AGRIBALKAN 2021

III. BALKAN AGRICULTURAL CONGRESS

29 AUGUST – 01 SEPTEMBER 2021,

EDIRNE, TURKEY

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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.

As third one, Trakya University hosted again in Edirne, Turkey in 2021. We would like to thank all participants for great interest to our AGRIBALKAN 2021 congress even in Covid 19 pandemy. There is a worldwide participation from 41 countries with 406 papers contributed by 988 authors. Our AGRIBALKAN Congress will be organized with 288 oral, 118 e-poster presentations.

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.

Prof. Dr. Erhan TABAKOGLU
Rector of Trakya University
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CONTENTS

VARIATION OF PM_{10} AND SO_2 CONCENTRATIONS IN HEATING DEGREE DAYS AND COOLING DEGREE DAYS IN KIRKLARELI, TURKEY

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ABSTRACT

Today, with the rapid increase of the world population, air pollution due to heating is increasing in cities. In this study, heating degree days and cooling degree days were determined in a one-year period in Kirklareli city centre. The variation and cumulative distribution of daily PM_{10} and SO_2 concentrations in heating degree days and cooling degree days have been compared. It has been determined whether the daily PM_{10} and SO_2 concentration values in the heating degree days and cooling degree days exceed the limit values set by The European Parliament and of The Council. The wind rose diagrams have been plotted in heating degree days and cooling degree days. In addition, polar plot has been produced for PM_{10} and SO_2 concentrations. The directions of transport of PM_{10} and SO_2 on heating degree days and cooling degree days were determined.

Keywords: PM_{10}, SO_2, heating degree days, cooling degree days, polar plot

INTRODUCTION

Air pollution has become a global problem due to the spread of pollutants throughout the atmosphere. Air pollution is considered a distinctive feature of city centres (Seinfeld, 1975). It has been stated that because of the anthropogenic emissions in these settlements reaching high values, the concentrations of pollutants cause deterioration of air quality and visibility and may reach threatening levels to human health. Thanks to the winds and atmospheric turbulences, the particles on the Earth are transported to the atmosphere (Wallace and Hobbs, 2006). It is very difficult to make a regular classification for air pollutants due to the large variety of substances in the air. Air pollutants can be divided into two general categories, as primary pollutants, and secondary pollutants. Pollutants emitted directly from sources are named primary pollutants; pollutants formed as a result of chemical reactions in the atmosphere are named secondary pollutants. Generally, air pollutants are compounds containing sulphur, nitrogen, carbon (except carbon monoxide and carbon dioxide), carbon monoxide and carbon dioxide, halogen compounds, particulate matter, radioactive compounds (Seinfeld, 1975). Particles that survive in the air through several processes are aggregates of many molecules, often different, sometimes similar (Vallero, 2008). Biomass burning and fossil fuel combustion, particularly coal, have been stated to be effective on particulate emissions worldwide (Wallace and Hobbs, 2006). The grater the weight of a particle, the more likely it will fall to the ground (Vallero, 2008). The particles produced during the gas-to-particle conversion process have smaller sizes compared to other particles, causing these particles to stay in the atmosphere longer than other particles (Wallace and Hobbs, 2006). Fine and coarse particle modes with different chemical compositions are separated from the atmosphere by different mechanisms. Major anthropogenic sources of particulate matter are included industrial processes, transport sources, fuel combustion, and non-industrial temporary sources (Seinfeld and Pandis, 2006).

Different types of urban air pollution have arisen due to the fact that a given urban atmosphere at any given time has few amounts of pollutants (primary and secondary). Low temperature, high concentrations of SO_2 and SO_4^{2-}, the presence of water and particles are typical properties of the first and oldest type of air pollution. The use of gasoline as motor fuel is characteristic of other types of air pollution (Seinfeld, 1975). Power plants, petroleum refineries, smelters, pulp, and paper mills are important sources of SO_2. After SO_2 is released into the atmosphere, it combines with oxygen to form the secondary pollutant SO_3 and then combine with water to form the secondary pollutant SO_4^{2-} (Lutgens and Tarbuck, 2016).

In this study, heating degree days (HDD) and cooling degree days (CDD) were determined in Kirklareli city centre in 2015. Daily concentrations of PM_{10} and SO_2 in HDD and CDD have been compared. Wind directions have been determined in HDD and CDD. Wind rose graphics have been created in HDD and CDD. In addition, polar plot was produced for PM_{10} and SO_2 concentrations in the HDD and CDD.

MATERIAL AND METHOD

Kirklareli is located in north-western Turkey. It is a border city neighbouring with Bulgaria (Figure 1). Kirklareli has a 180 km land border with Bulgaria. It also has a border of 60 kilometers with the Black Sea. Terrestrial climate is observed in Kirklareli city centre where PM_{10} and SO_2 measurements are carried out. Although agricultural production still maintains its importance throughout Kirklareli, industrial production is also developing (ÇDR, 2020).

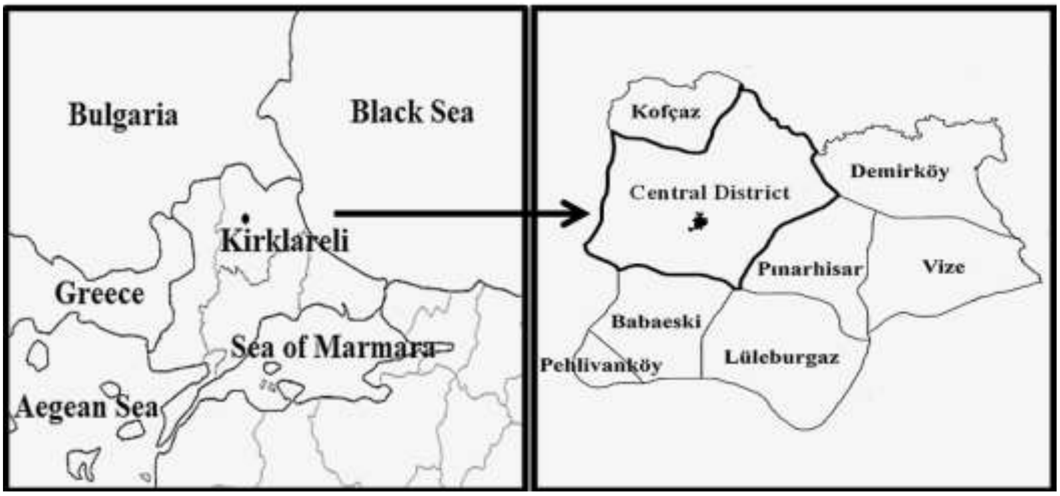

Figure 1. Sampling point

In this study, daily PM_{10} and SO_2 concentrations data obtained from the Air Quality Monitoring Station of Republic of Turkey Ministry of Environment and Urbanization were used (MEU, 2021). In addition, the wind speed, wind direction and temperature used in the study were obtained from Meteorological Service of Turkish State. Wind rose and polar plot were created with the R-Studio program using OpenAir statistical analysis package (Carslaw and Ropkins, 2012; Carslaw, 2019).

The energy required for heating in houses and industry in Kirklareli is obtained by burning coal, fuel-oil and natural gas, which are fossil fuels in solid, liquid and gaseous form (ÇDR, 2020). Considering that people living in Kirklareli city centre use coal, fuel-oil and natural gas for heating in houses and workplaces at temperatures below 18 ^oC, HDD and CDD have been determined in 2015 (Büyükalaca et al., 2001). HDD and CDD are relative methods that allow assessment of heating and cooling costs as well as weathering needs (Lutgens and Tarbuck, 2016). Degree days are defined as the difference between the daily mean outdoor temperature and base temperature. HDD and CDD are calculated according to Equation (1) and Equation (2), respectively (Büyükalaca et al., 2001; Işık et al., 2019).

$$HDD = (1\ \text{day}) \sum_{\text{days}} (T_b - T_m)^+ \qquad \text{(Equation 1)}$$

$$CDD = (1\ \text{day}) \sum_{\text{days}} (T_m - T_b)^+ \qquad \text{(Equation 2)}$$

In equations, T_b is the base temperature and T_m is the daily mean outdoor temperature.

RESULTS AND DISCUSSION

The European Parliament and of The Council (Directive-2008/50/EC) has set the PM_{10} concentration limit value for a day at 50 µg m^{-3} and stated that the daily value should not be exceeded for more than 35 times a calendar year (EU, 2008). It has been observed that 53.64% of daily PM_{10} concentrations in HDD and 31.13% in CDD exceed the 50 µg m^{-3} limit value. Values of daily average PM_{10} concentration are 64.70 µg m^{-3} in HDD and 29.28 µg m^{-3} in CDD. It is seen that the data set has a high range (minimum-maximum) in HDD and CDD. Standard deviation values of PM_{10} concentrations are 40.91 and 29.84 in HDD and CDD, respectively. Daily PM_{10} concentrations in HDD and CDD are shown in Figure 2. The lowest and highest values of daily average PM_{10} concentrations are 5.22 and 230.00 µg m^{-3} in HDD, respectively, and 2.39 and 110.61 µg m^{-3} in CDD, respectively. Cumulative distributions were examined to see the internal distribution of the PM_{10} concentration data set. For PM_{10} concentrations, values between 25% and 75% of the data set vary between in HDD 36.01 and 80.38 µg m^{-3}, while in CDD varies between 5.28 and 57.58 µg m^{-3}.

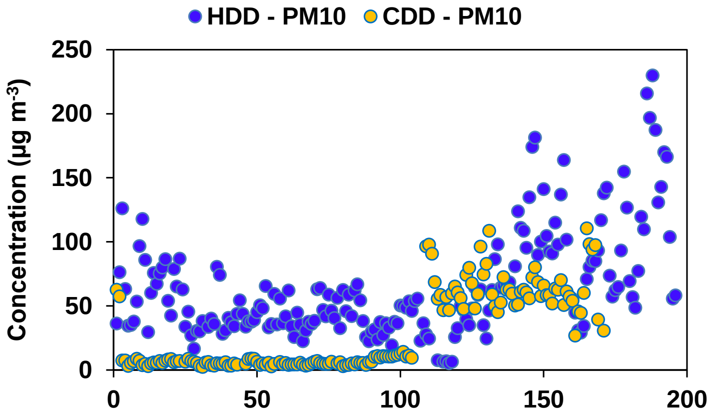

Figure 2. Daily PM_{10} concentrations in HDD and CDD

The European Parliament and of The Council (Directive-2008/50/EC) has set the SO_2 concentration limit value for a day at 125 µg m^{-3} and stated that the daily value should not be exceeded for more than 3 times a calendar year (EU, 2008). It has been observed that daily SO_2 concentrations exceed the 125 µg m^{-3} limit value for only 1 day (137.70 µg m^{-3}) in the HDD. In CDD, it has been determined that the values of daily average SO_2 concentration do not exceed the limit value determined. Values of daily average SO_2 concentration are 24.79 µg m^{-3} in HDD and 4.77 µg m^{-3} in CDD. It is seen that the data set has a high range (minimum-maximum) in HDD and CDD. Standard deviation values of SO_2 concentrations are 20.51 and 4.50 in HDD and CDD, respectively. Daily SO_2 concentrations in HDD and CDD are shown in Figure 3. The lowest and highest values of daily average SO_2 concentrations are 2.43 and 137.70 µg m^{-3} in HDD, respectively and 1.22 and 26.13 µg m^{-3} in CDD, respectively.

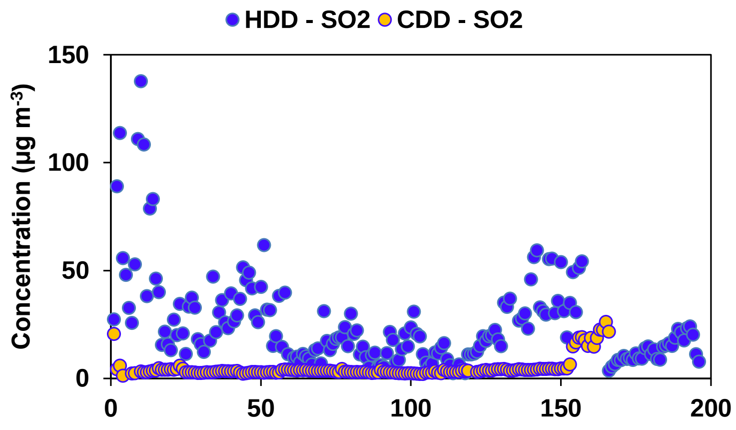

Figure 3. Daily SO_2 concentrations in HDD and CDD

Wind rose in HDD and CDD is shown in Figure 4. It has been observed that the wind in the HDD blows with approximately 25% frequency at the most, in the east (E) direction with 0-2 m s^{-1}, 2-4 m s^{-1}, and 4-6 m s^{-1} speed. It blows in the east-northeast (ENE) direction with approximately 22% frequency and in the north-northeast (NNE) direction with approximately 12% frequency. In CDD, the wind mostly blows with approximately 36% frequency in the east (E) direction. It has been observed that the wind speed is more than 6 m s^{-1} in the west-southwest (WSW) direction in the HDD and CCD.

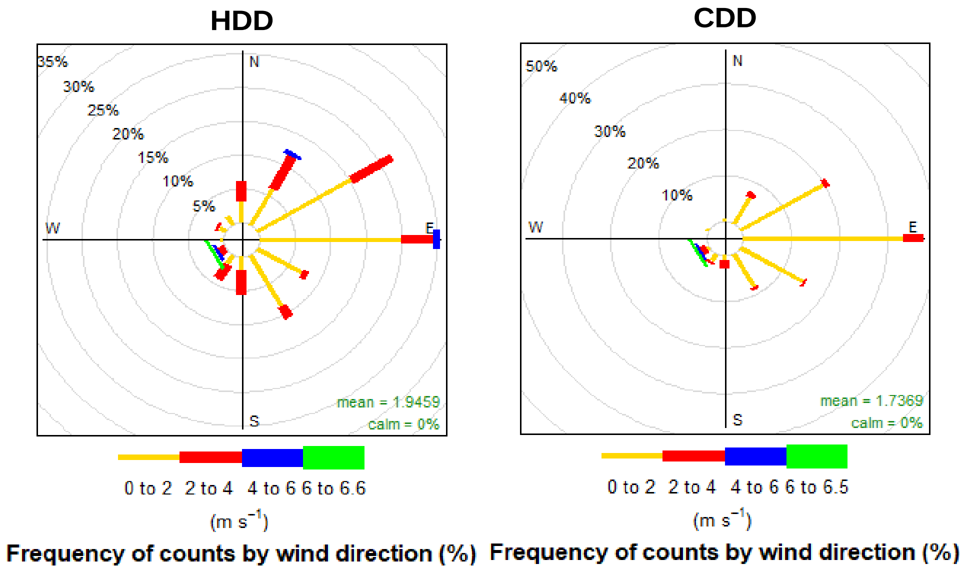

Figure 4. Wind rose in HDD and CDD

Polar plot for PM_{10} concentrations in HDD and CDD is shown in Figure 5. PM_{10} transport in the HDD has been seen to be higher in the west-southwest (WSW) and northeast (NE) directions, but high PM_{10} transport from all directions. It has been observed that the PM_{10} transport in CDD occurs mostly in the west-southwest (WSW) direction. Again, in this direction, it is seen that there are the highest concentration values for PM_{10}.

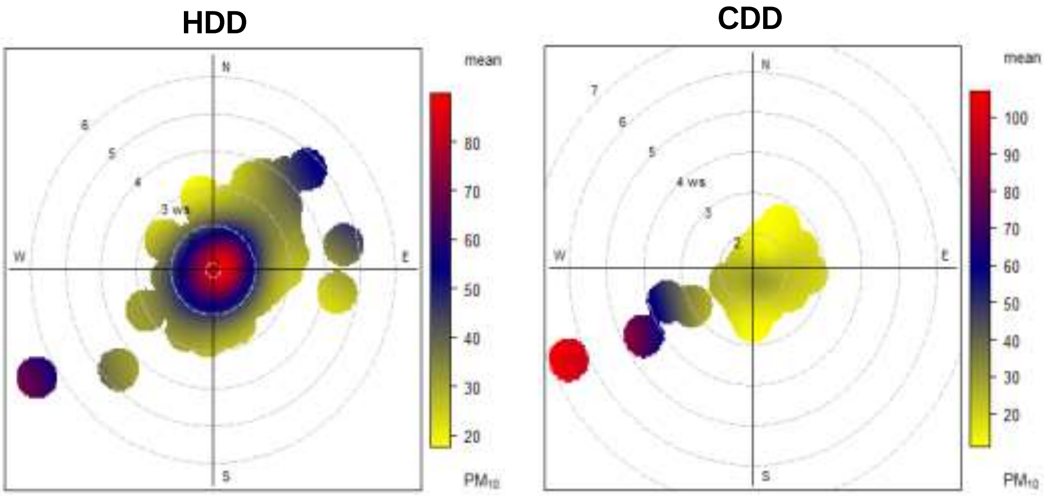

Figure 5. Polar plot for PM_{10} concentrations in HDD and CDD

Polar plot for SO_2 concentrations in HDD and CDD is shown in Figure 6. For SO_2, the wind directions where the most pollution is transported are north (NE) and north (N) in the HDD. In these directions, the highest concentration range for SO_2 was observed. The wind directions with the highest pollution for SO_2 in CDD are determined as west (W) and east (E) (5.5-6 µg m^{-3}). After these directions, it is seen that the wind direction where the most pollution is transported in the CDD is west-southwest (WSW).

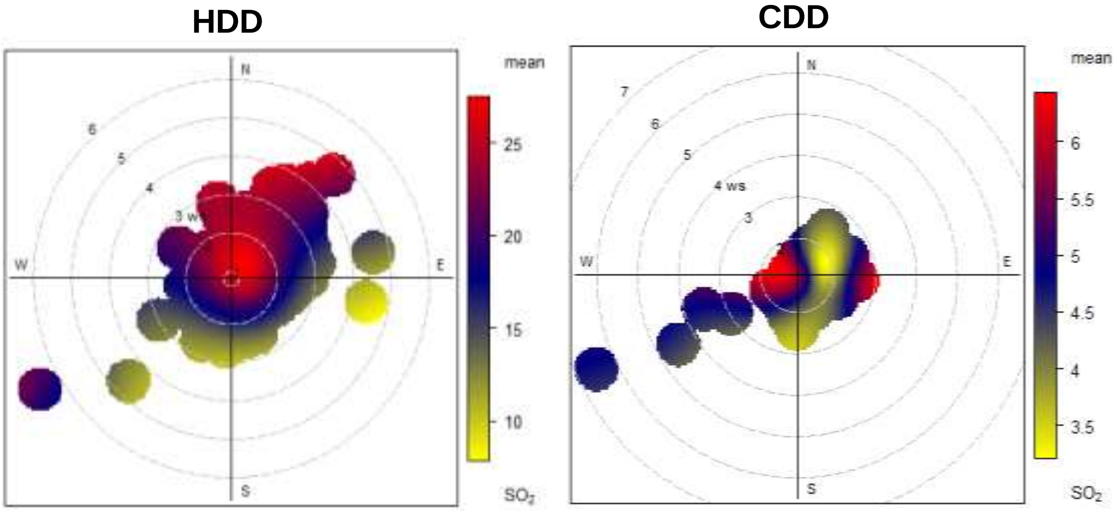

Figure 6. Polar plot for SO_2 concentrations in HDD and CDD

CONCLUSIONS

HDD and CDD were determined in Kirklareli city centre in 2015. The variation and cumulative distribution of daily PM_{10} and SO_2 concentrations in HDD and CDD determined during the sampling period were compared. The values of daily average concentrations of PM_{10} and SO_2 are 64.70 µg m^{-3} and 24.79 µg m^{-3} in HDD, respectively, and 29.28 µg m^{-3} and 4.77 µg m^{-3} in CDD, respectively. It has been observed that, respectively, 53.64% and 31.13% of daily PM_{10} concentrations in HDD and CDD exceed the limit value. It has been observed that daily SO_2 concentrations exceed the limit value by only 1 day in the HDD. During the study period, wind blew most frequently from east (E) in both the HDD and CDD. The highest concentration range for PM_{10} was observed in the west-southwest (WSW) direction in the CDD. The highest concentration range for SO_2 was observed in the north (NE) and north (N) directions in HDD, and in the west (W) and east (E) directions in CDD.

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I thank The Meteorological Service of Turkish State for wind speed, wind direction, and temperature data.

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ABSTRACT

Trial with 10 samples of garden peas was performed on the experimental fields of the Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria during three years period. Three of samples had afila leaf type (Echo-af., Kazino-af. and line 22/16- af.), while the other seven (Marsy-n., Plovdiv-n., line 22/16-n., Shugar dwarf-n., Vecherniza-n., line B4/34-n. and line 1/17-n.) – normal leaf type. In the stage of technological maturity of 10 plants from each sample the next indicators were evaluated: plant height (cm), height to first fertile node (cm), internode length (cm), tillers number, branches number, ineffective nodes number, total number of nodes, total number of pods per plant, 1 pod per fruiting handle, 2 pods per fruiting handle, pod length (cm), pod width (cm), pod weight per plant (g), green grains weigh per plant (g), % filled grains, % unfilled grains, average number of grains per pod. Regression analysis was applied and based on the values of the regression coefficient (R) it was found the most significant role in the formation of grain productivity was attributed to total number of nodes (R = 1.190), pods weight (R = 0.610) and height to first fertile node (R = 0.162). The analysis allows determining through which structural elements of the yield it is possible to more effectively increase the productivity of green grains from plants. Based on the regression dependencies, the effectiveness of the selection performance in the sampled garden pea can be successfully predicted.

Key words: garden pea, regression analysis, dependencies, grain productivity

INTRODUCTION

Peas (*Pisum sativum* L.) is one of the earliest food crops and is grown in almost all temperate regions of the world (Bashir et al., 2017). There is a great need to develop high yielding cultivars in order to achieve future improvement. The plant breeder depends upon variability present in the material for the improvement of quantitative characters and their mutual association with seed yield (Singh et al., 2018).

In the progress of selection, the study of the contiguity of breeding indicators, the identification of their contribution to the formation of productivity and yield are an important stage in the development of varietal models and the improvement of the selection process technique. In pea breeding, these issues are given close attention in connection with the creation of new varieties with a new combination of morphological and quantitative traits (Sinyushin, 2015). To improve the model of a pea plant with a new feature, an in-depth study of the crop dependence and its constituent elements of productivity is necessary (Vasileva et al., 2020). The study aimed at to determine the correlation dependences of some quantitative traits and grain productivity in garden peas samples.

MATERIALS AND METHODS

The study was conducted in the experimental fields of the Institute of Vegetable Crops Research Institute, Plovdiv, Bulgaria during the period of 2017-2019 with ten garden peas genotypes.

Three of them (Echo-af., Kazino-af. and line 22/16- af.) had afila leaf type, while the other seven (Marsy-n., Plovdiv-n., line 22/16-n., Shugar dwarf-n., Vecherniza-n., line B4/34-n. and line 1/17-n.) – normal leaf type. The experiment was performed by block method, four repetitions with a working plot area of 6.4 m2. The sowing was done out at the end of February on a high flatbed according to the scheme 80+20+40+20/4–5 cm. The peas are grown according to the technology adopted for the crop.

The main morphological (biometric) characteristics of the aboveground biomass were measured at the technological maturity of plants. For this, 10 plants were used from the four replications of the trial. Plant height (cm), height to first fertile node (cm), internode length (cm), tillers number, branches number, ineffective nodes number, total number of nodes, total number of pods per plant, 1 pod per fruiting handle, 2 pods per fruiting handle, pod length (cm), pod width (cm), pod weight per plant (g), green grains weigh per plant (g), % filled grains, % unfilled grains, average number of grains per pod were measured.

The biological requirements of garden pea and nutrients into the soil we had in a mind to prepare the fertilizer rates. So, in the first experimental year, 20 kg/da of triple superphosphate, 25 kg/da of potassium sulphate and 3 kg/da of magnesium sulphate were imported with basic fertilization, for the second year the doses were 30 kg/da, 25 kg/da and 5 kg/da, respectively. During the growing season, up to 30 days after emergence, 5 kg/da ammonium nitrate and 8 kg/da potassium sulfate were imported for the two years.

Regression analyses were used to process the data (Dimova and Marinkov, 1999). Additionally, GENES 2009.7.0 for Windows XP software was used also (Cruz, 2009).

RESULTS AND DISCUSSION

After the multiple regression analysis, an equation of the productivity model (the green grains weight) was compiled on average for the years of study. The analysis is statistically significant (Table 1).

Table 1. Regression analysis (ANOVA) of the green grains productivity in terms of quantitative indicators

	df	SS	MS	F	Significance F
Regression	16	652.8389	40.80243	22.78508	0.012687
Residual	3	5.372257	1.790752		
Total	19	658.2111			

The model type of the regression equation (1) is as follows:

(1) $$Y = -19779.9 - 0.127*X1 + 0.162*X2 - 0.230*X3 - 0.772*X4 - 1.254*X5 - 1.617*X6 + 1.19*X7 - 0.099*X8 - 1.049*X9 - 0.308*X10 - 1.662*X11 - 2.912*X12 + 0.610*X13 + 197.991*X14 + 197.91*X15 + 0.148*X16;$$

Where, Y is grain productivity; X1 – plant height (cm), X2 - height to first fertile node (cm), X3 - length of internode (cm), X4 - number of tillers, X5 - number of branches, X6 - ineffective nodes number, X7 – nodes total number, X8 - pods total number, X9 - 1 pod per fruiting handle, X10 – 2 pods per fruiting handle, X11 - pod length (cm), X12 - pod width (cm), X13 - pod weight per plant (g); X14 - % filled grains, X15 - % unfilled grains, X16 - average number of grains per pod.

The dependencies (expressed by the green grains weight) with the aboveground mass signs in pea samples are shown in Figure 1 (averaged for the period).

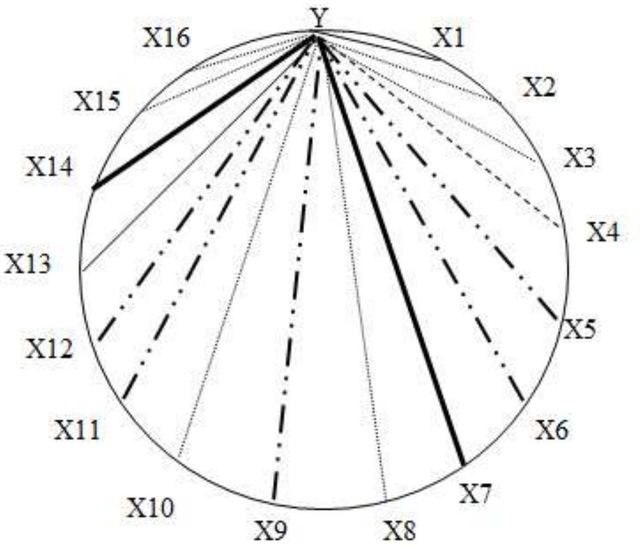

Figure 1. Regression dependencies (R) of the weight of green grains on plant Y with other quantitative traits

weak positive; average positive; strong positive;

weak negative; average negative; strong negative

Y - green grains weight (g); X1 - plant height (cm); X2 - height to first fertile node (cm); X3 - length of internode (cm); X4 - number of tillers; X5 - number of branches; X6 - ineffective nodes number; X7 - total number of nodes; X8 - total number of pods per plant; X9 - 1 pod per fruiting handle; X10 – 2 pods per fruiting handle (number); X11 - pod length (cm); X12 - pod width (cm); X13 - pod weight (g); X14 - % filled grains; X15 - % unfilled grains; X16 - average number of grains per pod.

Based on the values of the regression coefficient (R), it can be seen that the largest direct effect on the formation of the weight of green grains per plant has number of nodes (R = 1.190) followed by weight of pods per plant (R = 0.610). Positive but low values of regression coefficients of height to first fertile node (R = 0.162), average number of grains per pod (R = 0.148), the % filled grains (R = 0.126) and % unfilled grains (R = 0.043), over the study period indicate that these signs do not play a significant role in the productivity. Shrivastava et al. (2008) suggested that the number of pods per plant, number of seeds per plant must be taken to account during the course of selection for high yielding varieties in pea. Tofiq et al. (2015) studied that the simple correlation coefficient among characters of pea. It was observed that the character weight of seeds plant correlated positively and highly significantly correlated with number of pea pods per plant, weight of pods per plant, biological weight per plant and harvest index respectively.

Judging by the negative regression coefficients of the width and length of the pods (R = -2.912; R = -1.662), the number of ineffective nodes (R = -1.617), the number of branches (R = -1.254)

and 1 pod per fruiting handle (R = -1.049) it is clear that the increase in their values definitely has a negative effect on grain productivity. In such a situation, the length of internodes and the number of tillers have a slight negative effect on the weight of green grains per plant. Opposite to our findings, some researchers reported that the positive correlations were also determined between grain yield and pod number per plant, plant height, grain number per plant and days to flowering (Kosev and Mikic, 2012; Jeberson et al., 2016; Bhuvaneswari et al., 2017).
According to Sardana et al. (2007) the number of main branches and pods per plant show the greatest positive effect and therefore special attention should be paid to the number of generative organs during selection.
A comprehensive study of these relationships allows us to predict the results of the selection on certain traits and to improve the selection process. It also allows us to determine which structural elements of production can more effectively increase the overall productivity of green grains from plants. Based on the correlation dependencies established, the effectiveness of the selection performance in the sampled garden pea can be successfully predicted.

CONCLUSIONS

The results of the study show that the most significant role in the formation of grain productivity is attributed to total number of nodes (R = 1.190), pods weight (R = 0.610) and height to first fertile node (R = 0.162), and negatively have the width and length of pods (R = -2.912; R = -1.662), the number of ineffective nodes (R = -1.617), the number of branches (R = -1.254) and 1 pod per fruiting handle (R = -1.049). The described correlations between the quantitative traits showed the prospects for their use in the breeding process, depending on the tasks given.

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RELATIONSHIP BETWEEN REPRODUCTION PERFORMANCES AND COAT CHARACTERISTICS OF MONTBELIARDE COWS DURING HOT SEASON IN ALGERIA

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ABSTRACT

Our study aimed to study the relationship between reproduction performances and coat characteristics of Montbéliarde cows born in Algeria or imported from Europe, during the hot season in Algeria. Hair coat traits (hair coat color, Hair Weight, hair length, number of hair per unit area, hair total diameters and hair medulla diameters) were estimated in 18 imported cattle and 49 locally born cows. These traits were measured in an area of 20cm below the dorsal line in the center of the thorax. Results showed that significant effect of hair total diameter was observed on interval from calving to conception (IC) for imported MB cows, suggesting less incidence of heat stress on reproduction efficiency of cows with thin diameter hair coat. MB cows with short hair coat registered significantly more number of mating per conception (2,28±1,93 Vs 1,67±0,92) and IC (98,04±78,81Vs 74.53 ± 35.60 days) when compared to cows with long hairs. Hair works as a temperature regulator in association with muscles in the skin and may affect reproduction performances during hit stress season. It can be assumed that length and total diameter of hairs for MB breed appear to be related to their reproductive efficiency.

Key Words: Hair coat, Reproduction, Montbeliarde cow, Hot season

1. INTRODUCTION

Environmental conditions are changing, and therefore the only passport to the survival of an organism is adaptability (Udo, 1978). The hair is an aspect of environmental adaptation: this is the border between the animal and the surrounding physical environment and reflects the response of the animals to their environments. So coats of cattle are adapted to withstand the harsh summer weather of breeds' experiences. In addition, animal coat changes in response to seasonal environmental changes (Brinkmann et al., 2012).

Solar radiation considerably increases thermal load on the animal grazing during the day, particularly in summer months. The amount of radiant heat absorbed by the animal's coat is partly determined by color, length and conditions of its hair. Light from the invisible infra-red part of solar radiation is completely absorbed irrespective of coat color, but the visible portion of radiation is absorbed by the animals coat depending upon its color (Walsberg 1983).

Penetration of fur by solar beams is a function not only of the color but also of the structure of the coat (Hutchinson and Brown, 1969). From this relationship, it can be inferred that when European cattle are transferred from temperate climates to sub tropical regions, adaption problems will occur. However few studies reported relationships between hair characters and fertility of imported dairy cows, and association between place of birth, hair characters and reproduction performances.

The present study investigated the association between reproduction performances of imported and locally born cows of montbeliarde dairy breeds and coat characteristics in summer, which coincides with the hottest period in subtropical region, in Algeria.

II. MATERIALS AND METHODS

II.1. Animals

A total of 67 Montbéliarde cows were selected in a commercial farm and used in this study, among them 18 cows were imported and 49 locally born. Detection of estrus was made by visual observation and insemination by natural service. After morning and evening milking, cows were kept free with sires. Individual records included cow identification number, birth date, parity, dates of natural mating, abortion and calving dates for all cows. Consequently, number of services per conception (SPC), interval from calving to first service (ICM), and interval form calving to conception (IC) for all cows during the period of 2012 and 2013 were calculated. Animals were offered to having the hair cut at the same time (August) when the cows were under heat stress conditions. All the animals were housed in a covered industrial farm, without cooling system, having free access to water and the same environmental conditions.

II.2. Morphological characteristics of coat

Hair sample collection from individual cows was performed late August in summer season. Percentage of white coat color was measured by taking photos from both sides of each animal using a digital camera (Becerril and Wilcox, 1992), then transferred to special software (IPWin32). Hair samples were taken from the center of the thorax about 20 cm below the dorsal line, as close as possible to the skin, by means of an electrician's pliers adapted in such a way that all hairs within a 1 cm^2 area could be plucked out (DaSilva et al., 1988). Hair samples were stored in plastic envelopes and sent to the laboratory for hair measurements: Hair Weight (mg/cm^2) was weighed with an analytical balance; hair length (mm) was taken as the average length of the 20 longest hairs of the sample by using a thin metal ruler, according to the method of (Udo, 1978) ; number of hairs ($hairs/cm^2$) was obtained by direct counting of all hairs present in the sample ; an ocular micrometer scale of microscope (Leica DM1000) was used to measure hair total diameters and hair medulla diameters (μm) of the same hairs used for length measurement.

