cryptomeria, with the optimal exposure being 20 minutes - so germination increases by 11% compared to control.

Key words: Cryptomeria, seed propagation, ultrasound treatment, germination, ornamental plant.

INTRODUCTION

Japanese cedar or cryptomeria (*Cryptomeria japonica D. Don.*), is a coniferous evergreen tree of the *Cupressaceae* family. The plant is indigenous to Japan and southern China and endemic to Japan. It is a widely used timber species in the Far East (Dirr, 1990). Cryptomeria is also considered a sacred tree in Japan with great landscape value (Creech, 1984). The plant that the Japanese call Sugi is an allogamous, wind-pollinated conifer species that is frequently used for commercial afforestation in Japan. Approximatelly 45% of all the man-made forests in Japan are composed of this species (Saito, O., 2009; Forster, E.J. et al., 2021). The species currently is gaining popularity not only in the northeastern United States, but also in the hot and humid southeast. The tree grows very well in rich, deep, acidic, moist soil but will tolerate heavy clay during dry and wet periods (Dirr, 1990; Tripp, 1993).. The plant has a pyramidal crown, reaching 15 to 18 m in height outside their homeland (up to 60 m in Japan), with descending branches. There are many cultivars of Japanese cedar that have a wide range of ornamental characteristics and uses. Cryptomeria has no major insect or disease problems (Baker, J. and R. Jones. 1987), grows rapidly and makes an excellent evergreen screen (Dirr, 1990; Tripp, 1993). In addition, the

emerald green foliage exhibits little dieback or discoloration (Tripp, 1993). This species is recommended as a replacement for Leyland cypress *Cupressocyparis leylandii* (A.B. Jacks and Dallim.) Dallim. and A.B. Jacks] which has various disease and insect problems (Baker and Jones, 1987). In Bulgaria cryptomeria is used as an ornamental tree due to the beautiful shape of the crown, high cold resistance - withstands up to - 23^0C - and the relative tolerance to diseases and pests (Bogdanov, B. 1984). Its extremely limited distribution in our country in public and private parks and gardens is due to the lack of supply of the plant in garden centers and nurseries. The main part of the plants offered in these sites are imported from abroad and the price is quite high. These problems stem from the lack of developed technology for the production of planting material for the conditions of Bulgaria. One of the main points in the technology for the production of planting material is the question of how to propagate. The different ways of propagation should be studied in detail and the one that has the highest efficiency and can be used for mass production of planting material in the conditions of Bulgaria should be singled out. There is a lot of information in the specialized literature about the ways of cryptomeria propagation. Special attention is paid to their advantages and disadvantages. Researchers studying vegetative propagation in cryptomeria are unanimous that the most appropriate method is by stem cuttings. Jull, L.G. et al. (1994) demonstrated that cryptomeria stem cuttings can be rooted at all growth stages, but branch order from which cuttings are prepared is critical in achieving high rooting percentages. Although popularity and subsequent demand of cryptomeria have increased, little research has been reported in journals on factors influencing propagation of the species and related cultivars by stem cuttings (Doran, W.L. 1957; Waxman, S. 1962; Henry, P.H., F.A. Blazich, and L.E. Hinesley. 1992). For centuries, Japanese cedar has been propagated in Japan for forestry by seed and stem cuttings (Brix and van den Driessche, 1977; Ohba, 1993; Still, S.M. and S. Zanon. 1991.), and a body of practical knowledge on propagation and culture exists in the Far East. Some research concerning stem-cutting propagation of the species has been conducted in the United States. Information regarding factors, such as growth stage (timing) and auxin treatment, have been published (Black, D.K. 1972; Bogdanov, B. 1984); however, much of this information is conflicting and needs to be resolved (Dirr and Heuser, 1987; Lahiri, 1975; Nakayama, 1978; Orndorff, 1974). In addition, tree forms of Japanese cedar exhibit a well-defined branch order (branch position), which may influence rooting. For some conifers, branch order is an important factor affecting adventitious rooting (Black, 1972; Bogdanov, 1984; Miller et al., 1982) and warrants study in Japanese cedar.

Cryptomeria can also be propagated by seeds (Hartmann, H.T., et al. 1990). In order to obtain more and better seeds Moriguchi et al. (2004); Moriguchi et al. (2005) and Moriguchi et al. (2007) recommend that in production of seedlings of Cryptomeria have to abidance three main factors, namely: first the pollen contamination rates can be >30%; this may be influenced by the surrounding forest plantations; second, the self-fertilization rate in conifer seed orchards has been determined to be generally less than 5% and third, the paternal contributions of constituted clones have been found to differ significantly from the expected equal contributions; this may be influenced by the number of male strobili. Itoo, S. (1984) compared the properties of cryptomeria seeds obtained from a greenhouse (indoors) and outdoors and found that seeds obtained in the open place have better germination. On the other hand, the plants obtained from these seeds vary considerably in the main observed decorative characteristics. According to Mitsch, J. (1975) and

Russell, R.S. (1977), a short period of statification is required for normal seed germination - from 4 to 6 weeks - at low positive temperatures, after which the seeds are ready for sowing. The issues of seed propagation in most cases are related to the study of the possibilities for increasing the germination and viability of the seeds through different ways of pre-sowing treatment. Ultrasound treatment is one of these ways (Awad, T. et al. 2012; Chen, G. et al. 2012; Miano, A.C. et al. 2015;) Ultrasound technology has been used to enhance the quality of seeds in many agricultural crops. According to Aladjadjian, A. (2002) the effect of under ultrasonic action is due to the mechanical energy of the ultrasonic wave which transformed into the kinetic energy of the molecules in the seed. This energy is redistributed between the molecules and transformed into chemical energy, increasing the activity of chemicals in the seeds, as a result of which accelerates their growth and development. The effect of ultrasound treatment on seed quality parameters such as germination and vigour has not been sufficiently studied (Yaldagard, M., 2008). This physical way of affecting the qualities of the seeds is still poorly studied, and in cryptomeria there is no information about it at all. This work investigated whether ultrasound technology affected the germination and vigour of cryptomeria seeds.

MATERIALS AND METHODS

The study was conducted in the period 2020-2021. The experiments were set in the laboratory of the Department of Horticulture, Agricultural University - Plovdiv.

The seeds were collected from well-developed, healthy plants Cryptomeria japonica var. japonica. Syn: Cupressus japonica Thunberg ex Linnaeus f., Suppl. Pl. 421. 1782; Taxodium japonicum (Thunberg ex Linnaeus f.) Brongniart (Fu et al. 1999). from TP State Hunting Farm Krichim. The collection took place in early November 2020. The seeds were separated by hand. Then lightly dried at room temperature. The ultrasonic treatment was carried out in an ultrasonic bath Ultrasonic water bath NAHITA, model 620/1, manufactured by AUXILAB, S.L., Spain with ultrasonic wave frequency 220-240 v - 50 Hz and ultrasonic power 35 W. The experiment was set on February 26, 2021 at the Agricultural University of Plovdiv. Cryptomeria seeds were immersed in water and wrapped in gauze and treated with ultrasound for different periods of time. 180 seeds were used, which were divided into 5 groups of 36 each - one non-treated control and 4 groups treated for 5 minutes, 10 minutes, 15 minutes and 20 minutes respectively. Immediately after sonification, the seeds were sown in a peat-pearlite mixture, each in a separate cell on a 180-cell tray. The seeds are then left outdoors and grown under natural conditions. Germination began in mid-April and ended in early May 2021.

The following indicators were studied: Growth rate of the stem; Phenological observations: Seed germination (%); Plant height (cm); Diameter of the stem at the base (mm); Number of internodes (pcs.). The experiment was reported every week after the emergence of the first plant. The experiment ended 6 months after sowing (in August). All plants of each variant were analyzed. Seed germination was reported three months after sowing.

The height of the stem was measured from the soil surface to the top of the plant in cm. The thickness of the stem was recorded at the base, above the root collar using a caliper in mm. The obtained data were statistically processed by analysis of variance.

Figure 1. Seeds of *Cryptomeria japonica D. Don.*

RESULTS AND DISCUSSIONS

Table 1 presents the results of studies of the seed quality of *Cryptomeria japonica.* The absolute mass of 1000 air-dried seeds was 1.279 g. This indicator is influenced by the size and fulfillment of the seeds, as well as by the climatic conditions in the growing area. These data are a criterion for the ecological plasticity of a species and its suitability for acclimatization to the conditions of an area. The vitality of the seeds determines their potential ability to germinate. In the seeds used in this experiment, the vitality was 63,94%. Germination is the most important indicator of the suitability of seeds to form normal sprouts under optimal conditions over a period of time. In *Cryptomeria japonica*, total seed germination was determined on day 7-th - 24,44% (Table 1).

Table 1. *Cryptomeria* seed quality

Absolute mass per 1000 seeds (g)	Vitality,%	Germination,%	Germination energy,%	Embryonic root length (cm)
1,279	63,94	24,44	73,18	1,07

Germination energy indicates the percentage of normally germinated seeds under optimum germination conditions within a period shorter than that for germination. In *Cryptomeria japonica*, the germination energy was determined on day 5-th and was 73.18%, indicating that the seeds germinate jointly and give strong and viable seedlings and, respectively, more viable plants. The

average embryonic root length of *Cryptomeria japonica* is 1.07 cm, which is also evidence of seed viability and usability.

The rate of stem growth in seedlings is a criterion for the viability of plants and their successful future development. The growth rate of the Cryptomeria seedlings was monitored from the beginning of June to the beginning of August and is presented in fig. 2. From the data becomes clear that the plants from the control group have the lowest growth rate - their initial average height is 2.50 cm and the increase in height in the first week is only 0.16 cm. In the the next three weeks, the stem increase in height, or more precisely the differences in stem height for the seven-day reporting period, is minimal - 0.02 cm in the first week, up to 0.04 cm in the third week. Transplantation was carried out in early July. Very intersting is the fact that after this period of minimal growth, from mid-July, the plants not only do not grow, but also the height of their stems begins to decline intensively - 0.15 cm in the first week of July, 0.37 cm in the second week of the same month and 0.1 cm the third week of July. This decline in average height is due to the gradual death of the seedlings. All plants then die, most likely because of the contact of the root hairs with air oxygen during transplanting.

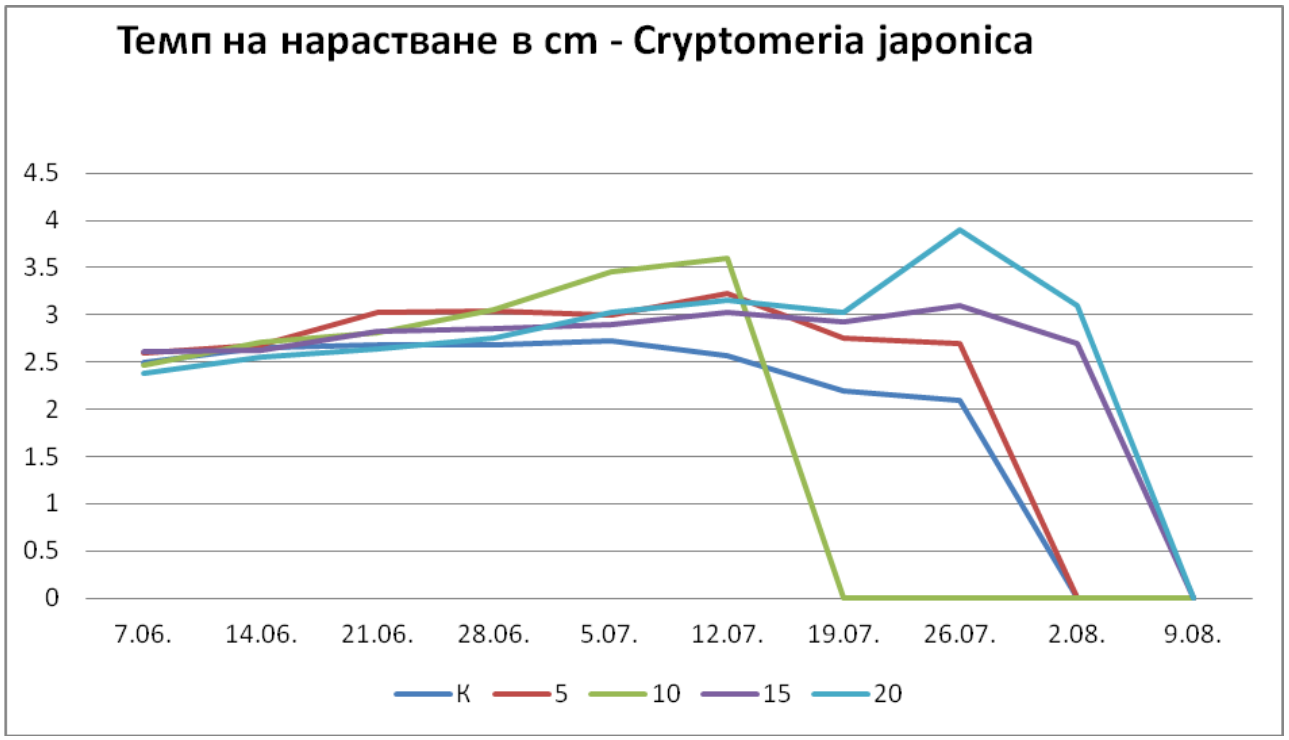

Figure 2. The rate of stem growth of Cryptomeria japonica

Seeds treated with different sonication exposure, give significantly taller and more viable plants, with more intense growth rate. The plants from the 5-minute exposure group have the most intensive growth rate at the end of June, with an increase in height for the seven-day period of 0.34 cm. Then follow periods of poor growth and height decrease. This process lasts until the end of July. In the beginning of August, the plants die. 10 minutes of ultrasound exposure leads to the death of plants in the third week of July, but until then they are vital and growing at an intensed rate - from 0.1 cm in the third week of June to 0.25 cm in the last week of the same month. The plants obtained from 15 minutes' sonication increase their height by an average of 0.2 cm in the third week of June. These plants are also characterized by an intensive growth rate in the second and fourth week of July - they grow by an average of 0.12 cm and 0.17 cm, respectively. This increase is due to the death of shorter plants and an increase in average height. During the remaining weeks of the experiment the growth of the plants is 0.02 cm, 0.03 cm and 0.04 cm. in the second and fourth week of June and the first week of July. Here there is also a reducing in the

stem height by 0.09 cm, in the third week of July. Plants received 20 minutes sonication are distinguished by the most intensive growth rate and the greatest vitality. Differences in stem height range from 0.08 cm in the third week of June to 0.87 cm in the last week of July. Again, the increase in the end of July is due to the death of shorter plants.

Table 2. Phenological observations in cryptomeria

Characteristics / Variants	germination			
	started		masse	
	date	No. days after sowing	date	No. days after sowing
К	19.04	52	26.04	59
5	21.04	54	26.04	59
10	19.04	52	26.04	59
15	19.04	52	26.04	59
20	19.04	52	26.04	59

Table 3. Phenological observations in cryptomeria

Characteristics / Variants	first stem branch			
	started		masse	
	date	No. days after sowing	date	No. days after sowing
к	28.06	122	28.06	122
5	28.06	122	28.06	122
10	21.06	115	28.06	122
15	28.06	122	28.06	122
20	05.07	129	05.07	129

Phenological observations on cryptomeria seedlings are presented in Table 2. The data show that pre-sowing sonication did not significantly affect the onset of germination of cryptomeria seeds - all experimental variants, except the variant treated for 5 minutes, germinate 52 days after sowing on 19.04. The same applies to the mass germination of seeds, which occurs 7 days after the beginning, on 26.04. or 59 days after sowing, both for treated and untreated seeds. The first stem branching occurs in plants obtained from 10 minutes seeds sonication, on 21.06. or 115 days after sowing (Table 3). Next are the plants obtained from seeds treated with 5 and 15 minutes sonication, which form the first stem branch 7 days later on 28.06. or 122 days after sowing.

Table 4. Phenological observations in cryptomeria

Characteristics / Variants	second stem branch			
	started		masse	
	date	No. days after sowing	date	No. days after sowing
к	-	-	-	-
5	-	-	-	-
10	05.07	129	05.07	129
15	-	-	-	-
20	-	-	-	-

Very interesting is the fact that plants growing from untreated seeds form the first stem branch within the same period. At the latest, the first stem branch is formed by the plants growing by 20 minutes seeds sonication - on 5.07., or 129 days after sowing. The second stem branch is formed only by plants from 10 minutes seeds sonication, on 5.07., 129 days after sowing, or 14 days after the appearance of the first stem branch (Table 4). The data illustrating how many of the plants form stem branches are very interesting (Table 5). The highest percentage of plants forming stem branches are in the variant of 15 minutes sonication - 8.33%. Next are the plants from the variant of 10 minutes sonication - 5.55%. Plants from the variant of 5 and 20 minutes sonication, as well as untreated ones have the same percentage of plants that formed stem branches - 2.77%.

Table 5. Characteristics of the stem in *Cryptomeria japonica* and germination

Characteristics / Variants	Stem height, cm	Number of stem branches, nb	Plants with stem branches %	Germination %
к	2,72	1	2,77	19,44
5	3,23	1	2,77	25
10	3,60	2	5,55	19,44
15	3,10	1	8,33	27,77
20	3,90	1	2,77	30,55

In Table 5 are presented also the data regarding the height of the stem and the germination. The greatest average height is reached by plants obtained from 20 minutes' seeds sonication - 3.90 cm. The plants from 10 minutes sonication are 3.60 cm or 0.3 cm lower, followed by plants from 5 and 15 minutes sonication respectively, 3.23 cm and 3.10 cm. The plants from the untreated variant have the lowest stem height - 2.72 cm, or 1.18 cm lower than the variant with the tallest plants.

CONCLUSIONS

1. Plants growing from 20 minutes seeds sonication are characterized by the most intensive and uniform growth rate.
2. The germination period of Cryptomeria seeds is not affected by pre-sowing sonication. Germination, both initial and mass, is not affected by the duration of sonication.
3. The germination percent, however is affected by the duration of sonication. 20 minutes of sonication results in highest germination rate - 30.55%. Lowest germination rate has the control group and the group treated for 10 minutes – 19.44%.
4. Cryptomeria was found to form first and second stem branches after 10 minutes' sonication. However, most plants form stem branches after 15 minutes sonication.
5. Greatest average height of 3.90 cm was reached by cryptomeria seedlings obtained from 20 minutes pre-sowing sonication.
6. The conclusion shows that 10 minutes pre-sowing sonication results in obtaining first and second stem branches; 15 minutes pre-sowing sonication results in obtaining the highest plant percentage of stem branches - 8.33%, and 20 minutes' sonication results in obtaining the highest plants - 3.90 cm average height. We can conclude that 20 minutes pre-sowing sonication of Cryptomeria seeds is recommended in seed propagation of this species. However, other indicators should be included in further studies in order to specify the exact duration of treatment.

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USE OF BENTONITE AGAINST AFLATOXICOSIS IN POULTRY FEEDING

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ABSTRACT

Aflatoxins are poisonous chemical compounds produced by some fungal species when suitable conditions are met. Aflatoxins cause significant losses in the quality and quantity of foods and feeds that can develop very easily in the environments where people live and in nature, and adversely affect the health of humans and animals. Contamination of feed used in animal feeding with aflatoxins before, during and after harvest constitutes an important problem affecting the health of animals, producers and consumers. The health and economic problems caused by aflatoxins have led researchers to control strategies for removing mycotoxins from the environment. Removal of aflatoxins from contaminated feed is an important problem and effective, inexpensive and practical decontamination methods are needed. In order to detoxify and reduce the toxic effect of mycotoxins, it is aimed to add various inert sorbent substances to the diets and thus to prevent the absorption of mycotoxins in the gastrointestinal region and to ensure their elimination from the body. In order to reduce the absorption of aflatoxins from the digestive system, some non-nutritive compounds and adsorbents are used in the diet. One of the most striking compounds among these adsorbents is bentonite, which has physical and chemical properties preferred for many sectors. In this study, the usability of a toxin-binding bentonite known as aluminosilicates against aflatoxicosis in poultry feed, and the possibilities of finding solutions to these questions and problems were investigated.

Keywords: Poultry feed, Aflatoxicosis, Toxin binder, Bentonite.

INTRODUCTION

The rapidly developing poultry sector in our country brings along some problems as well as socio-economic benefits (Ergün, 1995). One of the most important problems caused by the storage of poultry feeds and feed raw materials under inappropriate conditions is the toxicity caused by the mycotoxins produced by the molds growing in them (Çelik, 2007). In addition to health problems, mycotoxins cause great harm in plant and animal production and national and international trade. About 25% of the total agricultural production on earth, including basic foodstuffs, is lost due to molds or the mycotoxins they create. Mycotoxins, which cause great harm not only in terms of health but also in terms of socio-economic terms, have received a lot of attention at the national and international level, and due to the economic losses they cause and their significant effects on both animal and human health, they also draw attention in many branches of science. (Aksoy, 1990). Organic acids (propionic, benzoic, acetic and sorbic acids) and their salts (such as potassium sorbate and calcium propionate), organic dyes and chemical compounds such as copper sulfate and ammonia, mold inhibitors and adsorbent substances are used in the detoxification of feeds contaminated with mycotoxins. Toxin-binding clays, zeolites, bentonite and montmorillonites, which contain more than 80% aluminosilicate in their structures, are the most commonly used methods to prevent the negative effects of mycotoxins in feed (Öztürk et al., 1998;

Bintvihok, 2002; Qu et al., 2018). Among the methods developed to prevent or minimize the economic losses and serious health problems caused by aflatoxicosis cases, which are especially important in poultry nutrition, the use of bentonite, which is considered as a toxin binder, is more common than other methods.

AFLATOXINS IN FEED AND THEIR EFFECTS

Aflatoxins are secondary toxic metabolites produced by Aspergillus flavus and Aspergillus parasiticus present on or in food and feed. There are four main types of aflatoxins: B1, B2, G1, G2. Aflatoxins B1 and B2 fluoresce blue under ultraviolet (UV) light, while aflatoxins G1 and G2 fluoresce yellow-green. In addition, the presence of aflatoxins M1 and M2, the metabolic product of aflatoxins B1 and B2, in food and feed is an indication of their direct contamination. These aflatoxins were isolated for the first time from the milk of animals fed with aflatoxin feed and were named M for this reason (Anonim. 2010). Aflatoxins are the most well-known and most researched mycotoxins in the world and cause various diseases such as aflatoxicosis in farm animals, pets and humans all over the world (Hussain et al., 2010). The chemical structure of aflatoxin is given in Figure 1.

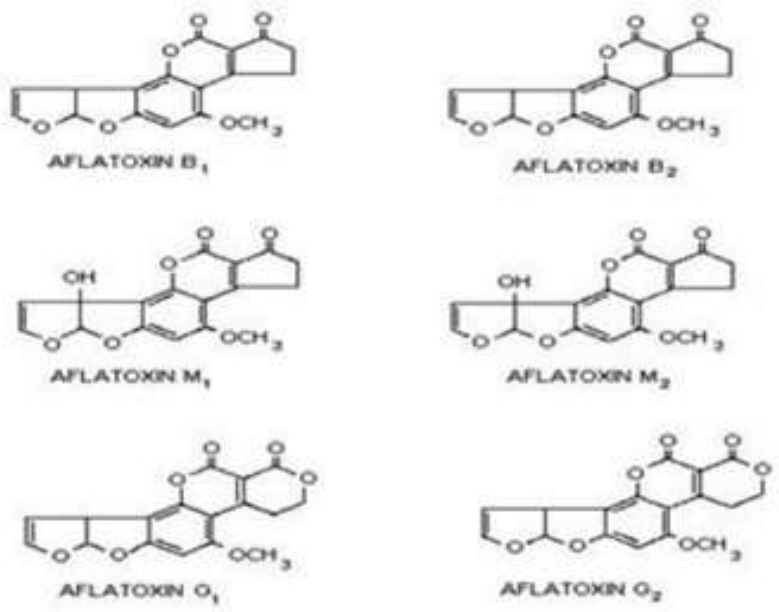

Figure 1. Chemical structure of aflatoxins

More than 300 mycotoxins have been characterized so far and this number is increasing over time (Riordan and Wilkinson, 2008). Especially aflatoxins, which are composed of toxic metabolites produced by molds of the genus Aspergillus, are the most frequently isolated mycotoxins in the field and the most harmful for human and animal health (Kumar et al., 2008). The toxicity caused by these is called aflatoxicosis. Aflatoxins cause negative effects such as decreased growth rate, increased mortality, decreased egg production, loss of appetite, decreased resistance to infections, liver damage, prolongation of blood clotting time, suppression of the immune system, anemia, and deterioration of fat, carbohydrate and protein metabolism in poultry. Some of the aflatoxin contained in the feed can also pass into the egg. Low levels of aflatoxin residues to be found in feed and therefore eggs are extremely important for breeding chicken or poultry businesses (Atalay, 2007). Aflatoxins can be the cause of acute or chronic intoxication after ingestion of metabolites in feed or food by humans or animals, as well as cancer and many other disorders, especially liver tumors (Oğuz, 1997; Robb, 1993). Since aflatoxins cause acute

and chronic poisoning in humans and animal species and are one of the most potent natural carcinogens, many studies have been conducted on the subject (Hamilton, 1982; Miller et al., 1984; Harvey et al., 1991; Robb, 1993).

USE OF BENTONITE AGAINST AFLATOXY IN POULTRY FEEDING

Clays consisting of the decomposition of volcanic ash and lava rich in aluminum and magnesium and predominantly containing montmorillonite, and in commercial terms, any clay with developed liquid absorbent and colloidal properties is called bentonite (Akbulut, 1996). The physical structure of bentonite is more important than its chemical composition. Bentonite has a wide range of uses due to its important properties such as showing colloidal properties when mixed with water, swelling in water and some organic environments, high plasticity and binding, as well as other properties. Basically; classified as alkaline (Na-bentonite), semi-alkaline (Na-Ca bentonite), alkaline earth (Ca-bentonite). Cation exchange capacity, viscosity, plasticity index, swelling capacity, granulation bonding and bleaching ability determine the usage areas of the product. For this reason, bentonite is used in various areas such as drilling mud additive, foundry sand binder and pellet making, bleaching processes in the food industry, as an additive in the pharmaceutical, paper and plastic industry, in petroleum refining, animal feed additive and toxin binder (Slamova et al., 2011; ÖİK Report, 2015). The cations contained in bentonite determine its ability to bind mycotoxins. Inorganic cations on the surface of bentonite can be easily replaced with long-chain quaternary ammonium compounds. The replacement of inorganic cations with organic cations causes a decrease in the hydrophilic property of the adsorbent, but an increase in its hydrophobic property (Abdel-Wahhab et al., 2002). It has been detected that as a result of the use of bentonite in broiler and laying hen rations containing aflatoxin, feed consumption, feed efficiency, live weight gain, egg yield and quality (Qu et al., 2018; Chen et al., 2019), heart, liver, kidney weight and health (Eraslan et al., 2003; 2005; Farag et al., 2017) improved. Montmorillonite, an aluminosilicate mineral clay, with its physical and chemical properties such as large surface area, strong adsorptive capacity and effective adhesion capacity (Segad et al., 2010) alleviates the effects of mycotoxins and can reduce the population of pathogenic bacteria in the intestines of poultry (Liu et al., 2018, Qu et al., 2018; Chen et al., 2019).

CONCLUSION

Considering the negative effects of aflatoxins on poultry health and losses in terms of the country's economy, it is seen that traceability is important. From production to consumption, foods and animal feeds should be analyzed and controlled in terms of aflatoxin with analytical methods. Consumption of food and feed containing aflatoxin higher than the accepted limits in accordance with legal regulations should not be allowed. It is necessary to increase the efforts to prevent the contamination of food and feed with molds and thus to prevent the formation of aflatoxin. In addition, it is important to keep the contamination under control, since the detoxification of aflatoxin is not sufficient. In addition, field studies such as research and application of effective, economical and applicable chemical substances should be carried out in order to prevent fungal infection and therefore mycotoxin formation in feed and foods.

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USE OF BENTONITE AS PERFORMANCE ENHANCER IN BROILERS

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ABSTRACT

In addition to the fact that the additives used in poultry compound feeds are expensive, their unconscious use causes adverse health effects both in animals and in humans who consume their products. In recent years, studies have been carried out on the use of new feed additives in animal nutrition, which do not harm animal health, increase various yields and are abundant in nature. Bentonite, which is one of the natural mineral resources, improves the performance of farm animals (live weight gain, feed consumption, feed conversion rate) and reduces the cost of animal products by increasing the amount and quality of the yield obtained from animals, since it binds the fungal or bacterial toxins in the feed of animals and prevents their absorption from the intestinal system. In this article, the usability of bentonite, known as aluminosilicates, as a performance enhancer in broiler feeds was evaluated.

Keywords: Poultry feed, Broiler, Feed additive, Performance enhancer, Bentonite.

INTRODUCTION

Thanks to the highly productive hybrids used by the poultry industry, it has an important place in animal production due to its ability to produce animal protein in the shortest time and in the most economical way. Due to the high rate of conversion of feed into meat and eggs, it is preferred more than other protein sources such as poultry products, red meat and dairy products to meet the current protein demand. The main goal in the poultry industry, which has developed rapidly in recent years, is to make a profitable poultry farming. In order to ensure profitability in the business, the egg must be obtained in the cheapest way. In poultry farming, obtaining animal products inexpensively can be achieved by reducing feed costs, which constitute 70% of the total cost. When the amount of feed costs in total costs decreases, the profitability of the enterprise will increase even more. This can only be achieved by preparing more suitable and economical rations for chickens (Ozen, 1989). With the prohibition of the use of antibiotics as performance enhancers in poultry rations since 2006, research has intensified on human and environmentally friendly alternative additives (probiotics, organic acids, plant extracts, plant essential oils and some compounds with no nutritional value) that can replace antibiotics. One of the additives used as a performance enhancer is bentonite, which has physical and chemical properties preferred for many sectors.

BENTONITE

Bentonite is a porous, soft and light-colored mineral consisting predominantly of montmorillonite clay mineral, formed as a result of chemical decomposition of volcanic molds and lavas rich in magnesium and aluminum. Bentonites, formed by the erosion of volcanic ash,

are composed of silicate layer as a result of the combination of octahedral aluminum and tetrahedral silicon layers with O2 atoms (Dakovic et al., 2003). Bentonites belong to the phyllosilicate group and are adsorbing agents with a layered crystal structure and variable composition. Bentonites are impure clays mostly composed of montmorillonite. Due to the montmorillonite content, bentonites swell and form thixotropic gels (Ramos et al., 1996; Diaz and Smith, 2005; Boudergue et al., 2009). Bentonite can be used in many different areas because it swells in volume in aqueous and some organic environments, shows colloidal properties when mixed with water, and has high binding and plasticity.

The main usage area of bentonites is the drilling sector; pellet making, pharmaceutical industry, bleaching works in the food industry, as an additive in the paper and plastic industry, animal feed additive (toxin binder), cement industry, ceramic industry, petroleum refining, fertilizer production and soil improvement, cat mat production and many other similar industrial areas it can be used. Bentonite's properties such as grain size and shape, surface area and chemistry, cation exchange capacity, color, plasticity index, viscosity, absorption and adsorption ability greatly affect its usage areas (Erdil, 2019). The cations contained in bentonite, which is used as a toxin binder in animal nutrition, determine its ability to bind mycotoxins. Inorganic cations on the surface of bentonite can be easily replaced with long-chain quaternary ammonium compounds. The replacement of inorganic cations with organic cations causes a decrease in the hydrophilic property of the adsorbent, but an increase in its hydrophobic property (Abdel-Wahhab et al., 2002).

USE OF BENTONITE AS A PERFORMANCE ENHANCER IN BROILER

There are many publications, expert opinions and medical products used in the market about bentonite clay with rich montmorillonite mineral in the world and in Turkey. Bentonite was formed over millions of years as a result of natural geological and biological events such as chemical decomposition or alteration of volcanic ash, tuff and lava rich in aluminum and magnesium. Therefore, it is a substance that can vary greatly in mineral content and quality in its composition. Due to these changes in their content, they have different physical and chemical compositions and/or humanoid properties such as high surface areas, electrostatic properties, biogeochemical cycles and the like (Pasha et al., 2008; Safaei Katouli et al., 2010; Hashemipour et al., 2010; Önal and Özgüven 2011; Çimen and Dereli, 2014; Gilani et al., 2016; Mishra et al., 2020). The physical structure of bentonite, which is formed by the decomposition of volcanic ash and lava rich in aluminum and magnesium, predominantly containing montmorillonite, and having advanced liquid absorbing and colloidal properties in the commercial sense, is more important than its chemical composition. Bentonite has a wide range of uses due to its important properties such as showing colloidal properties when mixed with water, swelling in water and some organic media, high plasticity and binding, as well as other properties. Basically; classified as alkaline (Na-bentonite), semi-alkaline (Na-Ca bentonite), alkaline earth (Ca-bentonite). Cation exchange capacity, viscosity, plasticity index, swelling capacity, granulation bonding and bleaching ability determine the usage areas of the product. For this reason, bentonite is used in drilling mud additive, foundry sand binder and pellet making, bleaching processes in the food industry, as an additive in the pharmaceutical, paper and plastic industry, in petroleum refining, animal feed additive and toxin binder (ÖİK Report, 2015; Akbulut, 1996). Different results have been obtained in studies with the addition of different levels of bentonite to poultry rations. In some of these studies, it was reported that although the live weight gain was adversely affected by the addition of sodium bentonite to the diets containing aflatoxin, there was no change in egg production and feed efficiency (Santurio et al., 1999; Rosa et al., 2001), as a solution to the contradictory results, the following studies were conducted. Most of them were supplemented with montmorillonite, thus increasing egg production and quality (Chen et al., 2019a; 2019b).