II.3. Statistical analyses

The mean and standard deviation were calculated and the tests for normality (Kolmogorov-Smirnov) and homogeneity of variances (Levene’s test) were performed for all parameters. Any variables deemed not normal were transformed by logarithmic transformation. A non-hierarchical classification (VL) method that uses the distribution of central trend statistics is performed to identify the different classes of hair coat traits (Sousa, 2000; Britoet al.,2005).

To estimate the effect of hair coat characteristics on reproduction parameters (SPC, ICM, IC) for montbeliarde breed (imported and locally born) during summer season we used a general linear model (GLM procedure). The comparison between means was performed by the LSD test (least significant difference). All analyzes were performed by SPSS 21.

III. RESULTS AND DISCUSSION

III.1. Effect of hair characteristics on reproduction performances

Hair total diameter of imported cows (<89.5 ; > 89.5 µm) are relatively larger when compared to those reported by Bertipagliaet al ., 2005 for Holstein breed (<55; 55-65; > 65 µm). Cows with large hair diameter recorded significantly reduced interval from calving to conception (82 vs 155 days) when compared to cows with thinner total hair diameter (table 2). The largest hair diameter, according to Maia et al., 2003, is an advantage in warm environments. Because animals are more able to lose surplus heat, they less suffer from certain physiological disturbances such as reduction in hypothalamic activity, which, in turn, can reduce thyroid hormone concentrations (Pugh, 2002).These hormones impact thermogenesis capacity and play an important role in certain physiological functions related to growth, reproductive efficiency and dairy production (West , 2003; Al-Haidary, 2004). Significant effect of total hair diameter on fertility of MB breed is probably the consequence of heat stress in Algeria.

For locally born MB, cows’ fertility was significantly affected by hair length. Individuals with long haired coats (length>15 mm) recorded shorter intervals from calving to conception (74.5 vs 98 days) and lower number of SPC (1.67 vs 2.28) when compared to cows with short hair (table 3). Under heat stress of hot season, the longer hairs with denser coats, thicker hairs pointed to the possibility for thermal transfer through the hair coat because the value of the effective thermal conductivity of the hair coat was greater.

Table 2: Effects of hair coat traits of imported cows on reproductive parameters

Effects	Montbéliarde (18)		
	SPC	ICM(days)	IC (days)
Coat color (%)			
Dark (≤62)	2,33±1,80	84,29±34,58	115,29±54,19
White(>62)	2,44±1,51	53,75±24,28	97,00±50,48
P-value	0,72	0,16	0,60
Hair Weight (mg/cm^2)			
Light (≤9,6)	2,44±1,74	79,00±32,22	113,71±57,94
Heavy(>9.6)	2,33±1,58	63,00±38,45	99,75±42,74
P-value	0,56	0,51	0,67
Hair length (mm)			
Short (≤16)	2,50±1,72	79,44±33,40	113,44±52,89
Long(>16)	2,25±1,58	45,00±21,21	87,00±50,91
P-value	0,75	0,21	0,54
Hair density ($hairs/cm^2$)			
Low(≤370)	2,33±1,73	70,20±32,17	109,00±43,98
High (>370)	2,44±1,59	75,67±37,67	108,33±60,63
P-value	0,59	0,13	0,32
Hair total diameter (µm)			
Thin (≤89,5)	2,56±1,81	60,29±30,03	82,00±31,45[a]
Large(>89,5)	2,22±1,48	95,75±30,02	155,25±46,64[b]
P-value	0,66	0,09	0,01*
Hair medulla diameter (µm)			
thin (≤54)	1,91±1,30	78,00±36,00	103,13±49,57
Large(>54)	3,14±1,86	60,33±27,93	123,33±63,36
P-value	0,15	0,47	0,59

SPC: number of services per conception**; ICM:** interval from calving to first mating**; IC:** interval from calving to conception; *** P** < 0.05;

Table 3: Effects of hair coat traits of locally born cows on reproductive parameters

Effects	**Montbéliarde (49)**		
	SPC	***ICM***	**IC**
Coat color (%)			
Dark ≤30	2,04±1,55	63,40±30,66	94,90±67,98
White>30	1,83±1,47	54,90±19,18	70,95±40,20
P-value	**0,43**	**0,24**	**0,11**
Hair Weight (mg/cm^2)			
light≤9	1,80±1,61	58,41±29,40	78,45±64,58
Heavy>9	2,17±1,46	65,70±33,29	97,25±62,37
P-value	**0,41**	**0,36**	**0,27**
Hair length (mm)			
Short (≤15)	2,28±1,93^a	67,87±37,81	98,04±78,81^a
Long(>15)	1,67±0,92^b	54,63±18,99	74,53±35,60^b
P-value	**0,02***	**0,10**	**0,03***
Hair density (hairs/cm^2)			
Low≤400	1,80±1,55	60,52±30,62	82,43±65,61
Dense>400	2,17±1,52	63,53±32,53	93,42±62,03
P-value	**0,77**	**0,67**	**0,63**
Hair total diameter (µm)			
Thin (≤85)	1,56±1,08	61,09±31,73	73,87±54,04
Large(>85)	2,42±1,82	62,84±31,26	103,79±71,35
P-value	**0,35**	**0,90**	**0,56**
Hair medulla diameter (µm)			
thin (≤49)	1,52±0,98	60,79±30,47	72,17±48,70
Large(>49)	2,55±1,90	63,33±32,85	107,72±75,79
P-value	**0,59**	**0,68**	**0,23**

IV. Conclusion

Our study investigated the relationship of hair coat and reproduction performances of Montbéliarde breed in the hot season. Results showed that reproductive performances were related to place of birth, hair length and total diameter for MB. Imported MB cows with thin hair diameter and locally born cows with long hair were had better reproductive performances. Our results suggested that hair characters can be used to select more adapted to hot climate and resilient animals.

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DETERMINATION OF PHYTOCHEMICALS AND ANTIOXIDANT CAPACITY OF EDIBLE DANDELION PLANT (*TARAXACUM OFFICINALE*) COLLECTED FROM KIRŞEHIR REGION

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ABSTRACT

In this study, the total phenolic and flavonoid content of the methanol extract of *Taraxacum officinale* (dandelion), which grows spontaneously in April and May in the Kırşehir region and is consumed as a fresh vegetable by the people of the region, was determined. Total phenolic and flavonoid substance amounts were determined as 36,53 mg GAE/g and 43,31 mg QE/g, respectively. Antioxidant capacity was determined by 1,1-Diphenyl-2-picrihydrazil (DPPH) Radical scavenging capacity method (DPPH), and IC_{50} value was calculated as 482,09 μgmL^{-1}. The Fe^{3+}-Fe^{2+} Reducing antioxidant power was determined and calculated as 42,652 µg AAE/mL ascorbic acid equivalent. As a result, it was determined that *T. officinale* plant, which grows spontaneously in arid and rural areas, has natural antioxidant characteristic as well as pharmaceutical characteristics. It is thought that it can be an important source to meet some of the daily antioxidant needs in the regions where it is grown, and there is a need for new studies to determine its industrial use and its effects on health.

Key Words: *Taraxacum officinale,* Phenolic and flavonoid substance, Antioxidant.

INTRODUCTION

Plants are very valuable that grow spontaneously and are used as foodstuffs depending on the season in their regions. Because such foods are free and easy to obtain (Ergün, 2021). In addition, many of them are used for therapeutic purposes as well as food. Such plants are also called medicinal plants or functional foods. The bioactive compounds in their structure are important for health. Especially phenolic and flavonid compounds and antioxidants have been reported to be effective against many diseases such as cancer, cholesterol and diabetes (Erlund et al., 2008).

T. officinale is a perennial weed in the Asteraceae family. İt is grows in arid areas that are uncultivable or fallow land. It is also called "Karahindiba" in Turkey. It is used fresh in the early spring period and dried in other periods. It has a bitter taste, young leaves are rich in fiber, minerals, vitamins and essential fatty acids (Escudero et al., 2003). In addition, the plant has been used as a medicinal plant since ancient times. For this purpose, more roots and leaves are used. In recent in vivo studies, it has been raported, its therapeutic effect in ulcerative colitis (Ding and Wen, 2018), its therapeutic effect on the liver (Cai et al., 2017), its effect on immunity

(Kim at al., 1998; Lee et al., 2012), diuretic effect (Clare et al., 2009) and antifungal, antiviral effects (Liang et al., 2020; Han et al., 2011).

In this study, it was aimed to determine the total phenolic and flavonid amounts and antioxidant capacity that the self-growing *T. officinale* plant in Kırşehir region.

MATERIAL AND METHOD

Supply of Plant Samples: The samples of *T. officinale* plant to be used in this research were collected from Kırşehir province. Samples were collected from the above-ground parts of the plant in May 2021. Samples were blended. Physical dirt on them was removed with distilled water. After washing, the drying process was carried out at room conditions and stored at +4 ^{o}C until use.

Preparation of plant extract: The plant sample was physically ground in a grinder. 10 gr was weighed and placed in a closed flask. 200 mL of methanol was added to them and mixed in a magnetic stirrer. The resulting extract was filtered. This process was repeated three times at regular intervals. Methanol was removed from the filtered extract by evaporator at 45°C. The extract were stored at + 4 ° C for the study. Then, a stock solution was prepared from the plant extract at a concentration of 1000 ppm just before the determinations (Ergün, 2021).

Determination of Total Phenolic Substance: Total phenolic substance determination was made according to the Folin-Ciocaltaeu method (Slinkard & Singleton, 1977). A standard graph was prepared using gallic acid. The total phenolic contents of the extract was determined as equivalent to gallic acid using the equation obtained from the standard gallic acid graph (mg GAE /g).

Determination of total flavonoid substance: The flavonoid contents of extract was determined as equivalent to quercetin by using the aluminum nitrate method (Nieva Moreno et al., 2000). Quercetin was used to prepare standard graphic. The total flavonoid contents of the extract was determined as equivalent to quercetin by using the equation obtained from the standard quercetin graph (mg OE /g).

Determination of DPPH• free radical scavenging activity: The free radical scavenging activity was determined by using the Blois (1958) method. 1,1-diphenyl-2-picrylhydrazil (DPPH•) (0.1 mM) solution was used as the free radical and 2,6-di-t-butyl-1-hydroxytoluene (BHT) (1000 ppm) as a standard. 20-100 µL of ektract was taken and their volumes were made up to 400 µL with methanol. Then, 1600 µL of DPPH• solution was added. Control was prepared using methanol under the same conditions After 30 min incubation of the prepared solutions in the dark at room temperature, the absorbance changes at 517 nm were measured against methanol (Optima SP-3000). Decreasing absorbances yielded the amount of free DPPH• solution remaining, ie free radical removal activity.

% DPPH● radical scavenging activity was calculated by the following formula:

% DPPH● radical scavenging activity=$[(A_0 – A_1)/A_0]\times100$

A_0: Absorbance of control reaction, A_1: Absorbance of plant extract and standard solutions

Determination of ferric reducing power (FRAP): The determination of the Fe^{3+} reducing power was made according to the method of Oyaizu (1986). BHT was used as standard. BHT and stock solutions were taken into a tube with a concentration of 10-50 µg/ml. Pure water was added to make a total volume of 1,0 mL. On top of these solutions, 2,5 mL of phosphate buffer (0,2 M pH 6,6) and potassium ferricyanide (1%) solution were added and kept in a water bath at 50 °C for 20 minutes. Then 2,5 mL of 10% trichloroacetic acid (TCA) was added and vortexed. 2,5 mL ultrapure water and 0,5 mL iron (III) chloride (0.1%) were added to 2,5 mL samples from vortexed tubes and the absorbance was read at 700 nm against the blank (Optima SP-3000). Results were calculated as ascorbic acid equivalent (µg AAE /mL).

RESULTS AND DISCUSSION

The young leaves of the *T. officinale* plant, whose roots and young branches are used for medicinal purposes (Yarnell and Abascal, 2009), are used in salads, beverages and vegetable dishes. Studies have shown that the leaves of Taraxacum officinale have a fibrous structure and are rich in minerals, vitamins and essential fatty acids (Escudero ve ark. 2003).

The amount of phenolic substance was determined as 36,536 mg GAE/g of the *T. officinale* plant (Table 1). While the value we found is higher than the value found by Şengül et al (2009) (15,50 mgGAE/g), they were lower than the values found by Lis et al. (2020) (187,70 ± 0,22 mg GAE/g). The total amount of flavonoids was determined as 43,310 mg QE/g (Table 1). The value we found and the value found by Garcia-Carrasco et al (2015) (28,32 mg QE/g) show close similarity.

Table 1. Total amount of phenolic and flavonoid substance

Extract	The amount of total phenolic (mg GAE/g)	The amount of total flavonoid (mg QE/g)
T. officinale	36,536	43,310

Antioxidants neutralize free radicals by transferring electrons or hydrogen atoms to DPPH when interacting with DPPH (Ergün, 2021). DPPH• (1,1-diphenyl 2-picrylhydrazil) is an organic radical with an absorbance at 517 nm. In this study, by measuring the absorbance decrease of the DPPH• radical at 517 nm, the amount of free DPPH• solution remaining, that is, the free radical scavenging activity, was determined. BHT was used as a standard in activity studies. Parallel to the increase in concentration (20–100 µg/mL), it was observed an increase in DPPH• radical scavenging activity (Figure 1).

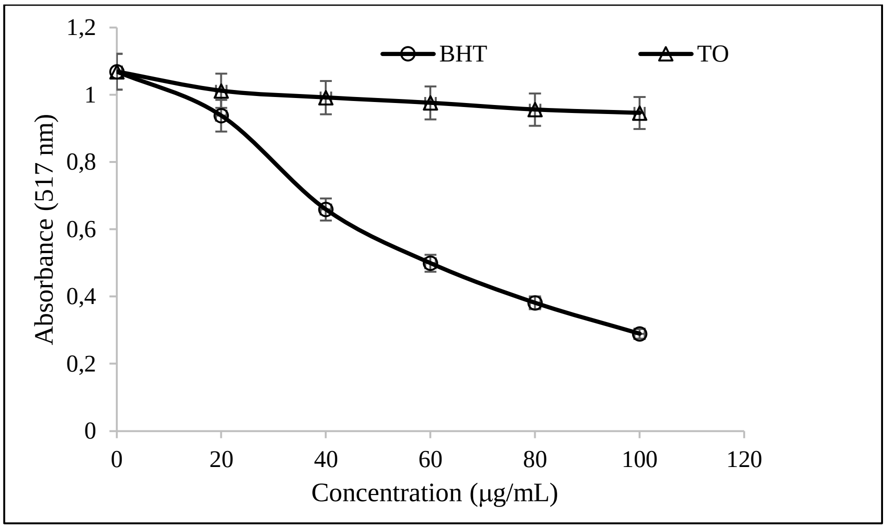

Figure 1. The DPPH• scavenging effect of extract and BHT at different concentrations (BHT: Butylated hydroxytoluene, TO: *T. officinale* extract).

DPPH• radical scavenging activities (%) of the extract and BHT were calculated (Figure 2). In the antioxidant analysis, DPPH• radical scavenging activity at a concentration of 100 µg/mL was determined to be lower than the standard used.

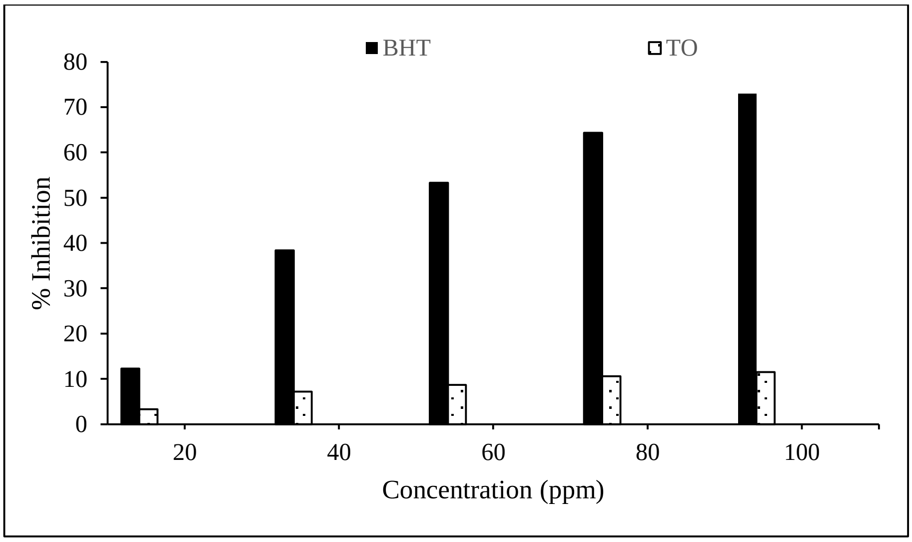

Figure 2. Comparison of DPPH• radical scavenging activities (%) of *T. officinale* plant extract with BHT (20-100 g/mL)

The concentration of extract and standard substance was determined as IC_{50}, which inhibited 50% of DPPH• radical scavenging (Table 2). This value was calculated by using graph of % DPPH • radical scavenging activity values versus studied concentrations. In similar studies, the IC_{50} value was reported as 81,05 ± 0,96 □g/mL (Aabideen et al., 2020) and 65,0 □g/mL (García-Carrasco et al., 2015). The value we found is greater than these values.

Table 2. *T. officinale* extract and of BHT IC_{50} values (BHT: Butylated hydroxytoluene, TO: *T. officinale* extract).

Extracts and standard	**IC_{50} (□g/mL)**
TO	482,09
BHT	58,89

The Fe^{3+}-Fe^{2+} reducing capacity of *T. officinale* extract and BHT was determined by FRAP method. The antioxidant effect of reducing agents in plants is based on the principle of giving a hydrogen atom and breaking the radical chain. In the measurements of Fe^{3+} reducing capacity, absorbances at 700 nm were determined and a graph was obtained by placing the absorbance values against the concentration (Figure 3). In this graph, increasing absorbance values show the reducing power capacity.

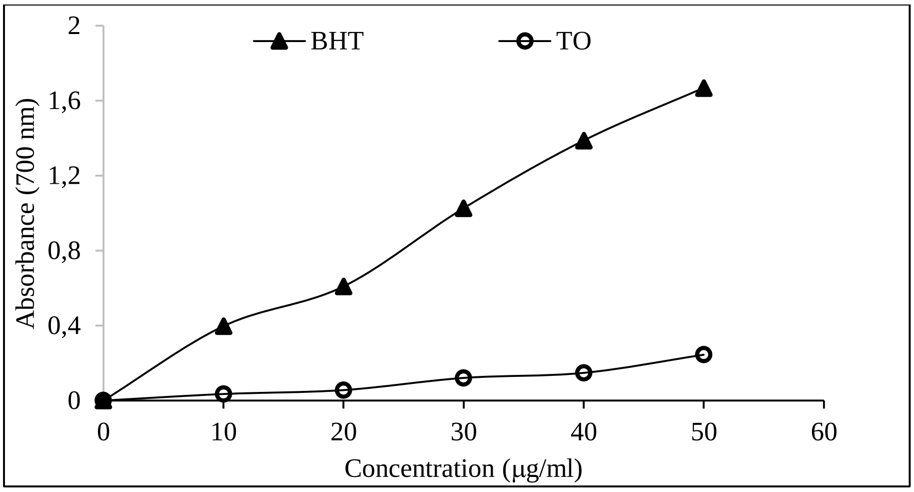

Figure 3. Comparison of Fe^{3+}-Fe^{2+}reducing power activities of extract of *T. officinale* with BHT (20-50 □g/mL) (BHT: Butylated hydroxytoluene, TO: *T. officinale* extract)

In addition, the Fe^{3+}-Fe^{2+} reducing power of the extract was calculated as ascorbic acid equivalent (µg AAE/mL) (Table 3). In a similar study conducted by Hu and Kitts in 2005, this value was reported that 66,70±3,33 µg AAE/mL .

Table 3. Fe^{3+}-Fe^{2+} reducing capacity of extract and BHT

Extracts and standard	µg AAE /mL
TO	42,652
BHT	1022,5

Conclusion

As a result of this study, it was concluded that the easily accessible and naturally self-growing dandelion plant, has antioxidant potential as well as nutritional and pharmaceutical values and this feature should be investigated with new studies.

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MINERAL, CRUDE ASH, AND CRUDE FAT CONTENTS OF SECOND CROP FORAGE PEA AND CEREALS MIXED CROPPING

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ABSTRACT

This research was carried out to determine some minerals, crude ash, and crude fat contents of second crop forage pea + cereals mixtures, which were sown after wheat harvest in Central Anatolia ecological conditions. The field experiments were conducted in the 2018-2019 years. Mixed cropping was carried out using forage pea at different densities (80, 100, 120 plants m^{-2}), and 25-50 % of the suggested densities of oat, silage maize, and Sudangrass. Results revealed that phosphorus (P) and magnesium (Mg) contents are increasing, but the crude fat content is in a decreasing trend depending on precipitation. Plant densities of forage pea did not cause any significant variation but cereal species caused significant differences in the examined characteristics. Phosphorus content was higher in pea + silage maize mixtures. Especially 25 % oat and pea mixtures had higher Calcium (Ca) content (10163,0 mg kg^{-1}) but increasing densities of cereals decreased Ca content in mixtures. The highest Mg content was 3692,1 mg kg^{-1} and it was recorded at 50 % silage maize + pea mixed cropping. Crude fat contents of forage pea and warm-season cereals (silage maize, Sudangrass) mixtures remained between 1,6 – 1,8 % but it was higher than 2 % at oat mixtures. The increasing ratio of the warm season cereals caused higher crude ash content in the mixtures and the highest value was determined as 11,68 % in 50 % silage maize and forage pea mixture. Forage pea and 50 % silage maize mixed cropping should be suggested for the high-quality second crop forage production in the region by considering the examined characteristics.

Keywords: Forage pea, Mixed cropping, Mineral content, Crude ash, Crude fat

INTRODUCTION

Forage legumes have been widely cultivated due to their high crude protein content. Forage pea (*Pisum sativum* ssp. *arvense*) is an annual forage legume, which contains nearly 20 % crude protein in its hay (Acikgoz, 2001), is highly digestible (Uzun et al., 2005), and has high lysine content (Manga et al., 2003). The cultivation area of the plant is increasing in Turkey due to these high-quality characteristics (TUIK, 2020). Although forage pea has high yielding and high-quality forage production capacity, it is mostly grown as mixed cropping due to common lodging problems (Tan et al., 2013).

Forage pea is generally sown in autumn in our country as a mixture with the erectly growing winter cereals like oat, barley, and triticale (Gocmen and Ozaslan-Parlak, 2017; Gulumser et al 2017). Moreover, it was observed in recent years that forage pea could be cultivated as a mixture cropping using warm-season cereals as silage maize and Sudangrass in irrigated areas of Central Anatolia at the second crop season after wheat harvest (Ileri et al., 2020). Maize and Sudangrass could alleviate the heat stress on forage pea because of their shadow effects and forage pea facilitate the growth of these cereals via nitrogen fixation (Geren and Alan, 2012; Hu et al., 2016). Therefore, high-quality and high yielded forage could be produced using these mixtures in the second crop season (Gulumser et al., 2017).

Crude protein content and digestibility are considered as the major forage quality characteristics but mineral, crude ash, and crude fat contents could also be quite effective directly or indirectly on animal production and welfare. For example; grass tetany risk increases under Mg deficiency (McDonald et al., 1995), deficient P content in consumed forage could cause pica or low production (McDowell, 1992), and excessive P may affect animal welfare negatively by causing renal calculus (Emerick, 1988). Greene (2000) indicated Ca deficiency does not have a direct negative effect but it is well-known that Ca/P and Ca/Mg ratios in forage could directly affect animal production (NRC, 1996). Besides crude fat content in consumed forage might be related to the fat content in animal productions as milk (Anil et al., 1998) and crude ash content is important because it indicates the total mineral content of forage (Eskandari et al., 2009).

Forage pea and cereals mixed cropping researches generally focus on forage yield, crude protein content, and digestibility. There is little information about the mineral, crude ash, and crude fat contents of these mixtures. Carr et al. (2004) determined the Ca content of pea-barley and pea-oat mixtures in a range of 6420 – 7000 mg kg^{-1} and P content between 1970 – 2800 mg kg^{-1}. In another research, Neugschwandtner and Kaul (2016) examined the stubble of pea-oat mixture and determined the Mg content between 913 – 3943 mg kg^{-1}. Other researchers indicated the crude ash and crude fat contents of forage pea – cereal mixtures respectively in the range of 3,19 – 13,58 % and 1,43 – 1,98 % (Gronle et al., 2015; Gocmen and Ozaslan-Parlak, 2017; Tsialts et al., 2018).

In our experiment we investigated the Ca, Mg, P, crude fat, and crude ash contents in the forage of forage pea and cereals (oat, silage maize, Sudangrass) mixture cropping, which were cultivated in the second crop season after wheat harvest in Central Anatolia conditions.

MATERIAL AND METHOD

The two-year field research was carried out in the experimental field of the Faculty of Agriculture, Eskisehir Osmangazi University in 2018 and 2019 years. Some minerals (Ca, Mg, P), crude ash, and crude fat contents were examined in the mixtures prepared by using different plant densities of forage pea and cereals.

Table 1. Meteorological data belong to experiment location*

Months	**Precipitation (mm)**			**Temperature (ºC)**			**Humidity (%)**		
	2018	**2019**	**LYA**	**2018**	**2019**	**LY**	**2018**	**2019**	**LY**
Jan	31,5	60,2	38,7	2,2	4,3	0,3	95,5	91,0	98,2
Feb	40,5	50,1	32,5	6,6	3,4	4,7	90,7	79,6	92,6
March	74,8	13,4	33,4	10,1	6,3	9,3	81,5	64,5	81,6
Apr	16,5	26,7	35,0	15,4	9,5	13,1	60,7	69,3	67,8
May	84,8	42,2	44,8	17,6	16,5	16,5	83,0	65,1	86,1
June	**72,5**	**45,7**	**30,6**	**20,6**	**20,9**	**20,4**	**80,7**	**67,9**	**83,3**
July	**38,3**	**33,5**	**14,0**	**23,0**	**21,3**	**23,3**	**71,4**	**62,3**	**75,8**
Aug	**25,0**	**2,4**	**7,8**	**23,5**	**22,3**	**22,9**	**62,2**	**61,0**	**74,1**
Sep	**4,3**	**5,0**	**14,4**	**19,1**	**18,1**	**20,0**	**62,9**	**62,1**	**68,1**
Oct	41,0	18,3	27,0	14	14,2	12,9	75,5	70,1	79,6
Nov	29,6	33,9	29,2	8,4	7,9	7,5	79,2	76,2	80,3
Dec	63,6	74,1	45,1	2,7	2,9	3,6	96,0	89,9	93,6
Tot. Ave.	***522,***	***405,***	***352,4***	***13,6***	***12,3***	***12,8***	***78,3***	***71,6***	***81,8***

*Turkish State Meteorological Service

LYA**:** Long years average

The typical continental climate conditions of Central Anatolia prevail in the experimental location, which indicates hot and dry summers, snowy winters. The mean annual precipitation of the Eskisehir is between 400-500 mm and total precipitation and average temperature were higher in the first experimental year (Table 1). Soil samples were taken from the experimental field in a depth of 0-20 cm and analyzed at the laboratory of the Soil Science and Plant Nutrition Department. According to the report, the soil was clay-loamy, slightly alkaline, slightly limy, poor in available phosphorus and organic matter, rich in potassium, and have no salinity problem.

Forage pea (*Pisum sativum* ssp. *arvense* L. cv. Tore) was sown using three different densities (80, 100, 120 plants m^{-2}) and separately in a mixture with oat (cv. Checota), silage maize (cv. Kilowatt), and Sudangrass (cv. Gozde-80). Cereal ratios were used by considering the 25 and 50 % of the suggestions, which were 180 kg ha^{-1} for oat (Basaran et al., 2018), 100000 plants ha^{-1} for silage maize (Turgut et al., 2005), and 20 kg ha^{-1} for Sudangrass (Acikgoz, 2001).

The experiment was arranged in a completely randomized block design using three replications. Sowing was carried out after the wheat harvest on 12 July 2018 and on 22 July 2019. Every plot consisted of 5 rows each 5 m long and had 30 cm row spacing (5m x 1,5m = 7,5 m^2). Mixtures were sown using experimental markers and irrigation was applied using sprinklers.

Harvest time was determined considering the pod-filling stage of forage pea (Fraser et al., 2001) and it was on 15 September in 2018 and 4 October in 2019. All plants were harvested using hand sickle from the middle $1m^2$ of the plots. Harvested samples were oven-dried at 60°C until reached constant weight and grounded using the experiment mill to pass through a 2 mm sieve. Analyses were carried out using near-infra-red spectroscopy (NIRS), which was calibrated for determining Ca, Mg, P, crude ash, and crude fat contents (Gronle et al., 2015).

All data were subjected to analysis of variance using SAS 9.3 statistical software and the means were separated using Tukey Multiple Comparison Test (SAS Institute, 2011).

RESULTS

Phosphorus content significantly varied between the years and among the mixtures but pea density did not cause any significant variation. The third-order interaction was also significant (Table 2). In 2019, P content was quite higher than in 2018. The highest and the lowest P contents were recorded at 50 % silage maize mixture (2178,2 mg kg^{-1}) and 50 % oat mixture (1714,3 mg kg^{-1}) respectively. However, the mixtures did not cause any significant differences considering monocrop forage pea in terms of P content (Table 2).

Calcium content showed similar variations with P, and it was statistically significant between the years and among mixtures, but not the pea densities (Table 2). Ca content increased in the second year and it was lower than monocrop pea at 50 % silage maize or Sudangrass mixtures. Other mixtures did not cause any significant variation concerning monocrop forage pea but a significant year × mixture interaction occurred because of the significant variation trends of the mixtures in both years.

The Mg content significantly varied among the mixtures and only the year × mixture interaction was significant but the other factors did not cause any significant variation in Mg content (Table 2). Mg content varied between 2471,6 – 3692,1 mg kg^{-1} among the mixtures and the highest value was observed in the mixture of 50 % silage maize.

Crude fat content was 2,11 % in 2018 but significantly decreased to 1,67 % in the second experimental year. Different pea densities did not cause any significant variation but the mixtures prepared using silage maize or Sudangrass decreased the crude fat content concerning monocrop forage pea (Table 2). Pea – oat mixtures had statistically similar crude fat content with monocrop forage pea and they were higher than 2 % in both oat densities (Table 2). The year × mixture interaction was also significant.

Crude ash content did not show a significant variation between the years and among the pea densities but the difference among the mixtures and three-order interaction were significant ($P \leq 0,01$). All mixtures of silage maize and Sudangrass were statistically in the same group but they were significantly higher than monocrop forage pea in terms of crude ash content. Crude ash contents of the oat mixtures did change significantly from monocrop forage pea (Table 2).

Table 2. P, Ca, Mg, crude fat, and crude ash contents

	P (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Crude fat (%)	Crude ash (%)
Year (Y)					
2018	1057,3 b	6233,4 b	3199,7	2,11 a	10,67
2019	2755,5 a	11440,2 a	3185,2	1,68 b	10,45
Pea density (P)					
80 plants m^{-2}	1937,2	8911,4	3281,7	1,88	11,19
100 plants m^{-2}	1940,9	9095,8	3198,6	1,91	10,00
120 plants m^{-2}	1840,9	8503,2	3097,1	1,90	10,49
Mixture (M)					
Monocrop pea	1861,7 abc	10292,8 a	3330,6 ab	2,29 a	9,81 cd
25% Oat	1783,3 bc	10163,0 a	3040,5 ab	2,15 ab	10,17 bcd
50% Oat	1714,3 c	9647,3 ab	2741,6 b	2,09 ab	9,46 d
25% Silage maize	2106,1 ab	8320,9 abc	3341,7 ab	1,84 bc	11,02 ab
50% Silage maize	2178,2 a	7333,4 c	3692,1 a	1,67 c	11,68 a
25% Sudangrass	1813,0 bc	8279,4 abc	2991,1 b	1,65 c	10,68 abc
50% Sudangrass	1888,2 abc	7820,8 bc	3209,9 ab	1,60 c	11,09 ab
Mean	*1906,3*	*8836,8*	*3192,4*	*1,89*	*10,56*
			ANOVA		
Y	*	**	ns	**	ns
P	ns	ns	ns	ns	ns
M	**	**	*	**	**
Y × P	ns	ns	ns	ns	**
Y × M	ns	*	**	**	**
P × M	**	ns	ns	ns	**
Y × P × M	*	ns	ns	ns	**

ns: the difference is not significant, *: P≤0,05, **: P≤0,01

DISCUSSION

Recommended P content is about 2000 mg kg^{-1} in the forage for proper animal nutrition (NRC, 1996). In the first year of the study, P content was below the recommendation but it was higher and 2755,5 mg kg^{-1} in the second year. This might be due to the delayed maturation caused by higher precipitation in the second year (Table 1) because P content decreases as the maturation of the plant increases (Bormann et al., 2004; Haan et al., 2007). Gulumser et al (2017) were also determined a higher P content in the forage in the years received higher precipitation. P content of the mixed croppings was not statistically different from monocrop forage pea but it was observed the P content increased up to recommended levels (2000 mg kg^{-1}) by mixed cropping of forage pea with warm-season cereals (Table 2). Various cereal ratios were stated not to cause significant differences in the P content of the pea cereal mixtures (Gulumser et al., 2017; Kaymak et al., 2021).

The mean Ca content was determined as 8836,6 mg kg^{-1} and it was higher than the recommendation (3000 mg kg^{-1}) made by Kidambi et al (1989). Besides, other researchers indicated the Ca content of pea and cereals mixtures between 6042 – 9270 mg kg^{-1} (Carr et al., 2004; Gulumser et al., 2017). It was observed that Ca content was also higher as similar to P content in the year which received more precipitation. Similar findings were also reported by Gulumser et al (2017) and Onal-Asci et al (2018). Monocropped forage pea had higher Ca content than all mixtures mathematically, but only 50 % silage maize and Sudangrass mixtures had lower values statistically than monocrop forage pea. This is mainly originated from the higher Ca content of the legumes concerning cereals, especially warm-season cereals (Carr et al., 2004; Kacar and Katkat, 2007).

Some researchers reported the Mg content of the forage pea and cereal mixtures between 730 – 3340 mg kg^{-1} (Gulumser et al., 2017; Kaymak et al., 2021). Mean Mg content was 3192,4 mg kg^{-1} in our research and it was consistent with the findings in the literature. There were not any significant differences between years and plant densities possibly due to the similar maturation levels at harvest because it is known that Mg content is related to the maturation level of the plant (Onal-Asci et al., 2018). Increasing cereal density could increase the Mg content in legume-cereal mixed cropping (Egritas and Onal Asci, 2015). In our research, increasing warm-season cereals density caused a slight increase in the Mg content of the mixture but this variation was not statistically significant (Table 2).

High-quality forage resources could contain about 1,2 – 5,8 % crude fat (Thiex et al., 2003). Moreover, the crude fat content might be affected significantly by climatic variations, especially precipitation, and altitude (Pozdisek et al., 2011; Tsialtas et al., 2018). Therefore, higher crude fat content in the second year might be related to higher precipitation in the second year (Table 1). Some researchers reported the variation among the pea-cereal mixtures was not significant (Carr et al., 2004; Pozdisek et al., 2011). In the research, pea-oat mixtures did not cause any significant difference in crude protein content considering monocrop forage pea but especially warm-season cereals caused a significant decrement. These results might be indicating that forage pea could display various responses due to cool- or warm-season cereals used in the mixed cropping in terms of crude fat content.

Crude ash content roughly indicates the total mineral content of the forage (Eskandari et al., 2009). This content changes between 7,9 – 8,3 % in the hay of forage pea (Kavut et al., 2016). Similar maturity levels during the harvest might be the main reason for non-significant variation between years and among the pea densities because crude ash content of forage pea could change significantly due to maturation level (Seydosoglu and Bengisu, 2019). Researchers reported that there were not any significant differences in crude ash content of pea-cereal mixtures (Gocmen and Ozaslan-Parlak, 2017; Seydosoglu and Bengisu, 2019) but we determined significant differences among the mixtures (Table 2). The difference mainly originated from the presence of warm-season cereals in the mixture, which were not examined in the other researches, because warm-season cereals have higher crude ash content concerning cool-season cereals (Carr et al., 2004; Kacar and Katkat, 2007).

CONCLUSIONS

Phosphorus, calcium, magnesium, crude fat, and crude ash contents of the second crop forage pea-cereal mixed cropping could be affected significantly from yearly climatic variations and could produce forage in higher quality in the years which were cooler and receive higher precipitation. Pea density of 80 plants m^{-2} could be used for such mixtures because increasing densities did not have any significant effect on the examined characteristics. Results showed that high-quality forage could be produced after wheat harvest in the Central Anatolia Region by mixed cropping of forage pea – silage maize or Sudangrass. In such mixtures, forage pea with a density of 80 plants m^{-2} could be sown with 50 % silage maize (50000 plants ha^{-1}) after wheat harvest in the region.

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CURRENT POSSIBILITIES OF MACROMINERAL USE IN LAYING HEN DIETS: A REVIEW

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ABSTRACT

This review aimed to discuss the strategies of macrominerals use in current laying hen studies, including the effects on performance and metabolic status, as well as requirement, metabolism, bioavailability, absorption, and excretion. In laying hens, calcium (Ca), phosphorus (P), magnesium (Mg) are essential minerals for growth, egg production, and formation of bone and eggshell. Meanwhile, sodium (Na), potassium (K), and chloride (Cl) have critical functions including controlling body osmotic pressure and water distribution, maintaining proper pH, regulating heart and other muscle function, and participating in oxidation-reduction processes. Also Sulfur (S) plays a variety of roles, including incorporation in amino acids, as well as enzymes and biomolecule metabolism. Deficiencies and excesses of certain elements in the diets of laying hens cause problems, mostly impacting performance, and egg quality. Thus, it is necessary to apply the strategies for supplying the minerals in modern laying hens to maintain their genetic improvement and prevent deficiency diseases in birds. The strategies can be implemented by providing sufficient and balanced minerals, using nanotechnology and organic forms such as metal ions with amino acid ligands, chelated amino acids, and proteinases, and also the use of enzymes like phytase to increase mineral bioavailability.

Key words: Bioavailability, egg quality, laying hens, macromineral, performance

INTRODUCTION

Minerals are catalysts or components of several enzymes and hormones, therefore, they are necessary for growth and many metabolic processes in the body. Minerals can be classified into macro and microminerals. Macrominerals are expressed in concentrations higher than 100 ppm, while microminerals are expressed in concentrations less than 100 ppm and can even be expressed in ppb values (Lukić et al., 2009). Currently, it is necessary to supply adequate minerals in modern laying hens to maintain their genetic improvement and prevent deficiency diseases in birds. As a result, due to genetic development potential variations and the usage of substances that interfere with macromineral utilization, the macrominerals suggested by NRC

(1994) may not be adequate. Moreover, under certain conditions such as hot stress conditions, laying hens may need to be added more than the minimum requirement. In addition, a balanced relationship among minerals such as Ca, P, Mg, as well as Na, K, and Cl, and mineral interactions and antagonisms are necessary for the growth, production, bone development, health, egg quality, and litter quality. Because both a deficiency and an excess of minerals in chicken diets generate problems, primarily impacting performance, and egg quality, both of which result in significant economic loss. For example, Mg, Ca, and P deficiency reduces feed intake, egg production, egg weight, eggshell thickness, and the concentrations of these minerals in the blood and bone (Belkameh et al., 2021). Meanwhile, high NaCl increases water intake and results in moist excreta. Thus, it causes a decrease in egg fertility and poor quality of the eggshell (Balos et al., 2016). Therefore, this review aimed to discuss the strategies of macrominerals use in current laying hen studies, including the effects on performance and metabolic status, as well as requirement, metabolism, bioavailability, absorption, and excretion.

Calcium and Phosphorus

Calcium (Ca) and phosphorus (P) are essential minerals in the poultry diet, especially for eggshell and bone formation, and an activator of enzymes and some hormone secretions. Ca is the most abundant structural element in eggshells and responsible for more than 90% of the shell formation in the form of Ca carbonate. Meanwhile, P is a component of phospholipids and nucleic acids, as well as an essential mediator of energy metabolism via ATP (Li et al., 2016). P is the second most abundant mineral in the animal body, representing around 80% of the total in the bone, and it is involved in gluconeogenesis, fatty acid transport, and protein synthesis (Suttle, 2010). In chicken, imbalance in dietary Ca and P impairs the growth rate and formation of bone and eggshell. The Ca to P ratio is commonly used to assess the intake of Ca and P balance. High dietary Ca and low dietary P showed negative consequences in laying hens performances. Elevating dietary Ca levels raises gut pH, which lowers P absorption and retention. As a result, for optimal egg production and eggshell qualities, the right dietary Ca to P ratio is crucial (Han et al., 2016). Walk et al. (2012), high dietary Ca raises gastrointestinal pH, which may inhibit pepsin activity in the proventriculus and gizzard.

Ca absorption and Vitamin D3 activity are influenced by gut health and kidney function Ca and P interactions with vitamin D3 are crucial because they influence Ca and P homeostasis (Yamamoto and Jorgensen, 2020). In chickens, vitamin D is required for calcium homeostasis and parathyroid hormone response. The parathyroid hormone promotes the conversion of vitamin D3 to $1,25(OH)_2D_3$ when the calcium level is low for an extended period. Thus, in the skeleton, $1,25(OH)_2D_3$ interacts with parathyroid hormone to increase Ca mobilization from bone, which helps to maintain steady blood Ca concentration and improves intestinal Ca absorption. Ca can be transported in the intestine via both active and passive transport mechanisms (Weglarz and Angel, 2013) (Figure 1). For P, renal excretion regulates the concentration of P in the body, with hormones and metabolic processes playing a role in maintaining P homeostasis. Because of the restricted regulation of P absorption from the gastrointestinal tract compared to Ca, it can be absorbed efficiently even when it is more than need. A substantial part of the available dietary P will be absorbed, but some will be excreted in the urine, which is controlled by the parathyroid hormone (Li et al., 2016).

Ca and P Requirement and Applications

The failure of normal skeletal calcification is caused by a deficit of either Ca or P. Rickets affects mostly young birds, whereas calcium inadequacy in laying hens leads to poor shell quality and osteoporosis. The cortical bone weakens and can no longer support the weight of the hen when calcium is mobilized from bone to overcome a nutritional inadequacy (Fleming, 2018). Calcium deficiency disorders are the most common among laying hens that are not appropriately supplied. Chronic hypocalcemia in laying hens is considered to cause partial paresis (weakness). In long-term Ca deficiency, hens use the bone source to replace Ca reserved in the medullary bone. This remolding process of the skeletal system results in a net loss of structural bone, leading to osteomalacia and eventually osteoporosis with a condition of paralysis known as cage layer fatigue (Hu, 2013). The NRC (1994) recommended the ratio Ca and on-phytate phosphorus (Ca/ non-phytate phosphorus) is 2.22 for 1 to 21 day-old, and 2.57 for 22 to 42 day-old broilers. Because of the high demand for bone and eggshell production, the Ca requirement of a laying hen is 4 to 6 times higher than that broiler requirement. Table 2 shows the calcium and phosphorus needs of Leghorn laying hens.

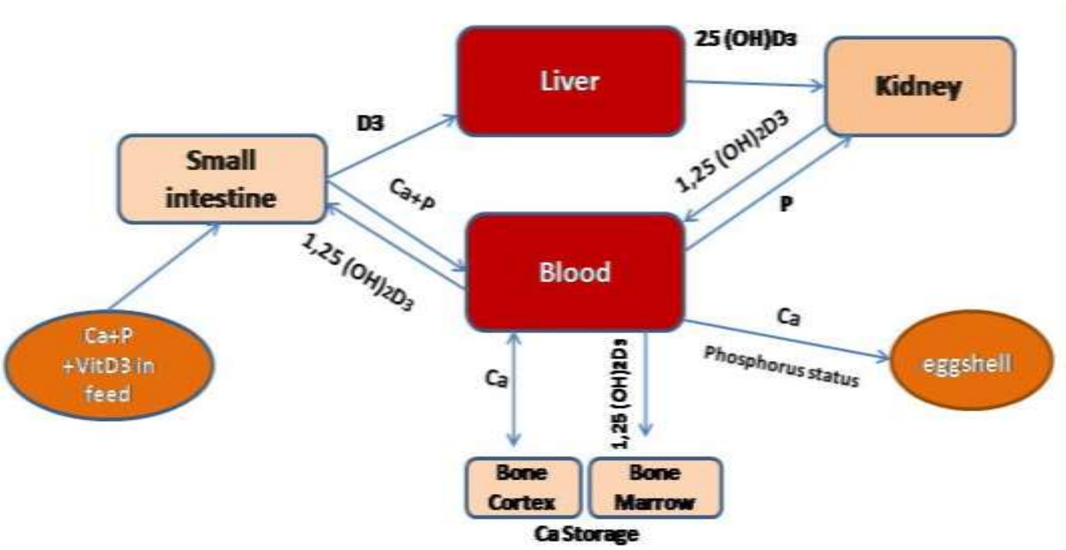

Figure 1. Metabolism pathway of Ca and P of laying hens

Most laying-hen lines have very few eggshell weaknesses during the first 35 weeks old. Meanwhile, egg production and shell thickness steadily decline due to a 50% decrease in Ca absorption in the intestine on week 40 compared to the first period, and the metabolic needs for Ca lead to osteoporosis in hens aged 38 weeks old or older. Therefore, Ca must be present in sufficient amounts and a well-balanced ratio (Carrillo et al., 2020). In addition, base on the laying phase, Rodrigues et al. (2013) also showed that 0.8 % Ca in pullets was sufficient to provide great performance and egg quality. The greatest results were obtained with a Ca level of 3.5% during the laying phase. Otherwise, base on different Ca sources, Ganjigohari et al. (2017) discovered that administering nano calcium carbonate (0.126–2.015%) could substitute calcium carbonate at a lower inclusion level without altering egg production and egg quality in laying hens. Nanotechnology can improve the bioavailability of the mineral, notably calcium, by increasing their surface area, which can improve absorption (Vijayakumar et al., 2014).

P is present in plant-based diets mostly as phytic acid (dihydrogen phosphate) and its salts (phytate). Monogastric animals lack endogenous phytase and phosphatase, which hydrolyze phytic acid to myoinositol phosphates and myoinositol to become P available. As a result, inorganic P, phytase enzyme, or both are commonly added to monogastric diets to meet phosphorus demands, and therefore the need is based on non-phytate phosphorus (Jing et al., 2018). By meta-analyzed, Ahmadi and Rodehutscord (2012) showed that 0.14 % non-phytate phosphorus was sufficient for layers fed with 400 FTU/kg phytase. Another result by Kim et al. (2017) discovered that a super dosing dosage at 20,000 FTU/kg phytase in Hy-Line Brown laying hen diets at 42 weeks to 47 weeks of age had a beneficial influence on egg production rate. They noted that the positive benefits of super dosing phytase have been linked to more P available and reduce its anti-nutritional impact and produce myoinositol. Increased phytate-P utilization may also enhance the utilization of energy, minerals, and amino acids.

Sodium, Potassium, and Chloride

All types of poultry have precise requirements for sodium (Na), potassium (K), and chloride (Cl) in the right quantities and balancing for physiological processes, such as acid-base homeostasis and metabolism process (Melo et al., 2020). According to Pohl et al. (2013), these electrolytes' functions are controlling body osmotic pressure and water distribution, maintaining proper pH, regulating heart and other muscle function, and participating in oxidation-reduction and catalysis as cofactors for enzymes. Na ions are the most abundant cations in extracellular fluid, while K ions are the most abundant cations in intracellular fluid. Cl has several functions including osmotic and acid-base balance, muscular and neurological function, part of hydrochloric acid, and solute movement within body fluids. In poultry, Ca is utilized as a component of a hormone and enzyme activator, as well as in the formation and replacement of the skeleton and eggshell (Ravindran, 2013). Na, K, and Cl can be introduced in organic forms, such as metal ions with amino acid ligands, chelated amino acids, and proteinases. As compared to mineral salts, organic forms are better absorbed by chickens. In chicken nutrition, organic mineral sources can assist prevent minerals from forming indigestible compounds as well as related mineral antagonisms in the gut, which can limit mineral absorption rates (Świątkiewicz et al., 2014). Another study that found the positive effect of the chelating mineral was Ozturk et al. (2014) who stated that there were changes in meat chicken mineral content by humic substance administration in drinking water.

Dietary electrolyte balance of Na, Cl, and K is crucial for the body's water management and blood acid-base balance control. The electrolyte balance is usually measured in milliequivalents per kilogram (mEq/kg), with a value of 250 mEq/kg regarded for normal physiological function. Three major factors influence the maintenance of this value: electrolyte balance and proportion in feed, endogenous total acidity, and renal activity level (Balos et al., 2016). The dietary electrolyte balance is calculated using the number of electrolytes represented as g/kg for each ingredient. Electrolyte balance in the diet = Na + K – Cl (mEq/kg). When Na^+ and K^+ levels in the diet are insanely high, an excessive quantity of H^+ is pushed out of the blood. In conversely, The intake of Cl^- reduces blood $HCO3^-$ concentration, resulting in acidosis and a reduction in blood buffering capacity (Figure 2).

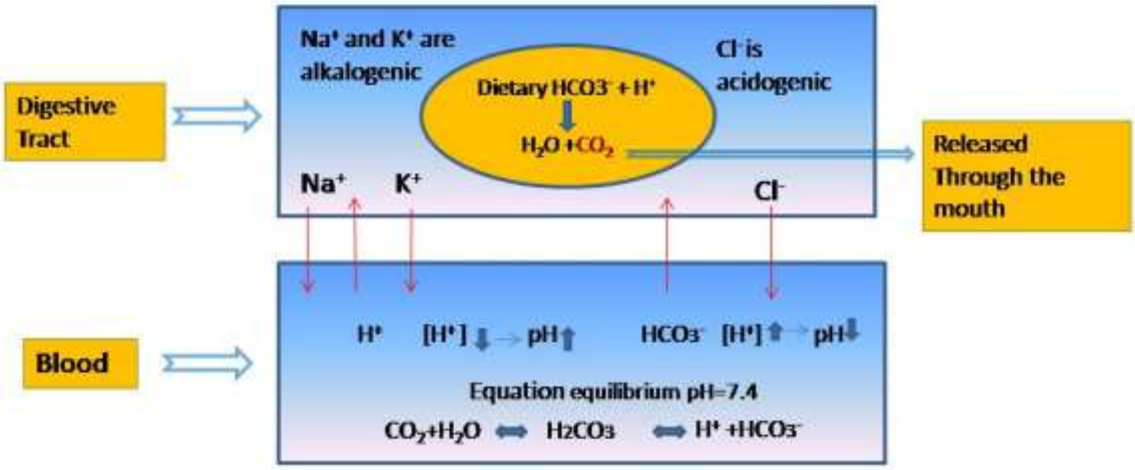

Figure 2. Blood buffering capacity mechanism

The minerals Na, K, and Cl are mainly absorbed in the small intestine. There are three primary pathways for Na absorption at the apical membrane. The sodium glucose-linked transporter (SGLT-1) is activated by the presence of glucose in the lumen in the first mechanism. The flow of sodium and glucose generates a gradient that causes sodium and water to migrate across the paracellular space, a process called "sodium drag." The next mechanism is Na absorption is linked to the sodium hydrogen exchanger isoform-3 (NHE3), which is distributed across the small intestine and swaps one Na ion for every proton. The last mechanism is the electrogenic sodium channels (ENaC), which are significant in the large intestine and are critical targets of the renin-angiotensin-aldosterone system, which controls blood pressure and fluid balance (Nighot and Nighot, 2018). Meanwhile, K^+ is quickly absorbed by active absorption in the gut mucosa. A total of 98% of K^+ absorbed through the gastrointestinal tract is contained in cells, with just 2% occurring extracellularly (Pohl et al., 2013). Excessive Cl is excreted in the urine, which is typically correlated with high Na and K levels (4). Cl is either actively absorbed through exchange processes like Cl/HCO3, Cl/OH, or passively absorbed through concentration gradients. Electroneutral sodium chloride absorption is reduced when the intestinal mucosa is activated by substances that increase intracellular second messengers. Electroneutral sodium chloride absorption is impaired when the intestine mucous is activated by substances that enhance intracellular second messengers. Thus, NaCl and KCl secretion is stimulated by transport proteins in the gastrointestinal tract and basolateral membranes (Kato and Romero, 2011).

Sodium Requirement and Applications

In all diets, common salt is utilized as a source of sodium and an appetite enhancer. Salt is supplemented to diets at levels ranging from 0.2 to 0.4%, but the salt dosage of more than

0.5% in the diet is considered toxic (Ravindran, 2013). Na insufficiency lowers osmotic pressure and consequent disruption in acid-base balance. Severe salt insufficiency symptoms include heart disease, low blood pressure, an increase in hematocrit, and reduced flexibility of subcutaneous tissue. Na deficiency also inhibits adrenal gland function, resulting in elevated blood uric acid and ultimately, shock and mortality. Excessive salt consumption increases water consumption and moist excreta. Thus, it causes a decrease in egg fertility and poor quality of the eggshell (Balos et al., 2016). As a result, the NRC recommends a minimum sodium requirement of 0.13-0.19% for the layer period to reduce this risk. The sodium requirement of Leghorn laying hens is presented in Table 2.

Meanwhile, in the case of postmolt laying hens, sodium administration of up to 0.15% resulted in improved performance and egg quality, particularly in the eggshell (Melo et al., 2020). However, under hot climates, supplemental salt with 0.2 to 0.3 % sodium bicarbonate is recommended (Ravindran, 2013). Moreover, Abbas et al. (2012) shown that dietary 1% of sodium bicarbonate improved immunity, enhanced nutrient digestibility and mitigated the detrimental heat stress effect on immunological reaction to the Newcastle disease virus under summer circumstances. Other studies with different sources of sodium were reported by Zhang et al.(2020) where dietary 0.1 % sodium humate can increase albumin quality and immunity. Youssef et al. (2013) also reported that up to 0.2 % sodium formate significantly increased egg production and feed efficiency in elderly laying hens (53-61 weeks) during the summer. Organic acid salt supplementation would lower the pH of meals and enhance protein digestibility by increasing digestive enzyme activity and decreasing harmful bacteria activity. When temperatures rise, birds accelerate the rate of respiration to enhance the rate of cooling effect, resulting in increased carbon dioxide loss and respiratory alkalosis. This can be noticed in high-producing layers as lower productivity and a drop in eggshell quality (Youssef et al.,2013; Ravindran, 2013).

Potassium Requirement and Applications

The most common symptom of K deficiency is hypokalemia with symptoms of muscle weakness, decreased intestinal tone, cardiac insufficiency and respiratory insufficiency, and failure, which can arise as a result of severe stress. Stress induces an increase in plasma proteins, which promotes adrenalin-mediated kidney excretion of K into the urine (Balos et al., 2016). High K consumption in the diet can alter the electrolyte balance in the body, as well as increase water intake and excreta moisture (Koreleski et al., 2010). The NRC (1994) recommends a minimum K requirement of 0.13-0.19% for laying hens to reduce this risk. The K requirement of Leghorn laying hens is presented in Table 2. However, under certain conditions such as hot stress conditions, K needs to be added more than the minimum requirement. Heat stress raises the amount of Na and Cl ions in the blood (Abbas et al., 2012) but lowers the amounts of K and phosphate ($PO4^{++}$) (Yosi et al., 2017). Potassium chloride, at a concentration of 0.2–0.5%, can assist maintain osmotic and acid-base balance while also increasing water intake. Furthermore, as the temperature rises, the K can be added up to 0.6-0.7% (Saeeda et al., 2019). Against heat stress, adding up to 0.4% potassium chloride to drinking water can hopefully avoid the drop in egg production (Dai et al., 2009).

Chloride Requirement and Applications

In general, the concentration of Cl in the feed should be 10-15% higher than the sodium concentration. For broiler in the whole growth period, recommended Cl levels range from 0.16 to 0.23 % and 0.25% for turkey (Ramos et al., 2019). Meanwhile, for laying hens, the suggested dietary Cl requirement is 0.15% (NRC, 1994). The Cl requirement of Leghorn laying hens is presented in Table 2. Cl deficiency is attributed to poor growth and bone mineralization, feather picking, cannibalism limb weakness, high mortality rate, dehydration, and elevated Cl levels in the blood. Excess salt in drinking water has more severe toxic effects than excess salt in feed. Tolerable Cl concentration in water from 0.015 to 0.018 % (0.25 to 0.30 % salt), however levels above 0.033% (0.54 % salt) were hazardous (Balos et al., 2016).

Furthermore, the quality of eggshells is impacted by Cl levels intake from diet and water because the eggshell formation is an ion transport process in which numerous ions move, maintain, and interact to produce calcium and bicarbonate ions. As a result, it passes across the uterine apical membrane and into the uterine fluid to produce calcium carbonate (Jonchere et al., 2012). This study founded that the breaking strength of eggs with dietary 0.20 and 0.25% Cl significantly lower than 0.10 and 0.15% Cl levels during 47 to 54 weeks. The level of 0.2% and 0.25% levels were considered to reach Cl^- and $HCO3^-$ balance and be able to meet the Cl and Na needs of the laying hens (Wang et al., 2020). Regarding the electrolyte balance among Na, K, and Cl, Silva et al. (2021), the electrolyte balance values that resulted in the lowest feed conversion ratio, increased yolk weight and generated greater uniformity were 1525, 1330, and 1250 in μeq/kg of feed, respectively. Furthermore, based on the regression equations, an electrolyte balance value of 1390 μeq /kg was determined as optimal for laying hens performances aged 30 to 46 weeks old. Another study by Dai et al.(2009) found that the supplementation of 0.2 % and 0.4% NaCl did not increases egg production during heat treatment, however, both treatments increased egg production after heat treatments. There was also a higher egg weight at 0.4 % NaCl supplementation on the fifth day of heat stress compared to 0.2 % NaCl. In comparison to the control group, they found that NaCl supplementation increased water consumption during heat treatment. An increase in water intake is normally followed by a decrease in feed intake and consequently, it can reduce egg production.

Magnesium

Magnesium (Mg) is one of the most common cations in the body and serves as a cofactor for several key enzymes involved in ATP-dependent activities, which energize many main metabolic processes (Pilchova et al., 2017). Mg^{2+} is a cofactor in over 300 enzymatic processes and is involved in a variety of key metabolic pathways such as macronutrient degradation, oxidative phosphorylation, protein synthesis, neuromuscular excitability, and parathyroid hormone secretion control (Schuchardt and Hahn, 2017). Mg appears to play an important part in eggshell and bone formation. Because its actions are strongly connected to Ca and P, maintaining the correct balance of Ca and P balance in diets is essential in poultry feeding (Shastak and Rodehutscord, 2015). According to McDonald et al. (2011), the addition of Mg to the chick rations also disrupted the Ca and P balancing that required for proper bone development during the first six weeks. According to Skrivan et al. (2016), Mg is a Ca antagonist therefore, increased Ca, Mg, and P ratios will decrease the excess Ca. Furthermore, Matin et al. (Matin et al., 2013) stated that the interrelationships between Mg and Ca and P suggest that hormones and enzymes involved with bone metabolism may be related to Mg metabolism. There can be a competition among ions for the active centers in enzyme systems as occurs with Mg and manganese in alkaline phosphatase.

Mg can move through the gut wall with three pathways: passive diffusion, solvent drag, or active transport. Due to the molecular similarities of Ca and Mg, vitamin D and its metabolites aided intestinal Mg absorption. The dominant location of Mg absorption in chicken has been observed to be between the lower duodenum and the lower jejunum. Mg is eliminated endogenously in urine and feces, and this is the major constituent of Mg body homeostasis (Shastak and Rodehutscord, 2015).

Magnesium Requirement and Applications

Considering the natural variation in Mg concentration in the ingredients (Table 1), Mg insufficient is not to be expected actual feeding. As a result, a better-balanced connection between Mg, Ca, and P in diets is needed to maintain performance production, bone health, and litter quality. It had been recommended that the Mg requirements of laying hens should not exceed 0.5 g Mg/kg (NRC, 1994). The Mg requirement of Leghorn laying hens is presented in Table 2. Mg deficiency reduces egg production, feed intake, body weight, eggshell qualities, and Mg concentrations in the blood, and bone (Belkameh et al., 2021). In growing laying hen, Mg insufficiency causes poor development and feathering, reduced muscular strength, incoordination, tremors, convulsions, and mortality (Shastak and Rodehutscord, 2015).

Dietary 300 or 600 mg/kg Mg significantly enhanced feed efficiency, egg weight, and eggshell quality in both normal and under heat circumstances (Gooya and Torki, 2018). Furthermore, Yang et al. (2012) found that Mg deficiency increases hydrogen peroxide formation and reduces the activity of catalase in chicks, and in comparison to controls, dietary $MgSO_4$ decreased heat stress-induced oxidative stress and improved performance due to the recovery of anti-oxidative enzyme activity. Gaál et al. (2004) found that MgO supplementation improved egg production, breeding egg quality, and hatching rate. Dietary 0.4% Mg citrate or 0.4 % MgO resulted in 9.68% more eggs than that control. Furthermore, Kim et al. (2013) found that dietary more than 4.2 g/kg Mg to 46-week-old laying hens enhanced eggshell strength and thickness. Another study by Lilburn et al. (2019) discovered that pullets and layer phase fed corn-soybean meal-based diets with or without 0.1% supplementary of MgO at 36 weeks had a positive influence on body weight at 18 weeks and 21 weeks at the beginning of egg production. The explanation for these positive effects might be related to a higher level of Mg in eggshells, as Mg is the second-highest proportion in the eggshells. It might also be linked to Mg's activity as a cofactor in important metabolic processes in the body, including macronutrient degradation, oxidative phosphorylation, and protein synthesis (Schuchardt and Hahn, 2017). Ca and Mg appear to have an antagonistic connection in terms of skeletal structure and eggshell thickness. In one research, high Mg supplementation up to 1.56 g/kg Mg combined with coarse-grained limestone at a Ca: non-phytate phosphorus (12.8) ratio significantly raised Mg content in the eggshell while lowering Ca concentration. Moreover, excess Mg can inhibit the action of the parathyroid hormone, lowering blood Ca and, laying hens performance and egg qualities (Skřivan et al.,2016).

Sulfur

Sulfur (S) plays a variety of roles, including incorporation in amino acids, as well as enzymes and biomolecule metabolism. Elemental S is related to a variety of antioxidant metabolisms, including Reactive Oxygen Species (ROS) scavenging and glutathione peroxidase (Battine et al., 2009; Kim et al., 2017). S can be obtained from a variety of sources, including synthetic sulfur amino acids (methionine, cysteine), ammonium sulfate, calcium sulfate, sodium sulfate, and elemental sulfur. Nonetheless, the absorption of S-containing amino acids is the primary source of S absorbed in the digestive system. Only a small amount can be absorbed in the form of hydrogen sulfide and with S from methionine and cysteine oxidation contributes to the body's acid-base balance. S is contained in amino acid or organic substances are necessary nutrients for chickens that cannot be synthesized in the body (Alam and Anjum, 2003; Park et al., 2017; Silva et al., 2014). S is included in the structure of biotin and thiamine, two B-vitamins that are important actors in energy metabolism. Sulfur has a structural function in mucins (sulfomucins), which are made up of proteins, glycoproteins, and lipids that protect the intestinal tract from the contents of the lumen (Richter,2011).

Sulfate S is absorbed up to 60% effective in the small intestine. S is transported across the apical membrane by a 2 $Na^+/SO4^{-2}$ cotransporter, and $SO4^{-2}$ is assumed to diffuse across the cell to the basolateral membrane. In return for 2 Cl^- or $HCO3^-$ anions, it is pushed over the basolateral membrane into the extracellular fluids. Sulfate is a powerful anion that can affect acid-base balance and dietary S can be converted to sulfide, which can impair the absorption of other minerals, especially Cu and Se (Goff, 2017). S is transformed into sulphate molecules and excreted in urine and feces (hydrogen sulfide) when there is an excess quantity. H_2S is mostly produced by anaerobic microbes decomposing S-containing amino acids, through bacterial sulfate reduction (BSR), or the by-products of the chemical reaction (Deng et al., 2018) (Figure 3). In addition, the case of toxic gas emissions from poultry operations, such as hydrogen sulfide (H_2S), potentially impacts the occurrence of various diseases. H_2S is rapidly absorbed into the bloodstream, where it dissociates, binds to haem molecules, and is partly metabolized to sulphate (SO_4) before being eliminated in the urine. As a result, excessive H_2S inhibits the enzyme cytochrome oxidase, which is required for mitochondrial respiration in cells (Wang, 2019). Therefore, the S supply in feed and water should be controlled to avoid excessive S content, especially if S-rich substances are used in the formulation.

Sulfur Requirement and Applications

The type of feed raw material used influences the overall sulfur content of the feed as well as the quantity of S supplementation. For instance, synthetic sulfur amino acids must be included in diets to ensure optimal performance due to the high use of soybean meal and corn. Soybean protein has a high protein content, making it suitable for animal food formulation; nevertheless, another limited element of soybean protein is an apparent absence of sulfur amino acid. Corn has a high lysine level but a low S-containing amino acid content, whereas soybeans have a high lysine content but a low S-containing amino acid content (Krishnan and Jez, 2018). However, S supplementation in the feed also needs to be considered, since acute S toxicity causes neurological abnormalities such as blindness, unconsciousness, and muscular spasms (Goff, 2017). Excess feeding of S causes vitamin D deficiency, increased S excretion in excreta, membrane permeability loss, and fluid accumulation around the breast (Alam and Anjum, 2003). High dietary S levels have been associated with increased calcium excretion and the complexing of intestinal calcium. Also when dietary canola meal with high levels of S is responsible for some of the leg problems and reduced feed intake. S content in corn is 1.1 g/kg whereas soybean meal is 4g/kg (Table 2). Broilers may tolerate dietary S levels up to 0.5 % without affecting performance while laying hens can tolerate even greater amounts (Leeson and Summers, 2005). However, according to the NRC (1994), the negative effect of S occurred at 14,000 mg/kg in the chick and at 8,100 mg/kg in laying hens.

The previous result by Lim et al. (2018) found that the use of organic S (0.0, 0.1, 0.2, and 0.4%) in the laying hen diet significantly improved egg production, albumen height, and haugh unit from 47 to 54 weeks of age. Organic S is necessary for the precursor of sulfur amino acid that supports egg production and albumen quality. Considerating in the dietary sulfur amino acid, Gomez and Angeles (2016) stated that the addition of 30 and 0.50% sulfur amino acid to laying hens at 68 to 83 weeks old with sorghum- and soybean meal-based diet produced the optimal egg weight, egg production, and feed efficiency. Conversely, Imik et al. (2006) demonstrated that the adverse effect of partially substituting corn with sorghum that containing low sulfur amino acid and high tannin with supplementation 0.05% inorganic S, 0.57% lysine, and 0.66% methionine had no effect on egg yield or weight. They explained that the absence of effect sulfur amino acids and inorganic S was due to low tannin concentration in diet that may cause the lack of efficacy supplementation inorganic S as a component of sulfur amino acid.