These differences can vary according to the source of the bentonite, which is obtained as a result of geological formations, the chemical and physical properties of the toxic binder, mycotoxin binding properties and animal material. (Qu et al., 2018). Magnoli et al. (2010) showed that AFB1 (50 μg/kg) liver toxin residue was reduced by 62.5% in chickens fed diets containing 0.3% sodium bentonite. In fact, there are numerous animal studies showing that bentonites can bind aflatoxins in digested feed and reduce or eliminate toxicity. For this reason, bentonite clays are routinely added to animal feeds for the prevention of aflatoxicosis, and they reported that it reduces the absorption and side effects of aflatoxins in the gastrointestinal tract. It has been proved that addition of bentonite to aflatoxin-containing broiler and laying hen rations improves feed consumption, feed efficiency, live weight gain, egg yield and quality (Qu et al., 2018; Chen et al., 2019a), heart, liver, kidney weight and health (Eraslan et al., 2003; 2005; Farag et al., 2017). Montmorillonite, an aluminosilicate mineral clay, with its physical and chemical properties such as large surface area, strong adsorptive capacity and effective adhesion capacity (Segad et al., 2010), alleviates the effects of mycotoxins and can reduce the population of pathogenic bacteria in the intestines of poultry (Liu et al., 2018; Qu et al., 2018; Chen et al., 2019a, 2019b). In a study conducted on laying hens and broiler chickens as a growth enhancer, it was determined that the addition of 1-1.5% bentonite to the feed increased the fattening weight by 35% and the egg yield by 15% in poultry (Ali, Çinku, Saeed, Jalil et al., 1996).

CONCLUSION

With the developing technology in recent years, mines have gained the feature of being raw materials in various industrial fields such as construction, chemistry, machinery, agriculture and animal husbandry. Making good use of existing mines, converting mines into products and trading them are factors that will contribute to the economic development of a country. Turkey has an important potential in the mining sector with its rich underground resources. Today, clays are widely used in areas such as adsorbent, decolorizer, molecular sieve, catalyst, ceramic raw material, paper, paint, plastic industries and animal feed, either in their original form or after being subjected to different processes. Bentonite, which is one of the clay minerals, is one of the possibilities that can be used for various purposes in canal cultivation in order to provide suitable health conditions and high yield due to its unique characteristics. As a result, the use of bentonite mineral, which is one of the mines of our country, in animal production will provide economic benefits. Studies on the use of bentonite to be used as a growth enhancer in broilers at different levels, under different conditions, with different ration compositions will shed light on the better evaluation of bentonite.

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STARCH-BASED FILMS: MAJOR FACTORS AFFECTING FILM PROPERTIES AND UTILIZATION OF STARCH-LIPID COMPLEXES IN FILM-FORMATION

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ABSTRACT

In recent years, research related to eco-environment protection are raised with an increase in environmental pollution threatening life. The fact that environmental pollution can be reduced by partially replacing petroleum-based plastics with biopolymer materials has attracted the attention of the packaging industry, environmental science and other disciplines. Among natural polymers, starch, one of the cereal-based plants' main components, has been widely used for the development of biodegradable films due to their edible and degradable characteristics, non-toxic, cost-effective, sustainability, abundance and perfect film-forming properties. The functional properties and efficiency of starch-based films such as optical, organoleptic, mechanical and barrier properties are highly dependent on the native characteristics of film-forming material. Therefore, many starch modification methods are used to obtain films with satisfactory mechanical and permeability properties. Native starch can be modified chemically (cross-linking, oxidation, starch-lipid complexation) and physically (annealing, heat-moisture treatment). Recently, with an increase in consumer concern about environmental protection, the starch-lipid complex formation has commonly been used as a modification method to produce starch-based films because lipids are environmental-friendly, provide flexibility to films, and have good hydrophobic properties that improve barrier and mechanical properties of starch-based films. In addition to these, the film properties can be altered by process temperature and time during film formation, plasticizer type, co-biopolymers existence, and storage conditions. Hence, the effects of reaction conditions of film formation including temperature, time, and pH is worthwhile for future studies on starch-based film formation. The current review focuses on the effects of major factors during film-forming and will also provide a general overview to the readers about the effect of starch-lipid complex formation on starch-based film properties.

Keywords: Starch, edible film, starch modification, amylose-lipid complex

INTRODUCTION

Petroleum-based polymers are the most often utilized materials in the food packaging sector. However, due to the rapidly declining crude oil resources, these polymers are gradually becoming less accessible and more expensive. In this regard, edible films are the best alternative to petroleum-based packaging. Edible films have accepted a sizable interest in recent years, due to the many advantages that have over petroleum-based films. The reason for the increased interest in edible packaging is the increasing consumer demand for safe and healthy foods and the

increasing awareness of the harmful environmental impacts of non-biodegradable waste (Hassan et al., 2018).

Edible films are biodegradable packaging materials obtained from natural polymers such as polysaccharides, proteins, and lipids. Among the polysaccharide, starch is the one that has drawn the most attention due to its low cost, abundance, and superior film-forming qualities (Agarwal, 2021). Starch-based films offer excellent physical properties, including being oxygen-impermeable, flavorless, and colorless. But, starch-based films often have poor mechanical and barrier properties. Additionally, due to their potent hydrophilic qualities, the using of them is restricted. These properties can be improved by adding plasticizers, additives or other polymers to the film solution. Glycerol, xylitol, sorbitol etc. are plasticizers commonly used in starch films (Pelissari et al., 2018). Moreover, these properties can also be enhanced by starch modification. Physical and chemical processes are typically used for starch modification. Pre-gelatinization, heat-moisture treatment, extrusion, and annealing are examples of physical modification. The chemical modification includes processes like partial enzyme hydrolysis, acid hydrolysis, starch oxidation, the formation of amylose-lipid complexes with fatty acids, and cross-linking (Shah et al., 2016). Among these methods, chemical modification causes an environmental problem due to using a chemical substance, except for the amylose-lipid complex formation method. Because, lipids are extensively employed in starch modifications as both food and environmentally beneficial compounds (Oskaybaş-Emlek et al., 2022; Shah et al., 2016). Amylose can interact with other molecules because of its structural properties; for example, amylose-lipid interactions. The majority of amylose molecules have a non-crystalline form and can be frequently complexed with natural substances like lipids (Cervantes-Ramírez et al., 2020).

This review focuses on starch and modified starch-based films and discusses the amylose-lipid complex formation mechanism which is one of the modification methods and its effect on the film properties.

EDIBLE FILMS

Edible films are thin layer coated on food, produced from biodegradable polymers and used to create a barrier between food and the environment. These films have many advantages, however (but ta sanki olumsuz bir şey gelecekmiş gibi bir durum var) the most important ones are their biodegradability and edibility. To provide these two features, the selected ingredients must be environmentally safe, odourless, tasteless, colourless, transparent, clear, and compatible with the foodstuff as much as possible not to cause any negative effects during consumption (Talens et al., 2010). These films exhibit many functions, such as separating food from the surrounding environment, acting as a barrier against moisture and gas transmission, prolonging shelf life by preventing food spoilage, and protecting food against physical impacts. These films also prevent aroma loss by protecting the volatile organic compounds in food and avoid light-induced chemical reactions until consumption (Mohamed et al., 2020; Otoni et al., 2017).

Two different methods, wet and dry, are used to produce edible films. The wet method is also often referred to as solution casting. It involves the steps of dissolving/dispersing polymers and other components in a proper solvent, pouring the solution over a smooth surface to form the film structure, and drying the poured film solution, respectively. This method is widely used in laboratory conditions because it is easily applicable. The main advantage of this method is that the films are produced at a low cost without using special equipment. In addition, in this method, the dissolution of the polymer in the solvent leads to better particle-particle interaction, resulting in a more homogeneous film. Another advantage is irreversible structural changes that occur at very high temperatures are prevented since the films can be produced at low temperatures. On the other hand, scaling up of film production process from the laboratory scale to the industrial scale

is difficult since several quality differences might emerge due to the difficulties in controlling process variables such as agitation speed, process temperature, and heating. This situation hinders edible film and coating development for commercial scales. In the dry method, films can be produced by extrusion and heat pressing without using solvents such as alcohol and water. The biopolymer is combined with plasticizers in the first phase and extruded in the next step; thusly, a film is prepared from the paste or pellets produced by injection moulding or thermal pressing (Chen et al., 2021; Hassan et al., 2018; Suhag et al., 2020).

COMPONENT OF EDIBLE FILMS

At least one component with adequate adhesion and the ability to form a structural film matrix should be used in the formulation of films. The major components used in edible film preparation are biopolymers such as polysaccharides, lipids, and proteins. These polymers are included in the film formulation independently or in composites according to the properties of the food products to which the edible films are applied and the advantages/disadvantages they provide (Mohamed et al., 2020).

Protein-based films are produced using both animal and plant-based proteins. Animal-based proteins mostly include casein, keratin, collagen, and whey protein, while plant-based proteins mostly include soy protein, wheat gluten, zein, wheat germ protein, and peanut protein. Since proteins may create stronger intermolecular covalent bonds, protein-based films have superior mechanical characteristics and they can also act as a strong barrier to oxygen. However, they have a high water vapour permeability because of their hydrophilic nature (Hassan et al., 2018). Lipids such as oils, waxes and fatty acids can be used in the production of edible films for foods with high moisture content, such as meats, seafood, fruits and vegetables. They can both prevent moisture loss and improve the product's appearance because these compounds have a hydrophobic character. Additionally, lipid films can also prolong shelf life by controlling oxygen transfer in the product they are used in. -But, lipid-based films have poor mechanical properties they are brittle and stick poorly to foods with hydrophilic surfaces. Therefore, they are rarely used to produce independent films and are commonly used by combining with hydrocolloid compounds (Chen et al., 2021; Hassan et al., 2018). Polysaccharides are frequently used to produce edible films or coatings owing to their low cost, simple availability, and effective film-forming capabilities. Although Edible films produced from polysaccharides generally act as a strong barrier against oxygen, flavour, and oil transfer due to their hydrophilic nature, they have poor mechanical characteristics and water vapour barrier properties. Alginate, pectin, dextrin, carrageenan, starch, chitosan, cellulose derivatives, and pullulans are polysaccharides that are generally used for film-forming (Cazón et al., 2017). In addition to these polymers, various plasticizers are added into film-forming solutions to provide the films with higher flexibility and various additives can be added or polymer modifications can be performed to improve mechanical, barrier, and physical properties of the films (Chen et al., 2021). In polysaccharide and protein-based films, rigid film structures are formed due to polymer-polymer interactions. The problems of unfavourable brittleness can be brought on by cohesive forces in polymer films. To prevent film brittleness, plasticizers which are low molecular weight compounds can be added to the polymeric film-forming components. Plasticizers enhance intermolecular space, thus, increasing film flexibility by decreasing intermolecular hydrogen bonding between polysaccharide or protein polymers. Additionally, they increase the film's elongation, strength, and processability, and reduce glass transition temperature (Tg) (Embuscado, M. E., & Huber, 2009; Sothornvit & Krochta, 2005). Plasticizers that are commonly used in film processing are glycerol, thymol, glycol, xylitol, sorbitol, etc. (Suhag et al., 2020). Additionally, fatty acids are also added to the film formulation as plasticizing, providing flexibility to the film (Jiménez et al., 2013;Schmidt et al., 2013; Thakur et al., 2016). The ideal plasticizers for starch-based films are OH-containing

species like water and alcohols because they closely resemble the structures of the polymers they plasticize. However, the plasticizing effect of water is very limited due to forming H-bonds between the components (Thakur et al., 2019). Among alcohols, polyols (glycerol, glycol, xylitol, sorbitol, etc.) are stated as more effective in plasticizing starch films (Nguyen Vu & Lumdubwong, 2016; Nordin et al., 2020; Talja et al., 2007; Zahiruddin et al., 2019).

STARCH AND STARCH-BASED FILMS

Starch is one of the polysaccharides which is frequently used in the production of films and coatings. Since it is inexpensive, naturally occurring, simple to use, and ecologically acceptable (Vellaisamy Singaram et al., 2021). Starch is a D-glucose polymer found in granular form in its sources and is chemically composed of amylose and amylopectin (Figure 1). A linear chain polymer called amylose is made up of 500-6000 glucose units connected by α-(1-4) D-glycosidic linkages. Its molecular weight varies between $1x10^5$-$1x10^6$ Dalton. Amylopectin, on the other hand, has a branched structure and is a polymer formed by connecting 25-30 glucose units with α-(1-4) and α-(1-6) glycosidic bonds. Its molecular weight varies between $1x10^7$ -$1x10^9$ Dalton (Agarwal, 2021).

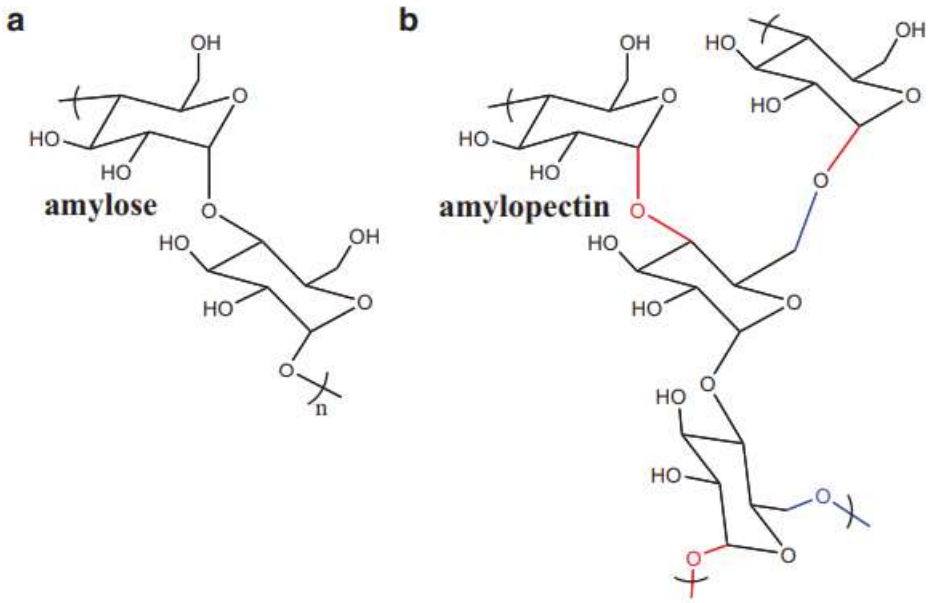

Figure 1. The structure of (a) Amylose and (b) Amylopectin (Lauer & Smith, 2020)

In comparison to amylopectin, amylose has a greater tendency to scatter, form gels, and recrystallize in the aqueous media. Moreover, because of its primary linear structure, the film formation and coating ability of amylose are better than amylopectin. Although the amylose: amylopectin ratio differs according to the source of starch, the amylose content of most starches is around 20-30%. Any kind of amylose-containing starch can be used to prepare a film (Cazón et al., 2017; Embuscado, M. E., & Huber, 2009; Ratnayake & Jackson, 2008; Skurtys et al., 2010). Besides its amylose content, starch produces stronger and better films due to its strong gelling capabilities and helical linear polymer structure (Pelissari et al., 2018).

The preparation of the starch film starts with the preparation of a viscous solution by heating starch granules in excess water. Thus, starch gelatinization plays a crucial role in transforming starch into biodegradable films by destroying the semi-crystalline structure and obtaining a homogenous film solution. Starch films are also superior in transparency and tasteless and are strong CO_2 and O_2 barriers (Hassan et al., 2018).

The starch granules do not dissolve in cold water, since the hydrogen bonds hold the starch chains together, when it is heated, water enters the structure of starch granules, and the granule becomes swollen. As a result of the interactions between water molecules and the hydroxyl groups of amylose and amylopectin, the granules' crystal structure deteriorates. Thus, reversible partial dissolution occurs. As the starch solution continues to be heated, water enters the amorphous area of the starch, and thus amylose leaks out and the double helix structures of amylopectin disrupt. This situation causes the loss of birefringence in the starch granule. As a result of this process, the solution's viscosity rises and it progressively loses its crystalline structure, turning into a gel. The temperature at which 98 % of the birefringence of starch granules is lost is known as the gelatinization temperature. The temperature at which gelatinization occurs, which depends on how long the branch chain of amylopectin is, ranges from 65 to 110 °C. While smaller amylopectin chains easily form hydrogen bonds with water molecules, longer chains interact with one another instead of water molecules, resulting in a higher gelatinization temperature (Agarwal, 2021; Cazón et al., 2017; Pelissari et al., 2018).

MODIFIED STARCH-BASED FILMS

Natural starch is not used alone in the production of films and coatings, since the films obtained from natural starch are not sufficient in terms of mechanical properties. However, the properties of these films can be improved by adding other polymers and plasticizers to film formulation or by modifying the natural starch. Natural starch can be modified as chemically or physically. One of the most efficient ways to significantly modify a starch film’s properties is physical modification. The physical modification includes methods such as heat-moisture treatment, annealing, pre-melting, and high-pressure application. It can be used safely as a starch modification method since it does not contain any chemicals and is often preferred (Shah et al., 2016). Some researchers reported that edible films obtained from rice (Majzoobi et al., 2015), sweet potato (Indrianti et al., 2018) and buckwheat starch (Sindhu & Singh Khatkar, 2007) modified by heat-moisture treatment have high tensile strength and elongation, but low water vapour permeability and solubility in water. In a study in which corn starch was modified by cold plasma treatment, researchers reported that increase in the tensile strength and hydrophobicity of the films, and a decrease in elongation and water adsorption (Sifuentes-Nieves et al., 2019).

Another method used in starch modification is chemical modification. Acid hydrolysis, oxidation, cross-linking, and the formation of amylose-lipid complexes are examples of use for starch modification chemically. Among them, crosslinking is an effective modification method used to enhance the mechanical properties of films. Based on previous research, modified starch by cross-linked has better film-forming properties than native starch (Lauer & Smith, 2020; Shah et al., 2016)

Mehboob et al (2020) reported that the sorghum starch film cross-linked with sodium trimetaphosphate is more soluble and transparent than the natural starch film and that its mechanical and water vapour barrier properties have improved. In the study in which rice starch was modified with hydroxypropylated and cross-linking methods, it was regarded that the mechanical, barrier and physical properties of the films were improved with the starch modification (Woggum et al., 2014). Narváez-Gómez et al (2021) researched the effects of modification of yam starch by oxidation and cross-linking on the mechanic and barrier properties of the films, they reported that water vapour barrier properties and tensile strength of films rose while elongation decreased. Dai et al (2019) examined the effect of different modification methods (esterification, crosslinking, and oxidation) on the filming properties and found that the mechanical and barrier properties of the film were improved in all modification methods.

AMYLOSE-LIPID COMPLEX FORMATION AND ITS POTENTIAL FOR EDIBLE FILM

Amylose-lipid complex formation is one of the preferred modifications because it does not pose an environmental problem. Amylose-lipid complex is amorphous or highly crystalline complexes formed by amylose and lipid, and are classified as resistant starch types V. It is naturally found in starch at low levels, or it might be formed by adding different fatty acids to starch during thermal processing (Panyoo & Emmambux, 2017). The formation of the amylose-lipid complex is based on the fact that amylose leaks out of the granule during the gelatinization stage and forms a complex with the fatty acids in the medium.

Amylose forms single helical complexes (- helix) with a variety of substances, including iodine, monoglycerides, phospholipids, and fatty acids, depending on its long, linear structure in the starch. The hydroxyl groups of the glucose units in this helical form are thought to be on the outside of the helix, whereas the hydrophobic regions are located in the inner part of the helix. Because of this, the hydrophilic groups stretch toward the outside of the helix while the hydrophobic components which will make up the complex are found within the helical structure. The aliphatic hydrocarbon chain of the fatty acids interacts with the hydrophobic region of the inside of the helix to fill the space within the helix in amylose-lipid complexes (Hasjim et al., 2013). Many parameters, such as the lipid types and molecular structures carbon chain lengths of the lipids, amylose content in the starch, and treatment of complex formation affect the formation of amylose-lipid complexes between amylose and fatty acids. The impact of these factors on complex formation has been examined in numerous research published in the literature (Kang et al., 2020; Liu et al., 2016; L. Wang et al., 2018; R. Wang et al., 2019).

Lipids, which are useful compounds for both food and the environment and used in starch modifications, are hydrophobic in nature, and act as an excellent barrier to water vapour in film formation but have poor mechanical properties (Oskaybaş-Emlek et al., 2022; Shah et al., 2016). To increase the water barrier properties of the starch film, lipids can be incorporated into the matrix of the film. However, their use alone may damage the mechanical properties of the film due to the phase separation (Thakur et al., 2017). Amylose lipid complex formation has been used in film production in recent years due to its advantages, such as improving the properties of the starch film and not causing environmental problems. The number of studies using amylose-lipid complex starches in the production of films is quite limited. R. Wang et al (2019) the production of potato starch-lauric acid complexes using various methods and their effect on the mechanical and barrier properties of the film. According to the researchers, the PS-LA complex-produced films have stronger tensile strength, lower elongation at break, and lower moisture permeability than the natural starch-based film. In the study of Field Liu et al (2018), the amylose-lipid complex was formed by ultrasonic treatment from sweet potato starch and lauric acid. It has been reported that the mechanical, optical and barrier properties of the films are improved by ultrasonic treatment. In another study by the same researchers, they examined the effect of saturated (stearic) and unsaturated fatty acids (oleic and linoleic) fatty acids on sweet potato starch-based films. They reported that sweet potato starch-saturated fatty acid complex films exhibit higher tensile strength, lower elongation at break, and lower water vapour permeability than sweet potato starch-unsaturated fatty acid complex films (Liu et al., 2016).

CONCLUSIONS

The use of biodegradable packaging material can reduce environmental pollution. The best example of these materials is starch, which is a polysaccharide. Starch is a readily available, inexpensive and environmentally friendly polymer, but the mechanical and water vapour barrier properties of starch films are poor. The properties of the films can be improved by the addition of

plasticizers or by starch modification. In chemical modification methods, it is more reliable due to the use of chemicals in the formation of the amylose lipid complex. Thus, studies have focused on this area in recent years.

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PREDICTION OF AGGREGATE STABILITY OF CULTIVATED FIELDS USING SOME SOIL PROPERTIES

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ABSTRACT

In this study, the relationships between water stable aggregates (AS) and basic soil properties were determined using 178 surface soil samples (0-20 cm) taken from cultivated agricultural fields araound Samsun Turkey. After analyzing AS, clay, silt, sand contents, soil reaction (pH), electrical conductivity (EC), organic matter (OM) content and exchangeable cations (Ca, Mg, K, Na) of the soil samples, linear regression models using stepwise analyses in SPSS program were done between water stable aggregates and some soil properties. The water stable aggregate stability values varied between 2.01% and 79.14% with a mean of 23.50%. While AS values had significant positive correlations with OM (0.514**), clay (0.495**), Ca (0.171*) and K (0.157*) contents, they gave significant negative correlations with pH (-0.312**), silt (-0.312**) and sand (-0.242**) contents. To predict AS values, 6 linear regression models were produced by stepwise analyses. The R2 values of the linear regression equations varied between 0.514** obtained using only OM as a variable and 0.805** obtained using the variables of OM, silt, clay, pH, K and EC. The values of AS can be predicted using the linear regression equation including less variables such as OM, silt, clay and pH with an R2 of 0.779**. İt was determined that OM, clay and silt contents are the most effective soil properties on water stable aggregates in cultivated fields.

Keywords: Aggregate stability, soil properties, linear models, prediction.

INTRODUCTION

Aggregation is an indicator of soil structure and results from the rearrangement of particles, flocculation and cementation (Gülser 2006). Aggregates improve soil quality by protecting soil organic matter entrapped in the aggregates from exposure to air and microbial decomposition, decreasing soil erodibility, improving water and air movement, improving the physical environment for root growth and improving soil organism habitat (USDA 1999). Breakdown of aggregates is the first step to crust development and surface sealing, which impedes water infiltration and increases erosion. Soil aggregation can change over a period of time, such as in a season or year. Aggregates can form, disintegrate, and reform periodically (Hillel, 1982). Determination of wet aggregate stability is generally difficult, time consuming process and needs labor works. The objective of this study was to predict aggregate stability of cultivated soils using the relationships between AS and soil properties in linear regression models

MATERIAL AND METHOD

In this study, 178 surface soil samples (0-20 cm) were taken from agricultural fields in Samsun, Turkey. After the soil samples were air dried and passed through 2 mm sieve, some basic soil properties were analyzed as follows; organic matter (OM) content was determined using the modified Walkley-Black method, particle size distribution by hydrometer method (Day, 1965), soil reaction (pH, 1:1 (w:v) soil:water suspension) by pH meter, electrical conductivity (EC25ºC)

in the same soil suspension by EC meter, exchangeable cations by ammonia acetate extraction (Kacar, 1994). Aggregate stability (AS) was determined for soil samples using a wet sieving method (Kemper and Rosenau, 1986). To estimate the AS values of the soils, a linear regression equation between AS and the soil properties was obtained with stepwise analyses using the SPSS statistic program.

RESULTS AND DISCUSSION

Descriptive statistics of some physical and chemical properties of the soils are given in Table 1. The clay content of the soil samples varied between 9.99% and 67.38% with a mean of 34.06%. Soil samples varied between strongly acid and slightly alkaline in pH (1:1), and non-saline according to the mean EC values (Soil Survey Staff., 1993). Soil OM contents varied between 0.20% and 9.40% with a mean of 2.02%. Rating of organic matter content results of soil samples showed that 10.67% of the samples is very low, 43.26 is low, 34.83% is moderate, 10.11% is high and 1.12% is very high in organic matter content (Figure 1).

Table 1. Descriptive statistics of aggregate stability values and some soil properties (n=178).

	Minimum	Maximum	Mean	Std. Deviation	Skewness	Kurtosis
AS, %	2,01	79,14	23,50	13,97	0,79	0,82
C, %	9,99	67,38	34,06	13,35	0,20	-0,67
Si, %	3,05	66,37	27,62	8,72	0,68	1,56
S, %	4,27	81,71	38,31	16,09	0,49	-0,63
OM, %	0,20	9,40	2,02	0,93	2,92	21,60
pH(1:1)	4,03	8,33	7,54	0,68	-2,31	6,10
EC, dS/m	0,11	2,95	0,64	0,45	2,77	10,12
Na, cmol/kg	0,08	5,64	0,60	0,79	4,11	20,95
K, cmol/kg	0,11	1,79	0,59	0,34	0,95	0,67
Ca, cmol/kg	1,67	52,53	23,25	9,71	-0,32	-0,04
Mg, cmol/kg	1,01	21,12	7,73	4,34	0,55	-0,11

OM: organic matter, AS: aggregate stability.

Aggregate stability of the soil samples used in this study varied between 2.01% and 79.14% with a mean of 23.50% (Table 1). Frequency distribution of the aggregate stability values is given in Figure 1. The results showed that AS in 17.42% of soil samples less than 10% is very low, 29,21% between 10-20% is low, 20.22% between 20-30% is moderate and 33.15% more than 30% is high according to the AS classification (Hazelton and Murphy, 2007). The mean of soil organic matter content of the group of soil samples within the different AS classification increased with increasing AS from less than % to greater than 30 % (Table 2). It indicates that increasing soil organic matter content increases the aggregate stability of soils.

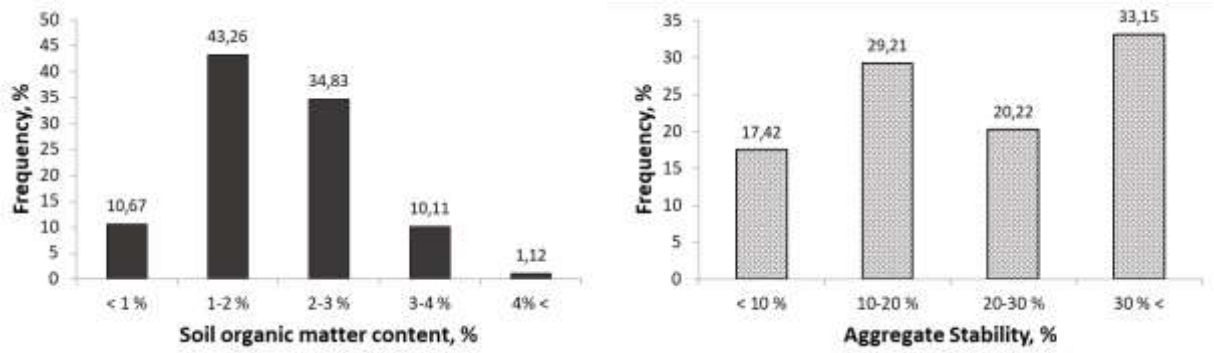

Figure 1. Frequency of soil organic matter contents and aggregate stability values of the soil samples.

Table 2. Mean soil organic matter content of soil samples in different AS classification group.

AS classification	number of soil samples	mean soil OM content, %
< 10 %	31	1,59
10-20 %	52	1,80
20-30 %	36	1,88
> 30 %	59	2,51

The correlation matrix between AS values and soil properties is given in Table 3. Aggregate stability values showed significant positive correlations with clay (0.495**), soil OM (0.514**), exch. Ca (0.171*) contents and significant negative correlations with silt (-0,312**), sand (-0.242**) contents, soil pH (-0.312**). In most studies, it was reported that AS values of soils increase with increasing clay, OM and Ca content (Oades, 1993; Gülser, 2006; Gülser et al. 2007; Candemir and Gülser, 2011; Gülser and Candemir, 2015). It is known that soil OM is metabolized by a variety of microorganisms to produce polysaccharides that act to bind soil particles into micro aggregates (Tisdall and Oades, 1982; Oades, 1993). While calcium ions associated with clay generally promote aggregation, sodium ions promote dispersion (Soil Quality Institute, 1996). Aggregate stability values of the soil samples reduced with increasing soil pH and exch. Na content.

To predict the aggregate stability (AS) values of soil samples, six linear regression models were obtained running the stepwise analyses in the SPSS programme with using soil physical and chemical properties (Table 4). Comparison of measured AS values to estimated AS values by the linear models are given in Figure 2. The AS values were estimated with a lower R value (0.514**) by using the model 1 including only soil OM content than the estimated AS values (0.805**) by the model 6 including selected soil properties by stepwise analyses. Using clay, silt, OM, pH, EC and exch. K content of soil samples in the linear regression model 6 gave the highest R value (0.805**) for the estimation of AS (Table 4). Candemir and Gülser (2012) determined that using clay and silt fractions together with EC, exch. Na, ESP, or SAR in the second order equations significantly increased the accuracy and reliability of multiple regressions to estimate saturated hydraulic conductivity. The model 5 used in predicting AS gave also had significant linear relationship (0.798**) as well as the model 6. In this case using model 5 instead of model 6 is also

possible with giving an advantage of using the less soil variable. The comparison of model 3 and model 5 is giving in Figure 2. The slope of regression line is becoming close the 1 when using the more soil variable in the linear models.

Table 3. The correlation matrix among the soil properties and aggregate stability.

	C	Si	S	OM	pH	EC	Na	K	Ca	Mg
AS	0.495**	-0.312**	-0.242**	0.514**	-0.312**	-0.020	-0.098	0.157*	0.171*	0.049
C		0.021	-0.841**	0.474**	0.347**	0.402**	0.171*	0.571**	0.720**	0.572**
Si			-0.559**	0.184*	0.210**	-0.012	0.002	0.003	0.134	0.161*
S				-0.493**	-0.401**	-0.327**	-0.143	-0.475**	-0.670**	-0.562**
OM					-0.123	0.053	-0.115	0.361**	0.286**	0.211**
pH						0.301**	0.211**	0.237**	0.578**	0.460**
EC							0.824**	0.350**	0.337**	0.602**
Na								0.176*	0.077	0.473**
K									0.546**	0.487**
Ca										0.465**

**significant at 0.01 level; *significant at 0.05 level.

Table 4. Linear models used to estimate aggregate stability (AS) values.

Model	Linear Regressions	R	R^2
1	AS = 7.893 + 7.742OM	0.514**	0.264
2	AS = 24.194 + 8.912OM – 0.676Si	0.660**	0.436
3	AS = 17.250 + 6.789OM – 0.643Si + 0.303C	0.708**	0.501
4	AS = 70.673 + 4.799OM – 0.469Si + 0.527C – 8.050pH	0.779**	0.607
5	AS = 68.134 + 4.799OM – 0.491Si + 0.627C – 7.547pH – 8.770K	0.798**	0.637
6	AS = 66.718 + 4.525OM – 0.495Si + 0.668C – 7.204pH – 7.784K – 3.895EC	0.805**	0.649

AS: aggregate stability, OM: organic matter, Si: silt, S: sand, K: exch. potassium, EC: electrical conductivity.

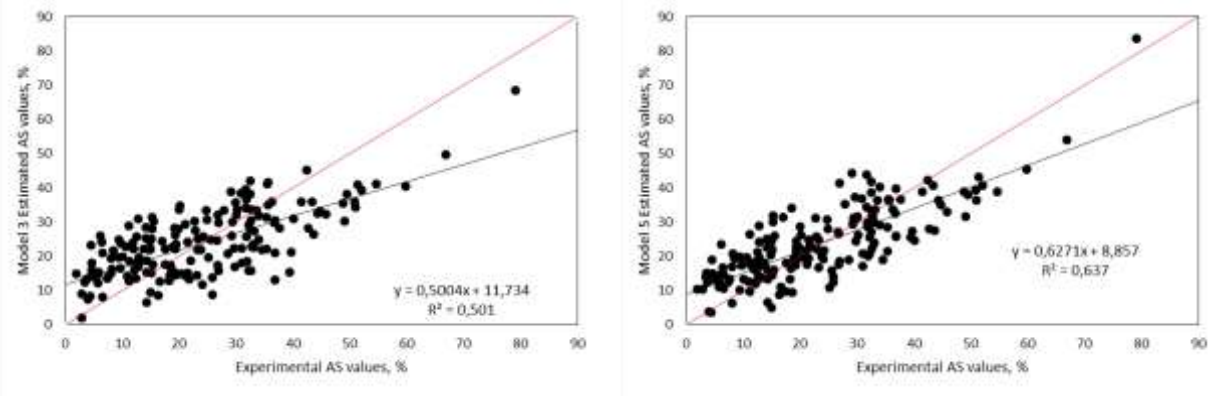

Figure 2. Comparison of measured aggregate stabilities (AS) with AS values estimated by the linear model 3 and model 5.

CONCLUSIONS

In this study, AS values of the soils had significant positive relationships with soil OM, clay, exch. Ca contents, and significant negative relationships with soil pH, silt and sand contents. Soil OM content had the significant effect on AS and the higher mean soil OM contents were obtained in the soil samples having the higher AS values. It was determined that including soil physical (silt, clay) and chemical (OM, pH, EC, K, Ca) properties together in the linear regression model gave the highest R value to predict AS values of the soil samples.

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USES AND IMPORTANCE OF WIDE HYBRIDIZATION IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) IMPROVEMENT

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is one of the most important oil plants in the world. Genetically resistant hybrids developed against broomrape, downy mildew and some other diseases that cause major problems in sunflower production as well as resistant to herbicides such as the Clearfield System (Imidazolinone (IMI)) and Sulfonlyurea (SU) herbicides, which control both broomrape and common weeds, have recently been used commonly in sunflower production in the world. These kind of new developments increased the profitability of sunflower production. Most of these contributions were derived from genes obtained from wild sunflower genetic resources. Wild Helianthus species preserve many genes required for cultivated sunflower for resistance to broomrape parasite, many diseases and for adaptation abiotic stress conditions. Many genes have been transferred to cultivated species via interspecies hybridizations in sunflower until today, and these wild species are used extensively for early selection in sunflower breeding to contribute significant contributions to increase genetic variation. Sunflower has been more tolerant to adverse climatic changes and has increased its cultivation areas and production in the Black Sea region in recent years due to the increase in the demand for oilseeds in the world.

Keywords: Sunflower, Wild species, *Helianthus*, Genetic resources, Wide Hybridization

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an oilseed crop native to North America. It is cultivated throughout the world, and most of its products are used as culinary or livestock feed. The adaptation of sunflowers to different climatic and soil conditions has enhanced its cultivation as an oilseed plant throughout the world. Black sea region countries contributed more for these rises because sunflower production has tripled in the region covering over 60% of world sunflower production. The growth of sunflower requires fertile soil, moderate rainfall, viable seeds, etc. Among the three leading oilseed crops, that is, soybean, rapeseed, and sunflower in the world today, sunflower is recognized as a major source of high quality edible oil and dietary fibre importantly used for culinary purposes. Due to the continuous increase in the human population, the demand for edible sunflower seeds, oil, and by-products has also increased, and to meet the demand, there is a need to intensify efforts to expand sunflower output.

Helianthus is a diverse genus comprised of 52 species with 18 annual and 34 perennials, all native to North Amerika (Shilling, 2006; Seiler and Marek, 2011; Kane et al., 2013). Most of *Helianthus* are perennial and annual weeds, and some species are ornamental like *H. argophyllus* and *H. debilis*. Wild sunflower populations occur in wide range of habitats that are characterized by variation in environmental conditions such as photoperiod, growing season, minimum/maximum temperatures, and precipitation.

Wild *Helianthus* species, i. e. crop wild relatives (CWR) represents a source for resistance or tolerance to insects, disease, and pests; for early maturity and resistance in unfavorable

environmental factors (soil salinity, acidity, and drought); and as source of cytoplasmic male sterility and fertility restoration (Faure et al., 2002; Seiler and Gulya, 2004; Breton et al., 2012).
Wide hybridization, including both interspecific and intergeneric hybridization, is one of the most important strategies for creating variations in plant species since it has the potential to combine useful traits, i. e. favorable morphology, disease resistance and some environmental tolerances that could not be achieved by crossing within a single species. According to Kantar et al. (2015), the term hybridization can be defined broadly as the offspring of individuals of genetically differentiated population, and the introgression as the sexual transfer of genetic material between genetically distinguishable populations.
Common sunflower (*H. annuus* L.) cultivars lack acceptable levels of genetic variations since hybrid breeding utilizes a comparatively narrow genetic base. Crop wild relatives have been used for decade for breeding, in particular, to transfer genes of resistance or tolerance to pests, diseases or abiotic stress from wild species to cultivated sunflower. The modern sunflower breeding programs directed towards high yield traits combined with resistance to abiotic and biotic stress. Thus, hybridization and introgression breeding have been of central importance in the exploration and utilization of wild germplasm in genetic improvement of sunflower. According to Seiler et al. (2017) sunflower represents the second most important crop based in hybrid breeding, after maize. Basic direction of the breeding objectives changes because of a new requirement, a new product quality, a new resistance to disease, or changes in agricultural practices, due to economic or environmental factors (Vear, 2016). At present, the success of sunflower hybrid breeding is associated with high seed and oil yield hybrids resistant to dominant diseases and tolerant to drought;
hybrids with changed oil properties;

confectionary hybrids;

herbicide resistant hybrids, and

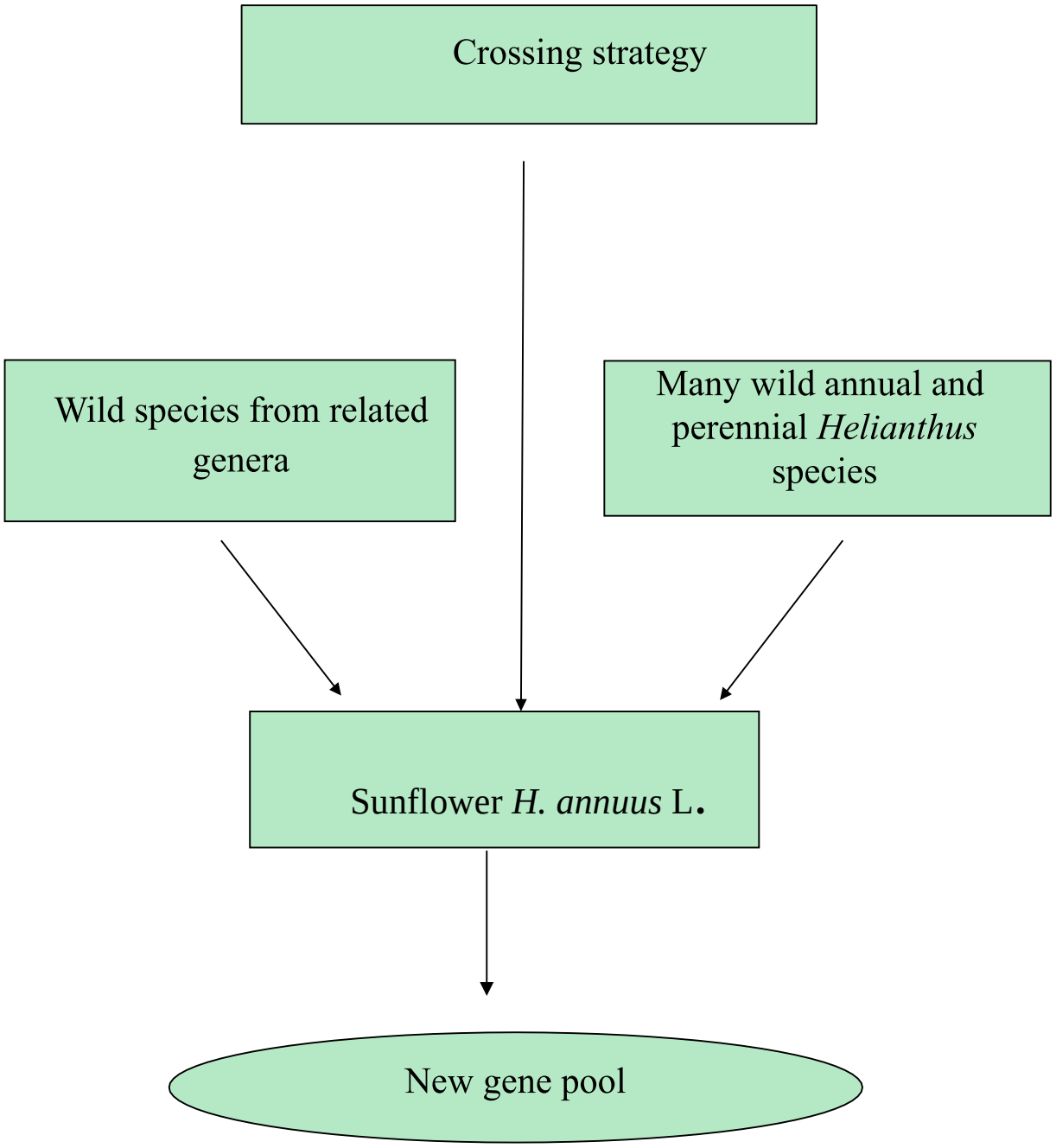
ornamental hybrids

Development of new sunflower genotypes via interspecific hybridization is a long, time-consuming and complex process that involves different knowledge and activities and is the result of collective efforts. Each of the aspect of plant breeding (of agronomic or food value) consists of many specific traits, each presenting its own range of variation. By manipulation a single trait, disregarding all others, it is more likely that no useful variety will be obtained. The challenge of plant breeding resides in improving all of the traits of interest simultaneously, a task made more difficult by the genetic correlations between different traits, which may be due to genes with pleiotropic effects, to physical linkage between genes in the chromosomes, or to population genetic structure (Breseghell, 2013). The key decision to the breeder is therefore retaining the favourable alleles while eliminating the rest during the backcrossing cycles. Identification of favourable alleles amongst unfavourable ones may be greatly facilitated by different molecular methods (DNA markers for QTL analysis, marker assisted selection (MAS), molecular breeding etc.).
In general, exploitation of available plant genetic resources in combination with recent development of genetic tools for the analysis of plant genome, could lead to considerable improvements in sunflower. Given the diversity of wild Helianthus species, the application of wide hybridization Helianthus x wild relatives represented a successful approach for development of new sunflower hybrids with a wide range of variability containing genes of interest from the donor species.
Herewith, we would like to present some of the results of a sunflower research program which objective was production and evaluation of new interspecific and intergeneric hybrids for

transferring desirable traits from wild relatives to cultivated sunflower lines, and developing germplasm pools having wild Helianthus genes in domestic background.

MATERIALS AND METHODS

Scheme 1 presented the summarized crossing strategy:



Scheme 1. The summarized crossing strategy for wild sunflowers.

The majority of wide hybridization studies have examined interspecific hybridization between closely related with *Helianthus* species. Therefore, here we highlight the key most important findings within intergeneric hybridization research in our program and emphasize results in the area.

Some of wild relative species from Asteraceae genera used as donors of useful agronomic traits

Helianthus mollis Lam. (Ashy, Downy sunflower) is a perennial diploid drought and disease tolerant species (Photo 1). *Echinacea purpurea* (L.) Moench which is one of the major medicinal herbs that have continuously gained commercial interest worldwide (Photo 2).

Photo 1. *Helianthus mollis* Lam. (Ashy, Downy sunflower)

Photo 2: *Echinacea purpurea* (L.) Moench

Thithonia rotundifolia (Mill.) Blake (Red Mexican sunflower) is an annual ornamental drought tolerant species that possesses allelopathic and medicinal properties (Photo 3). *Verbesina encelioides* [(Cav.) Bentham & Hooker fil. ex Gray] (crown beard) is an annual drought tolerant species with significant medicinal activities and allelopathic effects (Photo 4).

Photo 3. *Thithonia rotundifolia* (Mill.) Blake (Red Mexican sunflower)

Photo 4. Verbesina encelioides [(Cav.) Bentham & Hooker fil. ex Gray] (crown beard)

The crosses were made using conventional hybridizing method. The wild relatives were used as pollen donors. First- generation hybrid plants were verified using morphological and cytological methods, and F1 hybrids were back-crossed to common sunflower to obtain BC1 and BC2. The new traits were selected for and fixed after BC2 to produce introgression lines. Seeds from advanced plant generations were produced after self-pollination under a bag.

RESULTS AND DISCUSSION

The outcome of wide crosses in respect to individual traits can include intermediate phenotypes in the progeny compared to parental plants, hybrids can be a mixture of both parents or they can exhibit novel characteristics that exceed the parental range – transgressive segregation (Morgan et al. 2021).

The systematic revision of morphological characters of hybrid progeny includes whole phenotype characteristic (agronomic and morphological traits) of both parents and hybrid plants, and comparative analysis of other qualitative traits such as antioxidant capacity (free radical scavenging activity) as well as the presence of ascorbate and tripeptide thiol, glutathione, tocopherols, phenols and flavonoids. In addition, total lipids and variability of fatty acid composition is important to be investigated.

The results showed that F1 hybrid populations exhibited one-parent-origin and intermediate characteristics in similar proportions. The explanation for the observed ratio of traits in the progeny proposed that morphological characters that differ among closely related species showed a dominant inheritance pattern, whereas the additive effects of different genes responsible for quantitative traits can lead to hybrid intermediacy (Rieseberg and Carney, 2008; Morgan et al,. 2011). As we have already mentioned, we will now present some of the practical results of intergeneric hybridization with special focus on *Thithonia rotundifolia, Echinacea purpurea* and *Verbesina encelioides* plants. In the course of our study on wide hybridization Helianthus x wild species, we obtained a suite of diverse recombinants, which reveal intermediate morphology and phenotype, or even novel features such as tubular ray flowers. Advanced sunflower line produced after hybridization H. annuus x Verbesina encelioides with tubular ray flowers instead of the ray flowers on the sunflower inflorescence is given in Photo 5 and Photo 6.

Photo 5. *Helianthus annuus x Verbesina encelioides*

Photo 6. *Helianthus annuus x Verbesina encelioides*

The most obvious feature of the line included compact plant architecture (width is reduced due to short petioles in comparison to a classic sunflower), and upright leaves. Generally, compact sunflowers have some valuable advantages, which is, they allow higher plant densities, facilitate cultivation and thus, it could increase the yield. Therefore, the knowledge of such plant architecture in sunflower was essential in breeding procedures.

Advanced semi-dwarf sunflower line *H. annuus* x *Ecinacea purpureae* is the most remarkable feature of the plants was greatly reduced number of leaves and stature, resulting in a semi-dwarf growth habit (Photo 7, 8, 9, 10, 11). Another wild cross advanced line H. annuus x H. mollis could be possible for ornamental purposes (Photo 12). Advanced ornamental line *Helianthus annuus* x *Titonia rotundifolia* is given in Photo 13.

Photo 7. Helianthus annuus x Echinacea purpurea

Photo 8. Advanced ornamental line H. annuus x Echinacea purpurea

Photo 9. Advanced branched dwarf sunflower line H. annuus x Ecinacea purpureae

Photo 10. Ornamental sunflower line H. annuus x E. purpurea

Photo 11. Advanced sunflower line H. annuus x E. purpurea

Photo 12. Advanced line H. annuus x H. mollis

Photo 13. Advanced ornamental line Helianthus annuus x Titonia rotundifolia

CONCLUSIONS

In conclusion, herewith, we tried to illustrate the variety of activities related to wide hybridization as a breeding technique in sunflower. Since the beginning of the twentieth century, a number of techniques have been adopted by wide hybridization to broaden the possibilities of plant improvement. The traditional methods are still very important to achieve success in wide crosses and it is unlikely they will lose their momentum in this area. Today, there are novel technologies emerging in plant science research with the potential to supplement the older established methods and provide new information about the processes that hamper hybrid production.

Various categories of breeding goals can be distinguished for breeding programs. Plant morphology and architecture as well as flower shape and colour, internal qualities, which are nonvisible features that influence the performance of plants, are another focus of the plant

breeders. Development of varieties resistant to abiotic and biotic stresses as well as improved postharvest quality is of great importance since it has enormous influence on the product quality and through this, its competitiveness. Furthermore, the availability of genomic resources can lead to identification of candidate genes from related plants within genera and families. The fast development of technologies and an increase in information about plant genomes and backgrounds of reproductive barriers in plants have provided new resources for genetic and functional research in sunflower. The future challenges remain to translate the vast amount of data into knowledge that can be applied to facilitate hybridization process and that can be used by plant breeders.

The knowledge obtained in the current study constitutes a basis for further investigations in which wide hybridization may also be important for evolutionary studies and consideration of phylogenic relationships in *Helianthus* and related genera of *Asteraceae*. In future, we expect the true value of wide hybridization and the use of wild relatives for crop improvement of common sunflower to be realized, with implementation of new technologies supported by gene banks and information resources for breeding and research outcomes.

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DETERMINATION OF SOME MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF GALICJANKA ARONIA CULTIVAR (Aronia melanocarpa L.) GROWN IN EDIRNE REGION

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ABSTRACT

In this study, the pomological and biochemical properties of Aronia fruit, which is one of the berry types and is rich in antioxidant content, called the 'super fruit' were investigated. Fruit weight (0.97 g), fruit width (12.25 cm), fruit length (11.57 cm), leaf width (5.82 cm), leaf length (8.05 cm), L (16.86), a (0.48), b (0.25), important morphological parameters such as chroma (0.54) and hue (26.36) values were determined. In terms of bioactive ingredients, the soluble solid content was determined as 25.57%, pH 4.53, and titratable acidity value was 0.21. However, the highest amount of specific phenolic compounds was determined as chlorogenic acid with 268.144 mg/kg, while the highest amount of organic acid was malic acid with 3128.04 mg/kg. In addition, the vitamin C content was determined as 20,868 mg/100g. In this study, it was determined that Aronia fruit can be used as a good source of bioactive phytochemicals in the human diet as a result of the properties examined. In addition, it is thought that determining the quality criteria and biochemical characteristics of the Galicjanka variety grown in Edirne conditions may be beneficial in the development of our country's biodiversity and have an important place in the food industry.

Keyword: Aronia, pomology, phenolic compound, organic acid, vitamin C

INTRODUCTION

Aronia melanocarpa has been a fruit species preferred by consumers in recent years due to its high phenolic compound content, antioxidant properties and potential positive effect on health. Aronia, which has been the subject of many studies due to its rich biochemical content, is a fruit that contains the most antioxidants. For this reason, aronia, which is called the 'Super Fruit', is also a storehouse of vitamins and minerals. It contains all vitamins except B12 and vitamin D (Tokusoglu, 2017). Aronia, which has an important place among berry fruits, is botanically included in the *Roses* class. There are three types of aronia; *Aronia melanocarpa* (Michx) Elliot species called black chokeberrey, *Aronia prunifolia* (Marsh) species as purple chokeberry and *Aronia arbutifolia* (L.) Elliot species as red chokeberry. In general, the names berry and black chokeberry are used for aronia. Among the aronia varieties widely known in Europe, 'Viking' and 'Kurkumachki' in Finland, 'Aron' in Denmark, 'Rubin' in Russia, 'Hugin' in Sweden, 'Nero' in Czech Republic, Hungary 'Fertödi' in and 'Galicjanka' in Poland are among the most well-known cultivars (Poyraz Engin 2016). 'Aron' 'Viking', 'Rubina', 'Nero' and

'Moskva' can be shown among the most important cultivars of aronia, which is considered a functional food (Đorđević et.al., 2021; Wangensteen et.al., 2014; Kulling and Rawel 2008).

Aronia is among the fruits consumed fresh in the world as a fruit with high antioxidant content. In addition, it is widely used as a raw material in the food industry and pharmacy (Poyraz Engin, 2018). Aronia fruits contain very high levels of secondary metabolites such as anthocyanin, proanthocyanidin, flavonoid, flavanol, flavonol polyphenols and phenolic acids (Shahin et al., 2019; Hwang and Thi, 2014). It has been reported that black chokebery berries accumulate quite high amounts of anthocyanins compared to other berry fruits such as black currant, blackberry and elderberry (Jakobek et al., 2007).

Phenolic compounds, which are secondary metabolites, have an important place in determining the nutritional values of fruits and vegetables. Phenolic compounds are effective in the formation of unique color and taste of foods. Anthocyanins, which have an important place among phenolic compounds, have a say in the formation of taste and distinctive color in fruits and vegetables (Cemeroğlu 2004; Gündoğdu 2019; Kibar et al., 2021). Chokeberry extract or juice is known to have antioxidant, antiviral, antimutagenic, anticancer hepatoprotective, antiinflammatory, gastroprotective or antidiabetic activities within the parameters determined in both in vitro and in vivo studies. In addition, it is known that it is effective against immunomodulatory and radioprotective effects by eliminating free oxygen radicals with its high content of anthocyanins and procyanidins (Jurikova et al., 2017; Denev et al., 2012; Kulling & Rawel, 2008). These properties have increased the interest shown in the research of bioactive compounds as secondary metabolites due to their beneficial effects on human health (Borowska and Brzóska, 2016). Because of these properties, the popularity of aronia has increased in recent years.

In this study, morphological, physicochemical and biochemical analyzes of the fruits of the Galicjanka variety were carried out to determine the quality criteria. As a result of the analyses, statistical distributions and definitions of Galicjanka variety were made in terms of morphological and phytochemical characteristics.

MATERIAL AND METHOD

Material

Material in this study, fruit samples belonging to the Galicjanka variety grown in Edirne province were placed in suitable containers, labeled and transported to the laboratory in the cold chain. After the agromorphological properties of these fruit samples were determined, they were stored at -20 °C for biochemical analysis. Morphological and physicochemical analyzes were carried out in Bolu Abant İzzet Baysal University Faculty of Agriculture Laboratory. Phenolic compounds, organic acids and vitamin C analyzes were carried out at Bolu Abant İzzet Baysal University Scientific Industrial and Technological Application and Research Center.

Determination of Agromorphological Characteristics of Fruits

Fruit weight; 20 randomly picked aronia fruits were weighed separately on precision scales and their averages were taken. Fruit sizes; fruit stalk length and fruit stem thickness; The width and length of 10 fruit samples from aronia fruit samples were determined one by one with a caliper sensitive to 0.01 mm. Leaf width and length (cm) and leaf stalk length and thickness (mm); the average width and length of 5 galicjanka aronia leaves were measured with a ruler and digital caliper. Fruit skin color; galicjanka aronia fruit samples were measured in terms of L, a, b, chroma and hue with Konica Minolta CR-400 brand colorimeter (Ercişli et al. 2011). Water-soluble total dry matter (SSC); It was measured by hand refractometer from the juice of aronia fruit and determined as % (Karaçalı, 2002). Juice pH; 10 ml of the juice of 20 fruits was taken and measured with a pH-meter (Eşitken, 1992). Titratable acid (TA) in juice; samples

diluted from the juice obtained from 20 fruits were calculated according to the formula below (Karaçalı, 2002). TA = (NAOH spent (ml) x 0.1 x 0.067 (malic acid) x 100)/(Amount of juice used (ml)).

Analysis of phenolic compounds

In the study, 7 phenolic compounds were identified. Phenolic compounds identified by HPLC were described by Rodriguez-Delgado et al. (2001)'s method was used after being modified. 5 g of fruit sample was homogenized. The extract was filtered with a 0.45µm millipore filter and transf1erred to HPLC. Chromatographic separation was performed on Agilent 1100 HPLC system, using a DAD detector and a 250*4.6 mm, 4µm ODS column.

Analysis of organic acids

The method described by Bevilacqua and Califano (1989) has been modified. 5 g of pitaya fruits were taken, homogenized with sulfuric acid and centrifuged. Extraction was passed through a 0.45 µm membrane filter and SEP-PAK C18 cartridge. The acids were analyzed in high performance liquid chromatography (HPLC). Aminex HPX - 87 H, 300 mm x 7.8 mm was used in the HPLC system and the device was controlled by a computer with Agilent package program. The DAD detector in the system is tuned to 214 and 280 nm wavelengths.

Vitamin C (ascorbic acid) content

The method specified by Cemeroğlu (2004) was used. 5 g of fruit sample was completed with 2.5% M-phosphoric acid solution and centrifuged. The extract was passed through a 0.45 µm Teflon filter and transferred to the HPLC device. In HPLC analysis, vitamin C was carried out on a C18 column.

Statistical Analysis

Pearson correlation analysis was performed in SPSS 26.0 package program in order to determine the relationship between the data.

RESULTS AND DISCUSSION

Morphological and Physicochemical Properties of Fruits

Turkey is a country rich in flora. Having a temperate climate zone allows many fruit species to be grown easily. Due to the important phytochemicals in the composition of aronia fruits, it has recently become one of the prominent fruit species. In this study, ripe fruit samples of the galicjanka variety were taken and necessary measurements and analyzes were made to determine the morphological and physicochemical parameters of the fruits.

The annual average yield of the examined aronia cultivar was determined as 1.930 g per plant. The average fruit weight of Galicjanka variety was determined as 0.97 g from the morphological measurements (Table 1). It was determined that the number of fruits in the cluster was 13.73, fruit width was 12.25 mm and fruit length 11.57 mm. Similarly, the fruit stem length and fruit stem thickness were measured as 14.49 mm and 0.78 mm respectively. The leaf length of aronia plant was 8.05 cm, the leaf width was 5.82 cm, the leaf stalk length was 12.06 mm and the leaf stalk thickness was 1.51 mm. When we looked at the fruit skin color measurements, the L value was 16.86, a value was 0.4, b value was 0.2, chroma value was 0.54 and hue value was 3.33 (Table 1). In the physical chemistry measurements of the study, the SSC content was determined as 25.57%, the pH value was 4.53 and the TA amount was determined as 0.21% (Table 1).

In the pomological examinations made on some aronia cultivars, in the fruits of Nero cultivar; in the first year, fruit width was measured as 12.123 mm, fruit length 10.711 mm and

fruit weight 1.206 g, and in the second year the same pomological parameters were measured as 12.355 mm, 11,466 mm and 1.34 g, respectively. In fruits of the Viking variety; in the first year, fruit width and fruit length were determined as 10.729 and fruit length as 10.305 mm and fruit weight as 0.818 g in the first year. In the fruits of Hugin variety; as of the first year, fruit width was determined as 9.142 mm, fruit length 9.234 mm and fruit weight 0.646 g, and fruit characteristics in the second year; fruit width, length and fruit weight measurements were determined as 9.291 mm, 9.245 mm and 0.637 g respectively (Özder, 2020). Poyraz Engin et al. (2018) stated that the fruit size of the aronia fruit varied between 5 and 14 mm, and the fruit weight varied between 0.5 and 2 g. Ochmian et al. (2012) reported that fruit size varied between 12 and 17 mm, and 100 fruit weight varied between 32 and 112 g, SSC was %14-20. In the other research, the average leaf width of Nero, Viking and Hugin cultivars is 4.75, 5.10 and 5.10 cm, respectively has been determined. Leaf length measurements were determined as 6.93, 7.22 and 6.83 cm in Nero, Viking and Hugin cultivars respectively (Özder, 2020). In their study, Jong and Hop (1994) determined the leaf width and height measurements as 4 x 7 cm. Ochmian et al. (2012) determined that Viking and Nero cultivars had a larger and much larger surface area (32.8, 34.4 cm2 and 12.8, 16.5 cm2, respectively) compared to the leaves of Hugin and Galicjanka cultivars. In Russia, leaf width of Galicjanka cultivar was 43 mm and leaf length was 62 mm. It was reported that the leaves of different cultivars grown varied between 41 and 55 mm in length and between 23 and 34 mm in width (Skvortsov and Maitulina, 1982). In aronia fruit, the amount of titratable acidity Ochmian et al. (2012) determined in terms of citric acid and they found that it varied between 0.75 and 1.05% values. Kulling and Rawel (2008) stated that pH measurements were between 3.3 and 3.7 values. Hudec et al. (2006) reported in their study that the SSC values of aronia fruit ranged between 15 and 24%. The results we obtained from morphological and biochemical analyzes show great similarities with other studies. However, it was determined that some of the results of our study were higher and some of the results were partially lower. This is due to the diversity of varieties, climatic characteristics and cultural processes.

Table 1. Morphological and biochemical values of aronia fruits

Morphological Parameter	Quantity/Value	Morphological Parameter	Quantity/Value
Yield (kg)	1.930	Leaf stalk length (mm)	12.06
Number of fruits per cluster (piece)	13.73	Leaf stalk thickness (mm)	1.51
Fruit weight (g)	0.97	SSC (%)	25.57
Fruit width(mm)	12.25	pH	4.53
Fruit length(mm)	11.57	TA (%)	0.21
Fruit stalk length (mm)	14.49	L* value	16.86
Fruit stalk thickness (mm)	0.78	a* value	0.48
Leaf length (cm)	8.05	b* value	0.25
Leaf width (cm)	5.82	Chroma value	0.54
		Hue value	26.36

A positive but weak relationship was found between the yield and the number of fruits per cluster (Table 2). It has been observed that yield has a parallel relationship with especially fruit weight and fruit length, and this relationship has a strong correlation. It has been determined

that yield increases as fruit weight and length increase. A negative correlation was found between fruit width and fruit length, and it was obtained that fruit length shortens as fruit width increases (Table 2).

Table 2: The relationship between yield, the number of fruits per cluster, fruits weight, fruit width and fruit size of aronia.

	Yield	Number of fruits per cluster	Fruits weight	Fruit width	Fruit length
Yield	1	0.292	0.962	0.412	0.909
Number of fruits per cluster		1	0.542	0.992	-0.134
Fruits weight			1	0.646	0.760
Fruit width				1	-0.006
Fruit length					1

Biochemical Properties of Fruits

In this study, the amounts of gallic acid, caffeic acid, chlorogenic acid, p-coumaric acid, protocatechuic acid, syringic acid and rutin of the examined aronia fruits were determined by HPLC. It was observed that chlorogenic acid and caffeic acid contents of aronia berries were higher than other phenolic compounds. Among the phenolic compounds, the amounts of gallic acid and syringic acid were determined as the compounds with the lowest. Considering the amounts of determined phenolic compounds; gallic acid 4.862 mg kg^{-1}, protocatechuic acid 19.033 mg kg^{-1} (Figure 1), caffeic acid 142.137 mg kg^{-1}, syringic acid 9.137 mg kg^{-1}, p-coumaric acid 18.007 mg kg^{-1}, chlorogenic acid 268.144 mg kg^{-1} and rutin 26.010 mg kg^{-1} (Figure 2) was detected. In one study, it was reported that protocatechuic acid and caffeic acid could not be determined, but chlorogenic acid was determined as 0.171 mg g^{-1}, syringic acid 0.419 mg g^{-1}, p-coumaric acid 0.175 mg g^{-1}, and rutin 5.544 mg g^{-1}. In the same study, they found that the total amount of phenolic compunds was 13.88 mg CAE/g (Cvetanovic et al., 2018). In another study, the total phenol content was determined as 105 mg CAE/g (Jo and Imm, 2017). Kim et al. (2013) determined caffeic acid as 0.736 mg g^{-1} dry and chlorogenic acid as 79.0 mg g^{-1} dry in their study. In addition, Denev et al. (2019) determined the amount of chlorogenic acid as 8.3 mg g^{-1} and the amount of rutin as 7.8 mg g^{-1} in their study. Also, in other study it was found that the total phenolic content in aronia berries ranged from 0.69 to 2.56 mg g^{-1} fresh weight and 3.44 to 7.85 mg g^{-1} dry weight (Denev et al., 2012; Kulling & Rawel, 2008). Polat et al. (2017) reported that chlorogenic acid was the dominant phenolic compound in Viking, Nero and Rubina aronia cultivars and varied between 43.65 and 43.72 ppm. Researchers stated in other studies that the total amount of phenolic compounds varies between 2.000 mg $100g^{-1}$ and 8.000 mg $100g^{-1}$, and the phenolic content varies depending on the variety, climatic conditions and harvest dates (Sueiro et al., 2006; Benvenuti et al., 2004; Hudec et al., 2006; Oszmianski and Wojdyło, 2005). It has been reported that stress factors such as temperature, humidity and soil nutrients may be responsible for the difference in phenol content (de Matos Nunes et al., 2014; Nantongo et al., 2018).