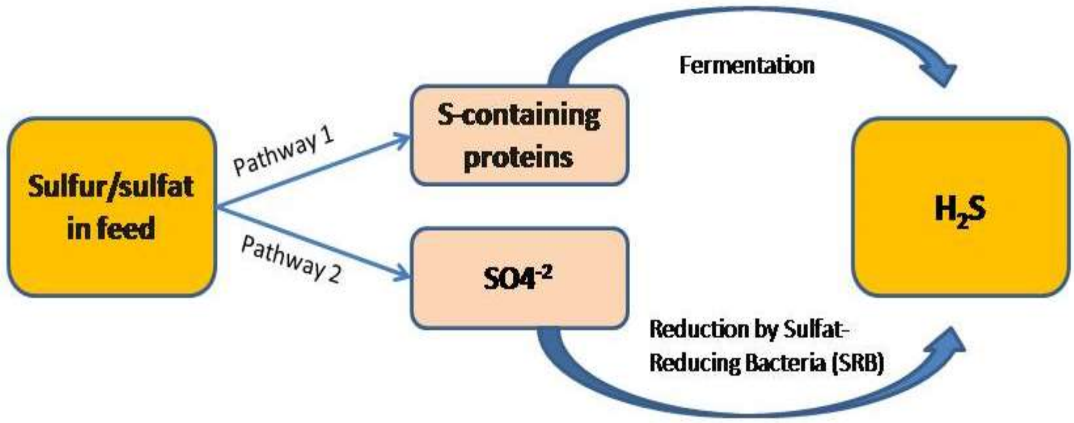

Figure 3. Production of H_2S pathways in colon or caecum of monogastric

Table 1. Macrominerals content in some ingredients (g/kg, as fed basis) (INRAE, 2021)

Ingredients	Ca	P	Phytate P	Mg	K	Na	Cl	S
Corn	0.40	2.50	1.90	1.00	3.10	0.03	0.50	1.10
Soybean meal (46% protein)	3.40	6.20	3.70	2.90	21.50	0.08	0.40	4.00
Barley	0.70	3.40	1.90	1.10	4.80	0.10	1.00	1.30
Millet	0.50	3.00	2.00	1.20	3.70	0.08	0.04	1.10
Sorghum	0.30	3.00	2.10	1.50	3.60	0.16	0.60	0.90
Wheat	0.60	3.10	2.00	1.00	3.90	0.05	0.80	1.40
Feather meal	11.70	7.70	0.00	0.80	1.20	1.21	1.90	16.70
Fish meal (Protein 62%)	44.80	27.60	0.00	2.20	7.60	10.85	17.00	7.10
Corn Gluten Feed	1.40	8.60	5.60	3.70	13.50	3.07	2.10	2.80
Corn Gluten Meal	0.30	3.70	3.00	0.50	1.20	0.84	0.70	5.90
Rice bran (Crude Fiber 5-11%)	0.80	15.00	12.80	3.80	8.20	0.50	1.00	1.80
Wheat bran	1.20	9.50	7.60	3.60	11.70	0.07	0.80	1.90
Calcium Carbonate	387.00	0.30	0.00	3.20	0.40	0.64	0.60	0.70
Limstone	350.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Magnesium Oxide	0.00	0.00	0.00	514.00	0.00	0.00	0.00	0.00
Sodium Bicarbonate	0.50	0.00	0.00	0.00	0.00	272.00	0.30	0.00
Copper Sulfate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	128.00

Table 2. Nutrient requirements of Leghorn-type chickens (NRC, 1994; Leeson and Summers (2005)

Minerals	White-Egg-Laying Strains				Brown-Egg-Laying Strains			
	0-6 Weeks	6-12 Weeks	12-18 Weeks	18 Weeks to the first egg	0-6 Weeks	6-12 Weeks	12-18 Weeks	18 Weeks to the first egg
Calcium (%)	0.9 (1.0)	0.8 (0.95)	0.8 (0.92)	2 (2.25)	0.9 (1.00)	0.8 (0.95)	0.8 (0.90)	1.8 (2.25)
Nonphytate phosphorus (%)	0.4 (0.45)	0.35 (0.42)	0.3 (0.40)	0.32 (0.42)	0.4 (0.45)	0.35 (0.42)	0.3 (0.38)	0.35 (0.42)
Potassium (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sodium (%)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)
Chlorine (%)	0.15	0.12	0.12	0.15	0.12	0.11	0.11	0.11
Magnesium (mg)	600	500	400	400	570	470	370	370

Note: The data in the parentheses is based on Leeson and Summers (2005)

CONCLUSION

Macrominerals studies in modern laying hens are growing attention. Numerous approaches were conducted not only supporting the adequate mineral, optimum performances, and egg quality but also considering laying hens health, animal welfare, and environmental protection. Therefore, the macrominerals strategies that can be implemented are:1) providing adequate and balanced minerals to maximize the genetic potential of laying hens. Since both mineral deficiency and excess in chicken diets lead to problems, mostly impacting performance and egg quality, 2) providing sufficient and balanced minerals, using nanotechnology and organic forms such as metal ions with amino acid ligands, chelated amino acids, and proteinases. The application of nanotechnologycan increase the bioavailability of minerals by enhancing mineral's surface area. Meanwhile, the organic mineral form can prevent the minerals from forming indigestible complexes with certain dietary components, as well as mineral antagonisms in the intestine, which can reduce mineral absorption rates, 3) using the enzymes such as phytase to improve mineral bioavailabilities. The beneficial effects of phytase have been related to the increased availability of P which may also promote the use of amino acids, energy, and other minerals.

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THE EFFICACY OF BENTONITE FEED ADDITIVES IN IMPROVING LAYING HENS PERFORMANCE AND EGG QUALITY: A REVIEW

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ABSTRACT

This review aimed to discuss the effect of dietary bentonite as feed additives on laying hens performance and egg qualities. The prohibition of antibiotics use could potentially have several negative effects on the animals, such as slower growth, lower egg production, higher illness rate, and death rate. Bentonite has been investigated as a potential replacement for antibiotic growth promoters in the maintenance of poultry health and production. The main benefits of bentonite are its low cost, safety, and convenience of use in animal feed. Bentonite is natural clays with a high water absorption capacity that is formed by the devitrification of volcanic ash. The efficacy of bentonite was revealed by numerous studies through several mechanisms including 1) increasing nutrient digestibility, and digestive enzymes in the gastrointestinal tract; 2) enhancing intestinal health by improving villus height and surface area of the jejunum which indicate a greater nutrients absorption. 3) providing necessary minerals such as calcium, magnesium, iron, iodine, selenium, and zinc, all of to promote the activity of enzymes and hormones for supporting the performance; 4) absorbing the aflatoxins that lead to liver damage, eliminate several enzymes activity and decrease the immune system. However, to ensure the safety for all animal species in terms of medicinal component binding and effectiveness, the EFSA recommends a maximum bentonite use of 20 g/kg in the diet.

Keywords: Absorption, Bentonite, Egg quality, Egg production, Laying hens

INTRODUCTION

Currently, many countries restrict the use of antibiotics as growth promoters (AGP) due to the presence of antibiotic residues in animal products such as meat, eggs, and milk, which can cause allergic reactions, intestinal microbiota imbalance, and antibiotic resistance in humans (Kumar et al., 2020). Prohibiting AGP has a number of negative effects on the animals, including declining growth, production, and egg quality, as well as a higher rate of disease and death, which could result in significant financial losses. As a result, industries and farmers need to look out for an antibiotic substitute.

Bentonite has been investigated as a possible replacement for antibiotic growth promoters in the maintaining of poultry health and production. Bentonite is a type of natural clay that is produced by the devitrification of volcanic ash and has a high water absorption capacity (Khanedar et al., 2013). The main constituents of bentonite are sodium montmorillonite (Na- montmorillonite) and calcium montmorillonite (Ca- montmorillonite), both of which have different characteristics. Sodium montmorillonite has a higher swelling capacity than calcium montmorillonite because it can absorb a significant volume of water and enlarge its original volume (Park et al 2016). In the poultry industry, bentonite used as a feed additive for improving growth performance and nutrient digestibility is widely recognized. According to Dos Anjos et al. (2015), the administration of bentonite can bind aflatoxins and eliminate their toxicity Furthermore, dietary montmorillonite enhanced intestinal health, which resulted in increased laying performance (Gilani et al., 2013; Gul et al., 2016; Chen et al., 2020). Therefore, this review aimed to discuss the physical and chemical properties of bentonite, as well as the efficacy of bentonite inclusion in laying hen diets on performance and egg quality.

Physical and Chemical Properties of Bentonite: Swelling and Absorption

Bentonite, a smectite mineral group, is comprised of various mineral substances, including quartz, feldspars, zeolites, cristobalite, and various clay minerals, but it is mostly composed of montmorillonite clay (Abdou, 2013). Montmorillonite is a useful clay mineral with remarkable swelling and adsorption capabilities. Because of this feature, as well as their low cost, high specific surface area, stability, and structural characteristics, clay minerals have been extensively attractive to manufacturers and scientists. (Motshekga et al., 2013). Smectite Montmorillonite's basic crystal structure is an octahedral alumina sheet placed between two tetrahedral silica sheets (1:2) with exchangeable cations between the layers (Figure 1) (Erdogan, 2015). Furthermore, because of the weak bonds, water, and other polar molecules can pass between the layers and affect mineral structure expansion. Silicon may be replaced by aluminum and possibly phosphorus in tetrahedral coordination. Meanwhile, aluminum can be replaced in octahedral coordination by iron, magnesium, chromium, zinc, or nickel lithium (Adamis et al.,2005). Clay minerals generally vary in nature and even clays from the same deposit have different clay minerals, although the most common oxides in bentonite are SiO_2 (48.16-49.87 %) and Al_2O_3 (14.86-14.98%) (Abdullahi and Audu, 2017). The detailed bentonite chemical oxide content is presented in Table 1.

Table 1. Bentonite chemical oxide content from a different source

Chemical oxide (%)	Bentonite Source	
	Ashaka	Tango
SiO_2	48.16	49.87
Al_2O_3	14.86	14.98
Fe_2O_3	4.80	5.12
CaO	1.16	1.81
Na_2O	1.66	1.43
MgO	2.08	2.08
K_2O	1.60	1.76
TiO_2	0.94	0.87
P_2O_5	1.06	1.01

Source: Abdullahi and Audu (2017)

In a previous study, the ability of montmorillonite clays to bind pathogenic viruses, including human viruses, has been well evidenced (Chattopadhyay and Puls,1999). According to Abduljauwad et al., (2019),clay nanoparticles have a high affinity for charged surfaces, which increases the non-specific adhesion attraction of cancer cells, therefore, they could be candidates for controlling cancer metastasis. Nanoclays have successfully been used to adsorb bovine coronavirus and bovine rotavirus (Clark et al., 1998) and may be effective in inhibiting the normal structure of COVID 19 (Mishra et al.,2020). Because of their high affinity and adhesive properties, nano clays can act as SARS-CoV-2 inhibitors by capturing the virus before it interacts with human hACE2. The nanoclays are linked to the S spike in SARS-CoV-2, and the molecular level simulation revealed the interaction between SARS-CoV-2 and nanoclays due to very high Cohesive Energy Density (CED) (Abduljauwad et al., 2020).

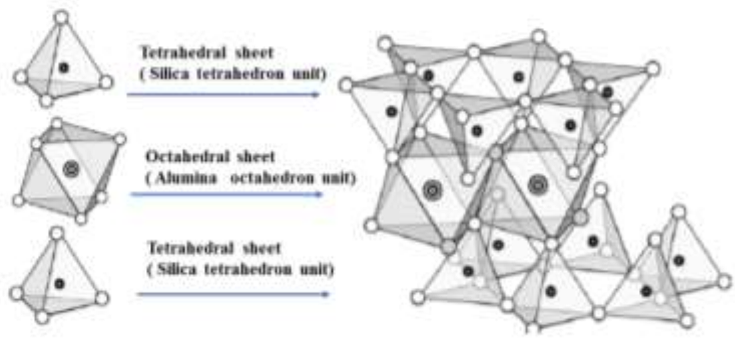

Figure 1. Moleculer structure of montmorillonite (Park et al.,2016)

Bentonite Effect on Egg Production of Laying Hens

Sodium bentonite is one of the bentonite types that have high absorbing and swelling capacity and it also has been used in many industrial applications. In the previous study, the inclusion of 0.5% sodium bentonite improved hen day production and egg weight compared to the control group (Yenice et al., 2015). Gilani et al. (2013) also reported that egg production and egg mass were improved significantly by dietary 10 g/kg sodium bentonite. Another finding by Gul et al. (2017) was that 2% sodium bentonite addition improved protein utilization, increased egg production and decreased egg defects in laying hens. These positive effects of bentonite on laying performances are related to its ability in improving nutrient digestibility and utilization and digestive enzymes in the gastrointestinal tract (Khalifeh et al., 2012; Damiri et al., 2012). It is supported by Ma and Guo (2008) that the addition of montmorillonite in the diet increased the activities of aminopeptidase, alkaline phosphatase and maltase in the small intestine. Bentonite has also been reported to be able to improve villus height for the jejunum and intestinal health of laying hens (Attar et al., 2017; Chen et al., 2020), which could imply a larger absorptive surface area for nutrients. As a result, it can provide maximum nutrients, particularly protein, energy, and minerals, which are the most important factors influencing egg production and quality (Godbert et al., 2019).

Furthermore, because of bentonite absorption and swelling capacity, it was effective in binding fungal and bacterial toxins and alleviating the negative effect in the intestinal tract (Moghaddam et al.,2008) through electrostatic adsorption and excreted through the gut (Qu et al., 2018). Also, Dos Anjos et al. (2015), stated that bentonite can effectively absorb aflatoxins

B1, which can reduce body weight and feed efficiency, increase liver damage, and decrease immune systems. Bentonite mechanisms for binding mycotoxins have been reported including electron-donating, selective chemisorption, ion interactions, hydrogen bonding, furan ring bonding, and coordination between exchange cations and carbonyl groups (Deng et al. 2010; Wang et al. 2018).

Additionally, clay groups contain anionic structure including alkali metal ions and trace elements, which are considered mineral supplies for animals (Suzanne et al., 2017) and promote egg production (Gul et al.,2016). Bentonite contains high minerals such as calcium, magnesium, iron, iodine, selenium, and zinc, all of which are required to promote the activity of enzymes and hormones for supporting the production (Smith et al., 2018). The activity of silicate minerals increased feed transit time through the gastrointestinal tract and nutrient metabolism, which could contribute to improved egg production, and feed efficiency (Safaeikatouli et al., 2012; Damiri et al. 2012; Saçakli et al., 2015).

Bentonite Effect on Egg Quality of Laying Hens

Previous studies by Choi (2018) found that dietary 0.5 % bentonite to 74-week-old laying hens improved shell thickness and Haugh unit. A Similar positive result was reported by Chen et al. (2019) that dietary montmorillonite 0.5 g/kg significantly increased shell thickness and albumen height. Also, according to Qu et al. (2018), dietary up to 1.2 g calcium montmorillonite/kg for increased yolk index, shell thickness, antioxidant, and immunological function. The greater egg quality could be attributed to bentonite's high mineral content, ion exchange capability, and calcium affinity (Inal et al.,2000). This explanation is supported by Choi (2018) and Gul et al. (2016), who stated that the most important content in sodium bentonite clay is calcium, which promotes calcium absorption in hens and assists in the improvement of eggshell qualities. In addition, according to Ma et al. (2009) dietary bentonite was able to increase alkaline phosphatase that has responsible for bone and eggshell mineralization as well as phytate degradation in the small intestine (Kriseldi et al., 2021). Because calcium is the major component of eggshells (98.2 % of calcium carbonate), the calcium availability is the primary factor in determining eggshell qualities (Shwetha et al., 2018). Calcium has a significant impact on egg albumen quality, whereas albumen height and haugh unit increased linearly with increasing calcium levels in the diet (Chang et al.,2019).

Although bentonite has been demonstrated to improve egg production and quality, it has non-specific absorbance qualities, therefore the quantity of dosage used must be monitored closely. For instance, bentonite is considered to bind pigments in feed rather than allowing them to be absorbed in the digestive tract (Kermanshahi et al., 2011), resulting in a reduction in egg yolk color (Heshemipour et al., 2010; Prasai et al.,2017; Elliott et al., 2019). Furthermore, bentonite may decrease the efficacy of oral veterinary medicines, therefore, for the effectiveness and safety for all animals, the European Food Safety Authority (EFSA) recommends a maximum dose of 20 g/kg diet of bentonite with a composition of more than 70% smectite, 10% feldspar and opal, and less than 4% calcite and quartz (EFSA, 2012).

CONCLUSION

Bentonite is a smectite mineral composed primarily of montmorillonite. Sodium montmorillonite has a larger swelling capability than calcium montmorillonite. Therefore, sodium montmorillonite is widely used in the poultry industry. In general, dietary bentonite can increase laying hen's performance and egg quality. However, because of non-specific absorbance, the EFSA recommends a maxımum bentonite use of 20 g/kg in the diet for effectiveness and animals safety.

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RESULTS OF APPLICATION OF DIFFERENT COLORS OF SHADING NETS OF QUALITY TOMATOES

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ABSTRACT

Extremely high temperatures leave a number of consequences for most vegetable crops, primarily disruption of physiological processes, reduction of total yield, reduced quality and quantity of products, higher occurrence of diseases, pests and great damage to plants. In order to reduce the negative impact of extremely high temperatures and the impact on yield in the experimental field PSS Sombor, an experiment was performed with tomatoes and the use of shading nets. We applied different colors of the shading net: green, blue, gray, black. Shading nets significantly reduce the permeability of UV rays and temperature. The experiments were performed with two varieties of " Kruna" and "Rio Grande" tomatoes using mulch foil in a "drop by drop" system. The varieties were planted by random selection, so that they could compare how the nets affect different varieties. The results of the experiment show that the application of shading nets affected the lushness, quality, yield of tomatoes as well as pest reduction. Statistical data showed that the Rio grande tomato variety gave better results, and a higher yield compared to the control. In the Rio grande variety, the blue net showed better than the control, while the black net also showed a significant statistical increase in yield compared to the control. In this way of production, a simple and mobile construction is a great advantage, as well as the possibility of long-term application. The disadvantages of shading nets are investments during the first year, but this application is recommended for the production of tomatoes on smaller areas because it contributes to stable and quality production.

INTRODUCTION

In the second half of the last century, science and profession began with serious warnings that humankind on the earth's ball threatened climate change. From the middle of the last century, especially since 1970, there has been a trend of increasing the average global air temperature. High temperatures strongly affect the intensity of transpiration. Plants respond to stress by decreasing the intensity of breathing and synthesizing nutrients, as well as by increasing the reflection of radiation energy. Physiologically, injuries to plants and plant parts obtained from high temperatures depending on the intensity and length of exposure. Direct injuries lead to an imbalance of metabolism and changes that are present as membrane injuries, loss of fat, protein, and denaturation of nucleic acids (Ilić Z., et al., 2007). In order to protect the plants and yields of the pepper from the undesirable effect of high solar radiation, it is used to shade the crops by using colored networks. Nets in color represent an additional tool in the management of light quality.

In order to enable the production of high - quality vegetables in vegetable production, the most modern technological solutions are applied. In order to contribute to the reduction of the negative impact of extremely high temperatures we used the shading nets in the trial field of Agriculture Extension Service "Sombor" (Serbia) as a special agrotechnical measure, characteristic for the production of tomato, which significantly contributes to the reduced negative effect of abiotic factors on the growth, quality and yield of tomato. At the end of the 1960s, shading net was used in Europe. The most famous factories were a location in England and Italy. Over time, this technology has found a way into other parts of the world. Nets, in addition to basic protective use, have the function of protection against birds, insects, and wind. The benefit of the color network includes an extension of harvest time, increased yields, improved quality and agro-economic performance of the yield (Shalom Y. and Gussakovsky E.E., 2004). Filtered sunlight through a photoselective net, shows the influence and quality improvement and yield (Oren - Shamir et al., 2001).

In order to solve the problem in the field of Agriculture Extension Service “Sombor” (Serbia), its implemented trial experiment with different colors of the shading net: Blue, green, black, grey. An experimental trial was set in the period from May 2021 to August 2020. The varieties of tomato was Kruna and Rio Grande. All plots were in a drip system “drop by drop”. The experiment is set in 2 repetions, 5 m^2 long.

Characteristics of shading net

Manufacturers offer nets that range from 10 to 90%. Recommended vegetable density are nets that shine from 30 to 40%. They are most commonly produced from high-density polyethylene and can be used for more seasons. Shading nets are made of UV - resistant material and are suitable for use over several seasons, minimum two. Setting up nets is relatively simple. When installing the structure, it is necessary to lift the pillars over which the net is clinging and stretching. Nets are manufactured in different diameters of 1.20, 2.00, 3.60, 4.00, 6.00 and 7.50 mm depending on the purpose.

Nets can be found in different colors. Although it is said to be a shadow net, it at the same time reduces the amount of light, the intensity of UV rays and the temperature. Shading nets have additional functions such as making cold for domestic livestock and pets. Trials filed with the use of shading net is set in 3 repetition, the size of plots 5m^2, in the “drop by drop” system with the application of a mulch foil. With each variant, a control plot was set up.

Picture number 2. Different dimensions of shading net

Picture number 3. Shading net (photo: V. Sabadoš)

MATERIAL AND METHODS

In order to enable the production of high - quality vegetables in vegetable production, the most modern technological solutions are applied. In order to contribute to the reduction of the negative impact of extremely high temperatures we used the shading nets in the trial field of Agriculture Extension Service "Sombor" (Serbia) as a special agrotechnical measure, characteristic for the production of tomato, which significantly contributes to the reduced negative effect of abiotic factors on the growth, quality and yield of tomato. At the end of the 1960s, shading net was used in Europe. The most famous factories were a location in England and Italy. Over time, this technology has found a way into other parts of the world. Nets, in addition to basic protective use, have the function of protection against birds, insects, and wind. The benefit of the color network includes an extension of harvest time, increased yields, improved quality and agro-economic performance of the yield (Shalom Y. and Gussakovsky E.E., 2004). Filtered sunlight through a photoselective net, shows the influence and quality improvement and yield (Oren - Shamir et al., 2001).

RESULTS AND DISCUSSION

Table 1. Results in 2020 years - first harvest

No.	*Rio grande*	*number of plants*	*number of fruits / I class*	*weight of fruits I class / kg*	*number of rotten fruits*	*weight of rotten fruits*	*yield I class t / ha*	*yield t / ha*
1	Green	24	420	29,40	163	9,80	61,20	81,60
2	Blue	24	613	37,35	50	3,35	77,70	84,50
3	Grey	24	450	33,10	73	5,20	68,90	79,70
4	Black	24	300	26,05	93	6,15	54,10	66,90
5	Control	24	385	30,60	161	10,70	63,70	86,00
	AVERAGE						**65,1**	**79,7**

No.	*Rio grande*	*number of plants*	*number of fruits / I class*	*weight of fruits I class / kg*	*number of rotten fruits*	*weight of rotten fruits*	*yield I class t / ha*	*yield t / ha*
1	Green	24	240	27,70	134	12,75	57,70	84,10
2	Blue	24	184	20,25	61	5,45	42,00	53,30
3	Grey	24	188	20,80	49	4,95	43,30	53,50
4	Black	24	320	41,40	80	7,80	86,20	102,50
5	Control	24	305	37,50	153	16,10	78,10	111,60
	AVERAGE						**61,5**	**81,0**

Table 3. Results in 2020 years - second harvest

Rio grande	*number of plants*	*number of fruits / I class*	*weight of fruits I class / kg*	*number of rotten fruits*	*weight of rotten fruits*	*yield I class t / ha*	*yield t / ha*
Green	24	175	9,80	103	7,30	20,40	35,60
Blue	24	188	8,80	192	7,70	18,30	34,30
Grey	24	194	10,00	143	7,55	20,80	36,40
Black	24	203	13,30	217	11,68	27,70	51,80
Control	24	30	1,32	151	6,96	2,70	17,20
AVERAGE						**18,0**	**35,1**

Kruna	*number of plants*	*number of fruits / I class*	*weight of fruits I class / kg*	*number of rotten fruits*	*weight of rotten fruits*	*yield I class t / ha*	*yield t / ha*
Green	24	11	0,65	36	2,54	1,30	6,60
Blue	24	21	1,32	58	3,60	2,70	10,20
Grey	24	56	4,92	52	4,20	10,20	19,00
Black	24	43	3,36	51	5,28	7,00	18,00
Control	24	13	0,98	33	3,03	2,00	8,30
AVERAGE						**4,6**	**12,4**

Table 4. Results in 2020 years - average of two harvests

red.br.	***Rio grande***	*number of plants*	*number of fruits / I class*	*weight of fruits I class / kg*	*number of rotten fruits*	*weight of rotten fruits*	*yield I class t / ha*	*yield t / ha*
1	Green	24	595	39,20	266	17,10	81,60	117,20
2	Blue	24	801	46,10	242	11,05	96,00	**118,90**
3	Grey	24	644	43,10	216	12,70	89,70	116,20
4	Black	24	503	39,30	310	17,70	81,80	**118,70**
5	Control	24	415	31,90	312	17,60	66,40	103,10
	AVERAGE						**83,1**	**114,8**

red.br.	***Kruna***	*number of plants*	*number of fruits / I class*	*weight of fruits I class / kg*	*number of rotten fruits*	*weight of rotten fruits*	*yield I class t / ha*	*yield t / ha*
1	Green	24	251	28,30	170	15,20	58,90	90,60
2	Blue	24	205	21,50	119	9,05	44,70	63,50
3	Grey	24	244	25,70	101	9,15	53,50	72,50
4	Black	24	363	44,70	131	13,00	93,10	**120,20**
5	Control	24	318	38,40	186	19,10	80,00	**119,70**
	AVERAGE						**66,0**	**93,3**

Based on the results from 2020, the Rio Grande variety showed a statistically significant increase on the blue network (118.9 t / ha) and the black network (118.7 t / ha) compared to the control, while the variety Kruna has a black network (120, 2 t / ha) showed an increase in yield compared to the control of 119.7 t / ha.

Table 5. Results in 2020 years - percentage of damage

Sorta	Control		Green net		Black net	
	Yield t/ha	% fruit damage	Yield t/ha	% fruit damage	Yield t/ha	% fruit damage
Rio grande	103,1	42,9	117,2	30,8	118,7	38,1
Kruna	119,7	36,9	90,6	40,0	120,2	26,5
AVERAGE	**111,4**	**39,9**	**103,9**	**35,4**	**119,5**	**32,3**
	Blue net		Grey net			
	Yield t/ha	% fruit damage	Yield t/ha	% fruit damage		
Rio grande	118,9	23,2	116,2	25,1		
Kruna	63,5	36,7	72,5	29,2		
AVERAGE	**91,2**	**30,0**	**94,4**	**27,2**		

In the cultivar Rio Grande we had the least damage on the blue net compared to the control, while in the cultivar crown we had the least damage on the black net.

Table 6. Results in 2020 years - fruit ratings

#	***Rio grande***	**pericarp thicknes s / mm**	**pericar p color**	**fruit weight / gr.**	**seed weight / gr.**	**box weight / gr.**	**pericarp weight / gr.**	**%Sac h mass**	**dry matter**
1	Control	8	crvena	121,9	11,6	12,7	94,2	4,0	3,6
2	Green net	7	crvena	113,7	9,1	12,6	91,2	4,3	3,1
3	Blue net	8	crvena	115,4	14,4	11,5	88,7	3,9	3,5
4	Grey net	8	crvena	105,7	13,1	10,0	79,6	4,0	3,8
5	Black net	9	crvena	130,1	11,8	14,7	101,4	4,1	4,1
	AVG	**8**	crvena	**117,4**	**12,0**	**12,3**	**91,0**	**4,1**	**3,6**

#	***Kruna***	**pericrp thicknes / mm**	**pericarp color**	**fruit weight / gr.**	**seed weight / gr.**	**box weight / gr.**	**pericarp weight / gr.**	**% Sach mass**	**dry matter**
1	Control	7	crvena	146,8	13,6	15,1	115,0	4,0	2,8
2	Green net	7	crvena	130,4	14,2	15,4	98,8	4,2	3,3
3	Blue net	6	crvena	166,8	8,0	17,0	139,7	3,8	2,7
4	Grey net	8	crvena	162,9	17,0	19,0	124,1	3,9	2,9
5	Black net	7	crvena	164,5	13,4	18,1	129,2	4,4	3,2
	AVG	**7**	crvena	**154,3**	**13,2**	**16,9**	**121,4**	**4,1**	**3,0**

In the cultivar Rio Grande we had the least damage on the blue net compared to the control, while in the cultivar crown we had the least damage on the black net.

CONCLUSION

The application of this technology is recommended for the production of tomato on smaller surfaces, but it is also interesting for the production of blue eggplant. The benefit of a network of colors includes an extension of harvest time (early and later maturation) yield increase, quality improvement and total agroeconomic yield performance. (Shahak Y. and Gussakovsky E.E., 2004). In addition, the importance of network applications is also reflected in the protection of plants from the hail and insects. **Black nets are naturally resistant to the sun's UV rays and are used in areas with the highest intensity of solar radiation.** In the production of shading nets, special chromatic additives are added to the materials. Along with the "protective SPF factor" from direct sunlight, the plant also received the most favorable light quality for growth. The excellent results we obtained with the application of black foil confirm that this foil is best for use both to increase yields and for a lower percentage of fruit damage. Also, the difference in the amount of yield in relation to the control is not negligible.The disadvantages of shading nets are a significant investment in production in the first year. The benefits of the shading nets are simple and mobile construction, the use of nets for many years, the possibility of using incentive means for purchasing shading nets, stable and quality pepper production. Agronomic professions and science responded in time and tried to reduce the negative impact of climate change and abiotic stress on plants by introducing new breeding technologies, tolerant varieties, and hybrids.

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MONITORING THE NUMBER OF PRINTS OF INJECTION MACHINES WITH WIRELESS CONNECTION

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ABSTRACT

The aim of this study is to examine the studies for online tracking of the number of prints of injection machines and to identify the problems with online tracking and to develop solutions for these problems. In this way, we wanted to both increase the efficiency for the production lines and develop a method based on this and also test its efficiency. If we compare the losses due to errors, it is seen that the production slows down and the quality decreases due to 10% employee-related, 10% material, 20% mold and 60% machine-based. For this reason, it has been decided to monitor the machines, since designing the control tool in a way that will reduce the error rate of the machine will greatly reduce the error.

Keywords: Injection, Communication of Objects, Thermoplastics, Polyamide

INTRODUCTION

American inventor John Wesley Hyatt, together with his brother Isaiah, officially patented the first plastic injection molding machine in 1872 and presented a simple machine design for different applications (Plastik enjeksiyon, 2020). Since then, injection technology has developed day by day and created today's injection molding machines.

Especially for the white goods and automotive industries, plastic injection processes are production processes that are widely used and studied. These systems, which enable the dry raw material to be in the desired shape and density by heat, pressure and mechanical processes, have high error rates due to their dependence on too many parameters. (Kamber, 2008).

Due to errors in the production line, lack of control and lack of follow-up, production efficiency decreases and efforts are made to increase efficiency. (Saraç & Kaya, 2008). These studies are based on reducing losses and increasing productivity through methods such as automating the production line, increasing the frequency of control and making performance monitoring continuous.In this project, a method that will enable performance monitoring to be done online not only from inside the production but also from the outside has been researched. It has developed a system proposal for online tracking of injection molding machines in order to analyze the convenience that this method will bring and what kind of effects it can create for production efficiency, and to investigate the usability of the method.

MATERIAL AND METHOD

INJECTION APPLICATIONS

Injection applications are one of the most common applications in the inclusion of molten raw material in production. These applications are based on the transfer of the raw material to the mold chamber with the help of a worm screw at a certain pressure after being melted with the help of heaters, and taking the desired shape by cooling with the help of cooling methods. For example, it is used in many industrial areas such as plastic injection machines, cable grouping processes, plastic mold production.

Vertical and Horizontal Injection Molding Machines

In the injection process, different tools and machines are used to include the raw material in the production as desired. Horizontal and vertical injection machines are the most common tools used for incorporating plastic raw materials into production. (Aldemir Plastik Kalıp, 2018).

In injection technology, machines are customized on 3 basic parts.

These parts are;

1) Injection Unit

2) Molding Unit

3) Vise Unit

The raw material is heated and melted in the injection unit. Although the temperature varies according to the raw material used, it melts in the range of 150-250 ºC. The raw material is transmitted to the molding unit by means of the pressure formed inside and with the help of the worm screw. Here, the hot raw material is cooled quickly with various cooling liquids from the channels in the mold and the cooled raw material is removed to the desired extent. (Çakır & Dallı , 2020)

PLASTICS USED IN INJECTION MACHINES

Depending on the usage area, the raw materials in the injection machines vary. The selection of raw materials is made among thermoplastics, considering its usefulness for injection processes according to heat resistance, flexibility and service life.

Thermoplastics are plastic materials that are solid at room temperature (Malzeme Bilimi, 2018). Considering their use, PP, PC, PE, PVC, PS, ABS (Fındık & Taşdemir, 2020) and Polysulfone are the most used thermoplastic materials (Hastek Plastik Sistemleri, 2020). Especially PVC and PE thermoplastics are preferred more because of their low cost, easy coloring and high strength properties (PVC, 2020). It has also been proven by studies that these polymers are not harmful to human health.