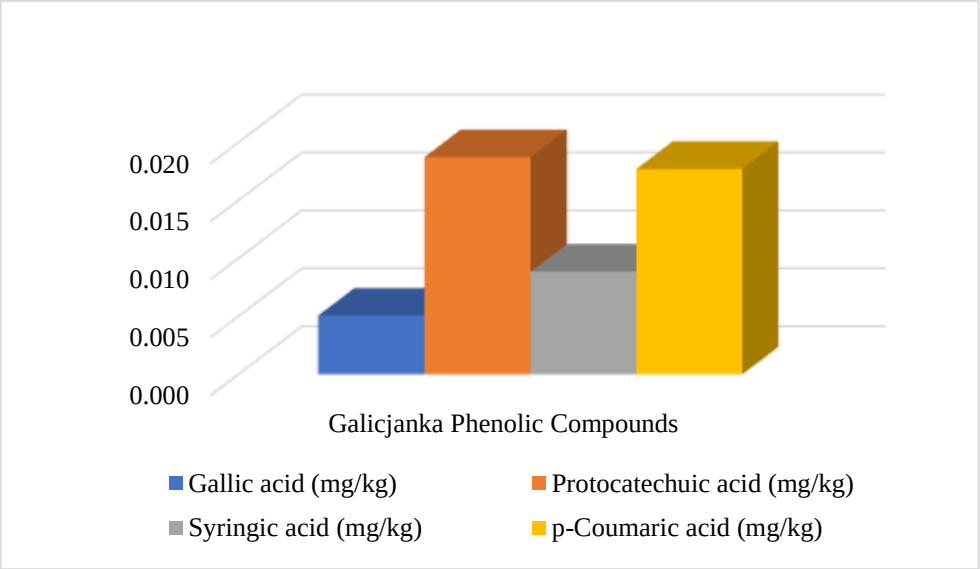

Figure 1. The gallic, syringic, protocatechuic and p-coumaric acid content of aronia.

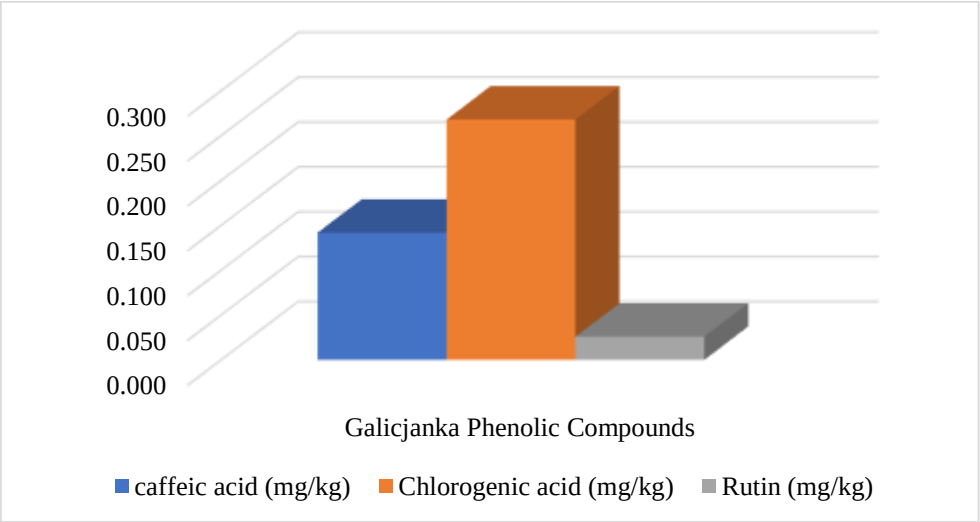

Figure 2. The caffeic acid, rutin and chlorogenic acid content of aronia.

Organic acids, which are frequently emphasized in terms of health, can affect many physiological events such as taste formation and ripening of fruits (Savran, 1999). The ripening of fruits depends on the organic acid-sugar ratio. Organic acids are effective in determining the unique taste of fruits. The concentrations of organic acids determine the taste of the fruit. According to this, low organic acid sugar content gives sweetness to fruits, and high sugar content gives fruits sour character. In the study, the amounts of malic acid, tartaric acid, citric acid, fumaric acid, succinic acid and vitamin C of organic acids belonging to the examined aronia fruits were determined by HPLC. When examined in terms of some important organic acids and vitamin C contents in the composition of aronia fruits; the amount of citric acid was 415.130 mg kg^{-1} (Figure 3), the amount of tartaric acid was 877.475 mg kg^{-1}, the amount of malic acid was 3128.044 mg kg^{-1}, the amount of succinic acid was 1452.059 mg kg^{-1}, the amount of fumaric acid was 20.443 mg kg^{-1}(Figure 3), and the amount of vitamin C was 20.868 mg kg^{-1} (Figure 4). has been done. Kalemba et al. (1995) reported that the amount of vitamin C in aronia ranged from 14 to 28 mg per 100 g of fresh fruit. In another study, it was reported that the ascorbic acid content ranged between 1.9–8.4 mg per 100 g (Skupien, 2005). Chokeberry

fruits generally have a low organic acid content ranging from 1 to 1.5% (Lehmann, 1990; Tanaka and Tanaka, 2001; Skupień et al., 2008). Ochmian et al. (2012), malic acid and citric acid were determined as essential acids in aronia fruits. They stated that the examined fruits were characterized by lower organic acid content. In the 'Galicjanka' cultivar, it was reported that it was 0.75 g per 100 grams of fruit.

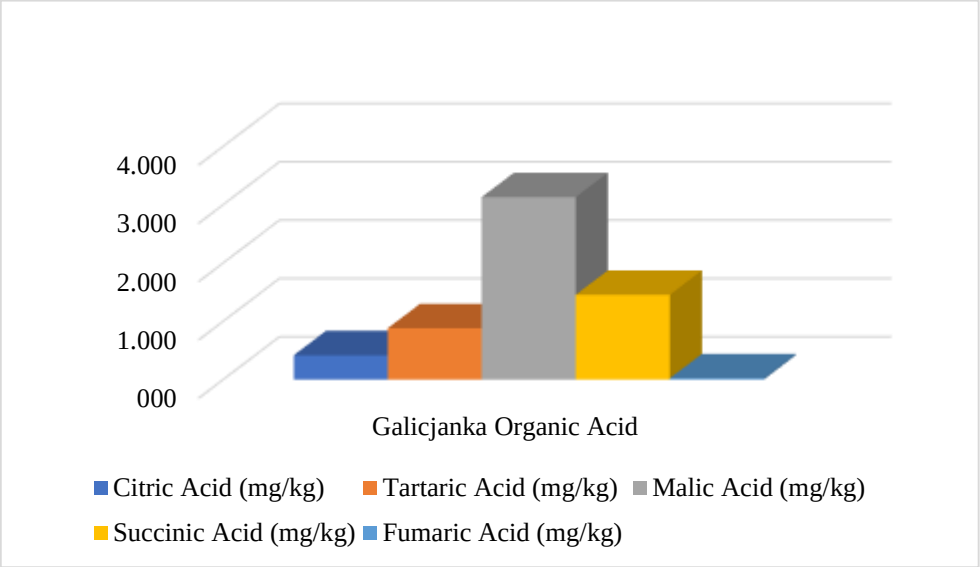

Figure 3. The citric, tartaric, malic, fumaric and succinic acid content of aronia.

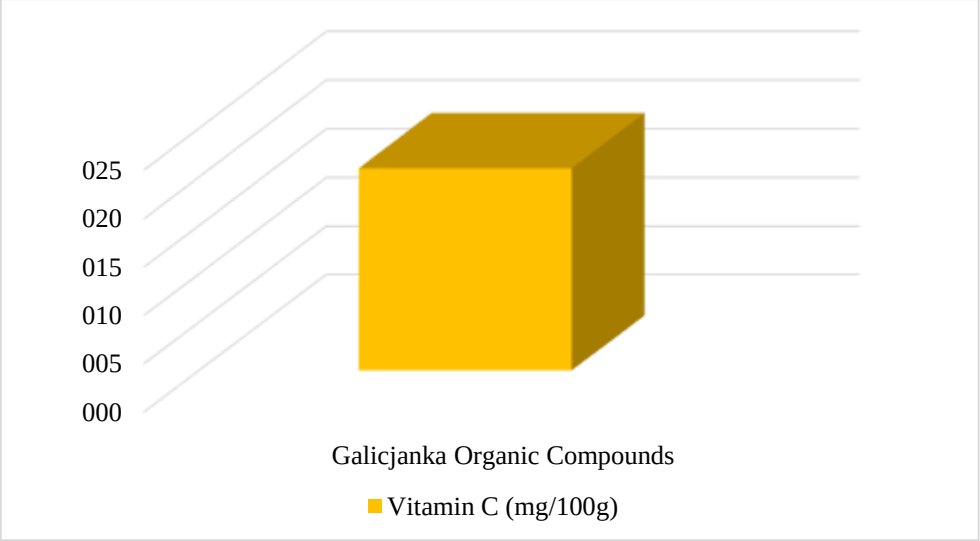

Figure 4. The vitamin C content of aronia.

A positive and strong relationship was found between caffeic acid and chlorogenic acid, rutin, citric acid, tartaric acid, malic acid, fumaric acid, SSC and vitamin C (Table 3). It was determined that especially tartaric acid and fumaric acid were significant in these relations. Syringic acid showed a negative relationship with the other components except pH, and a positive relationship was found with pH, provided that it was weak (%2). Very strong correlations were found between caffeic acid and tartaric acid (%99), between chlorgenic acid and rutin (%99), between citric acid and tartaric acid (%98), between fumaric acid and tartaric

acid (%99), and between malic acid and vitamin C (%100), and it was observed that they increased together. It was determined that the amount of water-soluble dry matter and the amount of vitamin C were negatively correlated with gallic acid and syringic acid (Table 3).

CONCLUSION

It is defined as a functional food due to the rich phenolic content and therapeutic effects in the fruits of Aronia melanocarpa and other Aronia species. For this reason, its use has become increasingly important in recent years. Phenolic acid compounds, organic acid and vitamin C contents are thought to be important in terms of pharmacological activity. For this reason, it has been concluded that studies on the bioactivity of fruit and growing should increasingly continue.

Table 3. The relationship between phenolic compounds, organic acids, SSC, pH and TA of aronia.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
Gallic (V1)	1	0.963	0.299	-0.653	-0.318	0.433	0.482	0.185	0.251	-0.256	-0.532	0.284	-0.237	-0.146	0.641	-0.438
Protocatechuic (V2)		1	0.544	-0.832	-0.052	0.658	0.699	0.442	0.501	0.012	-0.285	0.531	0.033	0.124	0.412	-0.181
Caffeic (V3)			1	-0.918	0.810	0.990	0.980	0.993	0.999*	0.846	0.649	1.000*	0.856	0.900	-0.540	0.727
Syringic (V4)				1	-0.511	-0.965	-0.978	-0.865	-0.897	-0.565	-0.294	-0.912	-0.581	-0.654	0.163	-0.395
p-coumaric (V5)					1	0.717	0.678	0.873	0.838	0.998*	0.972	0.819	0.996	0.984	-0.931	0.992
Chlorogenic (V6)						1	0.998*	0.966	0.981	0.761	0.533	0.987	0.774	0.829	-0.415	0.621
Rutin (V7)							1	0.950	0.969	0.724	0.485	0.977	0.737	0.796	-0.363	0.577
Citric (V8)								1	0.998*	0.903	0.734	0.995	0.911	0.945	-0.636	0.802
Tartaric (V9)									1	0.872	0.686	0.999*	0.881	0.921	-0.582	0.761
Malik (V10)										1	0.955	0.854	1.000*	0.994	-0.906	0.981
Succinic (V11)											1	0.661	0.949	0.915	-0.991	0.994
Fumaric (V12)												1	0.864	.907	-0.554	0.737
Vitamin C (V13)													1	0.996	-0.897	0.977
SSC (V14)														1	-0.853	0.953
pH (V15)															1	-0.971
TA (V16)																1

** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level V:Value

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SUNFLOWER THEME IN PAINTING

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ABSTRACT

From past to present, the artist has questioned both what is and what is behind the existing in the universe, which is in eternity and obscurity. In this process, the artist chose to reshape his work with his own subjective thought. In this context, the artist reflected his work by feeding from nature, and nature has been a very important factor in the development of the artist and the formation of his art. Sunflower paintings are evaluated in still life (still life) or landscape painting, one of the types of painting art, according to the way they are handled. Sunflowers, as the artist's source of inspiration and inspiration, take place in the works of artists both as a subject and as a material. In this process, the artists not only imitated the theme, but also used surrealistic expressions that reflected their emotions more intensely. In the 20th century, in the paintings of Gustav Klimt, Egon Schille and Guido Borelli, especially by Van Gogh, very influential paintings on the Sunflower theme appear. In this study, the works of Van Gogh, Gustav Klimt, Egon Schille and Guido Borelli will be researched, analyzed and analyzed by using the qualitative research method. Sunflower theme will be the subject of the research as an area where the artists covered in the research are fed.

Keywords: Sunflower, Painting, Art, Theme

INTRODUCTION

The art of painting continues its existence from the past to the present, by multiplying the forms of expression, and continues its search. These searches have led to the formation of different styles and have given birth to art movements and have survived to the present day. Artists have created their art by perceiving and internalizing what they see around them. Artists have produced and continue to produce their works by interpreting nature with their experiences and using their own personal styles. Artists have been influenced by nature in every period and nature has been an important factor in the formation of their art. While creating his works, the artist produced his works by evaluating the form/distortion, the work/object and the work/audience relationship, the perception of space/art from different angles.

When we look at the history of painting, it is seen that Sunflower takes place as a theme or a complementary element in the paintings of some painters. It is used as a formal element as well as with symbolic meanings. Symbolically, it is believed to mean happiness, optimism, longevity, luck and loyalty in different cultures. In Chinese culture, sunflowers are believed to bring lasting happiness and good luck.

In this text, the way in which the Sunflower theme is handled by the artists, what it means for the artist, and the Sunflower theme is explored with different approaches. Van Gogh, Gustav Klimt, Egon Schille and Guido Borelli's approaches to the Sunflower theme, which is an element of nature, are briefly discussed. Artists have been inspired by nature from past to present and have created their works. In this context, the works of the artists were researched, examined and analyzed.

VAN GOGH (1853-1890)

Van Gogh, one of the most important painters of the 20th century and known for his suicide attempts by cutting off his ear, experienced many turmoil in his life and reflected this in his works. The artist, who thinks that it does not work, has always sought a way out. This is especially evident when we look at his paintings, inspired by many artists such as his best friend, Gauguin. Paul Signac, one of the representatives of the pointillism period and the representative of realism. In addition to millet and expressionists, the influence of Japanese estamps is seen in Van Gogh's works (Alparslan, 2018: 212).

According to Gombrich; Since artists became conscious of the style, they have realized that hand skills are not required to produce art, as he stated, not to follow the traditional rules. Artists no longer wanted art with sufficient dexterity, and advocated the reflection of passions and the power that comes from within the artist (Gombrich, 2007: 551).

Impressionists sought to get rid of these traditional patterns. While Van Gogh was in this search, he found what he was looking for in the prints made on porcelain produced in Japan or China. He came under the influence of Japanese art by discovering the places where sharp, vivid and primary colors were sold. The characteristic of Japanese art, which influenced the artist, was the way it reflects nature and portrait. Undoubtedly, the main reason why Van Gogh went to Arles was to show nature in his works such as Japanese art. Because of his understanding of line and color, the simplicity of Japanese art influenced Van Gogh. Desiring to leave Paris and go south, he went to Arles, the Japan of France. Another reason for him to go to Arles is the desire to see a different light, to reflect the emotions, lines and colors of the Japanese artist on his works when he examines nature under the bright sky (Uysal, 2021: 1169).

Figure 1. Van Gogh, Sunflowers, 1889, Oil on canvas, 92.4 x 71.1 cm, Philadelphia Museum of Art, Philadelphia, ABD

Van Gogh ended his art in Paris in 1888 and moved to a yellow house in Arles. This period of Van Gogh's time in Arles is the period in which he used the color yellow in his art. The yellow color of his room made him feel at home for the first time (Uysal, 2021: 1170).

During his time in Arles, he predominantly used the yellow color in most of the flowers, people, vegetables and fruits in the works he produced. In the town of Arles, where he lives, there are beautiful fields of sunflowers stretching out to infinity. It is understood from his works that the landscapes in which the sunflower fields are dense in Arles greatly influenced the artist. Also, for him, the yellow has become a symbol of happiness.

Figure 2. Van Gogh, Four Cut Sunflowers, 1887, Oil on canvas, 60 x 100 cm, Kröller-Müller Museum, Otterlo, Hollanda

Dreaming of establishing a workshop for himself, the painter communicated with the French painter Paul Gauguin, whom he admired. He is excited to host the artist he admires in his house, he begins to decorate the room where he will stay and he paints many paintings to impress him. Paul was very impressed by Van Gogh's sunflowers, and their friendship continued even though there were tense and unpleasant events between the two later on.

Figure 3. Van Gogh, Sunflowers, 1888, Oil on canvas, 98 x 69 cm

Figure 4. Van Gogh, Sunflowers, 1888, Oil on canvas, 73 x 58 cm, Private Collection

There are many reasons why Van Gogh obsessively used too many sunflowers in his painting. Although Van Gogh's aim was to attract Gaugun's attention in the early days, he made sunflowers his symbol. Like Monet's lotuses, Van Gogh's sunflowers were among the artist's most important paintings and made him known worldwide with sunflowers.

Sunflower paintings are the first thing that comes to mind when we think of Van Gogh's symbol. It is because of his love for nature that he paints a lot of sunflowers. For the artist, who reflects the sunflowers, which follow the movements of the sunlight and move according to the angle of the sun, on his works, sunflowers represented a return to his essence. When it comes to Japanese art, flowering trees come to mind, and when Van Gogh is mentioned, Sunflowers paintings are one of the first to come to mind. The sunflower theme has a very important place in his art as it symbolizes the personality of the artist (Çığşar, 2020: 131).

The Sunflowers series, different from each other, consists of a total of 11 works in two series. In the first series, the artist depicted the sunflowers ripening in 1887 while he lived in Paris in 4 different paintings. In his second series, Van Gogh painted sunflowers in vases and consists of 7 paintings in total. The second series, in which the color yellow is dominant, is his most famous work. Yellow is the symbol of happiness for the artist. Van Gogh Sunflowers worked in series and became his favorite theme

Figure 5. Paul Gauguin, Vincent van Gogh Painting Sunflowers, 1888

Figure 6. Vincent van Gogh, Twelve Sunflowers in a Vase, 1888

At the end of 1880, he started to deal with the Sunflower theme in series in his paintings. He has always studied the sunflower theme and it has become one of his favorite themes. The flowers in the Twelve Sunflowers in the Vase show different stages of development. (Figure 6) In the lower left, the green small fresh sunflower hanging from the vase is in the same vase with the adult yellow petaled flowers and the brown big-headed flowers that have gone to seed. While the presence of many colors in his painting causes a harmony in his works, it also shows that different stages of life, youth, maturity and old age, pass together in the same life.

The distinctive symbolic content of Vincent van Gogh's paintings is of interest to anyone who wishes to discover it. Today, Vincent's presence in both the East and the West is a result of the increasing value of subjective creativity around the world. Van Gogh's work is seen as symbolic, confusing and confusing. It is seen that Van Gogh insisted on using many motifs such as the sun, moon, stars, wheat fields, cypresses, olive groves and sunflowers in his works. These images are used in a different sense each time.

GUSTAV KLİMT (1862-1918)

Gustav Klimt, who followed his dreams, created his own style by combining ornaments with the symbolist movement. It is possible to say that the symbolist painter Klimt shaped his works with the reflections of his memory and symbols and ornaments that can express these reflections.

As one of the founders of the art nouveau movement in Vienna, Gustav Klimt revealed a hitherto untreated formality in his works. He used new materials such as arabesque motifs, vegetal forms, curved linear rhythm, gold color or semi-precious stones in his art. He is an important artist of this movement (Gombrich, 2007).

Figure 7. Gustav Klimt, Adele Bloch-Bauer I, 1907

Figure 8. Gustav Klimt, Sunflowers in the Farm Garden, 110x110 cm, Oil on canvas, 1913

Most of Gustav Klimt's works include ancient patterns, sculptures, mythological characters, life figures, female portraits, landscapes, and allegories. Gustav Klimt, who is the representative of the art nouveau movement, used flower motifs or vine shoots in his paintings. He transformed them into geometric forms and then incorporated them into his asymmetrical compositions (Gombrich, 2007).

Klimt was influenced by Van Gogh's technique of simplifying form and using large areas of color. The painter has changed the composition order by reflecting this technique in his work. Colorism started in Vienna with French paintings taking part in exhibitions, and Klimt was one of the first to apply it. The artist used contrasting complementary colors within the color wheel as defined by Goethe (1749-1883). To show this, he reflected the flower theme in his Works (Kazanç, 2018: 105).

Klimt painted his work "Sunflowers in the Farmyard" with impressionist and pointistic touches. When we compare it with the color palette in the paintings made in the workshop, the sunlight in this work has added a bright and natural image to the painting and a lively atmosphere. It is seen that the artist creates texture with large and small dots and brush strokes in his work with the colors he uses.

Klimt's landscape works created an interior atmosphere suitable for the harmony of the painting by creating intermediate tones from local colors. Designers in today's fashion world were very impressed by these works and inspired their collections.

In Klimt's painting The Kiss; A kissing couple emerges from a field of flowers out of a multitude of patterns and shapes (Figure 9). Gilding, bright colors of flowers and rich ornaments of clothes predominate in the color scale. The work is conveyed in eye-catching colors. The authoritarian woman in Klimt's earlier works has become the submissive woman here. He surrendered himself to man, to his dominion (Yener ve Nacar, 2017: 117).

Figure 9. Gustav Klimt, The Kiss, 180x180 cm, Oil on canvas,1907

Figure 10. Gustav Klimt, Roses Under the Trees, 110x110 cm, Oil on canvas, 1904

Whether landscape or portrait, it was important to Klimt to use the creative form. In his works, he depicted the female figure in nature with portraits of vegetation. The symbolic meaning of female figures has a very important place in his works. The artist has never attributed outer appearance to inner feelings. He painted as an idealist. In his painting “Roses Under the Trees” he reflected the connection between nature and the subject.

Klimt's nature paintings are most clearly seen in the "Sunflower" painting, in which the phenomenon of metamorphosis is reflected. In front of the ornate, inaccessible, vegetal wall, a single flower in human form forms the main form of the painting. The connection between Klimt and Emilie Flöge is seen in this work. If we compare the photo taken by Klimt, we can better understand the relationship of form in silhouettes.

It is a very effective painting as the sunflower covers the center of the picture with its green leaves and branches and is depicted from the perspective of the bottom and directs the viewer's perception to the sunflower. The enormous effect of the sunflower from the point of view shows that the artist attributed a sacred meaning to the sunflower plant (Figure 11).

EGON SCHIELE (1890-1918)

The artist, who adopted the Schiele expressionism movement and is known for his often distorted, thinned, elongated hands, figures and portraits, was sometimes inspired by the young people who visited his studio and worked on their portraits.

The often tense and harsh images of Schiele's portraits, figures and landscapes are clearly visible. The themes of his works are love, loneliness, life and death. A recurring theme in his work has been growth and decay. These issues are most evident in the Sunflower study. It symbolizes the

Figure 11. Gustav Klimt, Sunflower, 110x110 cm, Oil on canvas, 1906-07

Figure 12. Emilie Flöge

Figure 13. Egon Schiele, Self-portrait, 32.2x39.9 cm, Oil on wood, 1912

Figure 14. Egon Schiele, Sunflowers, 110x110 cm, Oil on canvas, Private Collection, Viyana, 1907

existence of the cycle of life using bright, vigorous flowers as well as dark, wilted leaves (Göktaş, 2021: 133).

Schiele is one of the Austrian representatives of the art movement called expressionism. Expressionist painters gave up beauty by clearly displaying the individual's pain, misery, illness or passions in order to increase the effect of expression. For them, insisting on beauty and harmony in art is refusing to accept honesty.

Figure 15. Egon Schiele, Sunflowers, watercolor on paper, 56x83 cm, 1909

Figure 16. Egon Schiele, Sunflower II, oil on canvas,150 x 29,8 cm, 1909

Schiele's sunflower scenes are alienating portraits of man. Sunflowers was in his twenties when he made his works, but already showing a deep interest and understanding of the world and society around his. Schiele, who lost his father to syphilis at the age of fifteen, quickly took off the rose-colored glasses of his childhood and became an adult, or at least he tried. In short, his works are based on the thought infrastructure of a very mature person. In his self-portrait paintings, he pondered the problem of identity and showed his place in society. Likewise, sunflowers represent the state of his soul, not the landscape he sees in front of him. Along with Van Gogh, Klimt also influenced Schiele's choice of the sunflower motif. Knowing Klimt's and Van Gogh's sunflowers in their original form, Schiele added his own interpretation while processing this theme. Van Gogh's sunflowers are depicted in vases, while Klimt's sunflowers are depicted as sultry, surrounded by ornate dense foliage. On the other hand, like the habits in Schiele's other works, sunflowers can be seen in their natural state lying in the background that is left blank.

This distinctive approach of Schiele is seen more clearly in the painting named Sunflower II (Figure 16). A single sunflower made on a narrow long canvas takes up as much space as the total size of the canvas. As in the artist's other works, the air of stillness created by the empty background is also reflected in the way he uses the colors in the sunflower, and all attention is focused on the

sunflower. The matte yellows used in the stem and leaves of the flower, whose head is painted black, and the tones of green mixed with muddy browns show neither the full freshness nor the fadedness of the flower. Colors are generally repeated with close tones of each other, piece by piece. The bright red flowers woven on a dark soil background are applied in the form of round spots, reminiscent of Klimt's ornamental style.

Schiele's paintings can be interpreted as death and life intertwined. The connection between life and death is a subject that Schiele deals with very often in his works. Reflecting the visible in his work was never in Schiele's interest. It is possible to see this especially in the artist's sunflower paintings. He transferred what he perceived to his art, not his reality.

GUİDO BORELLİ (1952-)

Artist Guido Borelli da Caluso was born in Caluso, Italy in 1952, at the foot of the Italian Alps. Born into an artistic family (his grandfather is an engraver and furniture maker, and his uncle and aunt are painters), young Guido first discovered painting between the ages of 4 and 6. With the encouragement of his family, Guido's first art competition was a public competition at the age of 13. Although he did not receive this art award, he opened his first personal art exhibition at the age of 17. He studied art at the Accademia Albertina, founded in Turin in 1672, at the request of the King. Guido's exhibitions, which started with his first exhibition at the Ars Plauda Gallery in Turin, Italy in 1969, continued to open all over the world. In addition to these rotating exhibitions, there are permanent exhibitions in many places. (Borelli, G. pixels.com).

“I am a true lover of nature and all things gentle. My landscapes and houses combine reality with imagination. First, I collect all the views that I find interesting in my mind. Later, when I need a topic, these views resurface in my memory and I paint what I selectively remember about them, with personal changes in nature.” (Borelli, G., pixels.com).

Figure 19. Guido Borelli “A taller sunflower” 40x50 cm, oil on canvas, 2009

Figure 20. Guido Borelli “A beautiful sunflower” 61x61cm, oil on canvas,2010

Guido's style of painting is influenced by the Italian realist style that began in the 1900s, especially by the artists Boldini and De Nittis. Inspired by the movement of shadows, his oil paintings

reflect Italian landscapes, nature as focal points depicting the homes and villas of Northern Italy and the Alps. Today his work is highly appreciated and has collectors worldwide.

The artist made many paintings on the subject of Sunflower. Reproductions of these pictures made with different mediums are sold on the Internet. Sunflower fields, together with these fields, depicted the white houses in it close to reality. In A Beautiful Sunflower, the sunflower head near the right middle center is the focal point of the painting (Figure 20). Sunflowers, abstracted in the background, are lined up along the road. It is a painting that impresses the viewers with the form and beauty of the sunflower.

CONCLUSION

Art has been a communication tool for individuals from past to present. The way the artists describe the Sunflower theme, which has been the subject of many artists throughout the history of art, is different from each other. In this process, the artists tried to reshape their works by filtering their own thoughts. Thus, the artist was inspired by nature while producing his works and nature has been a very important factor in the development of his art. Sunflower theme, which is an inspiration for artists, seems to be handled by artists with a realistic, abstractionist, symbolist and ornamental approach.

While Van Gogh's works are expressed as being together in the same life, such as youth, maturity and old age, in a symbolic sense created with more vivid and bright colors, Egon Schiele's works with an expressionist approach, using harsher and brighter colors as well as dark, withered leaves. It symbolizes the existence of the life cycle.

Klimt, on the other hand, reached the most striking point in the recognition of women and flowers with his works and brought a new inner view to his paintings. Known for his landscapes, Guido Borelli combined his imagination with reality and preferred to depict Sunflower paintings realistically.

The sunflower theme has been an important tool in the artist-nature relationship. As an area where the artists discussed within the scope of the research are fed, each artist reflects the Sunflower theme to his art, his inner world, feelings, that is, his perception of life. In this process, the artist chose to reshape his work with his own subjective thought.

Sunflower with its beauty, effective form and symbolic meanings has influenced many painters in the art of painting and has been and will continue to be the subject of their works.

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COFFEE TOXICOLOGY AND LIVER HEALTH: EFFECTS ON PROCESSED COFFEE PRODUCTS

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ABSTRACT

The present study aimed to investigate the beneficial effects of coffee on the liver. The results show that coffee has beneficial effects on the liver and can reduce liver disease progression due to its antioxidant properties. Coffee contains antioxidant capacities of chlorogenic acid, hydrophilic components, hydrophobic components, lactones, and diterpenes. There are also rich amounts of potassium and magnesium in coffee. Roasting of the green coffee beans at high temperatures will make unique components due to the chemical reactions between carbohydrates and amino acids as Maillard reactions.

Caffeine with a purine derivative is found in several dietary sources, including tea, chocolate bars, coffee, cocoa beverages, energy, and soft drinks. Caffeine can pass all biological membranes due to the hydrophobic properties of caffeine. Three primary metabolites, such as theophylline, theobromine, and paraxanthine, are caused by metabolizing caffeine in the liver. Caffeine at normal consumption doses mainly acts among humans as an antagonist of adenosine receptors. Two cups of coffee per day should be consumed to show its beneficial effects. Coffee drinkers experience a lower incidence of advanced cirrhosis and fibrosis. There are also differences between males and females in their responses to caffeine due to changes in circulating steroid hormones.

Keywords: Caffeine, bioactive, coffee bioactive, processing

INTRODUCTION

Coffee contains antioxidant capacities of chlorogenic acid, hydrophilic components, hydrophobic components, lactones, and diterpenes. There are also rich amounts of potassium and magnesium in coffee. Roasting of the green coffee beans at high temperatures will make unique components due to the chemical reactions between carbohydrates and amino acids as Maillard reactions. Caffeine with a purine derivative is found in several dietary sources, including tea, chocolate bars, coffee, cocoa beverages, energy, and soft drinks. Caffeine can pass all biological membranes due to the hydrophobic properties of caffeine. Three primary metabolites, such as theophylline, theobromine, and paraxanthine, are caused by metabolizing caffeine in the liver (Ergin et al., 2021).

Coffea canephora (Coffee Rustica) and Coffea Arabica (Coffee Arabica) are the two main species. It has been reported that caf- feine content varies based on the type of tested coffee bean. It has been reported that Arabica beans have a coffee bean with 10– 12 mg caffeine/g. There is a coffee bean with 19–21 mg caffeine/g in the Robusta beans (Patay et al., 2016).
Seeds of green coffee are obtained by harvesting coffee fruits, that is, cherries, and pulp ex- traction. Green coffee seeds can then be processed or roasted for decaffeination. Only roasting will result in flavor and characteristic aroma of coffee in the seeds. The brewing method, including per- colation, electric coffee maker, French press, boiling, Italian coffee maker, or espresso machine, also can affect the coffee chemical composition as another factor (Ergin et al., 2021; Farah, 2012).

Instant Coffee is typically produced by adding hot water to the ground roasted coffee and using high pressure to extract the compounds, which can be solved in water. Heating is conducted to cool, centrifuge, and concentrate this soluble material, and freeze-drying is used for drying and re- ducing moisture by about 5%. The by-products of coffee processing, such as coffee husks, coffee pulp, spent coffee grounds, and silver skin, are massively untapped, representing a large source for value- added products. The pulp of coffee, which is about 28% of the fruit of coffee per dry weight, is an abundant by-product of the coffee cherry fruit. About 12% of the coffee fruit dry weight is in the coffee husks, while about 50 to 55% of the coffee fruit dry weight is in the coffee bean. There is research on these waste streams used to pro- duce biofuels (Ergin et al., 2021; Hughes et al., 2014).

Health Effects of Coffee Bioactives

The experimental evidence shows that there are high concentrations of CGA, such as polyphenols in the Coffee. Intolerance of glucose can be modulated. The development of NAFLD can be improved or decreased by CGA (Panchal et al., 2012). Despite the abundance of other caffeoylquinic, feruloyl quinic, and caffeoylquinic acids, 5-caffeoylquinic acid is the main chlorogenic acid found in coffee. There may be the transformation of the chlorogenic acids into phenolic acids such as ferulic, caffeic, and isoferulic moieties, and then, into colonic metabolites such as dihydrocaffeic acids. To study if there is a potential bioefficacy, phenolic metabolites of chlorogenic acids have been investigated, and since there are no completely clear results, there remains controversy (Ergin et al, 2021; George et al., 2008; Higdon and Frei, 2006).

Regarding cafestol and kahweol, there is an antioxidant activity in the coffee oil in mice models and cell models involving the upregulation of the key antioxidant enzymes (Gómez-Ruiz et al., 2007; Liang et al., 2007). From a different perspective, the main compounds that raise cholesterol in coffee are the two diterpenes. Filtered coffee is the most beneficial due to its reduction of kahweol and cafestol raising serum cholesterol, while caffeine and CGA are maintained. There is coffee brewed in a French press and Turkish Coffee in the nonfiltered, boiled coffee due to cardiovascu- lar disease and increasing lipoprotein cholesterol with low density (Ranheim & Halvorsen, 2005).

Roasting of the green coffee beans at high temperatures will make some unique components due to the chemical reactions be- tween carbohydrates and amino acids as Maillard reactions. The roasting process produces the Maillard reaction products (MRP) with color, flavor, and aroma in different brewed coffees, significantly affecting its antioxidant activity (Ergin et al,, 2021; Liu & Kitts, 2011).

Processing and Occurring Compounds in Coffee

Roasting of the green coffee beans at high temperatures will make some unique components due to the chemical reactions be- tween carbohydrates and amino acids as Maillard reactions. The roasting

process produces the Maillard reaction products (MRP) with color, flavor, and aroma in different brewed coffees, significantly affecting its antioxidant activity (Ergin et al., 2021; Liu and Kitts, 2011).

As a source of acrylamide, coffee should be examined deeply to figure out the contribution of diverse precursors. In the study by Kocadağlı et al. (2012), the contributions of sucrose decomposition and lipid oxidation to acrylamide formation in coffee during roasting were investigated.

Coffee is prone to various pests and diseases caused by myco- toxin contamination, which is highly important for consumers' health and economic loss. The primary compound of concern is ochratoxin A (OTA), which originated from the producing fungi Aspergilli ochra- ceus that often appears well within limits. Although some surveys revealed levels higher than the statutory limits, the situation is con- tainable, and contamination frequency was often high (Patersona et al., 2014). It is expressed that coffee husks are high in OTA and are fortunately extinguished during coffee processing (Ergin et al., 2021).

CONCLUSION

It has been reported that two cups of coffee per day should be consumed to show beneficial effects. There are 5 g of instant coffee and 10 g of whole bean coffee in one cup. It has been re- ported that four to six cups of coffee per day can have increasing beneficial effects. Well-designed, randomized, and controlled trials should be conducted to investigate the effect of different doses of bioactive components of coffee on patients and healthy individu- als to clarify the important controversially discussed points in the literature. Careful monitoring of coffee is essential to nurture this desirable commodity in the future.

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CITRININ MYCOTOXIN RISK IN BLACK AND GREEN TABLE OLIVES: THE QUICK SIMULTANEOUS DETERMINATION WITH OCHRATOXIN-A BY NOVEL EXTRACTION AND IAC-HPLC

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ABSTRACT

The mycotoxin citrinin (CIT) (and ochratoxin-A (OTA) are formed as a secondary meta- bolic product of the mould fungi Penicillium, Aspergillus, Pythium and Monascus on agricultural products. CIT and OTA were simultaneously identified using immunoafinity column-high performance liquid chromatography with fluorescence detection (IAC-HPLC- FD) (Ex.333 nm; Em:495 nm) after an optimized extraction procedure. The four categories of citrinin levels [0-0.55; 1.56-2.0; 0.66-2.64; 5.76- 14.55 µg kg-1 of CIT] and three categories of ochratoxin levels [0 - < 0.1; 0.1-0.25; 0.30- 0.46 µg kg-1 of OTA] were found in 88 groups of olive samples. Recovery studies [y= 21416x - 7919.4 (R2=0.9998) for citrinin and y= 0.0001x + 0.0074 (R2=0.9999) for ochratoxin A] were performed and the mean analytical recoveries detected in CIT and OTA in table olives ranged from 92.65 - 96.83% and 88.92 - 95.58%, respectively. Limit of detection (LOD) was equivalent to 0.05 µg/kg for both CIT and OTA. With the proposed method, CIT and OTA were both quickly determined in table olives and could be used to detect of mycotoxinic risks in a HACCP quality system of olive and olive-based food products.

Key Words: Table Olives, Mycotoxin, Citrinin, Ochratoxin-A, Extraction, IAC-HPLC

INTRODUCTION

Mycotoxin citrinin (CIT) is a toxic secondary metabolite, isolated from filamentous fungus Penicillium citrinum and is also produced by other species of Penicillium Aspergillus and Monascus (Tokusoglu and Bozoglu,2010; Betina, 1989). Due to antibacterial effects of citrinin, it was investigated as an antibiotic but relative toxicity studies showed that citrinin acting in animals as a nephrotoxin damaged the proximal tubules of the kidney and was implicated as a causative agent in human endemica Balkan nephropathy (Tokusoglu and Bozoglu,2010; Frank, 1992).

The LD_{50} (standardized measure for expressing and comparing the toxicity; the dose that kills half (50%) of the animals tested [LD = “lethal dose”]) of CIT has been reported as about 50 mg/kg for oral administration to the rat 35–58 mg/kg (ip.) to the mouse and 19 mg/kg (ip.) to the rabbit (Anon, 2008).

Black table olives are favorable and 85% of consumable olive is black type. Table olives are more consumed especially as breakfast foodstuff in all the Mediterranean therefore citrinin is a risk for food safety. Known researches on CIT levels in black table olives are limited in the food world. It is reported that 0.45–0.52 µg CIT/kg (average 0.5 µg/kg) was found in black table olives collected growing in areas and markets in Morocco (El Adlouni et al., 2006) and an average of 0.77 µg CIT/kg

was found in 69 black table olives at the Marmara and Aegean region in Turkey in the 2000–2001 period via pre-coated silica gel TLC plates and UV spectrometry in the survey by Heperkan et.al. (Heperkan et al., 2006).Tokusoglu et.al. (2010;2009,2008) reported that effective citrinin and OTA inhibition in black and green olives by optimized HHP processed.

The objectives of this study were to determine the presence of possible citrinin (CIT) levels and to evaluate the degradation levels of citrinin mycotoxin by high performance liquid chromatography (HPLC), to determine total mold-count and total aerobic-mesofilic-bacteria in controlled factory–uncontrolled black table olive market samples after high hydrostatic pressure (HHP) technology.

Preparation method of table olives prior to the HHP process

For 5 category of olives, the stones of olives were removed by a seeder (TM 01/12-inserted-blade cutter, İzm., Turkey), stoneless black table olives were homogenized with a blender (Waring, Torrington, CT, USA) and table olive pastes were obtained.

Fifty grams of each sample in a sterile stomacher bag (Fisher Scientific, Pittsburgh, PA) was homogenized via a stomacher (Inter- science, BagMixer, Cole-Parmer, London, UK) after pre-homogeniza- tion at an aseptically sterilized stainless-steel blender (Waring, Torrington, CT, USA). Homogenized samples were filled to each plastic cryo tubes (Genaxxon BioScience GmbH, Germany) (as 2 g) with aseptic microbiological conditions prior to HHP process. Following homogenized preparation, CIT loading in various concentrations was carried out prior to cryo tube filling of HHP (Tokusoglu et.al.,2010;2009,2008).

High pressure unit

The HHP experiments were carried out in a designed and constructed lab-scale unit (capacity 30cm^3) high pressure cell, where a mixture of deionized water and glycol was used as the isostatic pressure transducing medium. The equipment consists of a pressure chamber of cylindrical design, two end closures, a means for restraining the end closures, a pressure pump, and a hydraulic unit to generate high pressure for system compression and also a temper- ature control device. The pressure vessel was made of hot galvanized carbon steel and piston was hard chrome plated and polished to mirror finish (steel type heat treated special K) which was processed into the required sizes). The liquid was heated prior to pressurization to the desired temperature by an electrical heating system surrounding the chamber. The rate of pressure increase and pressure release was approximately 5–10 s for the designed system (Tokusoglu et.al.,2010;2009,2008).

HHP treatment conditions of olive pastes was justified (R^2= 0.999). Throughout the experiments the olive samples were subjected to HHP treatment for 5min at 250MPa/35±1°C. Pressurization times reported in this study did not include the pressure increase and release times. The unit is capable of operating up to 450 MPa pressure between 25 and 95°C. The cryo vials were placed inside the cylindrical vessel of the HHP equipment, the chamber was closed and the samples were kept for 2 min for temperature equilibration. Duplicate cryo vials filled with black olive paste were used for each experiment.

After pressurization treatment, the cryo vials were removed immediately and cooled in an ice-water bath (4 ± 1 °C) prior to enumeration (CFU ml^{-1}) whereas untreated control samples was held in ice-water bath at atmospheric pressure. The olive paste samples in unpressurized cryo vials were

enumerated as controls. HPLC and GC measurements of bioactive compounds were also carried out immediately after HHP.

CONCLUSION

CIT and OTA were simultaneously identified using immunoafinity column-high performance liquid chromatography with fluorescence detection (IAC-HPLC- FD) (Ex.333 nm; Em:495 nm) after an optimized extraction procedure. The four categories of citrinin levels [0-0.55; 1.56-2.0; 0.66-2.64; 5.76- 14.55 µg kg-1 of CIT] and three categories of ochratoxin levels [0 - < 0.1; 0.1-0.25; 0.30- 0.46 µg kg-1 of OTA] were found in 88 groups of olive samples. Recovery studies [y= 21416x - 7919.4 (R2=0.9998) for citrinin and y= 0.0001x + 0.0074 (R2=0.9999) for ochratoxin A] were performed and the mean analytical recoveries detected in CIT and OTA in table olives ranged from 92.65 - 96.83% and 88.92 - 95.58%, respectively. Limit of detection (LOD) was equivalent to 0.05 µg/kg for both CIT and OTA. With the proposed method, CIT and OTA were both quickly determined in table olives and could be used to detect of mycotoxinic risks in a HACCP quality system of olive and olive-based food products.

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HONEY POWDER AS INTERMEDIATE PRODUCT FOR BIOACTIVE SUPPLEMENT MANUFACTURING

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ABSTRACT

Honey, a natural biological bee-food evolved from plant and fruit nectar and of major benefit to human body as food and drug, contains high sugar such as fructose and glucose (80- 90%) and water, additional to small levels of proteins, minerals, organic acids, and vitamins and phenolic compounds. Owing to its unique aroma and taste as well as its numerous health-promoting properties, honey has been significantly consumed. In this research, honey and gum-arabic (as a carrier) was spray dried by Buchi laboratory type pilot drying unit. The procedure took 60 min with an inlet air temperature of 60ºC and an outlet air temperature not exceeding 36ºC by novel procedure of our developed and particles sizes were in the range of 5-25 μm. The invention relates to the food industry and can be utilized in the health industry for value-added health promoting supplements, for confectionery and ready-to food industry and for commerce products.

KeyWords: Honey powder, Spray-drying, Intermediate product, Food supplement

INTRODUCTION

Honey, a natural biological bee-food evolved from plant and fruit nectar and of major benefit to human body as food and drug, contains high sugar such as fructose and glucose (80- 90%) and water, additional to small levels of proteins, minerals, organic acids, and vitamins and phenolic compounds. In this process study, the aim was the effective honey powder production in food industry branches.

METHOD

This current patent application relates to honey powder. Spraying dryer or cylindrical drier is used in honey powder production. An additive agents of gum arabic are added into honey appropriately and the obtained mixture is fed into said devices and thus powder honey is obtained. In this research, honey and gum-arabic (as a carrier) was spray dried by Buchi laboratory type pilot drying unit. The procedure took 60 min with an inlet air temperature of 60ºC and an outlet air temperature not exceeding 36ºC by novel procedure of our developed and particles sizes were in the range of 5-25 μm. Honey was mixed with additives such as gum arabic agent, and the mixture was spray dried at inlet (60 C) and outlet temperatures (36 C), respectively, which are much optimized than the conditions employed in the other methods.

An improved method of production of spray-dried honey powder was performed. The product had reasonably good honey (REMIKHONEY) content (~52%), characteristic honey flavor, acceptable color (L: 77.87, a: −1.60 & b: 18.71) with a yellow tinge, and a free-flowing nature. By weight quantities of raw material (raw honey) that can be used (%) is as shown in Table.1.

Raw material	*By weight quantities that can be used (%)*
Honey	20 - 80
a member selected from the group consisting of milk, milk powder, cacao powder, whey and whey powder or combinations thereof	80 - 20

Table 1. By weight quantities of raw material (raw honey).

CONCLUSION

The effective and stabile honey powder as intermediate product for bioactive supplement in food industry manufacturing units and its shelf life was 18 month. The invention relates to the food industry and can be utilized in the health industry for value-added health promoting supplements, for confectionery and ready-to food industry and for commerce products.

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APPLICATION OF FLUORESCENCE SPECTROSCOPY FOR THE ANALYSIS OF DIFFERENT GARLIC ACCESSIONS AFTER 9 MONTHS' STORAGE IN A WIREHOUSE

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ABSTRACT

The present study aims to establish the application of fluorescence spectroscopy in the comparison of garlic accessions from different branches. The garlic samples were stored in a warehouse, under uncontrolled conditions. This will allow the method to be applied as a non-invasive rapid tool to establish the origin of unknown bulbs during storage.The experimental investigations were carried out locally in the warehouse for 8 accessions, 9 months' storage. The spectral setup for the generation of emission fluorescence spectra is mobile and has applicability in local product quality assessments. A system engineering approach based on the classical principles of modern optoelectronics was applied, during its adjustment (optical setting up). The results of the experiment will be able to be applied to the optimization of the analysis time of garlic samples, in a storage room, under uncontrolled conditions. This will support the breeding process in its initial stages, when it is necessary to qualify a large set of samples in a short time. Application of fluorescence spectroscopy for the analysis of different garlic accessions after 9 months' storage in a warehouse will be an applied tool in breeding programs.

Keywords: Fluorescence spectroscopy, Garlic accessions, Warehouse, Uncontrolled conditions, 9 Months' storage

INTRODUCTION

Garlic (*Allium sativum* L.) is a vegetable crop, substantial in nutrients and used primarily as a spice. It has healing properties. The greatest diversity of garlic forms is concentrated within Central Asia. This abounding genetic pool contains genes of interest for use in future genetic studies and breeding programs (Kamenetsky et al., 2007). Genotypic solution of garlic to storage duration has been confirmed in many studies (Shokrgozar et al., 2013; Darabi, 2018).

On the territory of Bulgaria, almost all regions of the country are suitable for growing garlic. In recent years, there has been an increase in the area, which is 560 ha (Agrostatistics, 2022).

Fluorescence spectroscopy is an important research tool in many analytical areas of science. Methods using this phenomenon of light are highly sensitive, do not take a long time, and do not require the expense and difficulty of working with radioactive tracers (Sikorska et al., 2019; Wolfbeis, 2012). The effects of light applicable to spectral analysis such as fluorescence, transmission and diffuse reflectance can be used for quality control of vegetable crops.

Advances in fiber optic technology offer outstanding opportunities for the development of a wide range of highly sensitive fiber optic sensors in many new application areas. Fiber-optic components have been successfully adapted to assemblies with micro-optics elements such as lenses, mirrors, prisms, gratings, and others (Dakin & Brown, 2006; Mitchke, 2010).

The effects of light applicable to spectral analysis such as fluorescence, transmission and diffuse reflectance can be used for quality control of vegetable crops, incl. with garlic. The spectral distribution of the emission signal in garlic mainly consists of two maxima in the visible range. The intensity and shape of the fluorescence emission spectrum at room temperature depend mainly on the concentration of the fluorophores and to a lesser extent on the structure, photosynthetic activity and arrangement of the cells in the tissue (National Research Council, 1968; Leo et al., 2007).

The aim of the present study is to establish the application of fluorescence spectroscopy for the analysis of different garlic accessions after 9 months' storage in a warehouse under uncontrolled conditions.

MATERIAL AND METHOD

The samples that are the subject of the study are Razgradski 11 (characterized by medium-large bulbs with closely spaced cloves. The bulb weighs 40 g), Plovdivski 157 (characterized by medium-large to large bulbs with loosely spaced cloves. Bulb is with weight 50 g), Strelkuvasch 4 (characterized by large bulbs weighing 60 g, with loosely spaced cloves), Razgradski 117 (characterized by large bulbs weighing 65 g, with loosely spaced cloves), Razgradski 80 (characterized by large bulbs weighing 60 g with loosely spaced cloves), Razgradski 4 (characterized by medium-large bulbs weighing 40 g, with closely spaced cloves), Razgradski 119 (characterized by medium-large bulbs weighing 45 g.) and Montanski 113 (characterized by large bulbs weighing 65 g, with closely spaced adherent cloves).

The experiment was conducted at the Institute of Vegetable Crops "Maritsa" - Plovdiv, with an experimental plot area of 4.8 m2, according to a scheme of 85+25+25+2525x6 cm according to the accepted technology for growing ripe garlic (Bachvarov et al., 1990). The garlic was planted in mid-October and harvested in mid-June, after which it was left to dry in a storage room. The agrotechnical measures were carried out in the optimal terms for the crop. For the analysis of different garlic accessions after 9 months' storage in a warehouse, fluorescence spectroscopy has been applied.

The research was carried out with a fiber-optic spectrometer, which allows the generation of emission fluorescence signals from 200 nm to 1200 nm. The device is used for fluorescence spectroscopy of solid samples with a photosensitive area of 1.9968×1.9968 mm.

The experimental setup includes a laser diode (emission wavelength 285 nm, optical power 16 mW, DC), a portable spectrometer model AvaSpec-ULS2048CL-EVO. The sample (garlic accessions) is placed on a duralumin stand, which allows reception of an emission signal from it under 180o by a U-shaped optical fiber. This reduces aberrations and allows the generation of a better quality emission fluorescence signal (Fig.1). The resolution of the spectrometer can be in the range of 0.06 - 20 nm, with that of the setup used for our experiment being 0.09 nm. It is preferable to use a laser diode (LED) as a source in the circuit, since its spectral width is very small. The LED used in the experiment has a relatively wide emission spectral width of about 30-40 nm and the angular distribution of its emission is in a large angular range of $+/-30^0$. The sensitivity of the spectrometer is in the range from 200 nm to 1200 nm. Its resolution is $\delta\lambda = 5$ nm.

The spectral setup based on fluorescence signals allows recording both the emission spectrum and the spectrum of the excitation source. The emission spectrum represents the wavelength distribution of an emission measured at a constant excitation wavelength. The excitation spectrum is the dependence of the emission intensity measured for one scanning wavelength against the excitation wavelength. This spectrum is represented as a function of the wavelength of the light intensity incident on the photodetector in the spectrometer.

For the particular circuit, the photodetector is of the CMOS model S9132 type.

The laser radiation is deflected from the source and hits the specimen. After the sample fluoresces, the emission signal is incident on a U-shaped optical fibre with a core diameter of 200 μm a step index of refraction, and a numerical aperture of 0.22. It takes him to the detector. In the spectrometer, the light signal is converted to electrical-digital using a USB 2.0 wire, downloaded to a computer with AvaSoft8 software, and exported to Excel. This allows analysis, processing, and visualisation of the results of the conducted research.

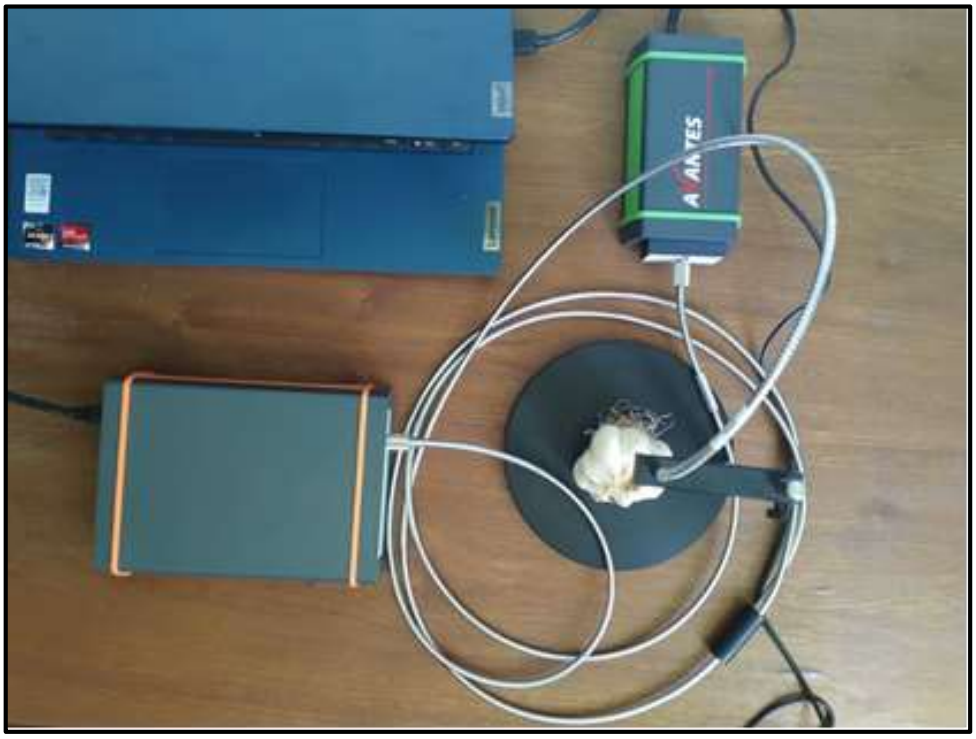

Figure 1. General view of the experimental installation used by fluorescence spectroscopy

RESULTS AND DISCUSSION

The optical properties of garlic are determined by its energy structure, which includes both the occupied and free electronic energy levels, as well as the energy levels of the atomic vibrations of the molecules or the crystal lattice. The possible transitions between these energy levels, as a function of photon energy, are specific to garlic, resulting in spectra and optical properties unique to it. Garlic contains particles smaller than the wavelength of visible light. Particles in the turbid medium, such as garlic, act as independent light sources, emitting incoherently, causing the samples to visibly fluoresce.

Therefore, fluorescence spectroscopy finds application for analysis in this vegetable crop. The optical parameters and spectral properties also change as a function of temperature, pressure, external electric and magnetic fields, etc., which allows obtaining essential information about changes in the chemical and cellular morphological composition of garlic.

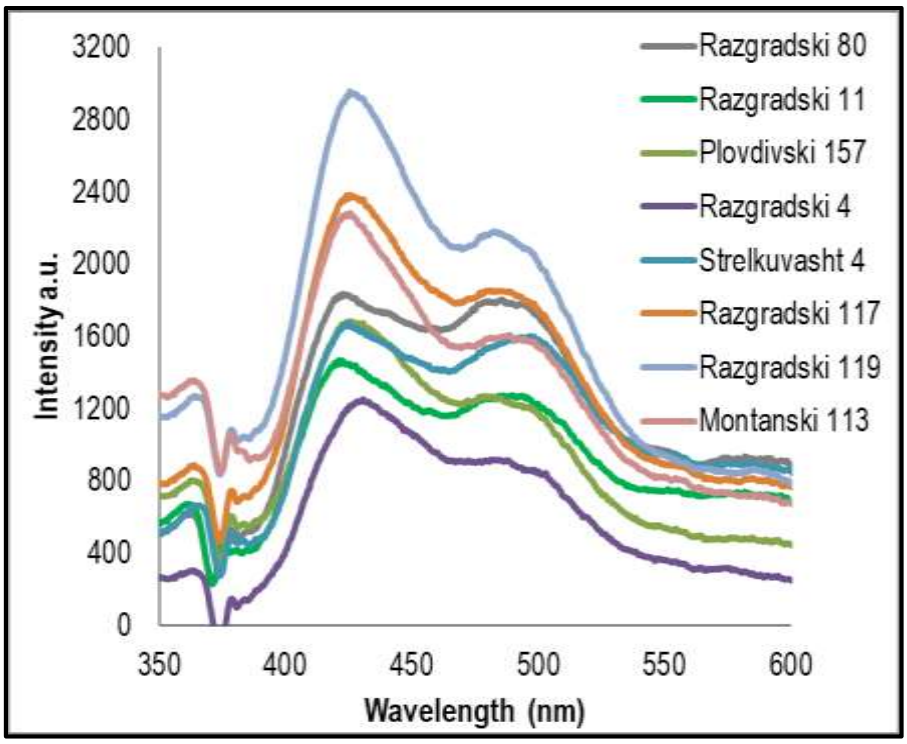

Figure 2. Difference in emission wavelengths in accessions

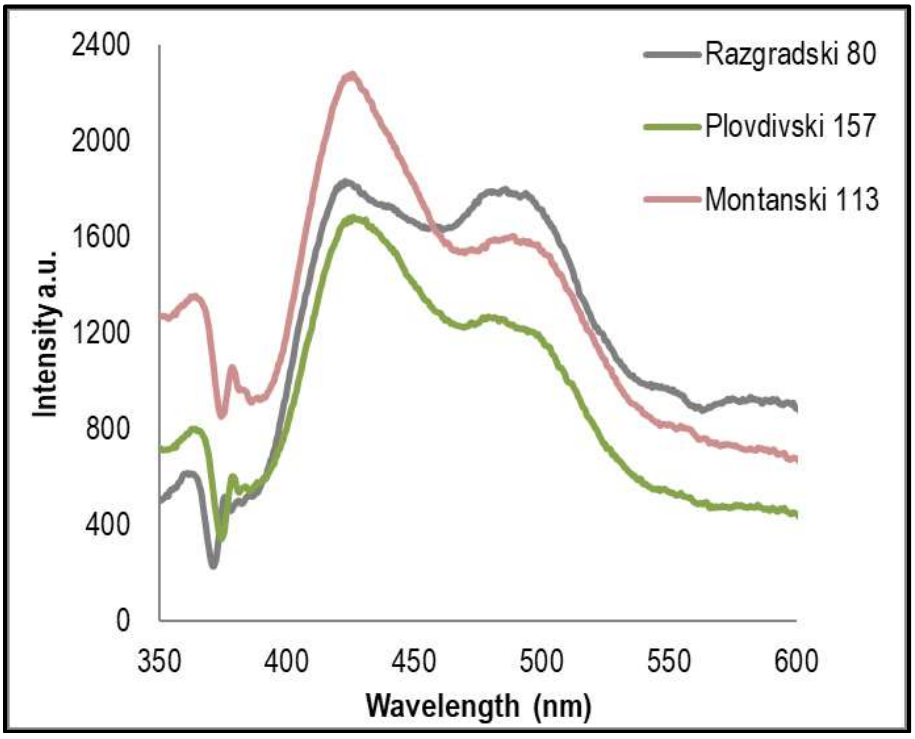

Figure 3. Difference in emission wavelengths in accessions breeding in a different geographical region of Bulgaria

A literature survey was conducted to conduct similar research. It turned out that until now the described experimental approach for analysis of different garlic accessions after 9 months' storage in a warehouse has not been applied nationally and internationally. This gives us grounds to claim that, for the first time, there has been an application of fluorescence spectroscopy for analysis of different garlic accessions after 9 months' storage in a warehouse under uncontrolled conditions.

Eighty garlic samples (10 samples of each genotype) after 9 months of storage in a warehouse were analyzed by the fluorescence spectroscopy method individually. In fig. 1, a clear difference in the spectral distribution and wavelength value of each genotype is observed. In fig. 2 there is a clear difference in the spectral distribution and value of the wavelength of genotypes bred in different geographical regions of Bulgaria,

The most suitable excitation wavelength was found to be 285 nm. This is due to the fact that there is only a small difference in the emission wavelengths between the genotypes bred in the same geographical region: Razgradski 11, Razgradski 117, Razgradski 80, Razgradski 4 and Razgradski 119.

The three main advantages of fluorescence spectroscopy are that the method is fast, does not require consumables, and can be performed in situ in the warehouse under uncontrolled conditions. The decision for local measurements was made to avoid damage to the samples during transport and thus, to ensure fluorescence analysis with high sensitivity.

The signal intensity is sufficiently high for genotypes grown in the same geographical region, which means that the method is applicable to the analysis of bulbs grown in the same geographical region stored in warehouses under uncontrolled conditions.

CONCLUSIONS

The fluorescence spectroscopy method is rapid in determining the origin of unknown garlic bulbs after 9 months of storage. This method has been shown to aid the breeding process when a large set of accessions need to be qualified in a short time. A systems engineering approach to the alignment (optical tuning) of a dedicated fluorescence spectroscopy applied research facility was found to be applicable to the characterization of garlic production after 9 months of storage.

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WEEDS AND THEIR MANAGEMENT IN SUNFLOWER FIELDS IN PAŞAELİ, TURKEY

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ABSTRACT

European part of Turkish territory, called Paşaeli, is an important sunflower growing area of Türkiye with half of sunflower production. A field study was carried out in three provinces of Paşaeli, namely Edirne, Tekirdağ, and Kirklareli to find out weeds and weed management methods in June, July and August of 2019. Five fields were chosen from a village of each province. The number of weed species were 9, 14, and 26 for Edirne, Tekirdağ, and Kırklareli, respectively out of 36 species. Wheat-sunflower rotation is the main system with some exceptions. Tillage after harvest and soil preparation with a second-class equipment in late March-early April are the main cultivations. Machine hoeing and herbicides are main weed control methods although it chances among provinces. Due to use of imidazolinone resistant varieties, imazamox is the most common herbicide followed by ACCase herbicides to control grass weeds. Xanthium strumarium was the most common weed in all observations but higher densities were found in August. It is concluded that more species in Kırklareli is result of less weed control implementation and less species in Edirne is result of more weed control implementation including mechanical control and herbicides.

Keywords: Edirne, Kırklareli, Tekirdağ, İmidazolinone resistant sunflower, mechanical control, ACCase

INTRODUCTION

Sunflower is the most cultivated and produced oilseed crop in Türkiye where is among the top ten producers of world. In addition, its production for snack is common in Türkiye but Paşaeli mainly produces for oil industry. The area harvested for sunflower and production have steadily increasd in Türkiye as well as world total (FAOSTAT, 2022). More than half of the sunflower seeds in Türkiye has been produced in the Marmara Region where is the study was conducted. Tekirdağ Province has produced the highest amoun with a share of 18.74% (Table 1). The other two provinces included in this study. Edirne and Kırklareli rank third and fifth with a share of 12.45% and 9.86%.

In the Marmara Region, sunflowers are grown using the dry farming system as in many other regions of Turkey. Therefore, the high fluctuation of yield and production depending on the precipitation and drought factors is one of the leading issues in sunflower production in Türkiye (Kaya, 2016). Apart from environmental factors, diseases, pests, and weeds pose a problem in the areas where sunflower is grown (Yeşilayer et al., 2016). Weeds can reduce sunflower yield upto 76% depending on environmental conditions, crop husbandary techniques, weed composition and densities, and duration of competition (Durgan et al., 1990; Carranza et al., 1995; Blamey et al., 1997; Erol, 2010; Lewis and Gulden, 2014; Mukhtar et.al., 2018). Furthermore, as less as 0.13 *Kochia scoparia* (L.) Schrad plant in a-meter row caused upto 14% yield loss (Durgan et al., 1990). Even quality of sunflower seed was reduced due to weed competiton despite no effect on sunflower yield was seen (Lewis et al., 2016) and effect on oil was observed (Nalejeva et al., 1972; Erol, 2010). Broomrape can reduce sunflower yields by up to 98% (Castejon-Muñoz et al., 1993).

Table 1. Top ten provinces of Türkiye with the highest sunflower production (ZMO. 2019)

Provinces	2013		2014		2015		2016		2017	
	Amount (Tons)	Share (%)	Amount (Tons)	Share (%)	Amount (Tons)	Share (%)	Amount (Tons)	Share (%)	Amount (Tons)	Share (%)
Tekirdağ	211.671	13.90	260.753	15.92	267.012	15.89	283.838	16.99	368.125	18.74
Konya	266.775	17.52	268.751	16.41	217.634	12.95	212.312	12.71	263.008	13.39
Edirne	175.857	11.55	258.568	15.79	226.573	13.48	222.064	13.29	244.655	12.45
Adana	100.677	6.61	89.565	5.47	134.361	7.99	166.524	9.97	195.225	9.94
Kırklareli	146.682	9.63	165.206	10.09	188.998	11.25	17.278	10.19	193.784	9.86
Çorum	47.739	3.13	38.297	2.34	53.189	3.16	59.069	3.54	75.157	3.83
Tokat	47.096	3.09	33.740	2.06	41.593	2.47	39.306	2.35	41.549	2.12
Eskişehir	43.101	2.83	35.520	2.17	29.281	1.74	30.553	1.83	39.993	2.04
Samsun	33.018	2.17	27.652	1.69	39.083	2.33	35.546	2.13	38.253	1.94
Balıkesir	27.837	1.83	26.483	1.62	30.609	1.82	30.555	1.83	37.923	1.93

In Türkiye, 231 plant species have been reported from sunflower fields of which three pteridophyte, seven parasites, and 28 monocotylodones (Uludağ et al., 2021). Weed species and their distributions in sunflower fields have been determined in different parts of Türkiye (Zengin, 1999; Karabacak and uygur, 2017; Asav and Serim, 2019; Özkil et al., 2019). The negative effect of weeds should be controlled, mitigated. Different weed control methods can be applied from mechanical to biotechnological (Blamey et al., 1997; İyigün, 1997; Yücel, 2011; Tursun et al., 2017; Kaya et al., 2018; Özen, 2021; Torun et al., 2021; Uludag et al., 2021). The suggested economic threshold for herbicide usage in sunflower fields in Ankara conditions were calculated as low as 0.61-1.97 cocklebur (*Xanthium strumarium* L.) m^{-2} (Başaran et al., 2017). Some years critical period for weed control starts very early such as the 2nd week or emergence of crop and lasts longer upto 10th week (Çoruh and Zengin, 2009; Kaya et al., 2020; Özkil et al., 2022).