COMMUNICATIONS IN INJECTION MACHINES

With the Industry 4.0 applications, the communication of objects has started to be implemented in all sectors and production lines (Akkuş, 2016). Data transfer between machines without the need for human intervention is called communication of objects. (Öztürk & Keçe , 2006). Ensuring the communication of the machines on the production lines provides great benefits in reducing the problems and losses, modeling the production, remote monitoring and process planning.

There are many factors affecting production in plastic injection machines. These factors vary from the raw material used, the pressure ratio, the mold wear or the employee error, so it needs follow-up for a better production efficiency. If the working patterns of the machines are followed through communication, the source of the error will be found faster and resolved faster.

FACTORS SLOWING PRODUCTION

The factors that slow down production in injection machines are human-induced factors, machine and material-based factors. Human-induced factors are factors such as improper implementation of production steps, use of the device by unqualified personnel (Yıldırım & Karaca, 2013). Machine-related factors are caused by the wear of the pumps, the malfunctioning of the control units, the pressure failure due to low pressure, and the abrasions caused by high temperatures. Material and mold-related defects occur due to the high granularity of the material, wear in the mold and clogging of the cooling channels. If we make a ratio, it is seen that production slows down and quality decreases due to 10% employee source, 10% material, 20% mold and 60% machine source. For this reason, it has been decided to monitor the machines, since designing the control tool in a way that will reduce the error rate of the machine will greatly reduce the error.

Control Methods Applied in Production

In order to prevent the production from slowing down and to speed up the detection of the error, the quality control units regularly keep checklists on the production lines, and these lists are compared with the daily output and the losses are tried to be calculated. The main factor in the formation of losses here is the frequency of control and the inability of the analysis to be comprehensive (Aygenteks, 2019). Instead, the establishment of a system where the number of units produced by the system can be monitored all day will both reduce the quality of manpower and cause a better analysis of the problems in production (Johnston, Kazmer, & Gao, 2009). Therefore, a tool that provides minute-by-minute traceability of prints should be considered. The vehicle to be made must ensure that the operation performed on the machine is transmitted continuously and accurately on the basis of units, without being dependent on people, so that we can analyze the system remotely.

DESIGNING THE CONTROL TOOL

In the light of all this information, it has become necessary to design a tool for continuous monitoring of the number of prints of the injection machine to improve control and analysis. For this reason, it was thought that it would be easier and less costly to detect the pieces in each print by making a counter between the start button that enables the machine to work and the machine.

The counter will count down depending on the injection working time, will output when the operation is finished, and will transfer the number of transactions to an online platform per minute via a communication tool (Wang & Zhou, 2000). If the print is not finished or at the time of an emergency stop, it will not count and the counter will send the same number. These data will be kept in days, hours and minutes. In this way, by taking these data, it will be determined which problem is caused in production when there is a decrease in number, and these problems will be analyzed better and solved more quickly. (Fei & Xiaowei, 2019). Through the online platform, printing and production will be traceable from anywhere.

CREATING THE DESIGN

A 12x12 Plastic box, a 5 Volt 1 Ampere adapter, 1 Arduino Uno R3, 1 4x4 keypad, 1 breadboard, 1 16x2 LCD Display, 1 in order to carry out the necessary operations in the design, reduce the cost and make it simple for the user. A potentiometer, a 1 KΩ resistor and an ESP8266 communication module are used for communication. In the design, a keypad is used so that the user can manually enter the duration of the print. A potentiometer was used to adjust the brightness of the screen used, and the ESP8266 Wi-Fi module, which is an ESP series IoT module, was used to transmit the number of machine prints in the background at each count. (Gömülü Yazılım, 2017).

Due to its easy coding and usefulness, the circuit made by using the Arduino Uno R3 control card was mounted in a box of appropriate dimensions and the injection machine was connected to the start button, allowing input per process.

ONLINE PLATFORM THAT DATA IS TRANSFERRED

ThingSpeak application was used to transfer all data. This application is an application that allows you to take data over IP and turn them into a table that keeps them constantly recording and can transfer the incoming data to Excel.

In addition, it is a useful online platform that can receive data from any number of different data sources at the same time with the created input channels and keep these data separately on a source basis as hour, day, month and year. To use this platform, it is sufficient to open an online account only. It also allows tracking of the same data source from different accounts. Considering these features, this platform, which can receive comprehensive and instant data, has been chosen for the online transmission of the tracking.

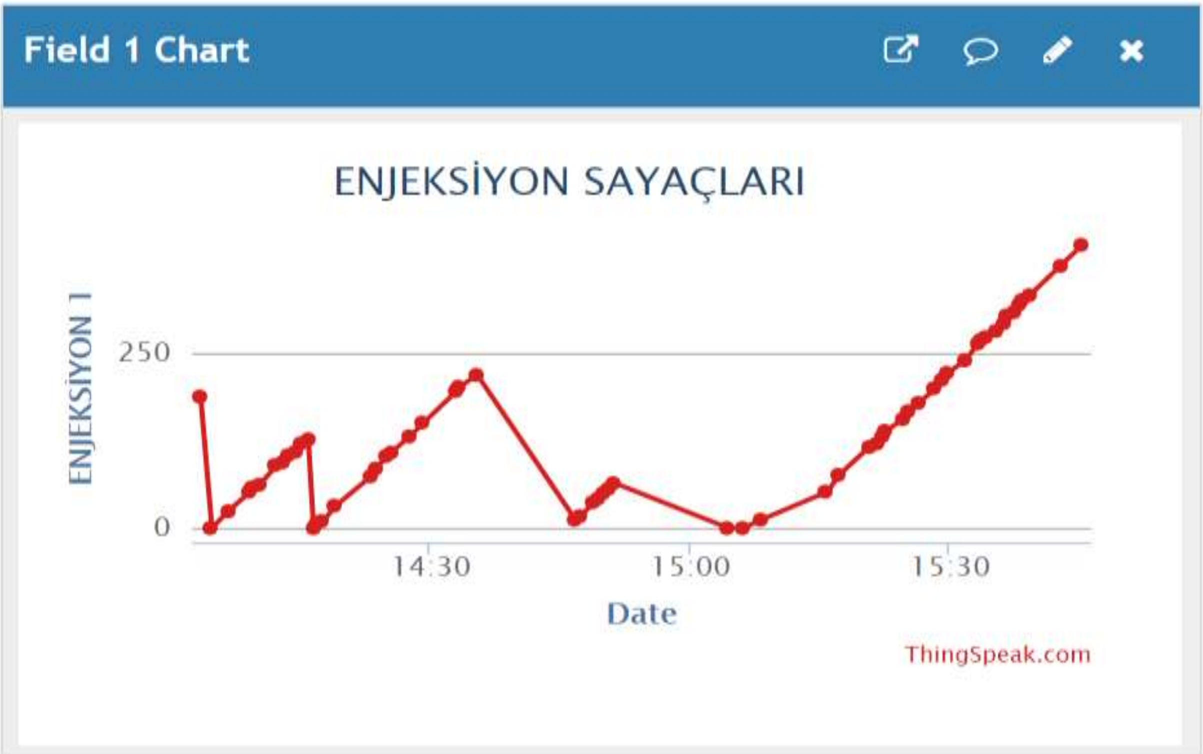

Figure 1. ThingSpeak Print Count Change Graph

TESTING THE MODULE ON THE MACHINE

The module made was connected between the buttons of the machine and the PLC, and it was watched for 2 hours and 45 minutes, giving a signal when each button was pressed, and it made a total of 1018 prints. The distribution of these prints and their hourly changes in Excel taken from ThingSpeak appear as in Table.1 below. During this time, the program was reloaded several times by changing the sending time.

Table 1. Injection Print Quantity Table

Date	Hour	Sent Signal Number	Number of Prints
12.04.2021	13:00:12	130	184
12.04.2021	13:00:42	131	186
12.04.2021	13:01:23	132	195
12.04.2021	13:02:03	133	203
12.04.2021	13:03:55	134	223
12.04.2021	13:04:31	135	233
12.04.2021	13:07:13	136	265
12.04.2021	13:07:59	137	272
12.04.2021	13:08:35	138	281
12.04.2021	13:09:36	139	288
12.04.2021	13:20:20	140	24
12.04.2021	13:20:41	141	31
12.04.2021	13:21:31	142	35
12.04.2021	13:22:27	143	45
12.04.2021	13:22:58	144	56
12.04.2021	13:24:39	145	74
12.04.2021	13:25:00	146	82
12.04.2021	13:25:25	147	86
12.04.2021	13:28:28	148	125
12.04.2021	13:28:54	149	127
12.04.2021	13:30:45	150	144

12.04.2021	14:32:59	151	196
12.04.2021	14:33:20	152	202
12.04.2021	14:35:22	153	219
12.04.2021	14:46:42	154	12
12.04.2021	14:47:18	155	17
12.04.2021	14:48:50	156	37
12.04.2021	14:49:31	157	42
12.04.2021	14:50:01	158	5
12.04.2021	14:50:42	159	56
12.04.2021	14:51:18	160	64
12.04.2021	15:04:23	161	0
12.04.2021	15:06:04	162	0
12.04.2021	15:08:12	163	12
12.04.2021	15:15:50	164	52
12.04.2021	15:17:12	165	76
12.04.2021	15:20:40	166	126
12.04.2021	15:22:38	167	139
12.04.2021	15:24:34	168	156
12.04.2021	15:25:20	169	167
12.04.2021	15:31:46	170	240
12.04.2021	15:33:14	171	264
12.04.2021	15:33:34	172	269
12.04.2021	15:34:21	173	273
12.04.2021	15:35:17	174	282
12.04.2021	15:36:13	175	293
12.04.2021	15:36:38	176	304

12.04.2021	15:37:34	177	309
12.04.2021	15:38:05	178	322
12.04.2021	15:38:25	179	326
12.04.2021	15:39:12	180	333
12.04.2021	15:42:44	181	375
12.04.2021	15:45:17	182	405

RESULTS AND DISCUSSION

Here, too, we see that the number of prints is very irregular. The same type of print on the same cable set gives very different numbers at very different times. With the work we have done here, we see that the production control is insufficient. Moreover, the deviations in the number of prints are regulated in the last 45 minutes because during the work, the pressure is reduced. A problem caused by the problem was encountered and after this problem was solved, it is seen that there is a more regular distribution of pieces in the prints. We understand from here that the system is more active and more effective in error detection and analysis in the continuous follow-up of the system. And by doing this online, we have observed that instabilities can be detected remotely.

In the study, the control process, which is normally done with the follow-up steps in the factory, was analyzed. With this developed method, the faster and more effective analysis and control of errors, by monitoring both from the factory and from anywhere with internet, minimizes losses in production, while providing a clearer and more comprehensive solution for optimization studies. It gives us the opportunity to analyze. This shows that continuous control is more advantageous and comprehensive than periodic controls under normal conditions. Thus, we have seen that the availability of continuously controlled online systems is more advantageous instead of traditional control in production.

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HONEY PLANTS ECOLOGICAL ASSESSMENT IN TREBESHINA-DHEMBEL-NEMERCKE MOUNTAIN RANGE

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ABSTRACT
In situ conservation of honey plants species is an effective strategy for the conservation of natural resources especially in under climate change impact. These species have also a great economic value as they are source of honey production, particularly in mountain areas where beekeeping is a promising economical source for locals. The knowledge about the list of honey plant of a certain area, their flowering period and their ecological requirements and optima is crucial for their conservation. This data could also assist local beekeepers to develop effective strategies in managing their farms and bee feeding. A study was conducted in the Trebeshinë-Dhëmbel-Nëmërçkë mountain range aiming the identification of honey plant grown wild in this area, assessing their flowering period and ecological requirements using Ellenberg-Pignatti indicators. A total of 121 honey plant species were identified in the studied area, the majority of them flower in the June –August. Based on the Elleberg-Pignatti ecological indicators the majority of these plant species require high light levels, temperate temperatures, sub oceanic to slightly continentality climate, dry to medium soil moisture and soils with neutral pH and with low to slightly rich nitrogen.

Keywords: Honey plants, ecologic indicators, Trebeshinë-Dhëmbel-Nëmërçkë mountain range, Albania

INTRODUCTION

In the climate change scenario, the conservation of biodiversity in general, and particularly of economically important species, has emerged as a major concern. Honey producing plants importance is related not only to food security and human nutrition as the basis for honey production, but also to the honey bee populations' survival.

According to the statistics the amount of honey produced in Albania, over years increased from 2886t to 4599t in the years 2010 and 2020, respectively. An increase in the number of beekeeper's farms was also recorded (INSTAT 2020). Since the honey production and the quality is higher in the farms located nearby and use wild plant resources, many beekeeper's farms are built in the mountain areas. The amount of honey produced by farms in the region of Trebeshinë-Dhëmbel –Nëmërçkë mountain range accounts for around 4.6 % of total honey production in the country (INSTAT 2020). The checklist of honey producing plant species in this mountain range is missing, this information would assist beekeeper's to better manage their farms. Beside well known factors as biotic factors, including anthropogenic activity, to date the status and the distribution of these plant species is potentially affected by the climate change impact and the land degradation, stressing the importance of the projects of their in situ conservation. Ellenberg's indicators are considered as an effective tool for the estimation of ecological conditions of plant species within a given flora. They can be especially useful in identifying ecological optima of plant species and assisting their conservation action plans. Indicators as Light, Temperature, Continentality, Moisture, Reaction, Nitrogen and Salinity are widely used to evaluate species optimal ecological preferences, this indices are given in a system of values (Diekmann, 2003). The first list of indicator values was given and then updated by Ellenberg (1974, 2001). Since the Ellenberg's list was designed for Central European Flora, the evaluation scale was slightly modified for indicators to adapt specific countries ecological conditions. Thus Pignatti (2005) in difference of Ellenberg modified the evaluation of Light, Temperature and Soil Moisture indices to 0-12 grade scale to adapt climate and ecological indices of Italy.

No database on specific species ecological requirements of plant species exist for Albanian flora, apart a set of data given for some species of Asteraceae family in Trebeshina Mt. (Peci et al., 2021). In this contexts, the current study aims to inventorying of honey producing plants species grown wild in Trebeshinë-Dhëmbel-Nëmërçkë mount range, assessing their phenology periods and ecological optima using Ellenberg-Pignatti indicators, in order to fill the key gaps in our knowledge on these species and assist their conservation plan and strategies.

MATERIAL AND METHOD

The study area, Trebeshinë-Dhëmbel-Nëmërçkë (TSHN) mountain range is located at the South of Albania, it is in the administrative district of Përmet and constitute a very important economy for local inhabitants. TSHN mountain range has a surface of 39570.4 ha, its altitude ranges from 170-2486m asl. The area has Mediterranean climate, the mean annual temperature is 13ºC, while the mean annual rainfall is 1200-1300mm (IGJEUM 2020). The geological formation is mainly calcareous.

The study was focused on the identification of plant species with potential use for honey production. The relevés were carried out in different periods of the year aiming to assess the flowering period of each of these species. The taxonomic identification of plant species was done according to Vangjeli, (2015), while data on their potential for honey production was obtained by Paparisto and Buzo, (2003)

Ecological indicators were used to assess the ecological requirements of these species for each of them six indices were considered such as: Light (L), Temperature (T), Continentality (C), Moisture (F), Soil Reaction (R), and Nitrogen (N) according to Pignatti (2005), since the climatic conditions of our studied area was closer to that of Italy.

RESULTS AND DISCUSSION

Based on the study results, in total 121 honey producing plant species were identified during relevés in the studied area and a checklist of this species, flowering periods and their potential amount of honey production was compiled and presented in the Table 1. The most dominant form among these plant species was herbaceous (71%), followed by trees (14.8%) and shrubs (13.2%). This species belong to 39 families, the majority of them belong to the Lamiaceae family (29 species) followed by Fabaceae (21 species), Rosaceae (8 species), Asteraceae (6 species), Boraginaceae (6 species), Fagaceae (4 species), Geraniaceae (3 species), Liliaceae (3 species), other 31 families are composed from 1-2 species (figure 1).

The vulnerability analysis of the plant species based on the Albanian Red List (2013) revealed that 9 % of them were classified as threatened according to IUCN categories.

Table 1. The list of plant producing species in the Trebeshinë-Dhëmbel-Nëmërçkë mountain range

No.	Species name	Family	Bloom phase	*Honey Production kg/ha
1	Acer obtusatum	Aceraceae	April-May	**439**
2	Acer tataricum	Aceraceae	April-May	**21.1**
3	Acinos alpinus	Lamiaceae	June-August	**54**
4	Aesculus Hippocastanum	Hipocastanaceae	April-May	**91.3**
5	Anthyllis vulneraria	Fabaceae	May-August	**21.74**
6	Arbutus unedo	Ericaceae	October-December	**99.75**
7	Asparagus acutifolius	Liliaceae	May-June	**36.4**
8	Astragalus glycyphyllos	Fabaceae	May-July	**11.6**
9	Asyneuma limonifolium	Campanulaceae	June-July	**24.5**
10	Ballota nigra	Lamiaceae	May-August	**104.1**
11	B. purpurocaerulea	Boraginaceae	April-June	**35.1**
12	Calamintha grandiflora	Lamiaceae	June-August	**36.9**
13	Calamintha sylvatica	Lamiaceae	May-October	**39.7**
14	C. sylvatica sp. ascendens	Lamiaceae	May-October	**128.3**
15	Centaurea alba	Asteraceae	June-August	**46.9**
16	Centaurea solstitialis	Asteraceae	June-August	**29.08**
17	Cercis siliquastrum	Fabaceae	March-May	**25.8**
18	Cichorium intybus	Asteraceae	May-October	**9.6**
19	Cirsium appendiculatum	Asteraceae	July-August	**100.6**
20	Cirsium candelabrum	Asteraceae	June-August	**54.15**
21	Cistus salvifolius	Cistaceae	May-July	**18.53**
22	Clematis vitalba	Ranunculaceae	May-July	**30**
23	Clinopodium vulgare	Lamiaceae	June-August	**58.24**
24	Colutea arborescens	Fabaceae	May-June	**25.8**

25	Cornus mas	Cornaceae	February-April	**5.59**
26	Coronilla varia	Fabaceae	May-August	**1.4**
27	Corylus avellana	Corylaceae	February-March	**20**
28	Crataegus monogyna	Rosaceae	April-June	**9.3**
29	Cynoglossum creticum	Boraginaceae	April-June	**66.4**
30	Cynoglossum officinale	Boraginaceae	May-August	**100.6**
31	Daphne oleoides	Thymelaeaceae	May-June	**10.45**
32	Dictamnus albus	Rutaceae	May-June	**14**
33	Dorycnium hirsutum	Fabaceae	May-June	**8.27**
34	Dorycnium pentaphyllum	Fabaceae	April-June	**1.6**
35	Echium italicum	Boraginaceae	April-August	**293**
36	Echium vulgare	Boraginaceae	April-September	**295**
37	Erica arborea	Ericaceae	March-May	**26.57**
38	Eryngium campestre	Apiaceae	July-September	**110**
39	Fagus sylvatica	Fagaceae	May	**20**
40	Fraxinus ornus	Oleaceae	April-June	**18**
41	Galium odoratum	Rubiaceae	May-July	**DD**
42	Geranium macrorrhizum	Geraniaceae	June-August	**14.46**
43	Geranium molle	Geraniaceae	March-October	**1.29**
44	Geranium sanguineum	Geraniaceae	April-June	**DD**
45	Glechoma hirsuta	Lamiaceae	March-June	**8.26**
46	Hedera helix	Araliaceae	February-April	**29.81**
47	H. nummularium	Cistaceae	May-August	**DD**
48	Hieracium pilosella	Asteraceae	June-October	**DD**
49	Hypericum perforatum	Hypericaceae	May-August	**DD**
50	Juglans regia	Juglandaceae	April-May	**DD**
51	Juniperus oxycedrus	Cupressaceae	March-April	**DD**
52	Knautia drymeia	Dipsacaceae	May-July	**7.1**
53	Lamium garganicum	Lamiaceae	April-August	**109.06**
54	Lamium maculatum	Lamiaceae	March-December	**146.45**
55	Lathyrus niger	Fabaceae	May-June	**101.1**
56	Lathyrus sylvestris	Fabaceae	May-August	**6.1**
57	Lembotropis nigricans	Fabaceae	June-August	**4.9**
58	Lotus corniculatus	Fabaceae	May-June	**1.37**
59	Malva sylvestris	Malvaceae	May-August	**18.9**
60	Medicago minima	Fabaceae	April-July	**2.56**
61	Micromeria graeca	Lamiaceae	March-July	**15.18**
62	Muscari neglectum	Liliaceae	March-June	**3.14**
63	Nigella damascena	Ranunculaceae	May-July	**DD**
64	Ononis spinosa	Fabaceae	May-September	**2.08**
65	Orchis morio	Orchidaceae	April-June	**DD**
66	Origanum vulgare	Lamiaceae	June-September	**105.69**

67	Ostrya carpinifolia	Corylaceae	April-May	**DD**
68	Oxalis acetosella	Oxalidaceae	April-June	**DD**
69	Paliurus spina-christi	Rhamnaceae	May-June	**17.15**
70	Papaver rhoeas	Papaveraceae	March-July	**35.36**
71	Phillyrea media	Oleaceae	April-May	**DD**
72	Phlomis fruticosa	Lamiaceae	March-May	**DD**
73	Plantago lanceolata	Plantaginaceae	April-November	**2.2**
74	Potentilla reptans	Rosaceae	June-August	**DD**
75	Primula veris	Primulaceae	May-June	**DD**
76	Prunella grandiflora	Lamiaceae	June-August	**38.37**
77	Prunella laciniata	Lamiaceae	April-August	**195.4**
78	Prunella vulgaris	Lamiaceae	April-October	**176.7**
79	Prunus avium	Rosaceae	April-May	**16.68**
80	Putoria calabrica	Rubiaceae	June-September	**30.52**
81	Pyrus amygdaliformis	Rosaceae	April-May	**7.5**
82	Pyrus communis	Rosaceae	April-May	**34.2**
83	Quercus cerris	Fagaceae	April-May	**8.12**
84	Quercus frainetto	Fagaceae	April-May	**DD**
85	Quercus pubescens	Fagaceae	April-May	**DD**
86	Rhinanthus minor	Scrophulariaceae	May-September	**9.1**
87	Rubus ulmifolius	Rosaceae	May-July	**32.9**
88	Ruscus aculeatus	Liliaceae	February-April	**DD**
89	Salix alba	Salicaceae	March-April	**79**
90	Salvia glutinosa	Lamiaceae	June-September	**168**
91	Salvia officinalis	Lamiaceae	April-June	**325.13**
92	Salvia pratensis	Lamiaceae	May-August	**85.64**
93	Salvia triloba	Lamiaceae	May-June	**264.33**
94	Salvia verbenaca	Lamiaceae	January-December	**208.68**
95	Salvia viridis	Lamiaceae	April-May	**22.54**
96	Satureja montana	Lamiaceae	July-September	**86.43**
97	Scabiosa crenata	Dipsacaceae	June-August	**47.42**
98	Sedum acre	Crassulaceae	May-August	**10.72**
99	Sideritis raeseri	Lamiaceae	June-August	**69.12**
100	Sorbus aucuparia	Rosaceae	May-June	**DD**
101	Sorbus graeca	Rosaceae	May-June	**DD**
102	Spartium junceum	Fabaceae	May-June	**11.7**
103	Stachys germanica	Lamiaceae	June-August	**262.12**
104	Stachys sylvatica	Lamiaceae	June-August	**83.23**
105	Stellaria media	Caryophyllaceae	March-September	**DD**
106	Symphytum tuberosum	Boraginaceae	March-April	**28.41**
107	Teucrium chamaedrys	Lamiaceae	April-May	**73.63**
108	T. polium sp. capitatum	Lamiaceae	June-August	**43.5**

109	Thymus cherlerioides	Lamiaceae	June-August	**112.75**
110	Thymus longicaulis	Lamiaceae	April-August	**93.72**
111	Tilia platyphyllos	Tiliaceae	May-July	**728.77**
112	Tilia tomentosa	Tiliaceae	June-July	**857.28**
113	Trifolium campestre	Fabaceae	April-August	**22.31**
114	Trifolium patens	Fabaceae	June-September	**6.65**
115	Trifolium physodes	Fabaceae	May-June	**38.21**
116	Trifolium pratense	Fabaceae	March-October	**80**
117	Trifolium repens	Fabaceae	May-July	**26.7**
118	Veronica persica	Plantaginaceae	January-December	**40**
119	Vicia grandiflora	Fabaceae	May-August	**39.68**
120	Vicia villosa	Fabaceae	March-June	**54.04**
121	Vitex agnus-castus	Verbenaceae	May-September	**220**

**data on estimated honey production in kg/ha are given according Paparisto and Buzo, (2003)*

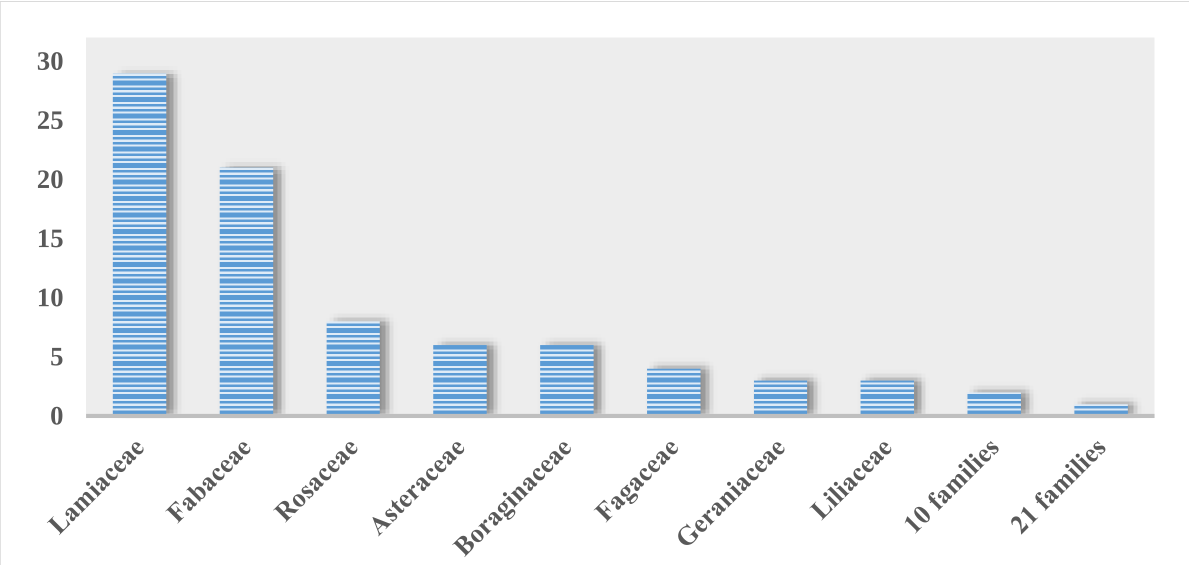

Figure 1. Family composition of honey producing plant species of the mountain range

Based on the data of Paparisto and Buzo (2003) about the estimated honey production in kg/ha of plant species, resulted that 23 of species in mountain range have a potential to produce over 100kg/ha (Table 1, Figure 2). While 10 of them such as *Tilia tomentosa, Tilia platyphyllos, Acer obtusatum, Salvia officinalis, Echium vulgare, Echium italicum, Salvia triloba, Stachys germanica, Vitex agnus-castus* and *Salvia verbenaca* are estimated to produce above 200 kg/ha, the highest potential is recorded for Tilia tomentosa which has an estimated honey production potential around 857 kg/ha (Paparisto and Buzo, 2003).

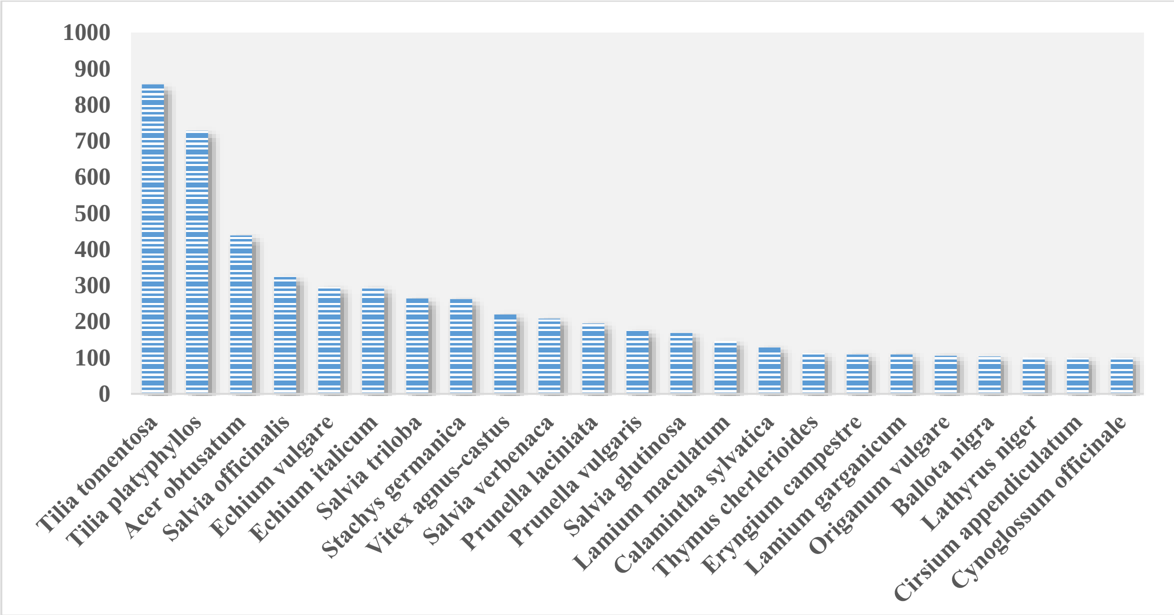

Figure 2. Species with higher honey production (kg/ha) found in mountrange *(*data according Paparisto and Buzo, 2003)*

Plant species bloom in different periods, the periods with the highest number of plants in flowering stage in mountain range resulted June –August (16 species), followed by April-May (14 species) and May-June (14 Species), May-August (11 species) (Table 1, Figure 3). The data on the flowering periods of honey plants will serve to beekeepers to better organize their bees feeding as well as serve to honey authenticity and security.

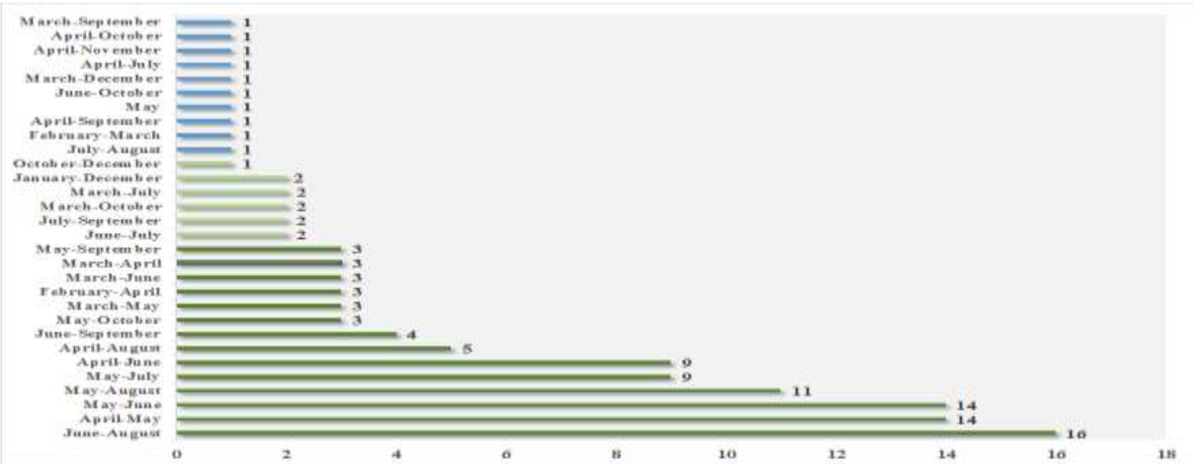

Figure 3. Blooming periods of honey plant species in mountain range

Considering the importance of honey plants and the threads of their population reduction and loss related to many factors especially to the impact of climate change and land degradation, in situ conservation activities could be a necessary option. The knowledge on the ecological preferences of species is relevant. Ecological indices were evaluated for each of the honey plants grown wild in the TDHN mountain range and presented in the figure 4 and figure 5. The indicator light (L) ranged from L=1 to L =12, for the light value the vast majority of species showed an optimum L=6 (18 species), L=7 (31 species) and L=8 (23 species). Thus reflecting the high demand for light of these species. The distribution of the values for the temperature indicator showed that the optimum values of temperature for the majority of species was T=6 (25 species) characteristic of low land areas of the mountain range and T=8 (27 species) for Mediterranean species. The continentality indices, most of species had a value of C=5 (46 species), reflecting sub oceanic to slightly continental preferences of the vast majority of honey plants. For the soil moisture indicator the majority of species showed value F=3 (36 species) and F=4 (34 species), indicating that the majority of honey plants require dry to medium soil moisture lands. The soil reaction values the majority of species showed value of R=7 (38), indicating the preferences for neutral soils of these species. The nitrogen indices values showed that species require low levels to slightly rich nitrogen soils, the majority of species showed value of N=2 (27 species) and N=3 and N=4 (18 species, each) (Figure 4, Figure 5).

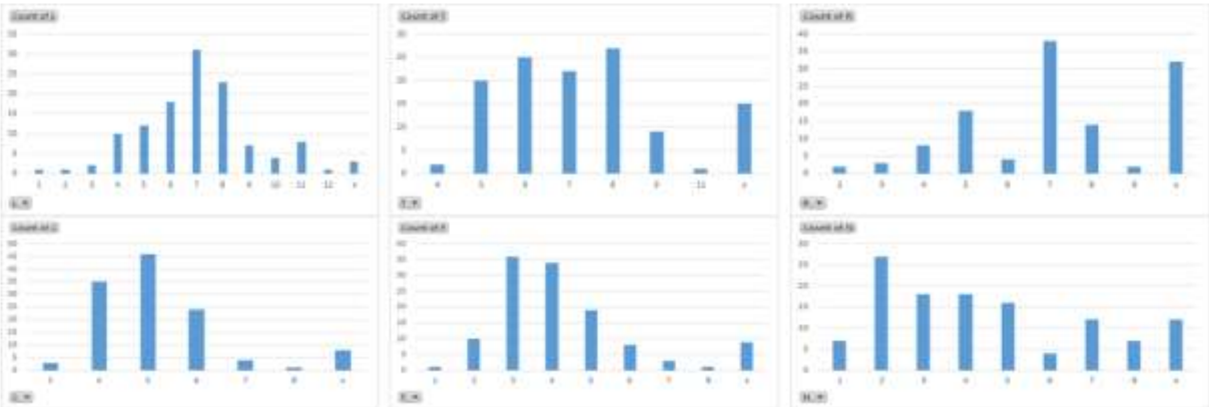

Figure 4. Distribution of species indicator values

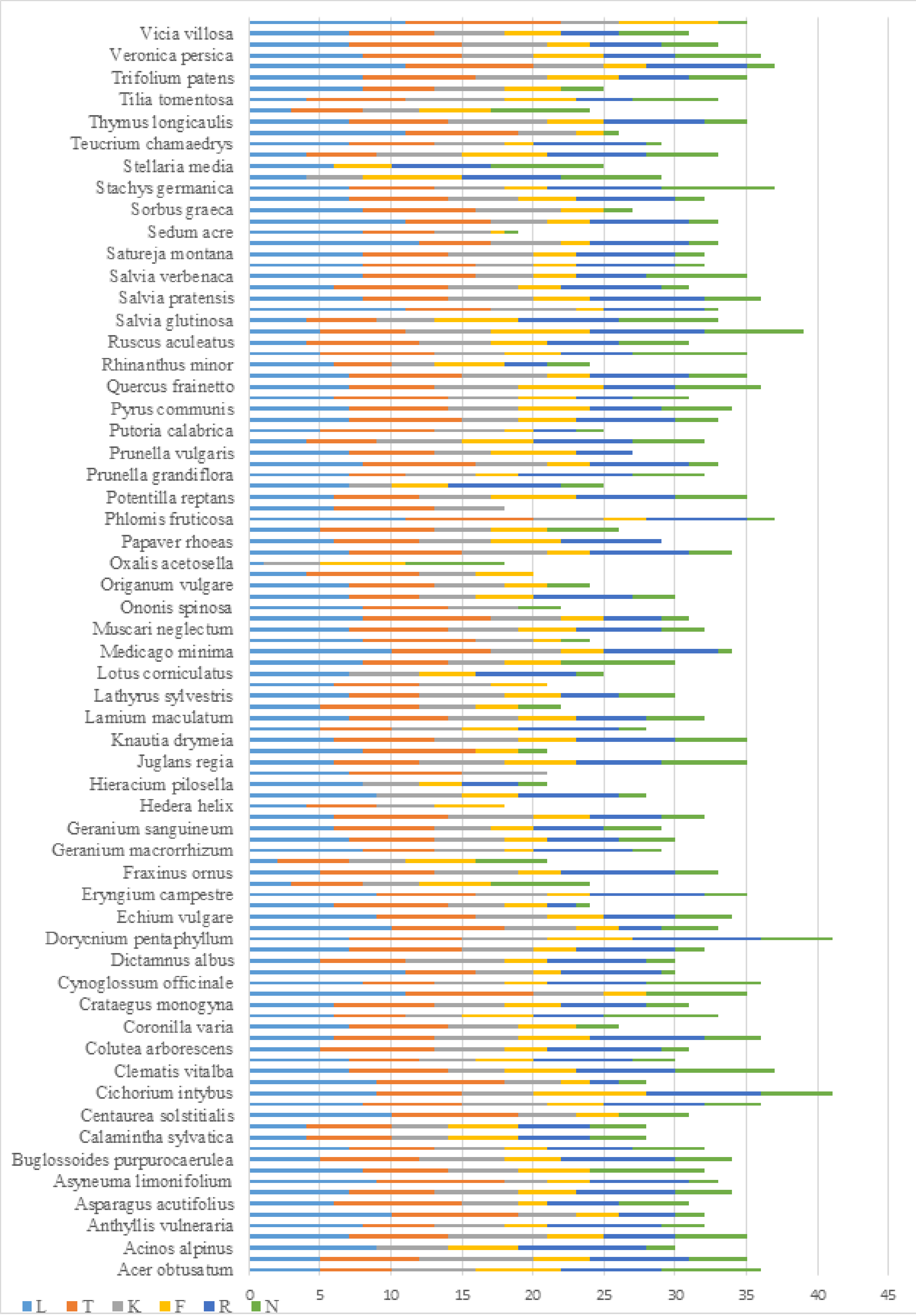

Figure 5. Ecological indicator values for each of honey plants in mountain range; L-light, T-temperature, C-continentality, F-soil soisture, R-soil reaction, N-nitrogen

CONCLUSIONS

Based in the study, the Trebeshinë –Dhëmbel-Nëmërçkë mountain range resulted rich in honey plants species, moreover 10 of them had high estimated honey production capacity. Based on the Elleberg-Pignatti ecological indicators the majority of these plant species require high light levels, temperate temperatures, sub oceanic to slightly continentality climate, dry to medium soil moisture and soils with neutral pH and with low to slightly rich nitrogen.

The obtained results on honey plants of the studied area reported for the first time in the current study, they provide a good basis for future the in situ conservation actions of these economically important plant species. Furthermore, the provided data on the list of honey plant species, their flowering period would assist beekeepers to better manage their bee’s farms.

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ASSESSMENT OF THE EFFECTS OF WATER STRESS AND SUBSTRATE ON FLOWERİNG, PODDİNG AND MYCORRHİZAE PRESENCE İN FABA BEAN LEGUME (*Vicia faba* L.)

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ABSTRACT

The bean is a legume rich in vegetable protein, carbohydrates, vitamins and fiber. It is one of the most cultivated vegetables in Mediterranean basin and the most influenced by water deficit. Our goal is to study the combined effect of water stress and growing substrate on bean productivity. The substrates used in this study are: (1) soil taken from a bean field (sandy clayey); (2) washed and rinsed sea sand and (3) a substrate made of a sand / peat mixture (rich in dry organic matter, phosphorus and potassium). The plants were followed for two months of the severe water stress application (10% retention capacity). The flowering precocity, weight pods per plant as well as mycorrhizae presence or absence were measured. The results obtained indicate that bean seems to express sensitivity to water stress action which is closely dependent on the substrate. In addition, peat composed substrate, rich in hydrophilic organic matter, allows a better plants water deficit resistance. In this state, plants are precocious, they flower quickly to avoid water stress and they are most productive in absence of any mycorrhization. The water restriction more affected the plants growing in the soil taken from the bean field compared to other substrates and acted as a brake on plants flowering and podding despite a significant colonization of the roots by arbuscular mycorrhizal fungi. This could result in the clay drying out and the development of a high suction pressure for the water which can oppose that of the roots of the plants. Sand culture is interesting under water stress thanks to its very filtering and very light texture. However, its lack of organic matter and its low water retention capacity are the cause of a low retention capacity of mineral elements, making it unfavorable to the development of mycorrhizae.

Keywords: *Vicia faba* L., water stress, substrate, mycorrhization, flowering, podding, seed yield

INTRODUCTION

Water and soil resources have an obvious and indisputable effect on plant nutrition and yield. Water is one of the ecological factors that most determines the growth and development of crops and its deficit plays an important role in inhibiting yields (Aldesuquy et al., 2014).

Soil is a living environment that supports plant roots and plays a fundamental role in water supply and plant nutrition (Girard et al., 2011). Substrate quality is one of the most important criteria influencing plant growth (Yasmeen et al., 2012). A good substrate has a set of physical and chemical properties that condition good and rapid plant growth (M'Sadak et al., 2016). The soil water retention properties are strongly influenced by soil texture. Substrate quality is one of the most important criteria influencing plant growth (Yasmeen et al., 2012). A good substrate has a set of physical and chemical properties that condition good and rapid plant growth (M'Sadak et al., 2016). Indeed, water retention is higher in fine-textured soils, containing levels of clay and finely divided constituents than in coarse-textured soils (Ouamer, 2010).

Organic matter plays an important role in microflora, fauna and soil structure. It controls aggregates stability, promotes water infiltration and therefore has a profound effect on soil moisture. It decreases runoff and protects the soil from erosion. Organic matter directly controls nutrients availability (Chabbi and Lemaire, 2007).

Arbuscular mycorrhizal symbiosis is considered a major component in relationships that plant maintains with its environment (Domergue, 2017). It concerns more than 80% of land plants and almost all cultivated plants (Lounès-Hadj Sahraoui, 2013). Many studies have also indicated that mycorrhizal interactions can strengthen the plant root system (Hamel, 2004), optimize its supply of water and mineral elements and improve its resistance to various abiotic stresses (Lounès-Hadj Sahraoui, 2013).

Our work objective is to study two factors interaction, water stress and substrate, and assess their effect on mycorrhization and bean production (*Vicia faba* L.), a legume rich in vegetable proteins but strongly influenced by the water deficit.

MATERIAL AND METHOD

The plant material selected for carrying out this experiment consists of broad bean seeds (*Vicia faba* L.) of Reina Mora variety of Spanish origin marketed in Algeria.

The experiment was conducted in a semi-controlled greenhouse. The previously disinfected bean seeds were sown in cells. Ten days after sowing, seedlings were individually transplanted to plastic pots, 18cm high and 21cm wide, filled with three different substrates (1) soil taken from a bean field (sandy clayey); (2) fine sea sand previously washed and dried and (3) a mixture of sand/commercial peat (85-90% organic matter)

Irrigation was carried out every three days always keeping the substrate at near field capacity. After three weeks, the growing plants undergo two water regimes until pods form:

- Control plants watered regularly (near field capacity)
- Severely stressed plants (10% field capacity).

The experiment was conducted in a randomized block factorial design with three replicates

To assess early flowering character, the number of floral clusters formed on the main stem of all plants was monitored daily at the same time for the first ten days.

The weight of pods per plant was determined at harvest after 60 days of water stress.

To demonstrate roots colonization by arbuscular mycorrhizal fungi (AMF), seedlings were removed from the pots, placed in a water basin for the removal of the substrate in order to not affect the root system. Washed roots were carefully fragmented into segments, clarified with 10% KOH (90°C, 40 min), then stained with 0.05% Trypan Blue (Phillips and Hayman, 1970). To observe the result obtained after the application of the protocol tested, roots segments were examined under an optical microscope.

RESULTS AND DISCUSSION

The results obtained indicate that the bean seems to express sensitivity to water stress action which is closely dependent on the culture substrate.

Water stress caused a drop in the number of flower clusters formed during the first ten days, both on the sandy substrate and on that composed of sandy-clay soil (Figure 1).

This fall is all the more important as the soil is rich in clay; on the sandy clay substrate this reduction reached 80% (S/T = 0.20), while on the sand, the number halved (51%) (S/T = 0.49). It should be noted that in absence of water constraint, the plants of the sandy clayey substrate retained the highest values of floral bouquets (6.5) (Figure1). On the peat substrate, water stress did not affect this parameter; the plants form the greatest number of floral clusters. This number even exceeded that of the control plants, an increase of 46% (S/T = 1.46) (Figure1).

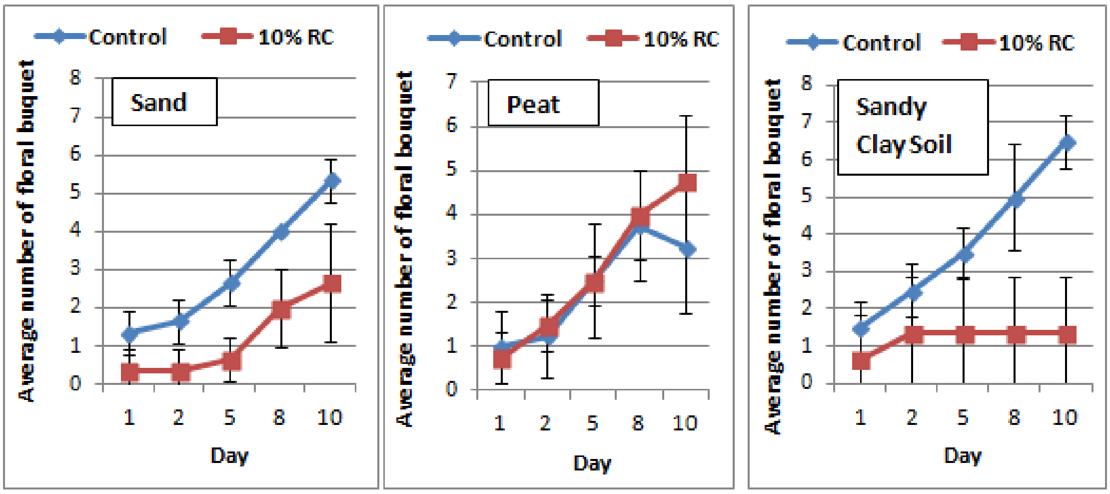

Figure1. Water stress effect on flower clusters formed number on the main stem of *Vicia faba* L. plants grown on different substrates

Results examination reveals variations in pod weight per plant under water stress (Figure 2 and 3). Under favorable conditions, plants in the clay-sandy substrate produce large pods (33.8g ±3.53) than those from peat (11.8g±4.94) and sand (7.97g±0.57). Under stressful conditions the plants were severely affected and failed to fruit (Figure 2 and 3). Those of sand experienced a decrease of 34% (S/T = 0.66), whereas on the peat, water stress had a positive effect by improving pod weight by 38% (S/T = 1.38) (Figure 2 and 3).

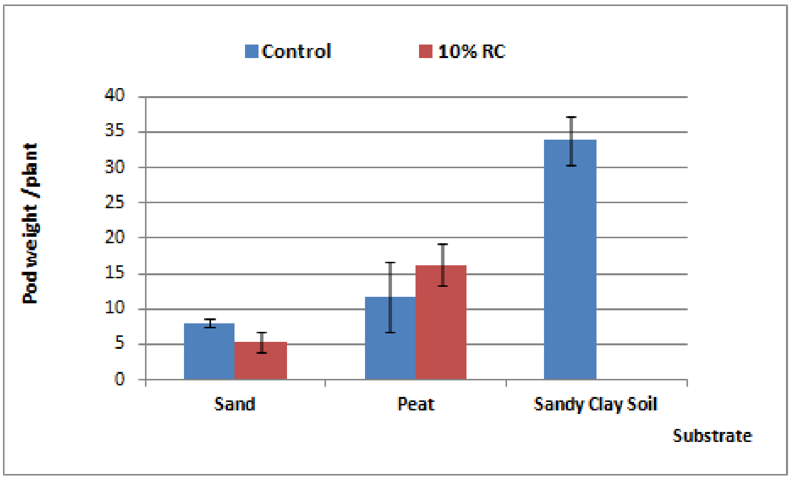

Figure 2. Water stress effect on pod weight per plant of *Vicia faba* L. grown on different substrates

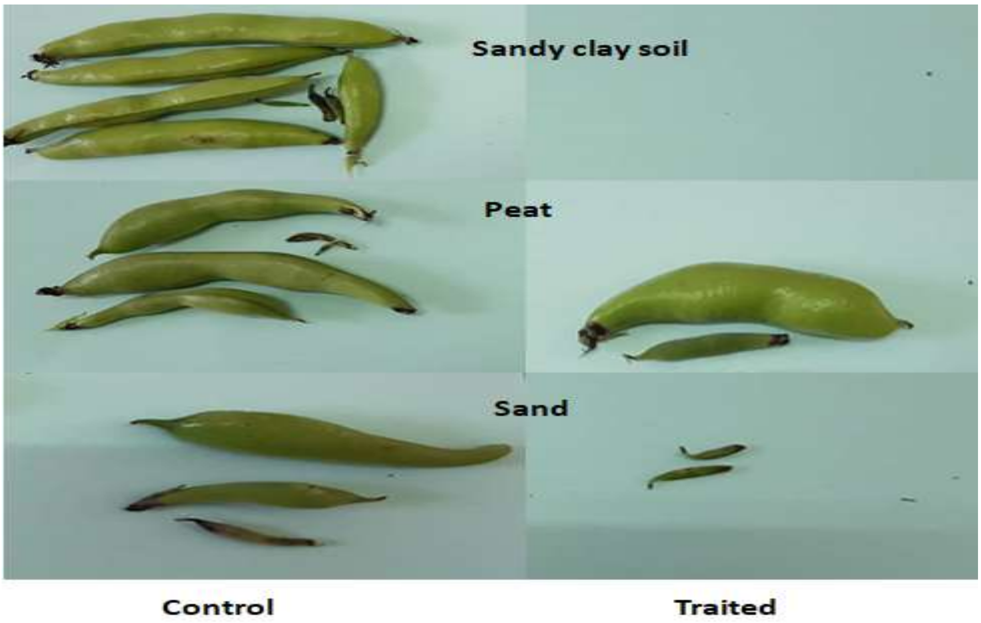

Figure 3. Water stress effect on pod yield of *Vicia faba* L. Plants grown on different substrates

Microscopic examination shows that root mycorrhization varies considerably with substrate properties (Figure 4). All plants (control and treated) in sandy clayey substrate were highly colonized by mycorrhizal fungi with typical structures (arbuscules, intraradical hyphae and vesicles) (Figure 4). In sandy substrate, only control plants were colonized. As expected, no colonization of roots was observed in peat soil (Figure 4).

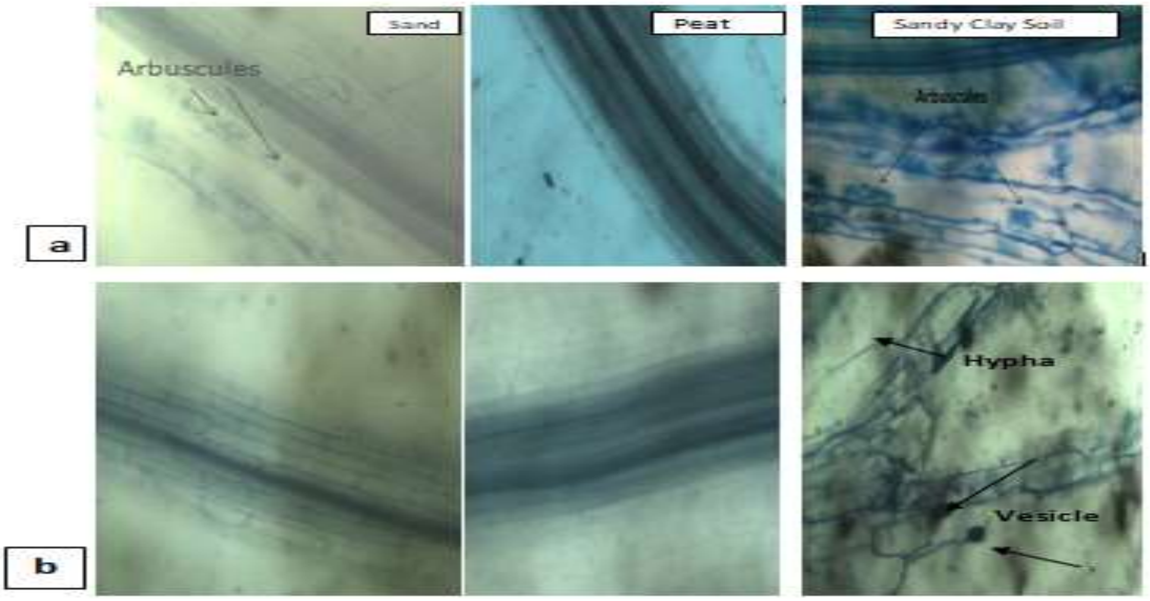

Figure 4. Water stress effect on mycorrhization of bean plants grown on different substrates (a: Control; b: Treated)

All the studies that have been carried out generally agree that the flowering phase is the most sensitive stage to to water stress (Duc, 1997, Khan et al., 2007; Alghamdi et al., 2015).

Drought has been shown to reduce flower size (Halpern et al., 2010), flowering speed (Koch, 2018), the flowers number per plant (Burkle and Runyon, 2016; Konfe et al., 2019) and it can cause closing flowers (Ruehr et al., 2019). Water limitation can reduce the capacity of plants to maintain turgor and transpiration, or to uptake nutrients, and can alter plant–microbial interactions in the soil, all of which can lead to decreased resources for leaves, flowers, fruits and seeds (Pinheiro and Chaves, 2011; Barber and Soper Gorden, 2014; Gallaghe and Campbell, 2017). According to Kambale Valimunzigha, (2006), water stress can act on growth regulators (auxins, gibberellins, cytokines, ethylene, abscisic acid) leading thus to physiological implications of various kinds such as flowers and buds abscision, dormancy buds and inhibition or flowering stimulation. Water stress can be influenced by other environmental factors that may act concurrently with water stress (Nielsen and Orcutt, 1996).

Soil structural porosity plays an important role in substrate-plant interactions, because it ensures water and air transfer as well as water storage used by plants (Chenot et al., 2017). Soil clayey fraction, with its very fine particles, is characterized by physicochemical properties which will give it a great capacity to trap water molecules and cation exchange (Yukselen and Kaya, 2008; Anaba et al., 2020). However, severe drying of the clay can cause changes in water availability at the roots. In fact, water retained in clay micro-aggregates would therefore not meet plants water requirements, which cannot exert sufficient suction to extract water from the pores (Tisdall and Oades, 1982).

Sandy soil has a weak structure, poor water retention and high permeability due to large pores presence (Bruand et al., 2005). It contains little organic matter and fine particles; thus its cation exchange capacity is low. In such substrate, water and nutrients can penetrate deep below the root zone and become unavailable to plants (Mahmoud Mohamed, 2011). However, it is interesting to note sand large pores provide a way for roots to anchor themselves in soil for the water and nutrients search necessary for their development during water stress (Whitmore and Whalley, 2009). Indeed it has been shown that roots length (not the mass) is greater in sandy soils than in clay soils (Hoad et al., 2001).

Organic matter also plays an important role in soil structure; water holding capacity and nutrient availability (Bot and Benites, 2005). Mineral deficiencies generally retard flower development and may even cause flower abortion (Kambale Valimunzigha, 2006). Fisher, (1969 in Turner, 2010) found that low levels of nitrogen in solution culture resulted in delayed opening of the flowers. While a delay in flowering as well as flowers abortion due to low levels of potassium in sand culture were reported by *Besford* and *Maw* (*1975*). Menary and Van Staden (1976 in Marschner and Marschner, 1995) revealed that *Phosphorus* deficiency also reduced the number of flower buds formed and delayed anthesis.
In our experiment, plants grown on peat produced highest yields under water stress, probably due to improved soil water properties as indicated by Chabbi and Lemaire, (2007) and Grosbellet, (2008) and also to fertilizer richness in nutrients, especially potassium (K) and phosphorus (P) which are flowering and fruiting essential elements (Konfe et al., 2019).

Water stress effects on yield components are similar to those on flowering. Sandy clayey substrate plants showed highest sensitivity to water stress reflected in fruiting absence compared to their controls which produced the highest yields and compared to other treatments. In sandy substrate, there was a decline by 34% in pod weight between the control and treated plants. These results confirm those of some authors including Halime et al., 2014; Harou et al., 2018 who mentioned higher yield decease by 60% in cowpea plants subjected to water stress during flowering and pod filling stages. According to Chiulele et al. (2011), Drought during flowering and pod filling stages has greatest impact as it directly affects yield.
Stress severity can be explained by a decrease in evapotranspirant surface due to leaves senescence causes of decreased photosynthetic rate metabolic activity (Dwivedi et al., 2008); grain filling process is then slowed down or even blocked (Araus et al., 2011; Radhouane et al., 2014). In tomato, it has been shown that water stress alters fruit water accumulation and impact cell division and expansion processes (Koch, 2018). In grapes, berries small size under water deficit conditions is associated with reduction in pericarp size (Ojeda et al., 2001).

The ability of plants to endure unfavorable conditions with preservation of active life and the ability to reproduce (often with a decrease in productivity) reflects Turner, 1997; Novitskii et al., 2011, Lan et al., 2019). Under our growing conditions, water stress did not affect plants grown under soil rich in organic matter. Plants have been shown to be resistant to water stress by producing larger pods than those of non-stressful plants. Plants good performance could be explained by water-retention property of organic matter, which reduces stress effect and thus promotes biomass, flowering and fruiting production. Thus, Bauer and Black, (1992) showed that organic matter tends to increase water retention, both at field capacity and at wilting point. In addition to being hydro-retentive, organic matter contains nutrients (N, C, and P) essential for plants proper development (Trumbore and Czimczik, 2008).Studies also show that nutrients availability around flowering is a critical factor for grain yield production (Tshiabukole, 2018).

Arbuscular mycorrhizal fungi (AMF) are important organisms for plants because they can enhance mineral uptake leading to increased production and resistance to environmental stresses (Barea et al. 1993; Van Der Heijden et al., 1998). Soil properties have been shown to affect mycorrhizal fungi (Johnson et al., 2010b), particularly in terms nutrient availability (Johnson et al., 1992a) and pH variations (Dumbrell et al., 2009; Bueno et al., 2017).

The results presented here demonstrate that there are differences in root colonization by arbuscular mycorrhizal fungus depending on substrate type. The peat allows better resistance to water stress in absence of any mycorrhization. This is perhaps due to high levels of phosphorus and nitrogen contained in organic matter reducing plants mycorrhization. According to Nowak (2004), mycorrhization increases plant growth under a low phosphorus diet; benefits that mycorrhizal fungi can offer are therefore lower when phosphorus used amounts are high.

On sand, water stress has negative effect on roots mycorrhization. The latter remained relatively low in control plants and did not elicit growth stimulating reaction which is mainly function of AM fungus ability to develop a large network of hyphae in rhizosphere (Tinker, 1978). This network can only propagate when root colonization by the AM fungus is sufficient to provide sugars necessary for fungus development and therefore for symbiosis proper functioning (Ho and Trape, 1973; Thomason et al., 1990; Juniper and Abbott, 1993).

Whatever the imposed water regime, all plants roots in sandy clay substrate exhibit significant colonization characterized by abundant presence of different mycorrhizal structures. However, this mycorrhization was found to be greater under favorable conditions than under unfavorable conditions resulting in good plant growth, good flowering and best yield. In treated plants, extensive roots mycorrhization did not elicit growth stimulating reaction. This could result in clay drying out and development of high suction pressure for the water which can oppose that of plants roots.

CONCLUSIONS

Drought is one of the abiotic factors that have the greatest impact on agricultural production. It occurs when soil moisture decreases to an amount that negatively affects the yield and profitability of agricultural production. The capacity of soil to retain water mostly depends on specific soil parameters, such as soil texture, soil structure, and soil organic matter. Sufficient soil organic matter is fundamentally important to availability of water and nutrients, carbon sequestration and crop production. Organic matter contribution can lead to increase of water retention in sandy soils, and its decrease in fine-textured soils.

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THE EFFECTS OF BEE FLIGHT AND POLLINATING DISTANCE ON SEED PRODUCTION AND QUALITY IN SEED SUNFLOWER PRODUCTION

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ABSTRACT

Cross pollination plays a significant role in the sunflower seed production. The population of natural pollinator species has dramatically decreased as a result of intense use of insecticide in farming areas. Therefore, the use of honeybee for pollination has become important issue for sunflower seed production.

This study was conducted in the farmer fields that were cultivated sunflower of Akcapinar village of Ezine district of Canakkale in 2020. To evaluate the effect of honeybee flight distance on the seed set, one hive per decare were placed in the near of four fields while another field was used as control. The planting of sunflowers was made as 10 rows of male-sterile, and 2 rows of restorer lines. The inter and intra row distance spaces were adjusted as 70 cm and 20 cm, respectively. In the study that has been conducted in farmer fields, three repetitive samples were harvested from 1st, 3rd, 5th, 7th and 9th rows that were after restorer line. These samples were shelled and seed set and the seed features were investigated in the labs of the Field Crops Department of Faculty of Agriculture of Canakkale On Sekiz Mart University.

Flight distances of honeybees did not influence on the seed set in the study. However, the distance to the restorer line has significantly affected the seed yield. The seed yield was increased in male-sterile plants which has been close to the restorer line. It was concluded that decreasing the number of rows of male-sterile line is beneficial for the sunflower seed production.

INTRODUCTION

Turkey has become a pilot zone in the production of sunflower seeds. Sunflower seeds are produced in very large areas in Çanakkale, Bergama, Karacabey, Konya and Afyon regions of Turkey.

In the production of sunflower seeds, male-sterility system is used, and male-sterile main lines and restorer lines are grew in seed production fields. Since the wind is not effective in the pollination, bee and insect activity is always needed for hybrid seed production in sunflower.

Due to the intensive use of pharmaceuticals in agricultural areas, natural pollinator species are constantly decreasing at both the species and population level. For this reason, honeybees are often used as pollinators in the production of sunflower seeds.

Honey bees use both pollen and nectar as their food source. Therefore, the pollen and nectar yield of the main and restorer lines is important in the production of sunflower seeds. On the other hand, for cross-pollination, the honeybees must visit both the male-sterile and the restorer lines. Male-sterile lines has usually single header while restorer lines are multi-heads.

While the pollen yield of the restorer lines is high, nectar yields are low because they have small and multi-headers. Male sterile lines have higher nectar yield compared to restorer lines.

For high seed yield, honeybees must visit both lines on each flight. Therefore, the pollen and nectar preferences of honey bees are effective in the decontamination and seed attitude of their behavior during the search for food.
In this study, sunflower seed production examined the effects of the proximity of the bushings to the field and the distance of the male infertile lines to the restorer line on seed yield.

MATERIAL AND METHOD

In the study, the production of sunflower seed production of a private seed company has been used as material. In the field with approximately 1000 decare, five different farmer fields have been selected, taking into account the conditions of uniformity, maintenance and production. The farmer's fields were selected from areas where a private seed company produced contracted sunflower seeds in Akçapınar village of Ezine district in Çanakkale. In the field selection, the distance between the fields was kept approximately 1 km and above, taking into account the distance of the bee flight.
In the production of sunflower seeds, all fields are watered with drip irrigation. Conventional breeding systems have been implemented. In the crop, the intra-row distance is 70 cm and the inter-row distance is 25 cm. 10 rows of male barren, 2 rows of restorer lines planted.
The shells are located 5 meters away from the fields and on one side. The headers selected close to the casings are selected from areas where the beehive flight distance is 30 m and headers selected far away are 150-200 m away. Each test unit consists of 5 plates. The field where no seed is produced without a shell is selected at a distance of 700 m from the casings. The activity of other pollinators was observed here, but more bees were visited.
The harvesting headers were made when they were dry. Table samples were taken from row 1, 3, 5, 7, and row 9 from row of the restorer lines in three replications.
After shelling, the moisture rate in the seed has been reduced to 8% and the yields are calculated accordingly. The blending and seeding characteristics were examined in Çanakkale Onsekiz Mart University Faculty of Agriculture Field Crops Department laboratories.
The data obtained in the study were analyzed for variance based on the split-split plot design. The main plots have created production fields, subplotss, distance to the hives, and sub-sub plotss have created a distance to the restorers and even to the distance. The analysis of the data uses the SAS statistical package program.

RESEARCH AND DISCUSSION

Header diameter

Significant differences have been found between farmer’s fields in the direction of header diameter (Table 1). This is due to the difference between the produciton techniques of the farmers.

Table 1. Variance analysis results for table diameter, full seed weight per header, empty seed weight per header, and seed hold rate data.

		MEANS OF SQUARES			
Variation Source	S.D.	Table diameter	Seed retention rate	seed yield	1000 seed weight
Recurrence	2	0,02	8,86	517,40	22,47
Field (F)	4	17,14*	827,70**	7170,80**	415,30**
Mistake 1	8	4,10	29,72	307,3	48,25
Distance to the Bee (DB)	1	13,66	420,95**	12745,90**	7,86
F*DB	3	4,89	2055,11**	29716,10**	179,07
Mistake 2	8	4,52	12,82	486,70	58,14
Restorer Distance (RD)	4	2,15	860,47**	15418,60**	12,99*
F*RD	16	3,06	65,65	1050,20**	78,92*
DB*RD	4	2,68	189,14**	2440,80**	41,53
F*DB*RD	12	3,11	82,44	1213,40**	26,64
Mistake 3	72	2,23	44,08	355,60	48,72
CV (%)		14,92	66,96	14,70	7,70

In the study, the average of the head diameter varied from 14,12 to 15,98 cm (Table 2). The highest head diameter values are obtained from Field 3 and the lowest values from Field 2. There was no significant difference between other treatments and their interactions for the head diameter.

Table2. Header diameter averages and multiple comparison results.