In Paşaeli area, weed species and their distributions have been determined in Edirne and Tekirdağ Provinces and control studies have been conducted (Arslan & Kara, 1997; Erol, 2010; Yay, 2015; Kaya et al., 2018). But there is no study in Kırklareli Province and no comparision weed flora and agronomic techniques in Paşaeli sunflower culture. The aim of current study is to determine crop husbandary applications and compare them with weed flora in sunflower production.

MATERIAL AND METHOD

This study was conducted in Balaban village of Uzunköprü District in Edirne Province (41.089293. 26.547830). Kofçaz District of Kırklareli Province (41.945747. 27.158770) and Alaybey village of Malkara District in Tekirdağ Province (40.860645. 26.997883) in 2019.

Weed observations were carried out in three different times in June, July, and August in five fields determined in each of these three locations. In each field, weeds were determined and their covarage as percentage were assessed in 10 random 3 m^{-2}-points that were set 3 meter on a row. Weed samples were taken for identification. The history of the fields and implementations in the study year, 2019 were determined asking or observing to producers. No intervention was made to the farmer during the season to be able to get direct views'.

RESULTS AND DISCUSSION

Wheat-sunflower rotation has been common system in all provinces for a decade. Barley replaced by wheat in field E3 and barley and rarely maize in field K4 in some years. Fallow was applied in field E1 in 2017, field E4 in 2018, and field E5 in 2017 and 2018.

Following the harvest in 2018, deep plowing was common practices in all provinces. Seedbeds were prepared using disc harrow followed by toothed harrow in Edirne; cultivator followed by toothed harrow (fields T1, T3, T5) or by rototiller and toothed harrow (fields T2 and T4) in Tekirdağ; and disc harrow (fields K1 and K2) rototiller (K3) and rototiller followed by disc harrow (K4 and K5) in Kırklareli from late March to early April. Seeds were planted mainly in mid April in Tekirdağ and early May in the other two provinces (Table 2). The most used seeds were imidazolinon resistant ones.

After sunflower harvest, in the provinces of Tekirdağ and Kırklareli, the stems are chopped with a shredding machine and mixed with the soil with a cultivator or rotary tillers.

Table 2. Farmer's practices in 2019

Field Code	Field Area (ha)	Planting Date (dd.mm.2019)	Variety sown*	Mechanical Intervention and date	Herbicide
E1	2.2	03.05	P64LC108	Hand hoe (June. July)	S-Metolachlor (312.5 g/l) +Terbuthylazine (187.5 g/l)
E2	2.5	07.05	P64LL62	Hand hoe (end of may. June) Plucking by hand (July. August)	Imazamox (40 g/l)
E3	1.9	30.04	P64LC108	Machine hoe (20th day of May) Plucking by hand (twice in July)	Imazamox (40 g/l) (June) and Quizalofop-p-ethyl (50 g/l) (Temmuz)
E4	2.8	20.05	P64LC108	Machine hoe (15th day of June)	Imazamox (40 g/l) (end of June)
E5	3.2	05.05	P64LC108		Imazamox (40 g/l) (early June)
T1	1.8	13.04	P64LE119	Machine hoe (early May)	Quizalofop-p-ethyl (50 g/l) Imazamox (40 g/l) (Middle of May)
T2	1.6	15.04	P64LC108	Machine hoe (early May)	-
T3	2.4	10.04	P63LE113	Machine hoe (early May)	Imazamox (40 g/l) and Quizalofop-p-ethyl (50 g/l)
T4	1.9	12.04	P64LC108		Imazamox (40 g/l) and Quizalofop-p-ethyl (50g/l)
T5	2.7	25.04	P64LE119		Imazamox (40 g/l) and Quizalofop-p-ethyl (50 g/l)
K1	6.0	04.05	P64LL62	Machine hoe (end of May)	Imazamox (40 g/l) (28th day of May)

K2	50.0	04.05	P64LL62		Aclonifen (600 g/l) (22th day of May) (This herbicide is for Monocots)
K3	1.3	03.05	P64LL62	Inter-row tillage with rototiller (Twice in June)	Imazamox (40 g/l) and Quizalofop-p-ethyl (50 g/l) (3rd day of June)
K4	9.0	25.04	P64LL62	Machine hoe (some time after herbicide applications)	Imazamox (40 g/l) and Quizalofop-p-ethyl (50 g/l) (5th day of May)
K5	9.0	28.04	P64LC108 Mas 80 Mas 87	Machine hoe (28th day of May)	Clodinafop-propargyl (240 g/l) (8th day of May) and Imazamox (40 g/l)

* The characteristics of the varieties can be seen at following literature: Pioneer. 2020; Maasseeds. 2019.

In this study, 9 species belonging to 8 families in Edirne, 14 belonged to 7 families in Tekirdağ, and 26 weed species belonging to 12 families in Kırklareli were identified with total of 32 weed species belonging to 12 families were detected (Table 3). The difference in the number of species among provinces can be attributed to difference among farming implementations and/or locations. Less intervention in fields including tillage and herbicide applications might lead less weed species in Krıklareli province comparing to the other two.

Table 3. Weed species identified iaccording to provinces

WEED		**PROVINCES**		
		Edirne	**Tekirdağ**	**Kırklareli**
Anthemis arvensis L.	Asteraceae			√
Anthemis cotula L.	Asteraceae			√
Anthemis pseudocotula Boiss.	Asteraceae		√	
Avena fatua L.	Poaceae	√	√	
Asperula arvensis L.	Rubiaceae			√
Amaranthus retroflexus L.	Amaranthaceae	√		√
Bromus sterilis L.	Poaceae			
Convolvulus arvensis L.	Convolvulaceae	√	√	√
Chenopodium album L.	Chenopodiaceae	√	√	√
Cynodon dactylon (L.) Pers.	Poaceae	√		√
Centaurea solstitialis L.	Asteraceae			
Digitaria horizontalis Willd.	Poaceae		√	
Digitaria sanguinalis (L.) Scop.	Poaceae			√
Datura stramonium L.	Solanaceae			√
Echinochloa crus-galli (L.) P.Beauv.	Poaceae		√	√
Lolium perenne L.	Poaceae			√
Lolium multiflorum Lam.	Poaceae			√
Lolium rigidum Gaudin	Poaceae		√	
Lithospermum officinale L.	Boraginaceae			√
Mentha sp.	Lamiaceae			√
Persicaria maculosa Gray	Polygonaceae			√
Phleum exaratum Griseb.	Poaceae		√	√
Polygonum bellardii All.	Polygonaceae			√

Portulaca oleracea L.	Portulacaceae	√	√	√
Rapistrum rugosum (L.) All.	Brassicaceae		√	
Rapistrum raphanistrum L. Crantz	Brassicaceae	√		√
Pennisetum glaucum (L.) R. Br.	Polygonaceae			√
Solanum americanum L.	Solanaceae	√	√	√
Silybum marianum (L.) Gaertn.	Asteraceae		√	
Volunteer wheat				√
Xanthium spinosum L.	Asteraceae			√
Xanthium strumarium L.	Asteraceae	√	√	√

The distribution of weed species was revealed with field observations. Xanthium strumarium and Convolvulus arvensis are the most common weed species in three provinces followed by Portulaca oleracea and Chenopodium album (Appendix 1). Lolium spp was common in Tekirdağ and Kırklareli while Digitaria spp in Edirne and Kırklareli. Amaranthus retroflexus, Echinochloa crus galli, Silybum marianum, and Solanum Americanum in Tekirdağ, Anthemis spp. and Cynodon dactylon in Kırklareli was recorded mainly. Xanthium strumarium was the densest weed species (Appendix1 and Appemdix 2). Weed coverage was higher in Kırklareli in general and least in Edirne. Less herbicide application and other methods for weed control were applied in Kırklareli comparing to the others.

Weed species have similarities beween two surveys in Tekirdağ province in 1996-97 (Arslan and Kara, 1997) and 2019 although the number of species and families only half of the earlier study, which is normal because of the difference in size between teo study areas. Chenopodium album ve Convolvulus arvensis had been the most common and dense species before 2000 (Arslan, 1998; Arslan and Kara, 1997) but Xanthium had been detected only half of the surveyed field, which was more common in 2019 as well as 2010 (Erol, 2010). Cynodon dactylon was denser in the earlier study in Tekirdağ (Arslan and Kara, 1997).

Less weed species were reported from sunflower fields of Edirne comparing to sueveys in Tekirdağ (Arslan, 1998; Arslan and Kara, 1997; Yay, 2015) as it was found out in current study. *Portulaca oleraceae*, *Xanthium strumarium,* and *Convolvulus arvensis* were important weeds in Edirne in 2013 and 2019. But, Cynodon dactylon was impoertant in 2013 while chenopodium albüm in 2019. In spite of differences earlier studies and the current one, important species did not changed much.

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Appendix 1. Weed coverage according to fields in three different estimation times

FIELD CODE	**PLANT NAME**	**Weed coverage (%)**		
		1st Count	**2nd Count**	**3rd Count**
E1	*Convolvulus arvensis*	4.20	7.33	11.00
	Solanum americanum	6.33	4.00	3.50
	Xanthium strumarium	18.30	15.00	23.60
E2	*Chenopodium album*	2.66	2.00	5.00
	Convolvulus arvensis	5.00	3.00	5.00
	Portulaca oleraceae	3.33	2.25	3.50
	Xanthium strumarium	7.33	4.75	15.50
E3	*Chenopodium album*	3.75	2.00	7.50
	Convolvulus arvensis		3.00	4.25
	Portulaca olaraceae	1.75	3.00	3.33
	Xanthium strumarium	4.75	3.22	9.12
E4	*Amaranthus retroflexus*		1.50	7.50
	Avena fatua	2.00		
	Convolvulus arvensis		4.00	3.50
	Portulaca oleraceae	2.66		
	Xanthium strumarium	1.88	3.57	6.85
E5	*Amaranthus retroflexus*	2.25	1.66	3.50
	Convolvulus arvensis	2.00	3.00	5.00
	Cynodon dactylon	2.66	3.00	5.00
	Portulaca olaraceae	2.33	1.66	5.00
	Xanthium strumarium	2.60	2.71	16.43
T1	*Anthemis pseudocotula*	3.00		5.00
	Avena fatua			2.66
	Convolvulus arvensis		2.50	4.00
	Chenopodium album		3.66	3.00
	Digitaria horizantalis	1.00		

	Digitaria cf.	1.50		
	Echinocloa crus-galli	1.33		
	Lolium rigidum	3.00		
	Portulaca oleracaea	2.50		
	Silybum marianum		5.00	
	Solanum americanum		3.00	4.50
	Xanthium strumarium	2.50		
T2	*Amaranthus retroflexus*	3.50	3.00	
	Convolvulus arvensis	4.00		4.33
	Chenopodium album		3.00	3.00
	Centaurea solstitialis	3.50	4.00	
	Digitaria horizantalis			5.00
	Digitaria cf.	2.00	2.00	
	Echinocloa crus-galli	3.00	5.00	3.00
	Lolium rigidum	2.50		
	Phelum exaratum			4.00
	Portulaca oleracaea	2.00		
	Silybum marianum		2.00	3.00
	Solanum americanum		3.33	
	Xanthium strumarium	1.00		
T3	*Amaranthus retroflexus*	4.00		
	Anthemis pseudocotula			1
	Convolvulus arvensis	3.00	5.00	4.33
	Chenopodium album	2.50	5.25	4.00
	Digitaria horizantalis			1.50
	Lolium rigidum	1.33	3.50	4.50
	Phelum exaratum			3.50
	Portulaca oleracaea	5.00		
	Rapistrum rugosum		4.50	
	Xanthium strumarium	2.33		
	Solanum americanum		3.50	5.00
T4	*Amaranthus retroflexus*	2.66	2.83	
	Convolvulus arvensis		2.00	9.00
	Chenopodium album	2.50	3.37	7.00
	Digitaria cf.	1.00	1.00	
	Echinocloa crus-galli	2.00	2.25	
	Lolium rigidum	2.00	2.00	
	Phelum exaratum			2.33
	Portulaca oleracaea	2.66	1.83	
	Rapistrum rugosum	3.00	3.00	3.00
	Silybum marianum		1.00	
	Solanum americanum		2.66	3.00
	Xanthium strumarium	1.75	1.37	
T5	*Amaranthus retroflexus*	1.33		
	Convolvulus arvensis		2.00	4.00
	Chenopodium album	2.33	2.33	3.50
	Digitaria horizantalis			3.33

	Echinocloa crus-galli		2.00	2.66
	Lolium rigidum	3.00		3.00
	Portulaca oleracaea	2.00		4.50
	Rapistrum rugosum		3.50	
	Silybum marianum		1.00	
	Solanum americanum		2.33	
	Xanthium strumarium	1.25		
K1	*Anthemis arvensis*	2.00	15.33	
	Bromus sterilis	5.00		
	Chenopodium album	45.00	41.66	16.66
	Cynodon dactylon		1.00	7.00
	Digitaria sanguinalis		5.00	
	Echinochloa crus-galli	60.62	2.00	2.00
	Persicaria maculasa		1.00	
	Pheleum exaratum		15.00	
	Polygonum bellardi	15.00		
	Portulaca oleraceae		1.00	
	Raphanus raphanistrum	15.50		
	Setaria glauca			32.00
	Xanthium strumarium	5.00	47.50	38.60
K2	*Amaranthus retroflexus*		5.00	
	Chenopodiumalbum	27.50	4.66	
	Convolvulus arvensis	31.66	1.00	
	Condrilla juneae	1.00	1.00	2.00
	Cynodon dactylon		1.00	
	Digitaria sanguinalis			5.00
	Echinochloa crus-galli	11.00	1.00	8.33
	Lolium multiflorum	1.50	1.00	
	Mentha cf.	3.50		
	Pheleum exaratum	3.50	1.00	
	Raphanus raphanistrum	34.20	1.50	
	Solanum americanum		19.28	29.00
	Xanthium strumarium		41.66	2.00
	Xanthium spinosum	1.00	1.50	
K3	*Amaranthus retroflexus*		8.50	1.00
	Anthemis cotula	2.00	2.00	
	Chenopodium album	41.25		
	Convolvulus arvensis			1.00
	Cynodon dactylon		16.66	2.00
	Digitaria sanguinalis		2.00	23.12
	Lolium rigidum	3.00		
	Mentha cf.	1.00	5.00	
	Pheleum exaratum	1.00	17.00	
	Raphanus raphanistrum	64.00		
	Rumex acetosella	1.00		
	Solanum americanum	1.00	44.50	23.33
K4	*Anthemis arvensis*	1.00	1.50	

	Chenopodium album	25.50	3.57	4.50
	Convolvulus arvensis	1.00	3.50	2.00
	Cynodon dactylon			1.00
	Digitarias anguinalis		3.50	16.75
	Lithospermum officinale	1.00		
	Lolium multiflorum		1.00	
	Rumex acetosella	1.00		
	Kendi Gelen Buğday	1.00		
	Pheleum exalatum	2.50		
	Portulaca oleraceae		2.00	1.00
K5	*Anthemis cotula*	38.33	2.00	
	Asperula arvensis		5.00	
	Chenopodium album	13.60	2.50	8.57
	Convolvulus arvensis	3.00		
	Datura stramonium		5.00	
	Digitaria sanguinalis		2.00	13.33
	Echinochloa crus-galli	7.50	4.80	4.25
	Lolium perenne	7.40		
	Portulaca oleraceae	2.00	2.00	1.00
	Setaria glauca		1.00	
	Xanthium spinosum		5.00	

Appendix 2. Densities of weeds in Edirne province fields

FIELD CODE	**PLANT NAME**	**Weed density (individuals/3 m²)**		
		1st Count	**2nd Count**	**3rd Count**
E1	*Convolvulus arvensis*	6	4	3
	Solanum americanum	4	3	2
	Xanthium strumarium	15	7	16
E2	*Chenopodium album*	3	2	2
	Convolvulus arvensis	1	1	1
	Portulaca oleraceae	3	5	2
	Xanthium strumarium	12	12	15
E3	*Chenopodium album*	6	3	3
	Convolvulus arvensis	0	1	4
	Portulaca olaraceae	4	2	3
	Xanthium strumarium	11	9	11
E4	*Amaranthus retroflexus*	0	2	3
	Avena fatua	1	0	0
	Convolvulus arvensis	0	1	2
	Portulaca oleraceae	4	0	0
	Xanthium strumarium	9	7	8
	Rapistrumraphanistrum	0	1	0
E5	*Amaranthus retroflexus*	3	3	2
	Convolvulus arvensis	2	3	2
	Cynodon dactylon	4	0	1
	Portulaca olaraceae	4	4	2
	Xanthium strumarium	7	8	10

THE STUDY ON SELECTIVE MARKERS FOR BROOMRAPE RESISTANCE IN SUNFLOWER

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ABSTRACT

Oilseed production is not enough for Turkey and this deficiency is compensated by importing oilseed or crude oil by paying higher amounts. One of the most important factors affecting the yield and quality of sunflower which is the most important oil crop in Turkey is broomrape parasite. Therefore, new developed sunflower hybrids should be resistant to broomrape. The transfer of these resistance genes to the cultivated sunflower from wild species is very important to obtain continued resistance. The use of molecular methods in breeding studies shortens the breeding period by providing an effective and accurate selection, as well as saving time for studies. In the present study, it is aimed to detect new Or5/Or6 source in wild sunflower species and to develop a molecular marker to be used for selection in transferring to cultivars. 9728A sunflower inbred line with having Or5 resistance gene and 2517B inbred line which is susceptiblewere used as parent lines and obtained F2 genotypes as a result of crossing were used for marker analyses in the study. Resistant F2 bulk (DB) and susceptibleF2 bulk (HB) were formed by combining gDNAs belonging to resistant and susceptibleF2 genotypes in equal amounts. For the detection of markers to be used in the selection of the selective Or5 and Or6 genes, 40 primers with high polymorphism were selected among the degenerate RGA primers. These primers were designed specifically for the conserved P-loop, kinase-2, kinase-3a, GLPLAL and LRR regions found in the resistance genes of various plants and 52 different PCR for performing in the combination. In addition, RGA172, RGA206, RGA192, RGA181 ORS1036, ORS665, ORS1114, ORS1021 markers reported for Or5 and Or6 in the literature were used.

Keywords: Sunflower, Wild species, Broomrape, Molecular markers

INTRODUCTION

The sunflower (*Helianthus spp.)* genus, which is native to North and Central America, consists of 51 species as 37 perennial and 14 annual ones. Sunflower (*Helianthus annuus* L.) is one of the most important oil crops for the world and Turkey. With the ever-increasing population, the vegetable oil need of Turkey is constantly increasing. The fact that sunflower oil is generally preferred in the use of vegetable oil in Turkey and then the importance of sunflower has increased with ever-increasing need for oil. Although sunflower has the ability to adapt to widespread areas, the cultivated areas are not larger and the seed yield does not increase as expected for many reasons. Therefore, it is necessary to take precautions to increase the cultivation of sunflower in large areas.

Besides being one of the most important vegetable oil sources, sunflower (*Orobanche cumana* Wallr.) broomrape parasite is one of the most important problems of Turkey in sunflower production. Broomrape is a serious problem of sunflower in Eastern Europe, the Balkans and the Mediterranean

(Parker and Riches, 1993). Numerous and small-sized seeds of broomrape quickly cause pollution in sunflower fields. Depending on the development stage of the sunflower during infection, the sunflower yield losses can vary between 5% and 100% (Miladinovic et al., 2014). Broomrape were observed in many countries such as Serbia, Hungary, Moldova, Greece, Tunisia, Israel, Iran, Kazakhstan, China, Mongolia and Australia, as well as the world's leading sunflower producing countries such as Russia, Ukraine, Romania, Bulgaria, Turkey and Spain (Molinero-Ruiz et al., 2009; Pacureanu-Joita et al., 2012; Amri et al., 2012; Kaya 2014; Miladinovic et al., 2014; Marinkovic et al., 2014). The most successful method for controlling broomrape in the world is the use of resistant varieties. Although resistant varieties have been developed with intensive efforts in this direction, the broomrape parasite has also evolved and continues to exist with new races with high virulence characteristics (Gonzalez, 2021) (Table 1).

Table 1. Broomrape races distributed in the world (Molinero-Ruiz et al., 2015; Gonzalez, 2021)

Country	Races in the past	Current races	References
Bulgaria	A, B, C, D, E	E, F, G, H	Shindrova, 2006; Batchvarova, 2014; Encheva 2018
China	A	A, B, C, D, E, F, G	Ma & Jan, 2014; Shi et al., 2015; Zhang et al., 2018
Germany	A, B, C, D	E, F	Zoltán, 2001; Hargitay, 2014; Molinero-Ruiz et al., 2014
Hungary	A, B, C, D	E, F	
Kazakhstan	Not known	C, G	Antonova, 2014
Moldova	B, C	E, F, G, H	Alanso, 1998; Gisca et al., 2013; Duca, 2014; Duca et al., 2017, 2019
Romania	A, B, C, D, E	F, G	Vrânceanu et al., 1980; Pacureanu-Joita et al., 2004, 2008, 2009; Risnoveanu et al., 2016
Russia	A, B, C, D	D, E, F, G, H	Kaya et al., 2009; Shindrova&Penchev, 2012; Antonova et al., 2013; Skoric, 2012; Antonova, 2014
Serbia	B, E	E, F	Mihaljčević, 1996; Masirevic et al., 2012; Miladinovic et al., 2014; Dedic et al., 2018
Spain	B, C, D, E	E, F, G_{GV}	González-Torres et al., 1982; Melero-Vara et al., 1989; Saavedra Del Río et al., 1994; Alonso et al., 1996; Molinero-Ruiz et al., 2008, 2014; Fernández-Escobar et al., 2008; Martin-Sanz et al., 2016; Malek et al., 2017
Turkey	D, E	F, G, H	Bulbul et al., 1991; Kaya et al., 2004; 2012; Molinero-Ruiz et al., 2014
Ukraine	A, B, C, D	E, F, G, H	Alanso, 1998; Burlov&Burlov, 2010; Hablak& Abdullaera, 2013; Pototskyi, 2014; Miladinovic et al., 2014; Maklik et al., 2018

Speed and efficiency of plant breeding could be increased with the use of MAS (Marker Assisted Selection) to develop new varieties. MAS systems to be used in breeding studies; defining of the degree of polymorphism, codominant character, and being testable and simple to implement (Bretting & Widrlechner, 1995). RGA markers are of great importance in determining disease resistance in plants and developing resistant individuals. Disease resistance is generally mediated by resistance (R) genes in plants (Staskawicz et al., 1995). Most of the R genes discovered in plants encode proteins composed of the nucleotide binding site (NBS) domain and leucine-rich repeats (LRRs) (Hammond-Kosack and Jones 1997; Ellis et al., 2000). NBS-LRR gene families are abundant in plant genomes and encode proteins necessary to trigger defense or protective systems (Dangl and Jones 2001; Meyers et al., 1999; Meyers et al., 2006; Holt et al., 2003). Since the initial discovery of the role played by NBS-LRR proteins in inducing disease resistance in plants, comparative genomic approaches have been widely used to isolate NBS-LRR genes that confer resistance to a broad spectrum of bacterial, fungal, nematode and viral pathogens (Bent 1996; Kanazin et al., 1996; Leister et al., 1998; Hammond-Kosack and Jones 1997; Dangl and Jones 2001; Meyers et al., 2003; Hulbert et al., 2001). Of the more than 40 R genes cloned from plants, the 75% of them are members of the NBS-LRR gene families (Hulbert et al., 2001). Several NBS-LRR gene families have been identified in sunflower and these gene families are effectively used in the development of molecular markers for resistance breeding (Gedil et al., 2001; Bouzidi et al., 2002; Radwan et al., 2003; Radwan et al., 2008).

New genetic resources are certainly necessary for setting up a successful plant breeding program and it is extremely important to use local and wild genetic resources rich in genetic variation. In the study, it was aimed to detect new Or5 and Or6 gene sources in wild sunflower species and to develop molecular markers to be used for selection in transferring them to cultivars. For this purpose, crossing populations were screened with 40 RGAP and 8 resistance-related markers selected from the literature.

MATERIAL AND METHOD

Material: Plant materials used for resistance to broomrape obtained from TTAE (Trakya Agricultural Research Institute) sunflower breeding program. In the selected crossbreeding population, 9728 A variety was used as resistant parent (DE) and 2517 B variety was used as susceptible parent (HE), which was resistant to Or5 and Or6 genes, hence to broomrape A-F races. Resistant and susceptible groups were formed as a result of the F2 genotypes, which are hybrids of them, as a result of the broomrape disease tests performed in TTAE. For this purpose, 30 F2 genotypes were used to form durable and 30 F2 genotypes were used to form susceptiblebulk.

gDNA Extraction: DNAs of leaf samples of plant material from each genotype were isolated using Doyle & Doyle's (1990) CTAB (Cetyl Trimethylammonium Bromide) methods. To create a durable and susceptiblebulk, the gDNAs of 30 genotypes were combined in equal amounts (25 ng/ul) to form durable F2 bulk (DB) and susceptibleF2 bulk (HB).

Molecular Marker Analysis: In this study, Or5 and Or6 genes, which were found to provide resistance to broomrape races in the Thrace region, where sunflower production is intense, were targeted. Therefore, 9728 A variety containing Or5 and Or6 genes was used as the resistant parent. 2517 B variety was used as susceptible parent. In the marker analysis, 8 markers in the reported literature and were selected for MAS (Figure 1, Table 2). These are the markers RGA172, RGA181, RGA192, RGA206 (Radwan et al., 2009), ORS1114, ORS1036, ORS665, ORS1021 (Imerovski et al., 2013). In addition, "Resistance Gene-Analog Polymorphism (RGAP)" markers using degenerate primers designed specifically for the conserved P-loop, kinase-2, kinase-3, GLPLAL and LRR regions in the R genes of various plants were also tested to identify new selective markers. Within

the scope of RGAP analyses, 40 RGA primers (Table 2) defined below were used as back-and-forth primers in different combinations, tested in 52 combinations and another marker associated with *orobanche* resistance was also tried.

PCR (Total 20 µl), 60 ng DNA 10 µl 2X Master mix, 1 µl F-R Primer (10 µM), 6 µl H2O were used. Initial denaturation at 94 °C for 3 minutes, denaturation for 45 seconds, binding at 40-60 °C for 1 minute continued with 35 cycles, 45 seconds of 72 °C synthesis and 10 minutes of final synthesis at 72 °C. For RGA 172, RGA 181, RGA 192, RGA 206, the bonding temperature was reduced to 68-58 °C with 1 minute intervals by applying touchdown, for ORS1114, ORS1036, ORS1021, ORS665, the bonding temperature was again reduced to 62-55 °C. PCR products were evaluated by running on 2% agarose gel and, if necessary, AATI capillary electrophoresis system.

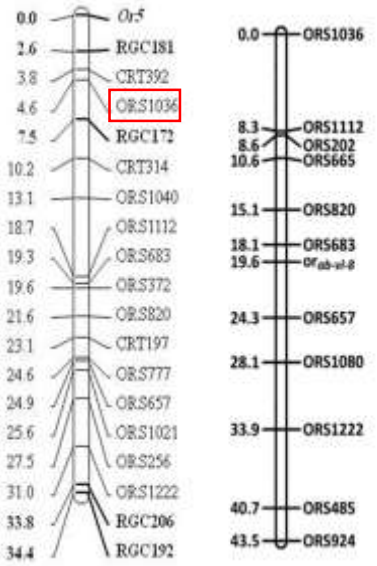

Figure 1: Sunflower *Or5* gene region markers and distances for genetic mapping (Radwan et al., 2009; Imerovski et al., 2016)

Tablo 2. The used primers in the study

Primer name	The array (5'-3')	Gene	Domain	Reference
CLRR-INV1	GCAGCAACTTGTGC	Cf9	LRR	Shi et al.(2001)
CLRR-INV2	TCTTCAGCTATCTGC	Cf9	LRR	Chen et al.(1998)
NLRR.INV1	TGCTACGTTCTCCGGG	N	LRR	Yan et al.(2003)
NLRR.INV2	TCAGGCCGTGAAAAATAT	N	LRR	Chen et al. (2007)
Cre3.LRR.r	CAGGAGCCAAAAATACGTAAG	Cre	LRR	Yan et al.(2003)
XLRR.f-r	CCGTTGGACAGGAAGGAG	Xa21	LRR	Chen et al.(1998)
XLRR.INV1	TTGTCAGGCCAGATACCC	Xa21	LRR	Shi et al.(2001)
XLRR.INV2	GAGGAAGGACAGGTTGCC	Xa21	LRR	Shi et al.(2001)
AS3.INV	CCIGAIGGIGAICGIG	N, Rps2	LRR	Yan et al.(2003)
RLRR.F-R	CGCAACCACTAGAGTAAC	Rps2	LRR	Chen et al. (1998)
S2	GGTGGGGTTGGGAAGACAACG	L6, N, Rps2	NBS	Leister et al. (1996)
AS1	CAACGCTAGTGGCAATCC	N, L6, RPS2	NBS	Leister et al. (1996)

AS3 (GLPLAL-1)	IAGIGCIAGIGGIAGICC	N, L6, RPS2	NBS	Leister et al. (1996)
Xa1NBS.f	GGCAATGGAGGGATAGG	Xa1	NBS	Shi et al. (2001)
Xa1NBS.r	CTCTGTATACGAGTTGTC	Xa1	NBS	Shi et al. (2001)
NBS.f1	GGAATGGGNGGNGTNGGNAARAC	N, RPS2	NBS	Yu et al. (1996)
NBS.r1	YTCAGTTGTRAYDATDAYYYTRC	N, RPS2	NBS	Yu et al. (1996)
Ptokin1	GCATTGGAACAAGGTGAA	Pto	Kinase	Chen et al. (1998)
Ptokin2	AGGGGGACCACCACGTAG	Pto	Kinase	Chen et al. (1998)
Ptokin3	TAGTTCGGACGTTTACAT	Pto	Kinase	Pahalawatta and Chen (2005a)
Ptokin4	AGTGTCTTGTAGGGTATC	Pto	Kinase	Shi et al.(2001)
RLK.f	GAYGTNAARCCIGARAA	LrK10	Kinase	Feuillet and Keller (1997)
RLK.r	TCYGGYGCRATRTANCCNGGITGICC	LrK10	Kinase	(Feuillet C, Keller B. 1997)
S2.INV	CAICAIAAIGGITGIGGIGG	N, Rps2	P-loop	Pahalawatta and Chen (2005)
S2(PLOOP)	GGIGGIRTIGGIAAIACIAC	Ploop	NBS-LRR	Hunger et al.(2003)
AS1.INV	CCTAACGGTGATCGCAAC	N, Rps2	P-loop	Lin and Chen (2007)
LM637	ARIGCTARIGGIARICC	L6, N, Rps2	P-loop	Kanazin et al.(1996)
LDD.AS	CCAIACATCATCMAGSACAA	Kinase-2	NBS-LRR	Hunger et al.(2003)
P1	GGIAAIACIACICTIGCI	N, Ploop	NBS-LRR	Hasançebi et al. (2009)
P15	IATGGAICCTTCIGCCATCCA	Kinase-2	NBS-LRR	Hasançebi et al., (2009)
P3N	GGIGGIGTIGTIGATGAT	Kinase-2	NBS-LRR	Hasançebi et al., (2009)
P17	IAGIGCIAGGGIIAGICC	Kinase-2	NBS-LRR	Hasançebi et al., (2009)
P8	ATCCTGGTGACIACICGI	LRR	NBS-LRR	Di Gaspero and Cipriani (2003)
P10	ATGICGCAAGTTGATIAG	LRR	NBS-LRR	Hasançebi et al., (2009)
Pto-Fen-S	ATGGGAAGCAAGTATTCAAGGC	Pto	Fen	Pahalawatta and Chen (2005a)
Pto-Fen-AS	TTGGCACAAAATTCTCATCAAGC	Pto	Fen	Pahalawatta and Chen (2005a)
RG2	ICCIAGIACYTTIARIGCIARIGGIAR	GLPL	NBS-LRR	Aarts et al. (1998)
NLRR.f	TAGGGCCTCTTGCATCGT	N	LRR	Chen et al. (1998)
NLRR.r	TATAAAAAGTGCCGGACT	N	LRR	Chen vd.. (1998)
Xa1LR.r	GAGATTGCCAAGCAATTGC	Xal	LRR	Lin and Chen (2007)

P172 (EL452113)	F:TACCATGTGTCTCCCTCTGATGT R:GATGTCCTTGATGTTCCCAATAA			Radwan et al. (2008)
P181 (EL455612)	F:TGGCATTAGATATGAGGTTTGGA R:TTGGACAAGAAGTCAAGTCAAGG			Radwan et al. (2008)
P192 (EL468211)	F:ACGATCAAAGTTCTGGGTTCATT R: AACGACGAACGATATTCTTACCC			Radwan et al. (2008)
P206 (DY908907	F:CGGTCTTCTATGATGTGGAACCT R: GGTTTCCTGACATTTCCCTTACA			Radwan et al. (2008)
ORS1036	F:CCCTTTCACTTCCTATTTTCTATTCA R:CTAAGAGGGGTCGGTATGATTTC			İmerovski et al. (2013)
ORS665	F: GCACATGAGGTATGGATCTCCT R: TGCAAATACAACTCGGGAAA			İmerovski et al. (2013)
ORS1114	F: AGATGGTGGCAGGAGAGTTAAAG R: GCAGAAACAGATCAGGAGGGTAT			İmerovski et al. (2013)
ORS1021	F: AACCCTAATCCAACCAGATACGA R: TACCACCAGCTCATCCTTAACCT			İmerovski et al. (2013)

RESULTS AND DISCUSSION

No DNA fragments to distinguish resistant/sensitive genotypes were observed in any of the RGA172, RGA181, RGA192, RGA206 candidate markers selected for the targeted resistance source Or5 gene region in this study. The same fragment amplified in the 443 bp region of the RGA172 marker in the resistant, susceptible parents and their bulk, the RGA181 marker amplified by 478 bp in the resistant parent and 450 bp in the susceptible parent, but only 450 bp bands were amplified in all of the resistant and sensitive F2 bulks. A single 357 bp DNA fragment was observed in both parents and F2 bulks with the RGA192 marker. As a result of the study, 2 bands of 138 bp and 206 bp were observed in both the resistant parent and the susceptible parents of the population with the RGA206 marker. Therefore, no DNA fragments distinguishing resistant/susceptible genotypes were observed.