Farmer's Field	Distance to Hive	RESTORER EVEN DISTANCE 1	3	5	7	9	Average
	Close	15,17	15,00	14,83	15,50	15,33	15,17
Field 1	Far	14,67	12,83	13,17	14,33	13,00	13,60
Average		**14,92**	**13,92**	**14,00**	**14,92**	**14,17**	14,36
	Close	15,33	15,33	16,83	16,33	16,00	15,97
Field 2	Far	16,50	16,17	15,17	15,83	16,33	15,98
Average		**15,92**	**15,75**	**16,00**	**16,08**	**16,17**	15,98
	Close	16,00	14,33	14,33	10,17	15,83	14,13
Field 3	Far	13,75	13,83	14,50	14,17	14,33	14,12
Average		**14,88**	**14,08**	**14,42**	**12,17**	**15,08**	14,13
	Close	15,83	16,25	15,67	15,25	14,17	15,43
Field 4	Far	14,75	14,67	14,00	14,75	13,25	14,25
Average		**15,29**	**15,46**	**14,83**	**15,00**	**13,71**	14,36
Field 5	without hive	**16,33**	**14,83**	**15,17**	**15,17**	**16,50**	15,60
Overall Average		**15,37**	**14,81**	**14,85**	**14,61**	**14,97**	14,92

Seed Retention Rate

Seed retention rate (%) is determined by discarding the empty grains after harvested headers have been threshed. The rate of seed retention has been significantly affected by the double interaction of these factors, with the farmers's fields, bee flight distance and even distance to the restorer.

The seed retention percentage is up to 80,85%, and in some applications it has dropped to 44,27. In all fields other than field 2, more seed retention rates were determined in the parcels close to the casings. On the other hand, as the restorer drifted away from the line, there was a significant decrease in seed retention. In the research, the restorer lines are the furthest of male infertile lines to the lines, ranked 5. from row 7, the other restorer line is approaching.

Table 3. Seed hold rate averages (%) and multiple comparison results.

Farmer's Field	Distance to Hive	RESTORER EVEN DISTANCE					Average
		1	3	5	7	9	
	Close	73,63	74,91	65,89	73,83	79,49	73,55
Field 1	Far	54,96	44,27	46,15	46,49	67,32	51,84
Average		**63,06**	**64,29**	**59,59**	**56,02**	**60,16**	**73,40**
	Close	71,05	64,52	46,34	63,66	61,21	61,36
Field 2	Far	83,89	79,34	73,45	81,01	79,23	79,38
Average		**80,85**	**77,47**	**71,93**	**59,89**	**72,34**	**70,22**
	Close	76,68	62,45	47,06	67,19	73,00	65,28
Field 3	Far	63,65	56,44	54,84	61,22	46,92	56,62
Average		**66,11**	**70,16**	**59,45**	**50,95**	**64,21**	**59,96**
	Close	79,71	78,23	59,10	75,92	81,68	74,93
Field 4	Far	78,24	73,53	68,56	69,41	71,71	72,29
Average		**78,65**	**78,97**	**75,88**	**63,83**	**72,67**	**76,70**
Field 5	without hive	73,63	74,91	65,89	73,83	79,49	73,55
Overall Average		**72,16**	54,96	44,27	46,15	46,49	67,32

Honey bees collect both nectar and pollen to feed. However, pollen collection is more effective in cross-pollination. When the nectar current starts in the male barren lines, visits to the restorer lines are reduced. So the cross, the same flight, both the main line and the restorer, are less likely to visit. As a result, as the restorer moves away from the line, the possibility of cross visits and seed retention decreases.

Seed yield

The efficiency of seed has been significantly affected by all the factors tested in the study, two-way and three-way interactions (Table 1).

Table 4. Seeds yield averages (kg/da) and formed groups.

Farmer's Field	Distance to Hive	RESTORER EVEN DISTANCE					Average
		1	3	5	7	9	
	Close	158,19	163,92	119,47	159,05	220,74	164,27
Field 1	Far	88,37	50,62	57,69	59,91	118,49	75,02
Average		**123,28**	**107,27**	**88,58**	**109,48**	**169,62**	119,64
	Close	142,58	100,45	73,17	111,26	108,00	107,09
Field 2	Far	202,50	170,06	138,41	169,50	168,59	169,81
Average		**172,54**	**135,26**	**105,79**	**140,38**	**138,29**	138,45
	Close	180,29	101,38	63,29	114,34	163,33	124,52
Field 3	Far	91,65	85,50	83,25	110,53	72,82	88,75
Average		**135,97**	**93,44**	**73,27**	**112,43**	**118,07**	106,64
	Close	165,60	170,79	88,18	160,37	179,55	152,90
Field 4	Far	169,28	137,50	109,61	115,69	131,70	132,75
Average		**167,44**	**154,14**	**98,89**	**138,03**	**155,63**	142,83
Field 5	without hive	168,75	131,93	100,83	132,34	177,75	142,32
Overall Average		**151,91**	**123,57**	**92,65**	**125,89**	**149,00**	128,60

The lowest seed yield in the study was obtained in the field 1, in the row of 3 male line (50,62 kg/da), in the highest seed yield, near bees in the same field, in the second row after the restorer line (220,74 kg/da). In all fields except field 2, seed yields are recorded lower than those that are far from the bees (control). As the flight distance grew longer, the cross visit was and seed yield was significantly reduced. On the other hand, as the restorer distanced from the line, seed yields were reduced. Higher seed yields have been recorded as a result of increased cross-visit in male sterile rows close to casings, but also close to the restorer lines. Similarly, in the field that is far from all hives, it was observed that the cross-visit had fallen and that the seed yield had decreased as the restorer moved away from the lines.

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INVESTİGATİON OF THE EFFECT OF DİFFERENT HARVEST TİMES ON SOME YİELD CHARACTERİSTİCS OF TURKİSH OREGANO (*Origanum onites* L.)

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ABSTRACT

This research was conducted to determine the effects of different harvest times (before-flowering, beginning of flowering, full flowering and end of flowering) on some yield characteristics of Turkish oregano (*Origanum onites* L.) in 2018-2019. The field experiment was carried out in randomized complete block design with three replications in Uşak province of Turkey. According to this study results, the plant height, fresh herb yield, dry herb yield and dry leaf yield varied between 36.18-42.17 cm, 10.95-14.44 t ha^{-1}, 3.55-5.56 t ha^{-1} and 1.68-2.24 t ha^{-1}, respectively. Considering the dry leaf yields obtained from this study, it can be suggested that the plants should be harvested during the full flowering period when Turkish oregano is cultivated in Uşak ecological conditions of Turkey for use as a spice.

Keywords: Turkish oregano (*Origanum onites* L.), harvest period, ontogenetic variability, yield

INTRODUCTION

The genus Origanum, which belongs to the Labiatae/Lamiaceae family, is represented in the world with 38 species, 6 subspecies and 17 hybrids and 22 species of which are naturally distributed in Turkey. One of the species belonging to this genus is *Origanum onites* L. (Syn. *Origanum smyrnaeum* L., Majorana onites (L.) Benth.; Lamiaceae), known as İzmir oregano. This species, known as "Turkish Oregano" in world trade, naturally distributed in the Mediterranean coastal regions of Greece and Turkey (Kacar et al., 2006; Toncer et al., 2009; Bozdemir, 2019).

Turkish Oregano (*Origanum onites* L.) is widely used as a spice and herbal tea in Turkey. Not only the essential oil of Turkish Oregano, but also its hydrosols are used in traditional medicine for the treatment of gastrointestinal disorders and diabetes. This species is one of the most cultivated oregano species and collected from nature in the Aegean region of Turkey. In addition, Turkey is the biggest oregano supplier all around the world (Yaldiz et al., 2005; Gungor et al., 2005).

Oregano essential oil has antibacterial, antispasmodic, antiseptic, antifungal, anticancer, antimutagenic, antidiabetic, antiviral, anti-inflammatory and antiprotozoal properties and is evaluated in traditional medicine. Desides these properties of essential oils, oregano also is used as spice and herbal tea, as well as an important raw material for the cosmetics and pharmaceutical industry (Baydar and Erdal, 2004; Kacar et al., 2006; Raut and Karuppayil, 2014; Ozdemir et al., 2017).

It is widely accepted that the yield and quality of cultivated plants are affected by many other agricultural practices, as well as the genetic potential of the plant and climate and soil factors. In many previous studies, it has been that the harvest time of medicinal and aromatic plants significantly affects yield and quality (Zawislak, 2011; Ozyazici et al., 2019, Sonmez, 2019). Uşak province is one of the leading provinces in oregano production of Aegean region, Turkey. In this respect, it is important to determine the most suitable harvest time for high yield in Uşak oregano cultivation. On the other hand, in the literature review, no study was found on this subject in the province of Uşak.

The objective of the present study was to determine the effect of harvest time on some yield characteristics of Turkish Oregano grown in Uşak ecological conditions of Turkey.

MATERIAL AND METHOD

The field experiment was carried out under the ecological conditions of Uşak province located in the Aegean Region of Turkey during 2019 growing seasons. The climate of the Uşak province was characterized by semi-arid climate. The climate is warm and temperate in Uşak and the winter months are much rainier than the summer months in province. Average temperature and total precipitation values are presented in Table 1. The highest temperature in the area was recorded as 24.4 C° in August in 2019. The total annual precipitation was 510.6 and 414.3 mm in long years and 2019, respectively (Table 1). Soil characteristics of experimental field were clay-loam with 7.87 pH, 2.34 kg ha^{-1} P_2O_5, 500 kg ha^{-1} K_2O, 14.3% lime, 1.16% organic matter.

Table 1. Some meteorological parameters of the experiment area

Months	Total Precipitation (mm)		Mean Temperature (°C)	
	LYA*	2019	LYA*	2019
January	58.4	87.9	2.6	1.6
February	58.5	17.3	3.5	5.4
March	51.1	20.8	6.4	7.8
April	57.7	40.1	11.0	10.5
May	43.1	36.9	15.7	16.7
June	24.1	37.3	20.3	20.9
July	15.5	8.9	24.0	22.8
August	9.5	0.4	24.1	24.4
September	17.2	22.1	19.3	19.4
October	44.9	6.4	13.8	16.6
November	56.3	47.9	8.1	11.6
December	74.3	88.3	4.1	5.5
Total	510.6	414.3	-	-
Mean	-		12.7	13.6

* Long Year Average

The experiment was established in randomized complete block design with three replications in 2018. Each plot was arranged as six rows at a 0.2 x 0.4 m spacing. *Origanum onites* L. cv. "Oğuz-2012" was used as plant material. The seeds were sown into a mixture of sand, forest soil and peat (1:1:1) on the first week of March, 2018. When the oregano seedlings reached 10-15 cm height, they were transferred to the experimental plots on the first week of May, 2018. Before planting seedlings on the plots, 100 kg ha^{-1} N and 50 kg ha^{-1} P_2O_5 fertilizer were applied. The same dose of nitrogen fertilizer was applied in 2019 when the plants started to grow in the spring. During the growing period the plants were irrigated in 2018, the establishment year, but no irrigated in 2019. Weed control was done by hand when required.

Although the plants was not harvested in 2018, they were harvested in 2019. In the study, the first and last rows of each plot and 0.5 m from both ends of the rows were excluded as border effect. Harvest dates were at before-flowering (on 10 of June), beginning of flowering (on 24 of June), full flowering (on 14 of July) and end of flowering (on 4 of August) stage of plants in 2019. The plants were cut at a height of about 10 cm from the soil surface and then weighed to determine fresh herb yield. Dry herb yield was determined by drying fresh herb samples in a shaded and airy place for one week. Dry leaf yield was determined after removing stems from leaves of dry herb samples.

The data obtained from the study were subjected to analysis of variance using the SPSS statistical program, and the differences among mean values were compared via the Tukey test ($p \leq 0.01$).

RESULTS AND DISCUSSION

The results of the study showed that the different harvest times had a significant effect on the on plant height, fresh herb yield, dry herb yield and dry leaf yield of *Origanum onites* (Table 2).

The plant height of *Origanum onites* varied between 36.18-42.67 cm depending on the developmental phase. The lowest plant height was obtained from plants harvested the before-flowering, while the highest plant height was obtained from plants harvested in the full flowering. However, plant height values measured in full flowering and end of flowering periods were almost the same (42.67 and 42.57 cm, respectively), and both harvest periods were in the same statistical group in terms of plant height (Table 2). This situation shows that the plant height of oregano increases significantly until the full flowering phase, and there is no significant different after this phase. Kacar et al. (2006), Ozyazici and Kevseroglu (2019) and Karaca Oner and Sonkaya (2020), were determined the plant height of oregano (*Origanum onites*) between 23.5-43.1 cm, 25.00-49.47 cm and 23.46-45.07 cm, respectively. The change of these results may be due to the different genotypes used in the studies and different ecological conditions of study regions such as rainfall, temperature etc.

Effect of different harvest periods on fresh herb yield of *Origanum onites* were found to be significant ($p \leq 0.01$). When the fresh herb yield values were examined, it was observed that the lowest value (10.95 t ha^{-1}) was recorded in the before flowering phase of plants and the highest value (14.44 t ha^{-1}) was recorded in the full flowering phase of plants. The mean value of fresh herb yield was determined as 12.56 t ha^{-1} for different harvest periyods (Table 2). The fresh herb yield values for *Origanum onites* had variations in many researches. The mean values of fresh herb yields were reported as 3.30-30.27 t ha^{-1} by Ozyazici and Kevseroglu (2019), 10.54-27.56 t ha^{-1} by Sonmez (2019) and 2.22-7.14 t ha^{-1} by Karaca Oner and Sonkaya (2020), under ecological conditions of Turkey's Samsun, İzmir and Ordu provinces, respectively. The results of this study for fresh herbage yield were lesser than those obtained by Ozyazici and Kevseroglu (2019) and Sonmez (2019) while being high than that of Karaca Oner and Sonkaya (2020).

The mean values obtained for dry herb yield in different harvest times were shown in Table 2. Similarly to fresh herb yield, the harvest date also was significant for dry herb yield ($p \leq 0.01$). The highest dry herb yield (5.56 t ha^{-1}) was obtained from the last harvest (end of flowering). The lowest value (3.55 t ha^{-1}) was determined at the first harvest (before flowering). The average value was 4.48 t ha-1 (Table 2). After drying, the fresh herb weight of oregano decreased by about 59-68%. The mean of dry herb yield was found to be 4.33-12.34 tons per hectare in a study conducted on *Origanum onites* in İzmir location (Sonmez, 2019). On the other hand, Baydar (2002) reported that the mean dry herb yield of *Origanum onites* was 2.30 t ha^{-1} under Isparta ecological conditions. It is thought that the difference in these results is due to the changes in the ecological conditions in which the studies were conducted as well as the cultivation practices such as fertilization and irrigation.

The dry leaf yield of *Origanum onites* was significantly affected by the changing harvest periods depending on the plant development stage, similarly to the fresh and dry herb yields (Table 2). The dry leaf yield values formed statistically two different groups. While the harvest values of the before flowering and beginning of flowering were in the first group, the other two harvest values (full flowering and end of flowering) were in the second group. The highest value of dry leaf yield (2.24 t ha^{-1}) was obtained from the last harvest periyod. However, there was no statistically significant difference between dry leaf yields of plants harvested at the full flowering and end of flowering stage. On the other hand, the lowest value (1.68 t ha^{-1}) was determined at the first harvest (Table 2). Contrary to this study conducted under dry conditions, it was stated that the dry leaf yield of *Origanum onites* was between 1.09-7.05 t $ha\text{-}^{1}$ in some previous studies conducted under irrigated conditions (Kacar et. al., 2006; Kizil et. al., 2009; Sonmez, 2019). İn parallel with this study, in previous studies on oregano (*Origanum vulgare* and *Origanum onites*), Karik (2007) and Ozyazici and Kevseroglu (2019) reported that they obtained the highest dry leaf yield during full flowering.

Table 2. The effect of different harvest time on studied characteristics of *Origanum onites*

Harvest Time	Plant Height (cm)	Fresh Herb Yield (t ha^{-1})	Dry Herb Yield (t ha^{-1})	Dry Leaf Yield (t ha^{-1})
Before Flowering	36.18 c	10.95 b	3.55 c	1.68 b
Beginning of	39.12 b	11.45 b	3.69 c	1.79 b
Full Flowering	42.67 a	14.44 a	5.10 b	2.23 a
End of Flowering	42.57 a	13.40 a	5.56 a	2.24 a
Mean	40.14	12.56	4.48	1.99
Harvest Time	**	**	**	**
CV (%)	7.13	12.63	20.61	13.56

**: significant at the $p \leq 0.01$ probability level

Means within columns followed by the same letter are not significantly different according to Tukey test

CONCLUSIONS

The results of this study revealed that that the different harvest times had a significant effect on the on plant height, fresh herb yield, dry herb yield and dry leaf yield of *Origanum onites*. Dry leaf yield is an important parameter in oregano cultivated to be consumed as a spice. Unlike many previous studies, according to the results of this study conducted in dry conditions (not irrigated), the dry leaf yield increased as the harvest date was delayed, but this increase did not reveal significantly in the period after the full flowering period. Considering the dry leaf yields obtained from this study, it can be suggested that Turkish oregano grown in Uşak ecological conditions as a spice should be harvested during the full flowering period.

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ORGANIC MATTER AS AN EXTERNAL FACTOR IN RESISTANCE TO WATER STRESS IN REINA MORA VARIETY OF BEAN (*Vicia faba* L.)

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ABSTRACT

The present study objectives are to analyze physiological responses to Reina Mora variety water stress of bean, introduced in Algeria, and assess growing substrate importance as an external factor promoting the resistance of the beans to water stress.

Experiments were carried out in a controlled greenhouse at Oran1 University, Algeria. After one month, young seedlings transplanted into pots containing three types well-watered substrates (Sand, Peat and sandy-clay soil) were subjected to moderate water stress at 40% and severe at 10% of the retention capacity for 60 days.

The criteria for resistance to water stress physiological evaluation concerned qualitative morphophysiological parameters (leaf orientation, leaf chlorosis and presence of leaf necrosis) and quantitative morpho-physiological parameters and their sensitivity index such as stem and root length, aerial and root dry weight, number and weight of pod/plant).

The results obtained show that severe water stress had a serious impact on plants cultivated in sandy-clayey soil growth and productivity, probable cause of clay drying out and development high suction pressure for water which can oppose that of roots.

Grown on peat, plants are more predisposed to protect themselves from water stress by lengthening their roots in search water than by changing their leaves orientation to reduce transpiration and maintain a high water potential which is reinforced by organic matter presence very hydrophilic and rich in mineral elements easily assimilated by roots. Water stress negatively affects plant growth and yield on sand. The leaves turn yellow and show necrosis, a response probably due to sandy soil particles which are spaced, dry, poor in nutritive substances and very draining.

Keywords: Water stress, substrate, bean, growth, yield, sensitivity index, resistance

INTRODUCTION

Faba bean (*Vicia faba* L.) is an important legume crop worldwide, ranking as the fourth most important grain legume after dry beans, dry peas and chickpeas (Lopez-Bellido et al., 2005). Faba bean (Vicia faba L.) is cultivated in many areas, mainly located in Mediterranean basin, western and eastern Europe, China, India, South America, Australia, its surface area accounting for: 240,000 ha worldwide (Faostat, 2014). Moreover, *Vicia faba* seeds represent a remarkable energy source, providing 44 Kcal 100 g-1 of fresh seeds, and they are rich in fiber, lysine rich proteins, mineral nutrients, vitamins and antioxidants (Crépon et al., 2010; Amalfitano at al. 2018). Notably, the high fiber concentration is essential for intestinal functions regulation as well as for blood glucose and cholesterol control (Macarulla et al., 2001). Water deficit in faba bean causes a significant reduction in internode length, number and size of leaves, shoot dry matter, number of pods per plant and seed production (Mohamad Zabawi and Dennett, 2010). Water can constrains plant growth also indirectly by influencing how plants subjected to water stress respond to nutrients (Song et al., 2010). Plant growth decline significantly under water stress due to the nutrient deficit that accompanies water shortage (Chapin, 1991). Fertilization provides a practical way to stimulate plant growth: it increases nutrient availability, enhances stress tolerance and encourages more efficient use of limited resources in infertile or dry environments (Wu et al., 2008). Organic manure has multiple benefits due to the balanced supply of nutrients, including micronutrients, increased soil nutrient availability due to increased soil microbial activity, the decomposition of harmful elements, soil structure improvements and root development, and increased soil water availability (Han et al., 2016). Pinitpaitoon et al. (2011) report that cultivation practices with organic manure improve soil properties, balance soil organic matter, *N* and *P* retention and availability (Gong et al., 2011; Meng et al., 2013), and finally, increase soil water use efficiency in dry-land farming (Wang et al , 2018).

To this end, the objective of this study was to analyze plants sensitivity of broad bean variety (Reina mora) on different substrates and at different soil water availability levels and to assess their effects on morpho -physiological characters as well as their productivity.

MATERIAL AND METHOD

Research was carried out on faba bean variety (*Vicia faba* L.) Reina mora grown in Spain and marketed in Algeria, in a greenhouse at Oran 1 university (Algeria). The seeds were disinfected by soaking them in a 5% sodium hypochlorite solution for 5 min and rinsed 3 times with sterile distilled water and sown in washed sand. On tenth day, the seedlings were transplanted individually in plastic pots (18 cm high and 14, 5 cm in diameter) and grown for 1 month, with regular watering before the treatment.

A 3 × 3 factorial experiment with a randomized complete block design, comprising three substrates types and three levels of available soil water, was performed in three replicates. The evaluated treatments included three substrates (1-sandy substrate, 2- sandy-peat mix substrate and 3- sandy clayey soil) and three water levels available in the soil (10, 40 and 100% water completely available).

At the end of the experiment at 100 days after sowing, qualitative morphophysiological parameters (leaf orientation, leaf chlorosis and presence of leaf necrosis) and quantitative morpho-physiological parameters and their sensitivity index such as stem and root length, aerial and root dry weight, number and weight of pod/plant) were measured.

Plant height and root length were measured with meter rod. Shoot and roots were separated and subjected to drying at 80 ºC to take their dry mass. Pods/plant number is evaluated by counting after harvest. Pods/plant was measured using weight balance. The quantitative physiological parameters sensitivity degree to water stress was calculated using the equation described previously (Zombre et al, 1994), as follows:

$$S\% = \frac{\text{(parameter measured on Control- parameter measured on Severe or Moderate)}}{\text{(parameter measured on Control)}} \times 100$$

The average sensitivity index is calculated using the method below:

$$S\% = \frac{(S\%_{Moderate} + S\%_{severe})}{2}$$

The higher it is more sensitive parameter and less resistant it is to water stress (Diallo, 2009).

RESULTS AND DISCUSSION

Qualitative morpho-physiological parameter

Plants do not have same ability to resist water stress. On the sand, we notice a change in leaves direction (vertical position) and chlorosis presence whose size varies with stress intensity. The leaves are moderately necrotic regardless treatment applied (Table1, Figure 1).

Table1: Qualitative morpho-physiological parameters (change leaf orientation, presence of leaf chlorosis and necrosis) for resistance to water stress of bean plants grown in different substrates

Substrate	**Sand**			**(Sandy-Peat) mix**			**Sandy Clay Soil**		
Retention Capacity (RC)	Control	40%	10%	Control	40%	10%	Control	40%	10%
Change leaves orientation	+	++	++	0	++	+++	+	++	0
Chlorosis	++	++	+++	0	0	+	0	0	+++
Necrosis	+	++	++	0	0	0	0	++	+++

0 = no effect; + = Low effect; ++ = medium effect; +++ = important effect.

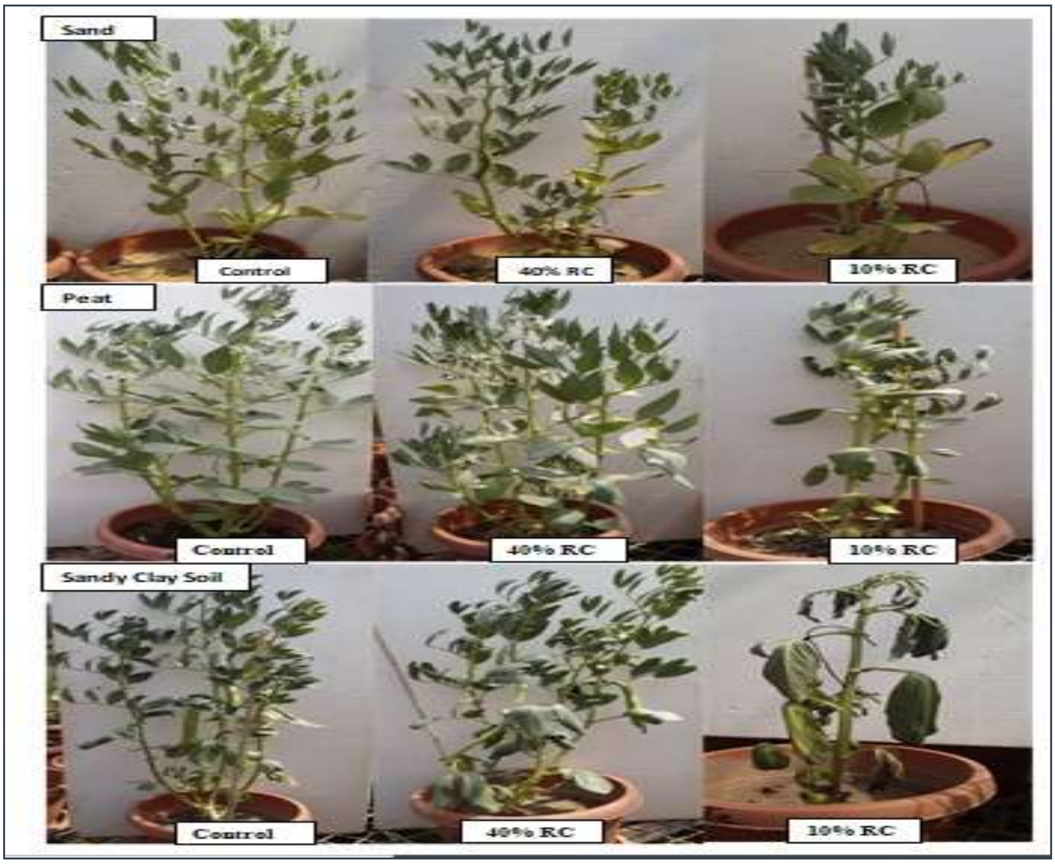

Figure 1: *Vicia faba* L. plants behavior under water stress and different substrates

On the (sandy-peat) mix substrate, stressed plants remained relatively green and little necrotic compared to their respective unstressed controls, which indicates their good resistance to drought probably due to their leaves orientation change to reduce transpiration and maintain high water potential reinforced by organic matter presence very hydrophilic and rich in mineral elements easily assimilated by roots (Table1, Figure 1).

On the sandy clayey substrate, plants leaves exposed to moderate stress (40% RC) have a vertical distribution to reduce transpiration. The green color loss (chlorosis) and necrosis appearance are seen to a moderate degree. Under severe stress (10% RC), plants show no defense reaction, leaves remain exposed to solar radiation, and maintain planophilic distribution which increases transpiration and facilitates water loss leading to plants withering (Table1, Figure 1).

- **Quantitative morpho-physiological parameters**

Plant Height

Figure (2a) explicates the variation in plants height of faba bean related to substrate type and soil moisture. Figure evidenced decrease in plants length with decreasing moisture content at 10% RC. Tallest plants (56.66 cm) were found on well-watered sandy clay substrate (100% RC). A decrease in soil moisture (10% RC) leads to a strong parameter decrease in this parameter which have registered only 26.57 cm against 33 and 40.6 cm recorded respectively on sandy substrate and peat substrate.

Root length

Effect of different substrates at different moisture levels on root length of plant is elucidated in figure (2b). It is illustrated that peat- rich substrate plants treated at 10% RC yielded long roots (31.25 cm) compared to their controls (29.5 cm) and compared to the other two substrates. In the sandy clay substrate, severe water stress (10% CR) strongly affects root proliferation which goes from 39.15 cm in the control to 24.33 cm under treated.

On sand, root length was not affected by water stress. Almost similar values were observed when plants grown with high-watering were compared to plants grown low-watering (Figure 2b)

Aerial and root dry weight

Figure (2c) elucidates the changes in plants aerial dry weight on different substrates and at different soil water content. In each treatment, all plants show a significant reduction in their aerial dry matter where water was maintained at 10% RC. However, this reduction was suggestively greater on peat substrate (6.67 g) than on the two other substrates sandy (3.87g) and sandy-clayey (3.28g). At 40% RC, the variations in dry weight in each treatment are not significant compared to those of control. Peat and sandy clayey Treatments exibited higher values than those under sand. This parameter was also higher on sandy-clayey substrate under well-watered conditions (Figure 2c).

It can be demonstrated from Figure (2d) that in soil with 10% available water, all plants root dry weight on all substrates was significantly decreased. Mean dry weight value of plants cultivated in soil with 100% available water was 5.42; 4.95 and 4.26g in sandy, peat-sandy and sandy clayey substrates; whereas that of plants cultivated in soil with 10% available water were 3.85; 2.66 and 1.75g respectively.

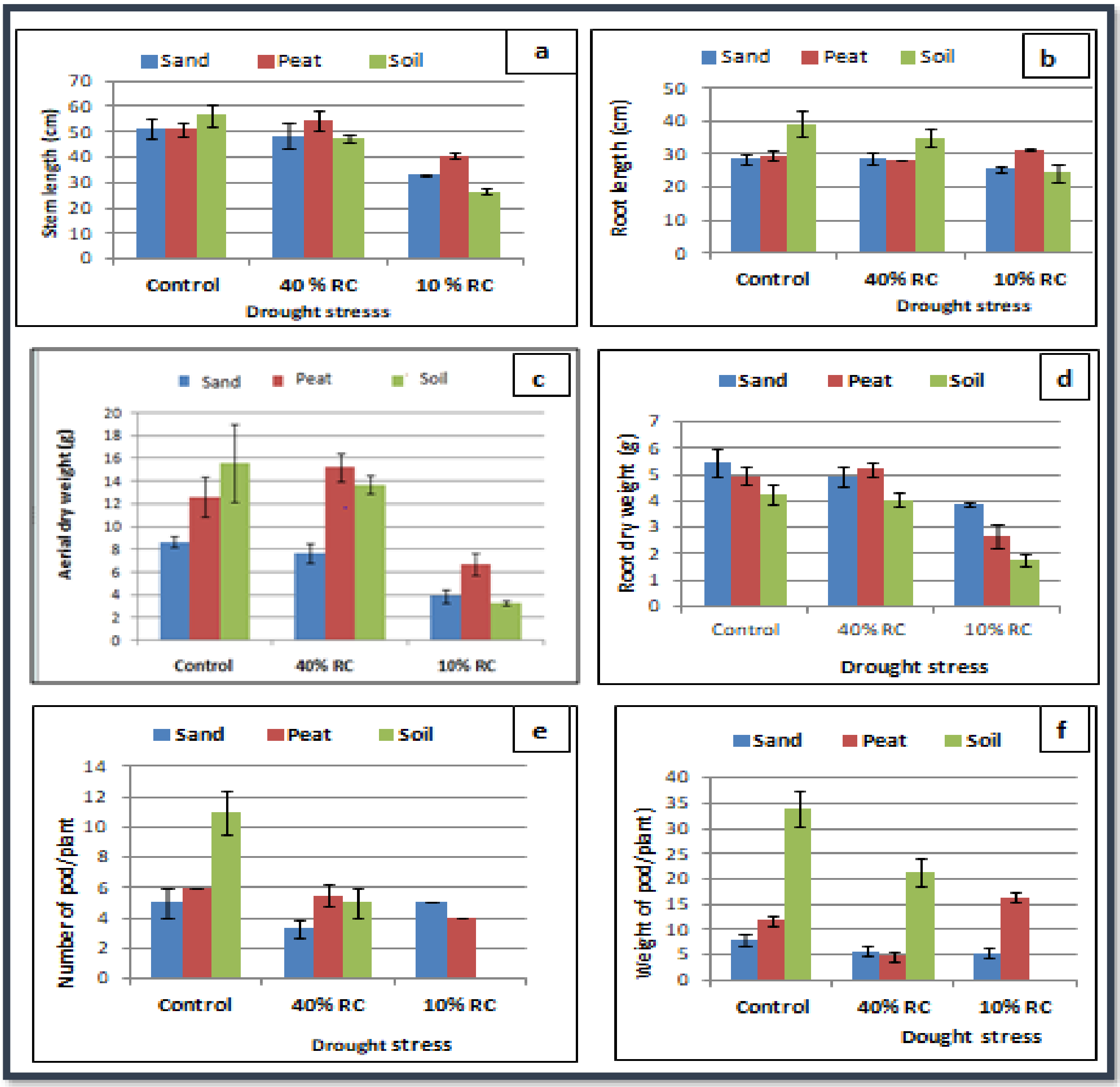

Figure 2: Stem length (a), Root lenght (b), Aerial dry weight (c), root dry weight (d), Number of pod/plant (e) and weight of pod/plant (f) of *Vicia faba* plants.

Number and weight of pod/plant

The pod *Vicia faba* L. plants production is determined at the end of the experience. Soil moisture had an impact on plant production on sandy clayey soil, as 100% water availability yielded 11pods/plant. This value drops to 5 pods/plant under moderate water stress (40% RC), i.e. a reduction in yield of 55% (Treated/Control=0.45). At 10% RC, plants do not produce any pods (Figure 2e).

Compared to previous treatment, plants grown on peat are moderately sensitive to severe water deficit and produce an average of 4pods/plant, while a maximum of 5pods/plant was produced by sand plants under same conditions (Figure 2e).

Figure (2f) examination reveals the variations in pods/plant weight. Compared with other treatments, seedlings grown on sandy clayey soil, under well-watered condition (100%

RC) and medium drought stress (40% RC) exhibited the highest pod/plant. The latter was severely declined at 10% RC; the seedlings do not bear fruit.
On peat, severely stressed plants (10% CR) are the most productive and display a high average of 16.3 g compared to medium drought stress (40%) and the control with respectively values of 4.8 g and 11.8 g. Strong decrement (7.9; 5.8 and 5.3g) in pod weight/plant was observed on sand in nontreated and treated soils (Figure 2f).