It has been reported that the Or6 gene region of the ORS1036 marker produces a selective band in the 240 bp region in Imerovski et al. (2013), and in the 245 bp region in Iuoras et al., (2004). In our study, it was observed that PCR products work and band formation based on the results of agarose gel electrophoresis. With the ORS1036 marker, 121 bp and 267 bp bands were amplified in the resistant parent, susceptible parents, and the same bands were amplified in the F2 bulks (Figure 2c), so no distinctive DNA fragments were observed in the resistant/susceptible genotypes.

It is known that the Or6 gene region of the ORS1114 marker produces a selective band in the 264 bp region in Imerovski et al. (2013), and in the 280 bp region in Iuoras et al., (2004). In our study, it was observed that PCR was successful based on the results of agarose gel and capillary electrophoresis images. As a result of the study with the ORS1114 marker, 257 bp in the resistant parent, 244 bp and 266 bp in the susceptible parent were observed, but the discriminant band profile observed between parents was not observed in the F2 bulks (Figure 2a), so no distinctive DNA fragments were observed in the resistant/susceptible genotypes.

It is known that the Or gene region of the ORS665 marker produces a selective band in the 281 bp region in Imerovski et al., (2013). In our study, it was observed that PCR products work and band formation based on the results of agarose gel electrophoresis image. With the ORS665 marker, no bands were formed in the resistant parent and the susceptible parent of the population. Similarly, no

distinctive DNA fragments were observed in the resistant/susceptible genotypes, as certain peaks did not occur in the F2 bulks (Figure 2d).

It is known that there is no correct correlation between fragment size and resistance genes for the ORS1021 marker, as presented in the study reported in the literature (Imerovski et al., 2013). Based on the agarose gel electrophoresis image, the PCR products were working and band formation was observed. As a result of the study, 106 bp in the resistant parent and 107 bp in the susceptible parent were observed with the ORS1021 marker. Since the same peaks were observed in F2 bulks (Figure 2b), it is thought that the ORS1021 marker does not distinguish the broomrape resistance regions.

In our study, in addition to the markers presented in the literature within the scope of RGAP analysis, 40 RGA primers were used as back-and-forth primers in different combinations and resistant/sensitive parents and bulks were screened. Thus, RGA markers associated with Or5/Or6 that can be used for MAS was determined. No DNA fragments distinguishing resistant/susceptible genotypes were observed in any of the analyzes with primer pairs matched in 52 different combinations (Figure 3).

Radwan et al., (2009) stated in their study that the markers that are planned to be used in the selection of broomrape-resistant genotypes are the markers associated with Or5 that provide resistance in all races from A to E. NBS-LRR genes in a cluster on linkage group 3 indicated that they play a central role in recognizing broomrape in sunflower and triggering host defense responses, and known resistance genes on linkage group 3 (Or1-Or6) are distinctive of NBS-LRR genes found in plants. Of the four NBS-LRR loci bound to broomrape, three were identified from *H. tuberosus* (RGA172, RGA181, RGA192), and the fourth from common wild *H. annuus* (RGA206). The closest marker (2.6 cM) to the Or5 locus is RGA181. RGA172 is located at 7.5 cm from the Or5 locus. On the other hand, RGA206 and RGA192 paired at 33 cM proximal to the Or5 locus.

Iuoras et al., (2004) found the ORS1114 marker useful for MAS in their research. In the 2013 study of Imerovski et al., however, Or5 and ORS 1114 marker did not show a correlation. Sunflower hybrids that are resistant or highly tolerant to broomrape attack contain resistant alleles at the Or5 locus in the LC 1093 inbred line, which have been used as resistant sources in all hybrid combinations. SSR markers applied to the crossover genotypes were unexpectedly segregated based on phenotypic characterization performed in the area of high broomrape infestation. The ORS 1114 marker identified the 280 bp band in 56% of genotypes characterized as resistant and was matched 74.3 cM proximal to the Or5 locus. ORS 1036 marker, 245-246 bp in 81% of genotypes considered resistant, identified the band and stated that it was mapped at 7.5 cm proximal to the Or5 locus.

Tang et al., (2003) placed Or5 in the telomeric region of LG3 and identified the ORS1036 marker 7.5 cM downstream of the nearest SSR marker. The paucity of DNA markers near Or5 may be due to the paucity of DNA polymorphisms, however, since Or5 and other broomrape resistance genes are introgressed from wild sunflowers, it makes it unlikely that the scarcity of DNA polymorphisms in the entrained DNA segments.

As a result, a marker test was carried out in these hybridized and inbred sunflower lines before wild sunflower species, but since a marker associated with Or5/Or6 that can be used for MAS could not be determined in the markers presented in the literature and tested for new development, no application could be made in wild sunflower species. The lack of success in the molecular marker study was attributed to the use of an broomrape population consisting of a mixture of different races in phenotypic disease tests and the lack of knowledge of which races were present in this different population.

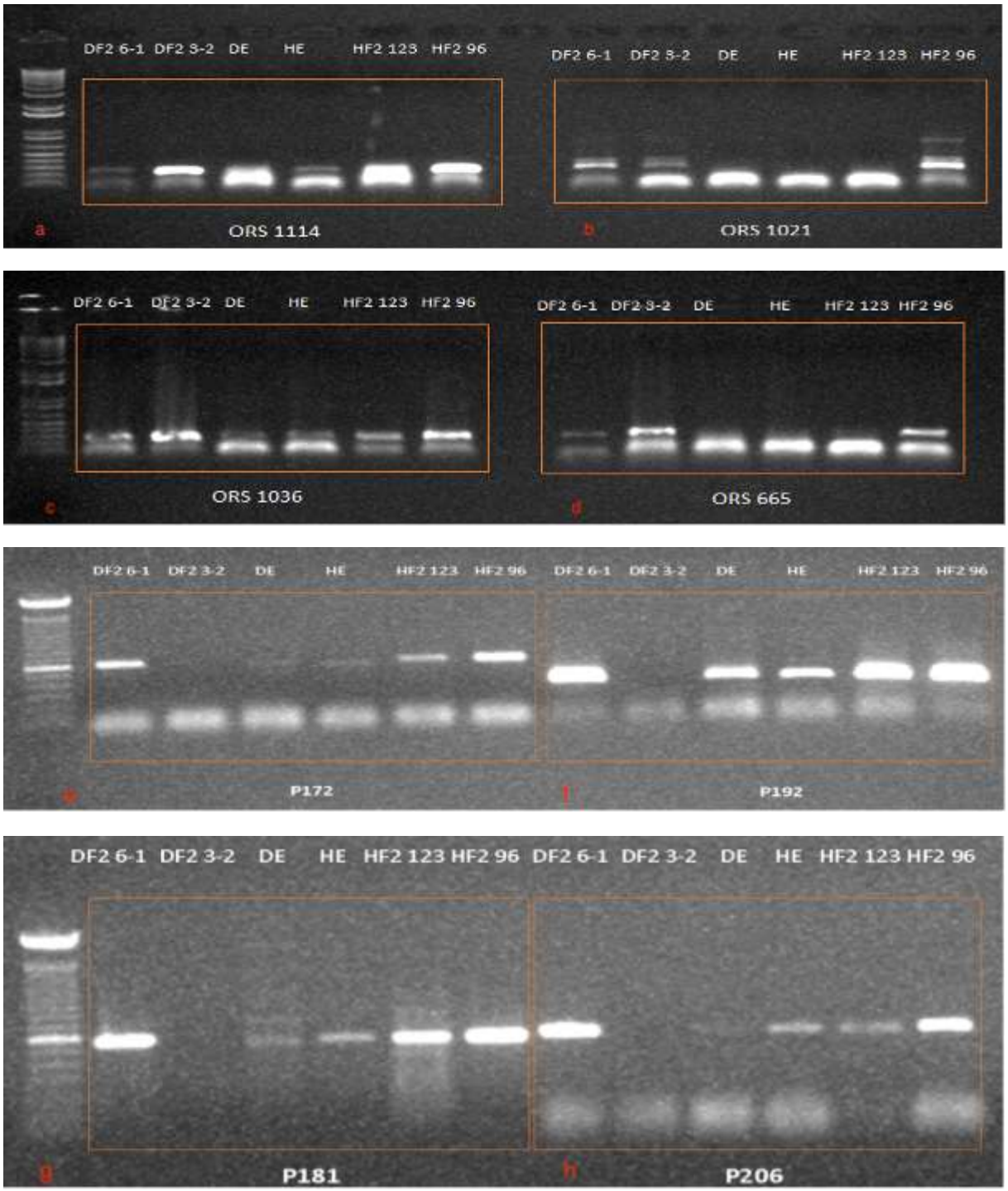

Figure 2. Each marker 6 six samples (Resistant Parent (DE), Susceptible Parent (HE), Two resistant F2 bulk (DB), Two susceptible F2 bulk (HB))

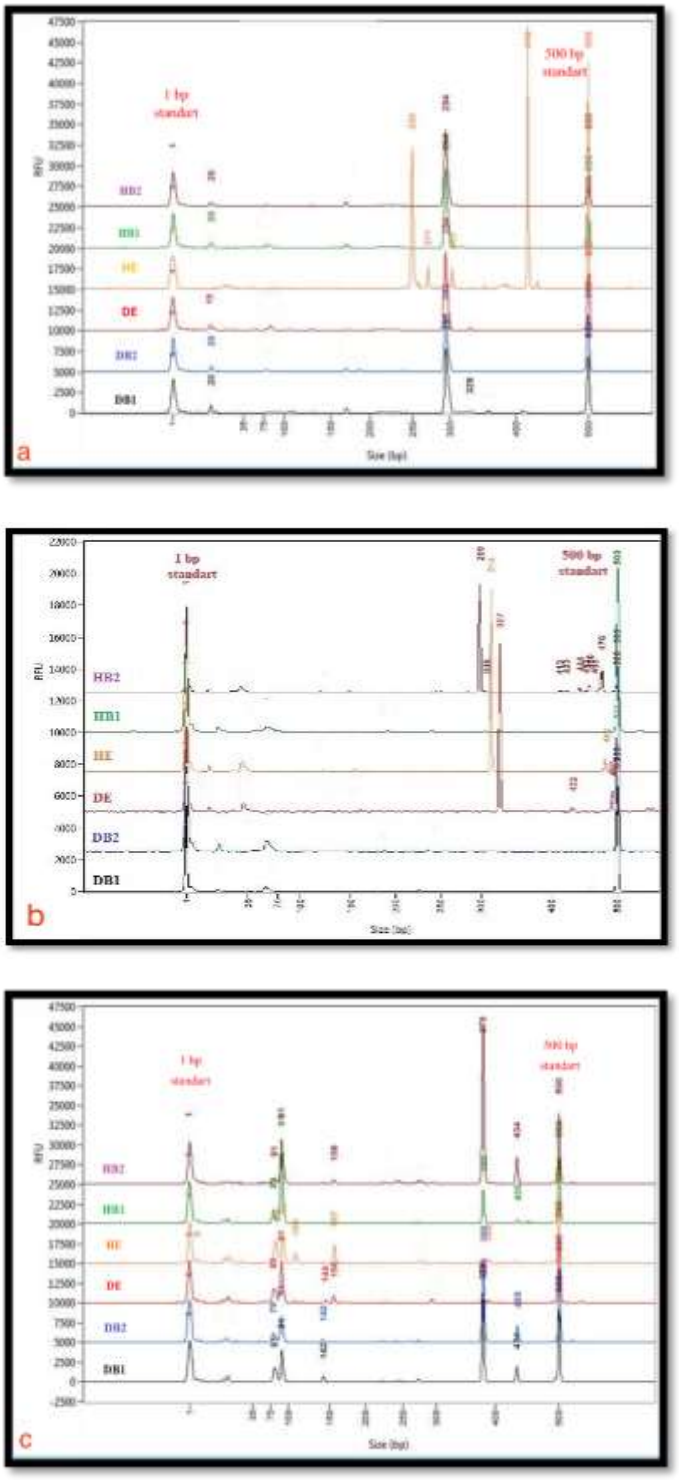

Figure 3. (a) RGA Ptokin 1 – PtoFen.AS, (b) RGA PtoFen.S-S2.INV, (c) RGA Ptokin 1 – PtoFen. PCR results obtained from S primers, capillary electrophoresis image (DE: Resistant Parent, HE: Susceptible Parent, DB: Resistant Bulk, HB: Susceptible Bulk)

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THE YIELD PERFORMANCES OF SOME NEW RICE LINES IN EDIRNE

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ABSTRACT

Rice is grown widely especially around rivers throughout Turkey. Turkey is 2nd rice producer country other than Italy in Europe. Because of having higher yielding and income per area rice is the most preferred crops in irrigated areas at Edirne province in Turkey. Edirne province has the largest rice planted area in Turkey. The study was performed to measure some rice candidate line and cultivar performances in Edirne 2021-2022 growing season. The candidate rice lines existed in yield trials with three control as Osmancık-97, Luna and Cammeo cultivars which are mostly planted and preferred by farmers recently in Turkey. The experiment results indicated that some candidate lines exhibited over yield performances than control cultivars. TRAGEN-500 candidate line performed better than the check varieties mean yield for comparing seed yields in Edirne location in both two years. The candidate lines were also evaluated and compared with control cultivars based on seed quality and other important agronomic traits results. As a result, some new rice lines had favorable results based this study and TRAGEN 500 promising one will send to registration trials to produce and then exist in Turkish seed market and others ones will be tested again future trials.

Key words: Paddy rice, Edirne, Seed yield, Adaptation, Yield traits

INTRODUCTION

Rice is the most consumable dish with bulgur existing all tables daily in Turkish cuisine (Sürek et al., 2011; Beşer and Sürek, 2012). Paddy rice cultivation has increased more in recent years and it reached one million tons' production and 130.000 ha planting area. Of course, Turkey is not existing top producer countries comparing Asian countries which is leading countries in rice consumption in the world. 2021/22 global rice supply increased by 2.2% to 753.9 million tons. In paddy production, China ranks at first with 213 million tons, while India ranks second with 195 million tons' production (TEPGE, 2022). However, Turkey is leading country with Italy in European continent and produce high quality rice with higher yielding cultivars.

Rice cultivation is mostly located in around rivers because the irrigation is dominated cost in the rice production. Edirne province exist first place because Meric river which is border line with Greece (Figure 1). Following Edirne province, Blacksea and South Marmara regions are other rice cultivation areas because Kızılırmak river in Blacksea region and Susurluk river in South Marmara are the main resource reservoirs for lower cost rice production. There is also rice production with some underground irrigation as well as water supply from lakes other than rivers. In recent years, Clearfield type cultivars became popular due to supplying reasonable and practical solutions to

control weedy rice which the most important issue in rice production (Konak et al., 2021). The study was conducted to evaluate to determine of yield performances some rice cultivars and candidate lines in Edirne.

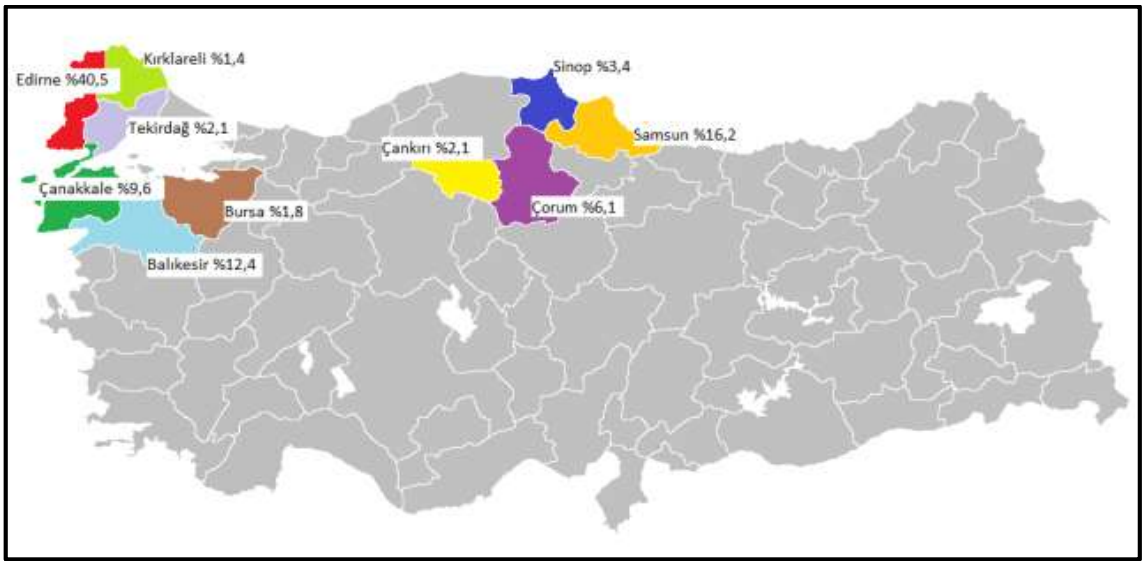

Figure 1. Provinces with the largest share in Turkey paddy production (2020, %) (TEPGE, 2022)

MATERIAL AND METHOD

Rice yield trials were conducted based on Randomized Complete Block Design by TRAGEN Research Company existed in Trakya University Technopark at the fields closer to Edirne city center in 2021 and 2022 in accordance with the Technical Instructions of the Seed Registration and Certification Center. Osmancık-97 and Cammeo as normal type and Luna as Clearfield varieties which are most popular ones in Turkey were used as standard in the experiments. In the trial, pre-germinated seeds were seeded in water, 500 seeds per square meter. In 2021 trial, the experiments were planted in June 2021 and harvested in 17 October 2021. In 2022 trial were planted in 20 May 2022, and harvested in 15 October 2022.

In the experiments, 30 kg of 20-20-0 fertilizer was applied per decare before seeding. At the beginning of tillering, 30kg/da Ammonium Sulphate (21% N) was applied as the first top fertilizer to the experiment. At the booting stage, 30 kg of Ammonium Sulphate (21% N) fertilizer was given as the second top fertilizer. Weed control was done chemically. The harvest was made as 5 square meters from each plot. Observations and evaluations during the growing period and after the harvest were made according to the TTSM Rice Technical Instructions. The plants were harvested by hand. Measurements and observations were done for days to flowering, days to maturity, Plant height, panicle length, panicle per square meter and yield and other requested yield traits.

RESULTS AND DISCUSSION

While there were 8 lines/cultivars in 2021, 13 lines/cultivars were in 2022. Since the number of common cultivars in both years was 6, the combined statistical analysis was performed for the six cultivars. There were promising results for seed yield in rice candidate lines comparing the controls both 2021 and 2022 and average of the years based on yield. Statistical analysis results for 2021, 2022 and combined were given in Table 1. Field observations of 2021 related to the trials were given in Table 2, field observations of 2022 were given in Table 3, quality values of 2021 were given in Table 4, and quality values of 2022 were given in Table 5.

The candidate variety named TRAGEN-500 showed a high degree of tolerance to the most important disease of rice, rice blast, in terms of leaf and panicle blast in both years (Table 2 and 3). Yield average is better than the standards mean in both years, grain size is bigger than that of Osmancık-97 variety, and closer to Cammeo grain size. Hread rice yield is higher either (Table 4 and 5). In both years, there was no problem effect negatively to the evaluation of the results of the trials as properly.

Table 1. Rice Cultivars yield trial results for 2021, 2022 and two year combined

Cultivars	2021 Kg/da	2022 Kg/da	Average Seed Yield (kg/da)	V.S.
Osmancık-97	942,8 BC	764,5 A	853,6 A	4
Luna	994,2 AB	676,8 BC	835,5 AB	5
Cammeo	842,7 D	732,4 AB	787,5 B	6
Osmancık-97/IR83260	1054,4 A	-	-	
IR83260/2/Osmancık-97	897,7 CD	-	-	
TRAGEN-500 (Candidate)	**986,1 AB**	**768,6 A**	**877,3 A**	**2**
Kızıltan//IRBL10/IRBL22 -3-1	-	742,0 AB	-	
Kızıltan//IRBL10/IRBL22 -5-1	-	746,5 AB	-	
Kızıltan//IRBL10/IRBL22 -6-1	-	740,0 AB	-	
Gönen/7721- 4-1	926,7 BCD	808,6 A	867,7 A	3
Gönen/ 7721-5-1	974,5 ABC	812,6 A	893,6 A	1
Gönen/7721- 7-1	-	752,2 AB	-	
Liao 007/002-1-1	-	730,1 AB	-	
Liao 007/002-3-1	-	634,1 C	-	
Liao 007/002-2-1	-	758,5 AB	-	
General Average	**952,4**	**743,6**	**852,5**	
Standard Average	**892,8**	**748,5**	**820,6**	
(LSD: 0.05) (kg/da)	84,2	85,5	61,3	
C.V (%)	6,2	8	7,1	
F	0,0022	0,015	0,0239	

Table 2. Rice cultivars yield trail field observations in 2021

Cultivars	Days to flowering (days)	Days to Maturity (days)	Plant Height (cm)	Panicle Length (cm)	Panicle per square meter	Grain number per panicle	Lodging (1-5)	Sterility (%)	Grain Shattiring (%)	Pest and Diseases			
										Leaf Blast	Panicle Blast	*Helminto sporium*	Other Diseases
Osmancık-97 (st)	80	128	93,8	13,5	385	142	7	7,4	1	3	5	-	-
Luna	83	125	78,1	16,2	360	152	1	7,0	1	5	5	-	-
Cammeo (st)	80	129	80,8	15,6	378	120	3	10,2	3	5	7	-	-
Osmancık-97/IR83260	81	120	99,6	14,9	412	145	5	8,4	1	1	1	-	-
IR83260/2/Osmancık-97	78	129	101,6	14,8	405	149	5	6,9	1	1	1	-	-
TRAGEN-500 (Candidate)	81	130	89,45	19,2	381	135	1	7,1	1	1	1	-	-
Gönen/7721- 4-1	78	128	81,8	16,8	342	130	1	9,4	1	7	7	-	-
Gönen/ 7721-5-1	77	125	86,45	17,3	360	129	1	10,3	1	3	7	-	-

Table 3. Rice cultivars yield trail field observations in 2022

Cultivars	Days to flowering (days)	Days to Maturity (days)	Plant Height (cm)	Panicle Length (cm)	Panicle per square meter	Grain number per panicle	Lodging (1-5)	Sterility (%)	Grain shattiring (%)	Pest and Diseases			
										Leaf Blast	Pancle Blast	*Helmintho sporium*	Other Diseases
Osmancık-97 (St)	79	129	95	14	356	138	7	10,2	1	-	5	-	-
Luna	83	121	75	17	412	147	1	8,7	3	-	5	-	-
Cammeo (St)	80	130	84	18	391	124	5	14	5	-	7	-	-
TRAGEN-500 (Candidate)	82	131	81	18	427	129	1	4,5	3	-	1	-	-
Kızıltan//IRBL10/IRBL22 -3-1	81	130	78	16	410	131	1	4,7	3	-	3	-	-
Kızıltan//IRBL10/IRBL22 -5-1	84	130	80	19	398	134	1	5,8	3	-	3	-	-
Kızıltan//IRBL10/IRBL22 -6-1	81	129	85	19	429	128	1	6,1	3	-	3	-	-
Gönen/7721- 4-1	77	124	77	18	384	119	1	10,3	3	-	5	-	-
Gönen/ 7721-5-1	76	126	87	17	396	126	9	13,1	2	-	5	-	-
Gönen/7721- 7-1	74	125	73	15	325	125	1	15,1	10	-	9	-	-
Liao 007/002-1-1	81	127	99	18	346	110	5	11,2	3	-	3	-	-
Liao 007/002-3-1	80	128	93	19	314	117	3	14,2	1	-	5	-	-
Liao 007/002-2-1	80	127	89	16	361	121	5	15,1	1	-	7	-	-

Table 4. Rice cultivars seed quality trait observations in 2021

Cultivar	Seed Length (mm)	Seed Width (mm)	Seed Shape Length/Width	Chalkines	Brown Rice. (%)	Total Rice (%)	Head Rice. (%)	1000 T seed Weight (g)
Osmancık-97 (st)	9,1	3,4	2,67	3	81,5	71,5	62	35,1
Luna	9,4	2,9	3,24	1	82,2	72,5	66	29,7
Cammeo (st)	10,2	3,5	2,91	1	80,1	71,4	59	40,8
Osmancık-97/IR83260	9,0	3,2	2,81	3	81,2	71,6	62	33,4
IR83260/2/Osmancık-97	8,9	3,3	2,69	3	80,4	70,1	64	34,1
TRAGEN-500 (ADAY)	9,4	3,6	2,61	1	81,2	71,2	61	37,6
Gönen/7721- 4-1	10,4	3,5	2,97	1	80,4	70,6	55	40,4
Gönen/ 7721-5-1	10,7	3,5	3,06	3	80,8	70,4	57	41,4

Table 5. Rice cultivars seed quality trait observations in 2022

Cultivar	Seed Length (mm)	Seed Width (mm)	Seed Shape Length/Width	Chalkines	Brown Rice. (%)	Total Rice (%)	Head Rice. (%)	1000 T seed Weight (g)
Osmancık-97 (St)	9,0	3,19	2,69	3	83,84	70,68	43	33,9
Luna	9,4	3,30	3,03	1	82,67	72,76	63	30,7
Cammeo (St)	10.1	3,64	2,77	1	83,62	69,68	50	41,7
TRAGEN-500 (ADAY)	9,3	3,60	2,58	1	81,04	67,69	58	37,6
Kızıltan//IRBL10/IRBL22 -3-1	9,8	3,73	2,63	1	81,08	68,27	56	38,2
Kızıltan//IRBL10/IRBL22 -5-1	9,3	3,50	2,66	1	80,18	67,49	56	38,4
Kızıltan//IRBL10/IRBL22 -6-1	9,3	3,50	2,66	1	80,88	68,78	55	38,3
Gönen/7721- 4-1	9,6	3,53	2,72	3	82,40	67,51	47	38,8
Gönen/ 7721-5-1	9,7	3,73	2,60	1	83,29	68,62	52	42,5
Gönen/7721- 7-1	9,5	3,60	2,64	3	81,76	69,36	37	40,1
Liao 007/002-1-1	9,6	3,43	2,80	1	83,31	70,72	53	37,0
Liao 007/002-3-1	10.1	3,30	3,06	1	82,77	70,56	49	38,6
Liao 007/002-2-1	8,5	3,30	2,57	3	81,74	69,96	58	31,8

CONCLUSIONS

In this study ıt was seen that, TRAGEN 500 candidate rice line exhibited higher tolerance to the rice blast as well as better seed yield than the standards and it has good seed quality. This candidate rice line will send to registration trials to produce and then exist in Turkish seed market 2023. Other promising ones will be tested again future trials will together new candidate lines.

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STRUCTURAL ELEMENTS AND GRAIN YIELD STABILITY OF WINTER PEA GENOTYPES

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ABSTRACT

Forage peas hybrid lines №PL, №6, №11, №12A, №13, №14, and varieties Taskent, Tore and Mir (standard) were studied in point of view structural elements of grain productivity. The study was performed in two locations both situated in the Central part of the Danube hilly plain region of Bulgaria (2017-2019). Location A (43º 23'N, 24º 34'E, 230 m altitude), podzolized soil subtype; location B (43.41º N, 24.61º E), haplustoll soil subtype. The variation in the structural elements of the productivity between the varieties and lines was found. Line №13 (163 cm) and Taskent (158 cm) are characterized by high plant height values. In terms of the height of the first pod, №PL showed the best results (116 cm; 89 cm), and the Mir variety formed a larger number of pods per plant (32; 40). Lines № 14, № 6 and № 12A are interesting in terms of grain productivity. Lines №PL and №14 had the highest productive capabilities with grain yield of 150.3 - 186 kg/da and 140.7 - 174.4 kg/da, respectively. Line №PL (bi=1.02) showed the best adaptive capabilities. Taskent (bi=1.23) and Tore (bi=1.27) cultivars were found unstable. №14 (bi=1.11) was high-yielding but variable, while Mir and №6 can be defined as stable (bi=0.80). GGE biplot analysis identified lines №PL and №14 as genotypes combining good stability and high grain yield. A strong positive correlation was found between grain yield and mass of 1000 grains, and grain weight per plant at both locations. The biggest effect on the grain yield had the genotype followed by the environmental factor. The data obtained can be used in future pea breeding programs.

Keywords: Pea, Variety, Selection, Elements of productivity

INTRODUCTION

Production of grain legumes is an important component of the grain complex of many countries in the world. One of the most valuable and in-demand crops of this group is pea, cultivated in temperate latitudes and occupying up to 85% of the sown areas of all legumes (Turusov et al., 2020). In modern conditions, against the background of increased requirements for the quality of the initial product, difficult tasks are set before the breeding. The selection of ready-made forms from the natural variety for crossbreeding is currently very limited, so breeding work should be aimed at combining valuable traits and properties from different samples in a new plant (Verbitsky et al., 2007; Zotikov et al., 2018).

A large role in this is attributed to the presence of genetically heterogeneous initial material, which possesses the most important economic-valuable characteristics that contribute to the improvement of seed productivity. At the same time, it is important to take into account the mutual influence of signs, which statistical methods help to identify, and in particular, the method of correlation analysis. Knowledge of the nature of the relationships between individual

elements helps the breeder to select valuable initial material when the direct assessment of genotypes by selectable quality is difficult or when the work involves splitting hybrid populations (Kondykov, 2011; Potanin et al., 2014).

The ability to estimate traits more accurately and differentiated, as well as to determine the level of their informativeness during selection, makes it possible to strengthen the effect of positive and eliminate the effect of negative properties of subsequent generations of plants (Shurkhaeva and Fadeeva, 2015; Kuzmina et al., 2017). The study of the relationship between productivity and its main constituent elements is of great importance in the work on the development of new varieties of grain pea. The presence of dependencies of a certain nature specifies the significance of each feature and the effectiveness of its use to achieve final results in the form of new highly productive genotypes. The creation of high-tech varieties implies the elimination of such shortcomings of firewood plants as stem drooping and seed shedding by introducing into highly productive genotypes such traits as fused cotyledon, moustached leaf type, limited stem growth type, etc. (Ponomareva, 2018; Putina and Besedin, 2019).

The aim of the research was to study the structural elements of grain productivity of winter forage pea genotypes and their productive possibilities for the conditions of Central Northern Bulgaria.

MATERIAL AND METHOD

The study was conducted on the experimental field of the Institute of Forage Crops – Pleven (2017-2019), situated in the Central part of the Danube hilly plain region of Bulgaria. Two locations were used, viz. location A (43º 23'N, 24º 34'E, 230 m altitude), podzolized soil subtype; location B (43.41º N, 24.61º E), haplustoll soil subtype. Forage peas hybrid lines №PL, №6, №12A, №13, №14, and varieties Taskent, Tore and Mir (standard) were studied for grain yield and elements of the productivity. The seeds were sown in plots in six repetitions with a plot size of 10 m2 for location A and in plots in two repetitions with a plot size of 2 m2 for location B. The sowing was done during October. The structural elements were recorded as follows: height, cm; height first pod, cm; number of grains/plant; number of pods/plant; 1000-seeds weight, g; grain weight/plant, g. Grain yield (kg/da) was recorded.

Experimental data were statistically processed using STATGRAPHICS Plus for Windows Version 2.1. The data for the quantitative signs investigated were processed by variance analysis. The yield stability was determined by the linear regression coefficient "bi" according to the method of Finlay and Wilkinson (1963). A GGE biplot model was applied that used a single value decomposition of the first two principal components (PC1 and PC2) (Yan, 2002).

RESULTS AND DISCUSSION

The efforts of selection to create new varieties and of agrotechnics to create technologies for their cultivation are mainly aimed at increasing the yield of agricultural crops. It is of great importance to test varieties and different agrotechnical options to establish the most suitable practices for the respective area (Dragov and Samodova, 2020).

Morphological characteristic

Plant height and first pod height (Table 1, Table 2) are associated with lodging resistance and to some extent determine suitability for mechanized harvesting. The variation in plant

height for location A was from 87 to 163 cm and for location B from 44 to 113 cm. The control variety Mir was superior to almost all Bulgarian lines except for line №13. The Turkish cultivars grown at location B have a stem length close to that of Mir. In the conditions of location A, Taskent significant exceeded Mir.

Table 1. Distinctive features of the investigated winter forage pea cultivars/lines (Location A)

Cultivar / Line	Height, cm	Height first pod, cm	Number of grains/plant	Number of pods/plant	1000-seeds weight, g	Grain weight/plant, g
Mir	140.0	92.0	32.0	9.0	135.0	4.42
№PL	124.0	116.0*	17.0	6.0	175.0**	4.96*
№6	87.0	40.0	25.0	8.0	155.0*	5.01*
№12A	103.0	75.0	27.0	10.0	151.0*	5.81**
№13	163.0*	89.0	33.0	11.0*	138.0	4.32
№14	91.0	78.0	18.0	6.0	201.0**	6.20*
Taskent	158.0*	84.0	21.0	6.0	151.0	4.47
Tore	138.0	77.0	31.0	8.0	103.0	4.23
Average	125,5	81,37	25,5	8,0	151,125	4,93

Significant * at $P < 0.05$; ** at $P < 0.01$;

Table 2. Distinctive features of the investigated winter forage pea cultivars/lines (Location B)

Cultivar / Line	Height, cm	Height first pod, cm	Number of grains/plant	Number of pods/plant	1000-seeds weight, g	Grain weight/plant, g
Mir	108.0	71.0	40.0	10.0	113.0	3.57
№PL	95.0	89.0*	21.0	7.0	141.0*	4.01*
№6	44.0	37.0	29.0	8.0	125.0*	4.03*
№12A	80.0	58.0	34.0	11.0	121.0	4.69*
№13	113.0	68.0	23.0	7.0	111.0	3.48
№14	69.0	60.0	23.0	7.0	162.0**	5.01**
Taskent	99.0	62.0	28.0	10.0	108.0	3.19
Tore	110.0	61.0	30.2	8.0	84.0	2.23
Average	89,75	63,0	28,52	8,50	120,62	3,77

Significant * at $P < 0.05$; ** at $P < 0.01$

The first pod height trait varied less than the plant height, with the values of line №PL being the highest in both locations (116 cm; 89 cm), and cultivar Mir occupying the second position. Lines №6, №14 and №12A form a height of first pod below the average for the studied group of samples (81 cm; 63 cm). Only line №6 has a straight stem and a height of the first pod, which defines it as particularly suitable for mechanized harvesting. The productivity or grain mass of a single plant is a complex trait due to the interaction of the genotype with different soil, climatic and agrotechnical conditions. Due to the multi-component nature of its structure and exposure to various external factors, it is difficult to lead a team according to this indicator.

The trait number of pods is closely related to the weight of a grain per lant and is one of the main elements of productivity. Lines №12A (10) and № 13 (11) are characterized by a greater number of formed pods in the conditions of location A, and for location B Taskent (10) and №12A (11), respectively. The number of pods per plant is a derived quantity determined by the number of fertile nodes, the number of pods per plant and the number of grains per pod. The number of grains was found maximum at №13 (33), Mir (32) and the Turkish variety Tore (31) in location A. Most of the samples form 21-25 grains. In lines №PL and №14, the number of grains per plant does not exceed 17-18. In location B, a partial similarity in the performance of Mir (40) and Tore variety (30) was observed. It is noticeable that №6 forms more grains compared to location A, and for №13 the trend is reversed.

The mass of 1000 grains is also of great economic importance. At the same time, the use of grains with a small mass reduces their sowing rate, which allows saving seed material. Selection work aimed at obtaining genotypes with too large grains can lead to negative changes in morphological and productive parameters and to an imbalance in productivity elements. Productivity is a rather variable feature, depending on both external factors and varietal characteristics, and is one of the characteristics determining the quality and quantity of yield. In the conditions of location A, the values of the main structural elements mass of 1000 grains and weight of grains per plant are higher compared to location B. Their variation for location A is as follows: from 103 g (Tore) to 201 g (№ 14) g per mass of 1000 grains, and from 4.23 g (Tore) to 6.20 g (№ 14) per mass of grains per plant. Despite the lower values of these indicators in location B, it can be said that there is no significant rearrangement of the pea samples. It is noteworthy that in terms of weight per 1000 grains lines №14, №PL and №6 demonstrate a significant superiority over other genotypes, and in terms of productivity №14, №12A and №6. They are of breeding interest and are suitable as parental components in combinatorial selection to obtain high-yielding varieties of winter forage pea. The Turkish variety Tore can definitely be referred to the small-seeded genotypes.

The results related to the yield are directly dependent on climatic conditions. From the two-factor variance analysis, it is clear that the year factor has a significant influence on yield formation (Table 3). The high value of this parameter is due to the differences in meteorological conditions during the experimental years and different locations. The variety as a factor of variation has the strongest effect on grain yield, the reason for which is the ecological plasticity and genotype of each variety. The influence of the two factors (year and variety) were significant. The influence of the genotype-environment interaction factor was significantly lower.

Table 3. Two-way ANOVA of seed yield

Source	df	SS	MS	F-count	F-table		P-value
					5%	1%	
Location	1	12,675.00	12,675.00	47.55**	4.20	7.64	0.0000
Genotype	7	19,158.33	2,736.90	49.46**	2.36	3.36	0.0000
G*L	7	3.873.3	553.33	5.89**	2.36	3.36	0.0003
Error	28	2.630.83	93.958				
Corrected Tota	47	33.550.00					

Significant * at P < 0.05; ** at P < 0.01; ns = not significant

Tables 4 and 5 present the obtained grain yields of the winter pea varieties and lines tested. The average yield of the group of samples for location A was 148.1 kg/da, and for location B it was 116.2 kg/da. The proven highest yield compared to the Mir variety (control) from both trials was realized by №14 (174.40 kg/da; 140.70 kg/da) and №PL (186.00 kg/da; 150.30 kg/da), followed by №12A (150.30 kg/day; 121.00 kg/day). The excess reaches 40% for line №14 compared to Mir. From other varieties, only Taskent in terms of grain yield approaches the sample average.

Table 4. Grain yield from winter forage pea cultivars/lines (Location A)

Cultivar/Line	Grain yield kg/da	To the Standard, %	To the Standard, kg/da
Mir	132.7	-	-
№PL	186.0*	+40.2	53.3
№6	150.0	+13.0	17.3
№12A	150.3	+13.3	17.6
№13	129.6	-2.3	-3.1
№14	174.4*	+31.4	41.7
Taskent	148.8	+12.1	16.1
Tore	127.0	-4.3	-5.7
Average	148.1		
STDEV	20.5		
Max	186.0		
Min	127.0		

Significant * at P < 0.05

Table 5. Grain yield from winter forage pea cultivars/lines (Location B)

Cultivar/Line	Grain yield kg/da	To the Standard, %	To the Standard, kg/da
Mir	107.1		
№PL	150.3*	+40.3	43.2
№6	119.0	+11.1	11.9
№12A	121.0	+13.0	13.9
№13	104.4	-2.5	-2.7
№14	140.7*	+31.4	33.6
Taskent	120.3	+12.3	13.2
Tore	86.9	-18.9	-20.2
Average	116.2		
STDEV	20.3		
Max	150.3		
Min	86.9		

Significant * at $P < 0.05$

The results obtained in the study are in agreement with the studies of Walker et al. (2011) for lupine, according to which three components determine grain yield – number of pods per plant, number of grains in a pod and weight of grains per plant. The authors consider that the number of grains is mainly determined by the factors of temperature and humidity during flowering, since both parameters influence the supply of assimilates, and they define the weight of the grains as a slightly variable trait.

Phenotypic stability

When assessing the stability and adaptability of the genotypes with regard to the grain yield indicator, of particular importance for the reliability of the results is the presence of significant interaction between the studied genotypes and the environment in which they were grown. The phenotypic stability of varieties in terms of yield is a reflection of the interaction between the genetic characteristics of the individual and the environment in which it develops. Varieties, which are characterized by higher phenotypic yield stability, have less expressed genotype-environment interaction and better general adaptability, as well as relative constancy in the manifestation of their genetic features (Dyakov and Borsukov, 2014; Ivanov et al., 2018).

Genotype-environment interaction is the equivalent of genotypic variation on phenotypic plasticity. This quality is best expressed by the regression coefficient bi. The higher the value of the coefficient, the more sensitive the variety is to changes in environmental conditions (Desheva and Deshev, 2021). When comparing the linear regression coefficient bi characterizing the ecological stability of individual genotypes, significant differences were observed (Figure 1). The genotypes Taskent (bi=1.23) and Tore (bi=1.27), as well as № 14 (bi=1.11), which has a high grain yield, are characterized by higher variability and

responsiveness. Mir and № 6 with a phenotypic stability coefficient bi=0.80 can be defined as stable. With the most favorable combination (high grain yield and parameter bi close to unity), determining a good adaptability, line №PL stands out (bi=1.02).

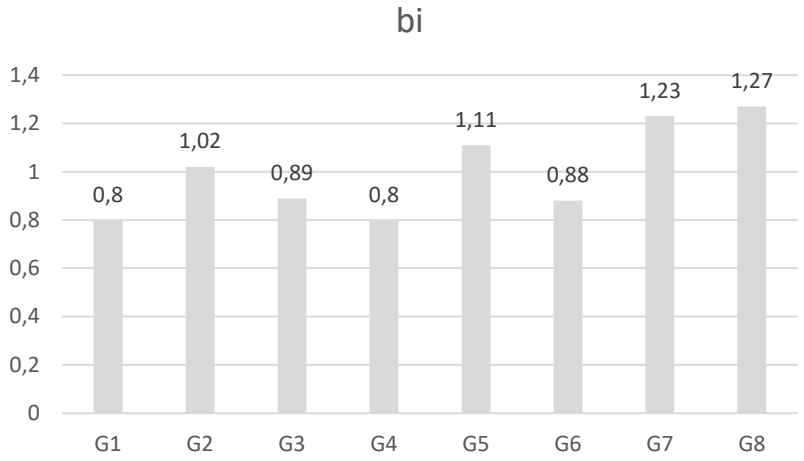

Figure 1. Yield stability according to Finlay-Wilkinson (b_i coefficient of linear regression)

G1-Mir; G2-№PL; G3-№6; G4-№12A; G5-№14; G6-№13; G7-Taskent; G8-Tore

GGE biplot analysis

The GGE biplot is represented as an irregular polygon (Figure 2). The points defining its peaks are the projections of the genotypes that showed the best results in the breeding locations located closest to them. The coordinate system is divided into four quadrants with positive or negative values of PC1 and PC2. Varieties are ordered on the abscissa based on their average performance for the trait, with those above the overall average for the given conditions to the right of the ordinate, and those below the overall average to the left. The figure reveals that, among the studied samples, pea №14 is very stable and highly productive and confirms the result obtained from the evaluation of trait stability by the bi parameter. Line №PL can be attributed to №14. It is distinguished by the highest grain yield and very good stability.

The projection of varieties relative to the ordinate axis regardless of direction determines their stability. Lines №6, №12A and variety Taskent are located in the negative part of PC2 and relatively close to the y-axis. Their location indicates that they are responsive with average grain yield. №13, Mir and Tore variety have negative values of PC1, which characterizes them as the lowest productive, and their distance from the abscissa axis suggests a pronounced instability of the trait.

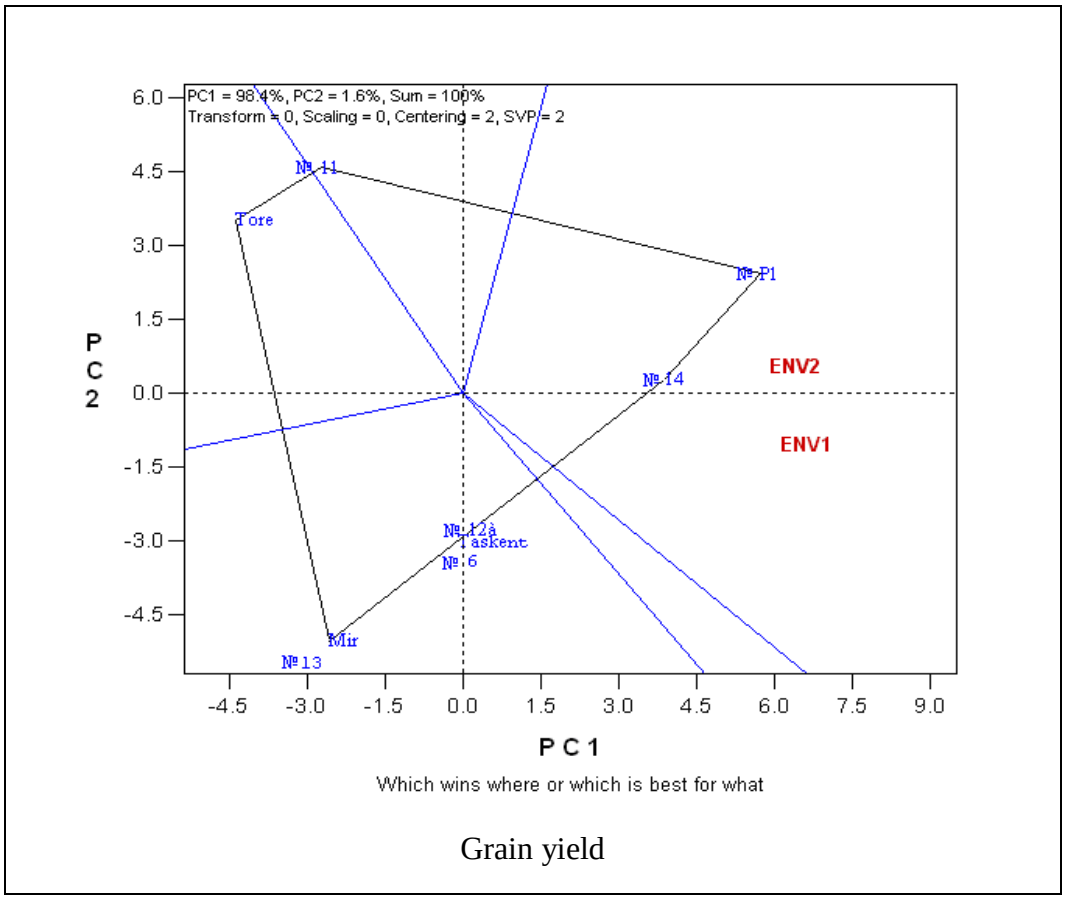

Figure 2. The GGE biplot based on the grain yield (ENV1 - Location 2, ENV2 - Location 1)

Correlation analysis

A strong positive correlation of grain yield with the mass of 1000 grains (r=0.881; r=0.875) and with the weight of grains per plant (r=0.732; r=0.676) was found in both locations (Table 6). This confirm that on the productivity side, the selection should target forms that form well-grained and heavier pods. The correlation of the yield with the height of the first pod is also positive, although weak (r=0.252*; r=0.251).

Table 6. Coefficient of Pearson between grain yield and distinctive features of the winter forage pea cultivars/lines

Coefficient of Pearson	Height, cm	Height first pod, cm	Number of grains/plant	Number of pods/plant	1000-seeds weight, g	Grain weight/plant, g
Location A	-0.528	0.252	-0.938	-0.700	0.881	0.676
Location B	-0.476	0.251	-0.586	-0.323	0.875	0.732

CONCLUSIONS

Line №13 (163 cm) and Taskent (158 cm) are characterized by high plant height values. In terms of the height of the first pod, the favorite was №PL (116 cm; 89 cm), and the Mir variety formed a larger number of pods per plant (32; 40). Lines №14 and №PLwere distinguished by higher pod weights (210 g, 162 g; 175 g, 141 g). Lines № 14, № 6 and № 12A are interesting in terms of grain productivity. Lines №PL and №14 had the highest productive capabilities with grain yield of 150.3 - 186 kg/da and 140.7 - 174.4 kg/da, respectively. Line №PL (bi=1.02) showed the best adaptive capabilities. Taskent (bi=1.23) and Tore (bi=1.27) cultivars were found unstable. №14 (bi=1.11) is high-yielding but variable, while Mir and №6 can be defined as stable (bi=0.80). GGE biplot analysis identified lines №PL and №14 as genotypes combining good stability and high grain yield. A strong positive correlation was found between grain yield and mass of 1000 grains, and grain weight per plant at both locations.

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INFLUENCE OF THE ULTRASOUND TREATMENT ON THE SOWING PARAMETERS OF *COLUTEA ARBORESCENS* L. SEEDS

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ABASTRACT

The main goal of the present study was to establish the possibilities for improving the sowing qualities of the seeds of *Colutea arborescence* L. after applying different sonication with ultrasound. The seeds were sonicated pre-sowing for 2, 4, 6 and 8 minutes with ultrasound, the untreated seeds were used as a control. Germination energy, germination, mean germination time, uniformity of germination, length of embryo root, length of hypocotyls and fresh weight of seedlings were determined. The highest germination was found after sonification for 6 minutes. The development of seedlings was with the best values when applied in 4 and 6 minutes. A polynomial regression of period for treatment and germination energy and germination, with high coefficients of determination R^2=0.99 and R^2=0.78, respectively was found. A strong positive correlation was observed between the fresh weight of the seedling and the length of the embryonic root.

Keywords: seeds, ultrasound, germination, seedling, sowing quality,

INTRODUCTION

The species of the genus *Colutea* form a rather diverse group of 12 species. They are distributed in a very large range, more massively in Africa and in Australia, and most of them are small trees or shrubs (Moghaddam et al., 2017). The species *Colutea arborescens* L. originates from Europe and North Africa. It is mainly used as an ornamental plant in landscaping, but it is also used to control erosion on various terrains. It has a bushy habit and a strongly branched stem, and in some cases, it can reach a height of up to 3 m. The decorative value is primarily determined by the fruits, which are swollen pods and acquire a papery texture when ripe (Dorbič and Španjol, 2017; Bsbi List, 2007; RHS, 2017). Although rare, this plant is also used for animal feed (Papachristoua et al., 1999).

Regarding ecological requirements, Oliverova and Veronika (2016) reported that *Colutea arborescens* L. is a light philic, thermophilic and xerophytic plant. Sukopp and Wurzel, 2003 point out that concerning climate changes and rising temperatures, this species, due to its higher heat requirements and in connection with its origin, from southern regions, is very suitable for landscaping in urban environments.

Ürgenç and Çepel, 2001) emphasize that the seeds of several tree species cannot germinate even if placed under favourable conditions of the necessary temperature, humidity and oxygen. According to the authors, this is due to the presence of dormancy of a different type, caused by a variety of reasons. Pijut and Paula (2008) reported that the seeds of *Colutea*

spp. they do not germinate easily, due to the poor permeability of the seed coat. To facilitate the germination process, they prevent these seeds from being pre-treated. Beikmohammadi et al. (2012) found dormancy in *Colutea* seeds related to their hard seed coat, which must be overcome to improve their germination.

The pre-sowing treatment of the seeds with physical agents, including ultrasound, is very suitable for breaking dormancy and improving seed quality (Panayotov, 2015). Meisam and Eteghadipour (2017) reached a similar conclusion, pointing out that ultrasound is a very promising technique for breaking dormancy and at the same time increasing the germination rate. Miao et al. (2016) stated that by applying ultrasound, the time for water absorption by the seeds is reduced by 25% and from there the germination is further accelerated.

The main goal of the present study was to establish the possibilities for improving the sowing qualities of the seeds of *Colutea arborescence* L. after applying different sonication with ultrasound.

MATERIAL AND METHODS

Experiments with seeds of *Colutea arborescens* L. were conducted in the Department of Horticulture at the Agricultural University-Plovdiv, Bulgaria. Harvesting of seeds at full maturity from the middle parts of eight plants located in the city of Plovdiv took place in September. The climate of this region is typical Moderate continental. After forming an average sample of the seeds from the experimental plants, the seeds for the experiment were separated.

Ultrasonic bath Nahita, model 620-1 of the company Auxilab, S.L. from Spain with parameters: frequency 40 kHz, bath volume 0.6 l, working with the electricity of 220 V and frequency 50-60 Hz was used for the pre-sowing treatment. The following sonication durations with ultrasound of the *Colutea arborescens* L.seeds were investigated:

1. Control;

2. Sonication with ultrasound for 2 minutes;

3. Sonication with ultrasound for 4 minutes;

4. Sonication with ultrasound for 6 minutes;

5. Sonication with ultrasound for 8 minutes.

By 100 seeds, in four replicates, in 10 cm diameter, Petri dishes with moist filter paper were placed in a germination chamber. The number of germinated seeds was counted daily. Germination energy and germination were determined as described by Beikmohammadi et al. (2012) and according to the requirements of the ISTA rules (2013).

To monitor the morphological characteristics of the seedlings, at the time of the establishment of germination, 15 seedlings from each replicate were measured for the length of the embryo root and the length of the hypocotyls. The germination time of 50% of the seeds

was also established. The Mean germination time was calculated by the method of Battle and Whittington (1966) using the equation

$$M.G.T. = \frac{\Sigma (G \times T)}{F}, \text{ where}$$

T – day in which the germination took place;

G – number of germinated seeds on this day (T);

F – final number of germinated seeds.

According to the method of Strona (1966), described by Panayotov (2015), uniformity of germination was determined.

The results were analysed by analysis of variance (ANOVA). Correlation and regression relationships between some indicators were determined (Fowel and Cohen, 1992).

RESULTS AND DISCUSSION

The application of ultrasound significantly improves the sowing qualities of the seeds of *Colutea arborescens* L. Table 1 shows the results for seed viability. As the duration of sonication increases, the germination energy grows steadily up to 8 minutes. It is the highest during the processing of 4 and 6 minutes - 14.67%, which is 6% above the control. A decrease towards them was found at 8 min, but also values were significantly above control. The importance of germination energy is seen in the fact that its higher values indicate that a greater part of the seeds have germinated in a shorter period and, accordingly, it is assumed that they have higher vital indicators (Panayotov, 2015).

A significant increase was also observed for germination, a sign by which the effect of a given pre-sowing treatment of the seeds is evaluated in the most detail. With increasing duration of treatment, it increases uniformly and is highest after sonication of 6 minutes, reaching 92% or approximately more than 50% compared to untreated seeds. Next is the one for 4 minutes. Compared to these two variants, at the other two durations tested, 2 and 8 minutes, germination was lower but also much greater than that of the control. The differences between the variants are statistically significant, in 4 and 6 minutes having the highest level of evidence (p=0.1%). The observed stimulatory effect of ultrasound on seeds according to Rifnaet al. (2019) is due to its mechanical effect, forming a very large number of small holes in the seed coat, which helps to improve humidity, and at the same time improves the porosity and the associated increase in oxygen availability.

The effect of ultrasound on the germination time of 50% of the seeds was weaker. However, it can be pointed out that the seeds treated for 6 and 4 minutes reached 50% germination the fastest. Slightly weaker results compared to these sonications were found for durations of 2 and 8 minutes. Correlations between the germination time of 50% of the seeds with the percentages of germinating energy and with the germination are high and negative, and their correlation coefficients are r=-0.98 and r=-0.81, respectively. From this, it can be

indicated that with a shorter time for 50% of seeds to germinate, the germination processes are accelerated and, accordingly, the germinating energy and the germination will be higher.

Table 1. Viability of the seed of *Colutea arborescens* L., after ultrasound treatment

№	Variants	Germination energy (%)	Germination (%)	Time for 50% of seed germination (days)
1	Control	8,00	38,66	13.00
2	2 min.	12,00	54,67	12.00
3	4 min.	14,67	82,66	11.66
4	6 min.	14,67	92,00	11.55
5	8 min.	12.00	42,67	12.00
p=5.0%		7,38	15,74	1,44
GD p=1.0%		10,69	22,79	2,09
p=0.1%		16,04	34,19	3,14
		*r= -0.98	*r=-0.81	

*correlation with Time for 50% of seed germination

On Figures 1 and 2 are shown the regression lines of the changes in the vital state of the seeds, caused by the steadily increasing duration of treatment to ultrasound. They are of polynomial type, both for germination energy and germination. By means of the coefficients of determination, the percentage of dispersion of the resulting variable, which is due to the action of the factor variable, is established. These coefficients are high, for germination energy is R^2=0.99, and for germination is R^2=0.78 and, respectively, in 99% and 78% of the cases, the ultrasound will cause the indicated changes in the seeds.

The mean germination time indicates the average time it took for a seed to germinate (Copeland and Donald, 2001). A certain improvement in this indicator was found after treating the seeds of *Colutea arborescens* L. with ultrasound (Table 2). An improvement is reported for all variants. Values ranged from 11.49 days for 2 min sonication to 13.04 for the control. At the same time, the variation between individual processing times is low. Most days for germination of one seed have been necessary after applying 4 minutes of ultrasound, but the results were still better than those of untreated seeds. The uniformity of the seeds was also higher as a result of the application of ultrasound except for 8 minutes of treatment where the inhibitory effect was significant. It is the highest for 6 minutes of sonication – 8.11%, followed by that for 4 minutes. Mathematical evidence of the differences was established, except for the 2-minute variant.

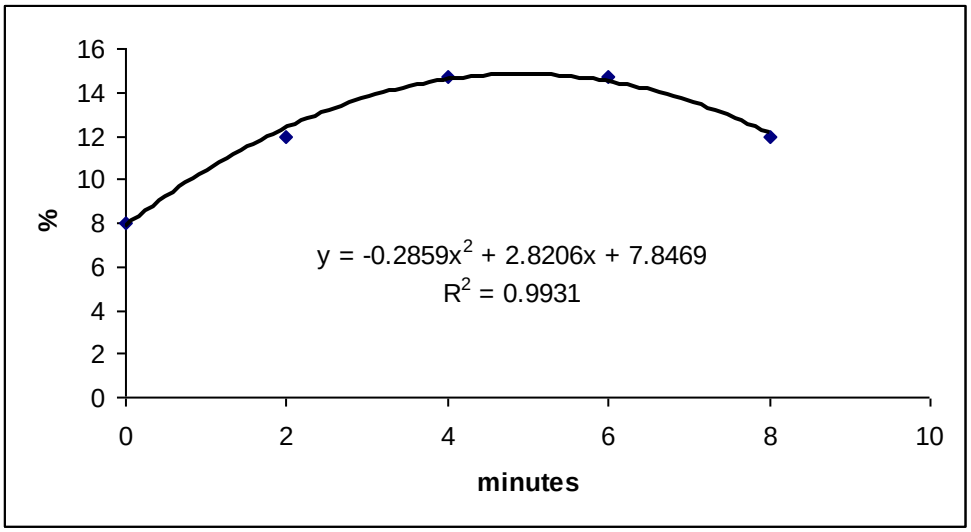

Figure 1. Regression dependence of germination energy of seeds of *Colutea arborescens* L., after treatment with ultrasound

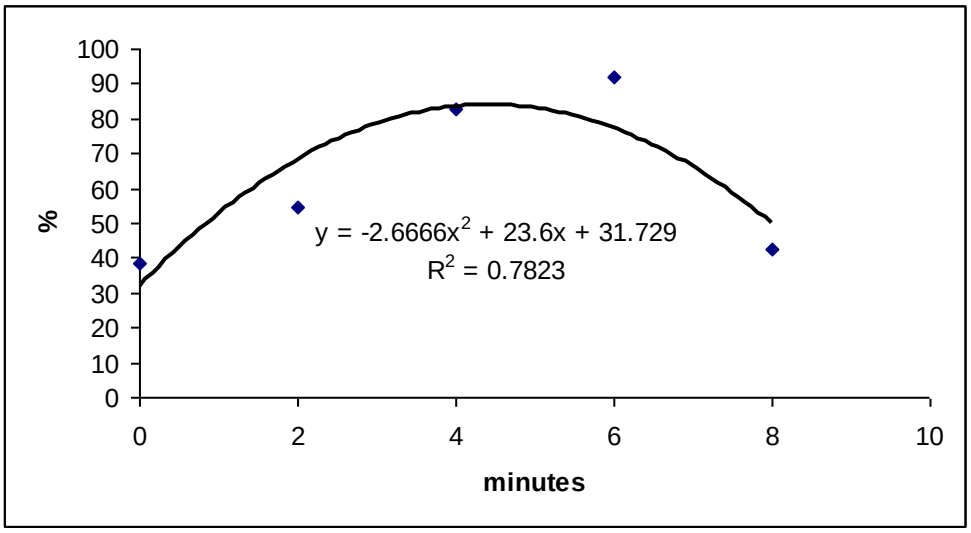

Figure 2. Regression dependence of seed germination of *Colutea arborescens* L., after treatment with ultrasound

Seed vigor, which indicates the ability of seeds to develop under a wide range of environmental conditions, is directly related to seedling morphological development (Black et al., 2006). The data in Table 3 show that the application of ultrasound improved embryo root growth. It has the longest length after treatment 6 minutes and reaches 3.61 cm, which is 1.37 cm above the control. Values for the shorter duration of 4 minutes are also close - 3.16 cm. On the length of the hypocotyls, the stimulating effect is also significant, which gradually increases to the variant of 6 minutes. At 6 and 4 minutes of treatment, its length is greatest, 3.07 cm and 2.90 cm, respectively. It was lower than the other durations for the variant of 8 minutes but also higher than that of untreated seeds. The mathematical proof of the differences is at the highest level of significance.

Figure 3 presents the data on the daily temp of germination of *Colutea arborescens* L. The earliest germination was observed at 2 and 8 minutes, as early as day 6. The percentage of germination after 9 days is significant, especially when sonication is 6 minutes - 9.33%. In this variant, the peak is highest on days 12 and 14. A clear peak for 2 and 4 minutes of ultrasound was established on day 11. The slowest rate for the seed was observed after processing for 8 minutes. The seeds from the control and those from 2 minutes complete germination the earliest, and at 4 and 6 minutes it continues until day 20.

Table 2. Sowing parameters of *Colutea arborescens* L. seeds, after ultrasound treatment

№	Variants	Mean germination time (days)	Uniformity of germination (%)
1	Control	13,04	6,06
2	2 min.	11,49	6,15
3	4 min.	12,61	7,29
4	6 min.	11,55	8,11
5	8 min.	11,85	4,92
p=5.0%		1,41	1,08
GD p=1.0%		2,04	1,56
p=0.1%		3,06	2,34

Table 3. Morphological behaviors of *Colutea arborescens* L. seeds, after ultrasound treatment

№	Variants	Length of embryo root (cm)	Length of hypocotyls (cm)
1	Control	2,24	1,98
2	2 мин.	2,56	2,60
3	4 мин.	3,16	2,90
4	6 мин.	3,61	3,07
5	8 мин.	2,44	2,32
p=5.0%		0,16	0,21
GD p=1.0%		0,24	0,3
p=0.1%		0,36	0,46

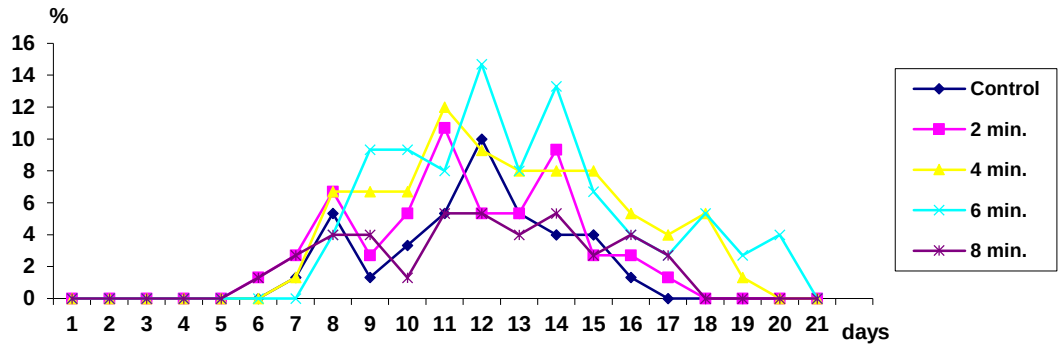

Figure 3. Daily temp of germination of *Colutea arborescens* L. seeds after sounding with ultrasound

CONCLUSIONS

The seeds of *Colutea arborescens* L. significantly improve their vitality under the influence of ultrasound treatment. The germination energy and germination were the highest at 6-min sonication, followed by those at 4-min. Regression dependences on the duration of ultrasound application on these two indicators are polynomial type and the coefficients of determination are high.

Under the influence of ultrasound, the uniformity of seed germination increases, while the influence of sonication on the mean germination time is weaker. The growth and development of the seedlings were improved the strongest from 6 minutes of ultrasound treatment.

For the seeds of *Colutea arborescens* L. it can be recommended that a suitable duration of ultrasound application is 6 minutes.

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