Mean Sensitivity index to water stress

The Mean Sensitivity index of morpho-physiological parameters is shown in Table 2, a negative percentage means an increase over the control.

Table 2: Mean sensitivity index of quantitative physiological parameters to water stress of *Vicia faba* L. plants grown in different substrates.

Treatment / Parameters (%)	Sand	Peat	Sandy Clay Soil
Stem length	20.42	7.40	34.82
Root length	4.68	-0.68	24.45
Aerial dry weight	33.15	13.23	45.84
Root dry weight	19.05	20.54	32.03
Number of pod/plant	16.67	20.83	77.27
Weight of pod/plante	30.33	10.59	70.46

The mean sensitivity index to water stress shows a variable behavior plants depending on culture medium (Table 2).Indeed, bean plants grown on sandy clayey soil experienced a reduction in all studied parameters compared to other substrates, probable cause of clay drying out and high suction pressure development for the water which can oppose that of plants roots. On the same substrate, yield parameters (number and weight of pods/plant) were the most strongly affected, recording respectively 77.27 and 70.24% reduction compared to control.
On the peat-rich substrate, the stressed plants root length showed a slight increase (0.68%) compared to unstressed controls. This deep root extension may be necessary in search of water. In addition, the peat organic matter, very hydrophilic and rich in mineral elements, has been favorable to growth, production and plants resistance to water stress.
In sand, water stress negatively affects plant growth and yield. This effect is due to soil particles relatively spaced, dry, poor in nutrients and very draining, allowing water to drain quickly under the effect of gravity.

The results obtained, from the experimentation that we carried out on certain qualitative and quantitative plants morpho-physiological parameters of the bean (*Vicia faba* L.) showed responses variation according to different combinations of soil texture and available soil moisture. Sandy-clayey substrate plants are more resistant to moderate stress (40% RC); their leaves are green with little necrosis and show vertical leaves distribution. Under severe stress (10% CR), plants show no defense reaction and maintain planophilic distribution of their leaves. On the (sandy-peat) mix substrate, stressed plants remained relatively green and little necrotic compared to their respective unstressed controls, which indicates their good resistance to drought probably due to their leaves orientation change to reduce transpiration and maintain high water potential. Diallo, (2010) showed that more pronounced rice leaves of Nerica variety curling seems to be a trait linked to plants resistance to water stress. Many authors report that plants adapt their architectural development to the available resources, and differ in plasticity to adapt to abiotic stresses (Ruiz-Ramos et al., 2006). Sunlight interception by plants depends on plant architecture. It is a function of the leaf area index and the angular distribution of leaves (Roujean, 1996). Under water stress, the daily paraheliotropic movement of leaves modify their angular distribution and changes the exponential interception of sunlight inside the canopy (Archontoulis et al., 2011). Leaves change their angular distribution from planophile to vertical position as a result of water stress in soybean (*Glycine max* L. Merr.) (Atti et al., 2005), common bean (Yu and Berg, 1994; Boutraa and Sanders, 2001; Pastenes et al., 2004) and switchgrass (*Panicum virgatum*) (Xu et al., 2012).Using this mechanism, leaves were able to diminish the incidence of direct sunrays and to reduce plant energy load, transpiration and temperature, leading to less sunlight being intercepted (Durigon et al., 2019).

The mean sensitivity index variation of parameters as a function of substrates and water supply levels reveals a resistance polymorphism of bean plants to water stress. Thus, sandy clay substrate plants show a high sensitivity to water stress. All physiological parameters such as plants height, roots length, areal and root dry weight as well as yield parameters (number and weight of pods / plant) underwent a strong reduction under severe water stress. This trend observed in bean plants supports observations made by several authors on different plant species including faba bean (Adid et al., 2017); rice (Diallo et al., 2010) and maize (Shahzad et al., 2019).

Soil texture indirectly affects also plant growth through its influence on soil water supply (Longwell et al., 1963) and on the supply of nutrients such as nitrogen (Wilsie et al., 1944). Plant water deficits probably reduced rates of photosynthesis related in part, to the water capacity and water movement characteristics of the soil (Smith, 1970).

On the peat substrate, measurements made on morphophysiological parameters lead to conclusion that they all show low sensitivity index. This reduction is smaller the more drought tolerant the plants are. This result could be due to organic matter presence rich in hydrophilic substances retaining water. Shahzad et al., (2019) report that organic substrates addition resulted in improved plant vegetative (height, fresh and dry biomass of maize plants) growth. This improvement maybe due to direct nutrient supplementation or by indirect amendment of soil physical characteristics i.e. soil structure and water retention capacity, bulk density, penetration resistance and porosity, infiltration rate (Hati et al., 2008). Positive response of productivity with integrated application of manure and synthetic fertilizers have been reported by many researchers (Bandyopadhyay et al., 2003; Ghosh et al., 2006; Hati et al., 2000). Recently, Wang et al., (2020) indicate that additional organic manure inputs increased yield to a high and sustainable level, due to the improvement of soil water-nutrient uptake when additional organic manure applied (Wang et al., 2011; Hou et al., 2012).

CONCLUSIONS

T stress dramatically decreased the growth and biomass production of S. davidii seedlings, Drought stress dramatically decreased the growth and biomass production of S. davidii seedlings, Drought stress dramatically decreased the growth and biomass production of S. davidii seedlings, Drought stress dramatically decreased the growth and yield of *Vicia faba* seedlings. Thus, limited water application in combination with appropriate organic matter supply could improve faba bean cultivation in sandy or clayey soils.

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RHIZOBIA INOCULATION AND DMPP NITRIFICATION INHIBITOR HAD EFFECT PHENOLOGICAL AND MORPHOLOGICAL CHARACTERS OF BEAN (*Phaseolus vulgaris* L.)

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ABSTRACT

The field experiment was conducted during 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 the year were significant for all of the investigated characters except for emergence time. Bacteria inoculation was increased all of the morphological characters and grain yield and decreased phenological characters. 50 kg ha^{-1} DMPP nitrogen fertilization types and doses were provided the highest values for investigated characters and grain yield. The use of nitrogen ihnibatörs may be an important practice to improve the bean crops. With the use of DMPP, the amount of nitrogen fertilizer and the number of applications of nitrogen fertilizers can be reduced.

Key Words: Bean, DMPP, phenological properties, morphological properties, yield

INTRODUCTION

N plays an essential role in plant production, regarding both its economic and ecological aspects. On the one hand, N affects yield level and quality like no other plant nutrient, and on the other hand, nitrogenous compounds in the hydrosphere (NO_3^- accumulation in groundwater) and atmosphere (release of nitrous greenhouse gases) may have numerous unwanted effects on the environment (Zerulla et al., 2001)

The microbial mechanisms underlying the key biogeochemical processes of the N cycle in soil have significant environmental and ecological effects, both through nitrification, where ammonium (NH_4^+) is converted to NO_3^-, a major potential source of N loss to surface and groundwater, and denitrification, where NO_3^- is converted to the potent greenhouse gas N_2O and other gasses (Myhre et al., 2013; Qin et al., 2017). An efficient strategy to reduce the N losses and pollution associated with fertilization is the use of additives that block or delay nitrogen-associated microbial processes such as nitrification inhibitors (Rodrigues et al., 2019). Therefore, the use of nitrification inhibitors (NIs) has become an increasingly important strategy in recent decades to help reduce N_2O emissions (Smith et al., 2015). Hundreds of nitrification inhibitors are known, but only a few so far have gained commercial importance for practical use, such as dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) (Liu et al., 2013). Among nitrification inhibitors, 3,4-dimethylpyrazole phosphate (DMPP) has been reported by many authors as the most efficient in slowing nitrification and reducing N_2O losses (Weiske et al., 2001a; Weiske et al., 2001b; Liu et al., 2013). The duration of its action depends on temperature and humidity conditions (Pasda et al., 2001). It can remain effective in upper soil layers even after heavy rain (Fettweis et al., 2001).

Like other legumes, the common bean can benefit from BNF through symbiosis with nitrogen-fixing rhizobia (Hungria and Kaschuk, 2014). There are many works on growth and yield of bean in response to Rhizobia. Küçük ve Kıvanç (2008), Bulut (2013) and (Şen (2018) indicated that rhizobia bacteria inoculation and nitrogen fertilization increases the yield in beans. Odabaş and Gülümser (2001), Bildirici (2003) and Bilen (2003) reported that bacteria application and nitrogen fertilization increase plant height. Şahin (2018) and Şen (2018) reported that bacteria application and nitrogen fertilization increase flowering time, harvest maturity time, plant height, first pod height, main branch number and pod height.

This study aims to investigate the effects of rhizobia and different nitrogen fertilizer types and doses on yield and some phenological and morphological characters for bean.

MATERIAL AND METHOD

The field experiment was conducted during 2017 and 2018 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey (39°48' N; 30°31' E, 798 m above sea level). Climatic data for long-term and experimental years are shown in Figure 1. Long-term annual total precipitation is 104.1 mm and it was 143.4 and 170.2 mm in the experimental years, respectively. The annual average temperature was 19.64 °C in 2017 and 20.1 °C in 2018. The soil of the experimental area was organic matter content of 1.44%, with lime 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 1.65%, with lime 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%.

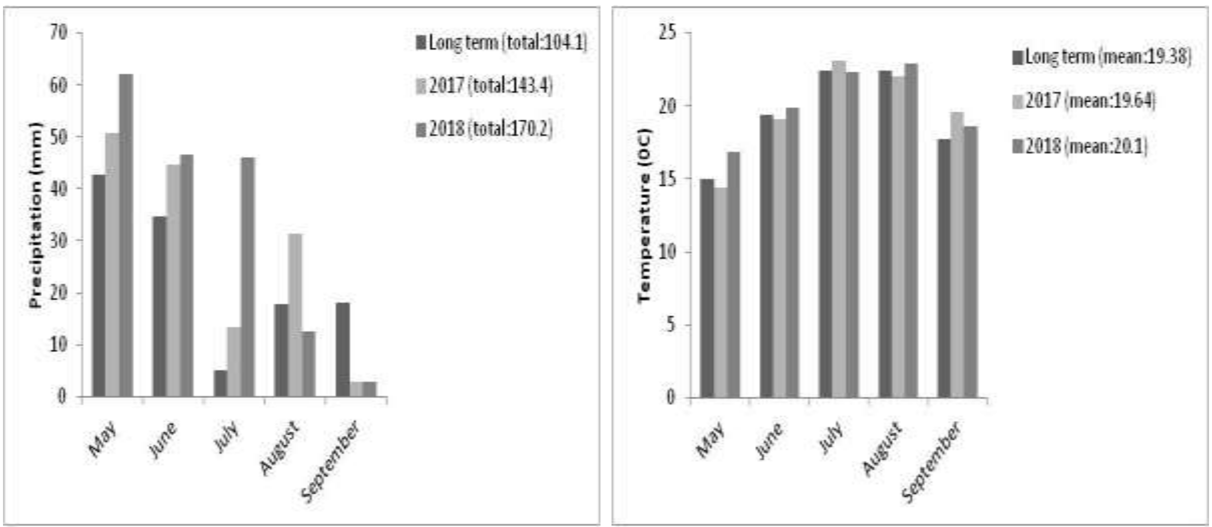

Figure 1. Climatic data of the research area

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 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 a refrigerator at + 4^{o}C until use**.** The basal fertilizer application of 60 kg ha^{-1} TSP (triple super phosphate (43-44 P_2O_5%) was given to each plot at the sowing. The harvest time of chickpea was on 13 September 2017 and 27 August 2018 in the first and second years, respectively. Emergence time (day), flowering time (day), pod formation time (day) and harvest maturity time (day) were determined in all the plots. The plant height (cm), first pod height (cm), main branch number, main branch diameter (mm) and pod height (cm) were evaluated on 5 randomly selected plants in each plot. Each plot was harvested, blended and grain yield (kg ha^{-1}) was estimated.

The variance analysis was subjected to 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 the year were significant for all of the investigated characters except for emergence time (Table 1,2). Differences between the rhizobia were significant were all of the investigated characters except for flowering time and first pod height. All investigated characters were significant for nitrogen fertilization except for pod formation time and first pod height. While flowering time had higher values in all of the plots in 2018, these traits showed lower values in 2017 (Figure 2B). For this reason, year x nitrogen fertilization interaction was significant. While pod formation time higher Rh$^-$ in 2017, Rh$^+$ showed the lower values (Figure 3A). For this reason, year x rhizobia interaction was significant. While emergence time higher Rh$^-$ on control plots in both of the years, Rh$^+$ showed the lower values (Figure 2A). While harvest maturity time, plant height, main branch diameter and grain yield higher Rh$^+$ on 50 kg ha^{-1} DMPP plots in 2017, Rh$^+$ showed the lower values on control plots in 2018. (Figure 3B, 4A, 5A, 6). While main branch number and pod height were higher Rh$^+$ on 25 kg ha^{-1} AS plots in 2018, Rh$^-$ showed the lower values on same plots in 2018. (Figure 4B, 5B). For this reason, year x rhizobia x nitrogen fertilization interaction was significant.

Flowering time, pod formation time and first pod height were higher second year but the other characteristics were a higher first year (Table 1,2). The flowering time and pod formation time were delayed due to more precipitation in May and June (especially in May) in the second year (Figure 1). Şehirali (1988) reported that more rainfall extends the flowering period in beans. Harvest maturity time was shorter in the second year. The higher temperatures in August was caused the plants to mature in a shorter time in the second year (Figure 1). High temperatures and drought after flowering were shortened the grain filling time (Wiegand et al., 1981). Plant height was higher first year than the second year but the first pod height was higher second year than the first year.

Table 1. Effects of rhizobia and different nitrogen fertilization on some traits of bean

Treatments	ET (day)	FT (day)	PFT (day)	HMT (day)	PH (cm)
2017	13.03	49.52 B	61.97 b	134.87 A	52.31 A
2018	13.26	52.32 A	62.75 a	132.15 B	48.72 B
Mean	13.14	50.92	62.36	133.51	50.51
Rh^-	13.74 A	51.07	62.75 A	132.80 B	48.11 B
Rh^+	12.55 B	50.77	61.97 B	134.22 A	52.92 A
Mean	13.14	50.92	62.36	133.51	50.51
Control	13.02 B	51.18 A	62.18	131.18 E	42.41 E
25 kg ha^{-1} AS	13.25 A	50.93 A	62.87	132.68 D	45.96 D
25 kg ha^{-1} DMPP	13.10 AB	51.00 A	62.31	133.18 C	51.36 C
50 kg ha^{-1} AS	13.25 A	51.50 A	62.18	134.50 B	54.96 B
50 kg ha^{-1} DMPP	13.12 AB	50.00 B	62.25	136.00 A	57.88 A
Mean	13.14	50.92	62.36	133.51	50.51
Year	ns	**	*	**	**
Rhizobia	**	ns	**	**	**
Nitrogen fertilization	**	**	ns	**	**
Year x rhizobia	**	ns	*	**	**
Year x nitrogen fertilization	**	*	ns	**	**
Rhizobia x nitrogen fert.	**	ns	ns	ns	**
Year x rhizobia x nitrogen fert.	**	ns	ns	**	**

ns: non-significant, *: $p \leq 0.05$, **: $p \leq 0.01$. Means in the same column with different letters are significant.ET : Emergence time FT: Flowering time PFT: Pod formation time HMT: Harvest maturity time PH: Plant height

Table 2.Effects of rhizobia and different nitrogen fertilization on some traits of bean

Treatments	FPH (cm)	MBN	MBD (mm)	Pod H. (cm)	GY (kg ha^{-1})
2017	12.26 b	2.92 a	7.12 A	12.28 A	1885 A
2018	14.61a	2.88 b	7.04 B	12.08 B	1425 B
Mean	13.43	2.90	7.08	12.18	1655
Rh^-	13.00	2.83 B	6.79 B	12.04 B	1312 B
Rh^+	13.87	2.97 A	7.38 A	12.33 A	1999 A
Mean	13.43	2.90	7.08	12.18	1655
Control	12.01	2.88 BC	6.02 E	12.11 BC	1162 E
25 kg ha^{-1} AS	14.63	2.83 C	6.56 D	12.05 C	1446 D
25 kg ha^{-1} DMPP	13.43	2.91 AB	7.18 C	12.15 ABC	1693 C
50 kg ha^{-1} AS	13.40	2.92 AB	7.42 BC	12.26 AB	1783 B
50 kg ha^{-1} DMPP	13.71	2.95 A	8.23 A	12.33 A	2193 A
Mean	13.43	2.90	7.08	12.18	1655
Year	*	*	**	**	**
Rhizobia	ns	**	**	**	**
Nitrogen fertilization	ns	**	**	**	**
Year x rhizobia	ns	**	**	**	**
Year x nitrogen fertilization	ns	**	**	**	**
Rhizobia x nitrogen fert.	ns	**	**	**	**
Year x rhizobia x nitrogen fert.	ns	**	**	**	**

ns: non-significant, *: $p \leq 0.05$, **: $p \leq 0.01$. Means in the same column with different letters are significant. FPH: First pod height MBN: Main branch number MBD: Main branch diameter Pod H: Pod height GY: Grain yield

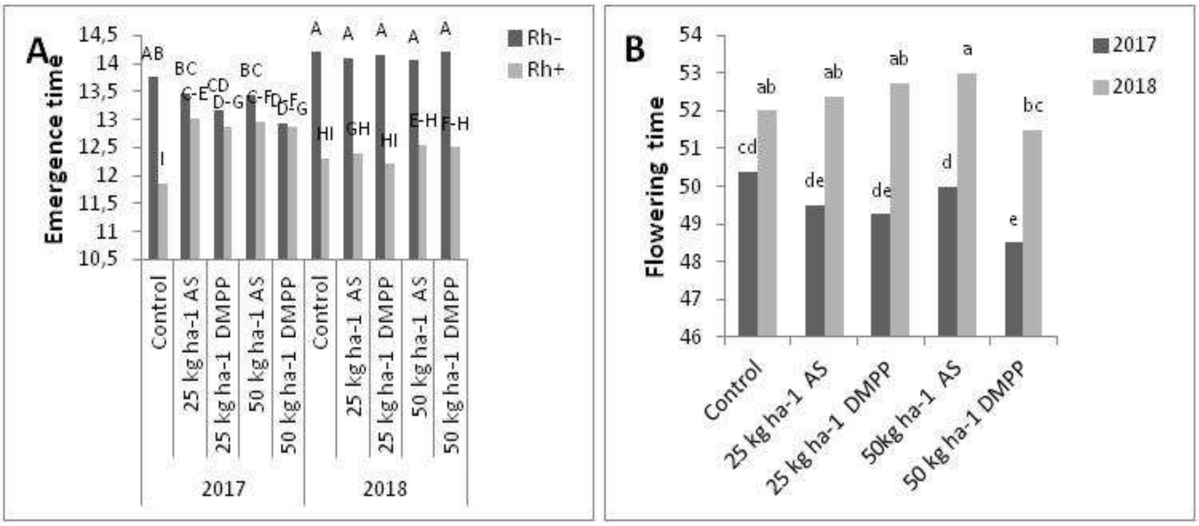

Figure 2.The interaction between rhizobia and different nitrogen fertilization on emergence time (A) and flowering time (B) of bean. Letters on each bar represent significance level at P < 0.05

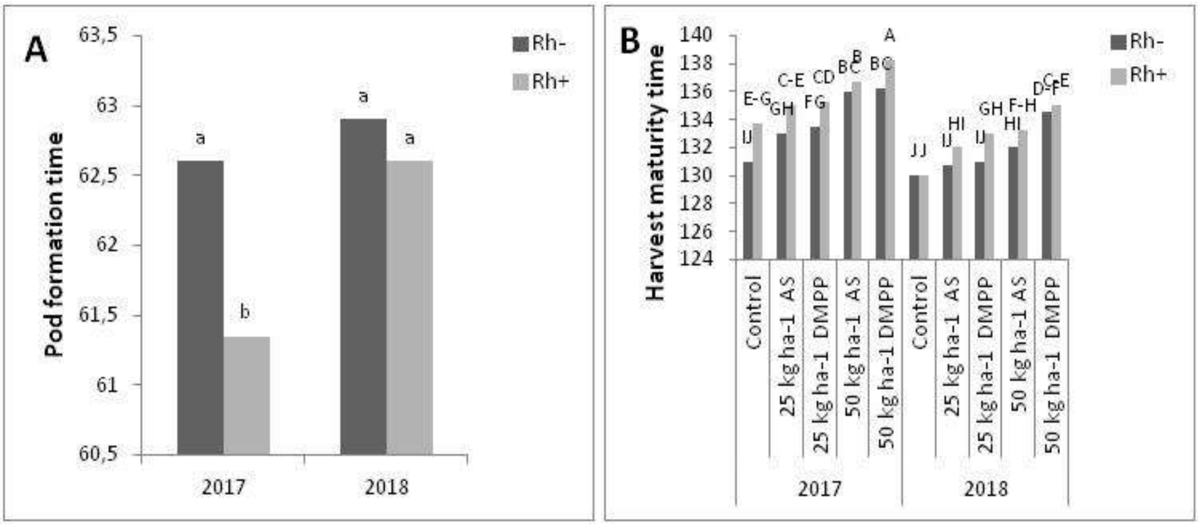

Figure 3.The interaction between rhizobia and different nitrogen fertilization on pod formation time (A) and harvest maturity time (B) of bean. Letters on each bar represent significance level at P < 0.05

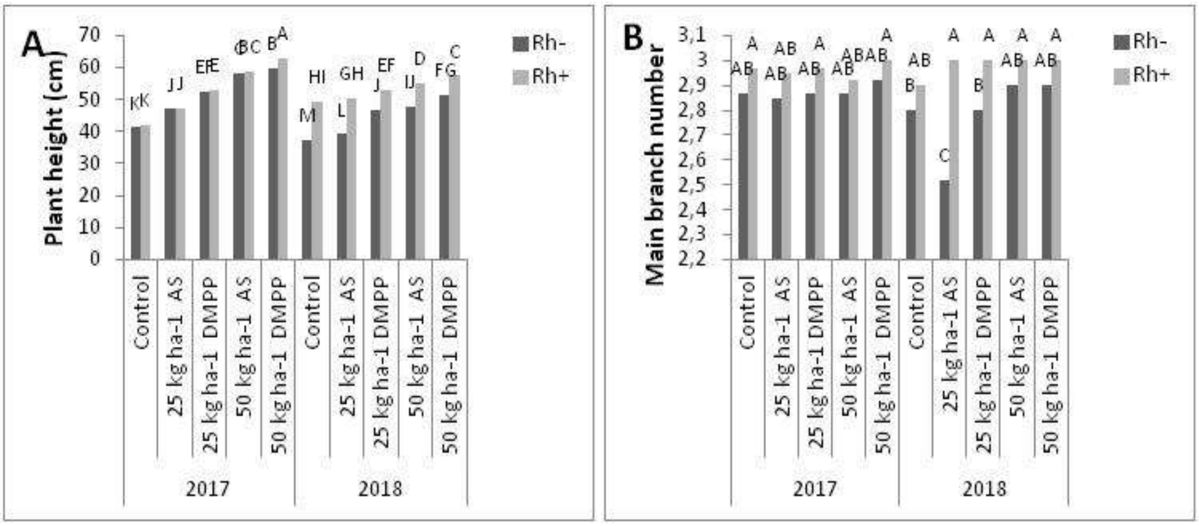

Figure 4.The interaction between rhizobia and different nitrogen fertilization on plant height (A) and main branch number (B) of bean. Letters on each bar represent significance level at P < 0.05

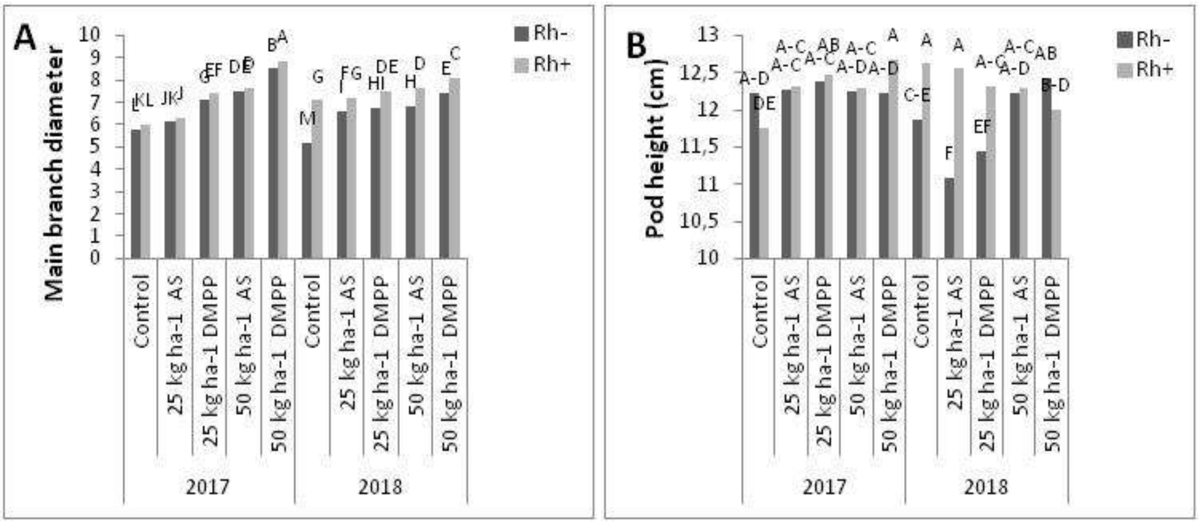

Figure 5.The interaction between rhizobia and different nitrogen fertilization on main branch diameter (A) and pod height (B) of bean. Letters on each bar represent significance level at P < 0.05

Pekşen (2005) and Ülker (2008) reported that plant height was significantly affected by environmental conditions and significant differences may occur between the same genotypes in different years and locations. Main branch number, main branch diameter and pod height were higher in the first year. The main branch number depends on the genotype and growing conditions in beans (Aytekin and Çalışkan, 2015). Other researchers were reported that significant differences between the years for main branch numbers due to climatic differences (Pekşen, 2005; Karakuş et al., 2005). Grain yield was lower due to total high temperature especially grain-filled period in the second experimental year (Figure 1, Table 2). Warland et al. (2006) reported that grain yield was reduced when the temperature is increased by 1.5 ^{o}C.

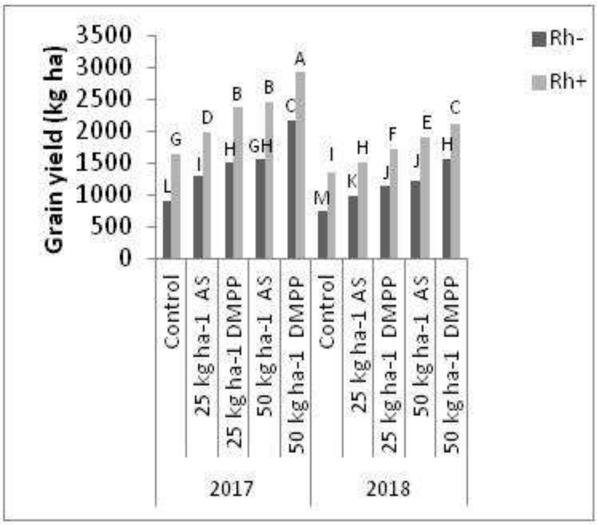

Figure 6. The interaction between rhizobia and different nitrogen fertilization on grain yield of bean. Letters on each bar represent significance level at P < 0.05

Rhizobia inoculation was decreased emergence time and pod formation time but harvest maturity time was increased by bacteria inoculation. Agba et al. (2013) reported that pod formation time was decreased rhizobia inoculation and Pekşen (1992) indicated that emergence time was decreased by rhizobia inoculation in chickpea. Plant height, main branch number, main branch diameter and pod height were increased by rhizobia bacteria inoculation. Rhizobia, which is one of the bacteria that plant growth-promoting rhizobacteria, increases plant growth by producing hormonal substances (Çakmakçı, 2005). Plant height, main branch number and diameter and pod length increased with the effect of bacteria in our study. Bildirici (2003), Bilen (2003) Uyanöz (2007), Altunkaynak ve Ceyhan (2018) ve Şen (2018) reported that nitrogen fertilization and rhizobia inoculation were increased plant height. Agba et al. (2013) indicated that rhizobia inoculation was increased main branch number and Ahmed et al. (2007), Rahman et al. (2008) and Akman (2017) reported that bacteria was increased pod height. Karadavut ve Özdemir (2001) indicated that rhizobia bacteria inoculation was increased plant height and main branch number in chickpea. Şahin (2018) reported that plant height, main branch number and pod length were increased by rhizobia inoculation in bean. Çakmakçı (2005), Bayraklı et al. (2017) and Barros et al. (2018) reported that grain yield was increased with rhizobia inoculation.

Emergence time and harvest maturity time were not affected by nitrogen fertilization types and doses. The shortest emergence time and harvest maturity time were observed in control plots. Zenawi and Mizan reported that emergence time was not affected by nitrogen fertilization. The shortest flowering time was observed on 50 kg ha^{-1} DMPP. Flowering time was shortened with DMPP application. The highest plant height, main branch number, main branch diameter, pod height and grain yield were obtained 50 kg ha^{-1} DMPP. Coelho et al. (2018) indicated that plant height was increased by nitrogen inhibatörs. 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, morphological characteristics and grain yield may be higher in 50 kg ha^{-1} DMPP plots in our study. Pasda et al. (2001) positive effect of DMPP on yield is more clear when precipitation was higher.

CONCLUSIONS

Flowering time, pod formation time and first pod height were higher second year but the other characteristics were higher first year due to climatic conditions. Phenological characters were affected differently by rhizobia and nitrogen fertilization application. Bacteria inoculation was increased all of the morphological characters and grain yield and decreased phenological characters. 50 kg ha^{-1} DMPP nitrogen fertilization types and doses were provided the highest values for investigated characters and grain yield. 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 more observed. The use of nitrogen ihnibatörs may be an important practice to improve the bean crops. With the use of DMPP, the amount of nitrogen fertilizer and the number of applications of nitrogen fertilizers can be reduced.

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SECTORAL USE OF WATER AND ECONOMICAL POLITICS OF WATER

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ABSTRACT

The journey of water in the atmosphere and the earth, called the water cycle, started with the industrial revolution and continues with both economic cycles and social transformation, and this process does not improve when we assume that only 2.5% of the world's water is fresh water and 95% of it is in glaciers. With the crisis of capitalism in 1970, it was aimed to ensure the circulation of capital and thus the consumption economy came to the fore with the globalization of capital. In the capitalist system, countries are located on production, consumption and circulation. Gaining the most profit with the least cost paved the way for rapid capitalism in the world and this situation caused the disruption of the natural cycle with rapid urbanization, rapid population growth and rapid consumption. When we look at the distribution of water consumption by sectors in developed countries and underdeveloped countries, water use in the industrial sector is the highest in developed countries, while it shows the highest water use in the agricultural sector in undeveloped countries. In this context, in order to increase efficiency in water use and to ensure sustainability, measures should be taken to ensure efficiency in agricultural water use, which is the area where the most water is consumed, gray water should be used where possible in domestic water use, rainwater harvesting should be done. At the same time, rapid urbanization should be prevented and on-site investment should be provided so that water use between basins should be balanced.

Key Words: sustainability, water efficiency, saving on water, neoliberalism, interdependence

INTRODUCTION

Water, which is an indispensable source of life, has become both a scarce resource as a result of the fact of globalization that has occurred for the sake of the circulation of capital after the structural crises of capitalism, as well as rapid urbanization for the revival of the economy, the inability to control the population growth rate and the environmental pollution. It has become a "commodity". Particularly, the physical homogeneity brought by rapid urbanization has increased the migration from rural to urban areas and unfortunately, accelerated the infrastructure problems. While this turned water, which is our main source of life, into a sellable commodity, in a sense, the fact that the water was priced in a sense led us to save money in water use. Especially when it comes to accessing the water source, the race of the countries has increased the dependence on the country where there is a kind of resource, while the interdependence of the central countries in terms of establishing new standards in reaching the water source and ensuring the sustainability of the water has been obliged.

Neoliberal concepts such as transparency accountability that arise with globalization have actually emerged in order to produce policies that prevent any crisis in any situation and to ensure sustainability in the current situation without borrowing from the future.

Integrated water management is a perspective that has both sociological, legal and political and economic pillars by preventing water from disappearing from the basin. In this sense, all stakeholders should work together as the gear of a wheel by considering how to manage the water that is exhausted and indispensable in every sector most efficiently and how alternative resources can be implemented.

In this study, the situation of water in the world and in Turkey, the determination of water use in the sector, the elements of water efficiency, the economy of water in the context of sustainable development goals as a requirement of neoliberal policies and the pricing of water without profit will be emphasized.

MATERIAL AND METHOD

In this study; water efficiency, sustainable water use, water cycle, neoliberalism and interdependence start from the concepts. The economic politics of water were demonstrated through neoliberal policies. The situation in Turkey is revealed from the UN Sustainable Development Goals in the title of water and sanitation and climate action. Again, the pricing of water was discussed while revealing the economic situation of water. In order to use water efficiently, data on urban water use, industrial water use, agricultural water use were used. The situation in the world and Turkey in sectoral water use has been compared. In addition to the UN data, a literature review was also carried out. Cases on the use of water resources in the literature are used in the study.

RESULTS AND DISCUSSION

Sustainable development; It refers to economic growth that continues continuously based on the consumption of renewable resources and causes limited damage to the environment (Anonymous, 2018).

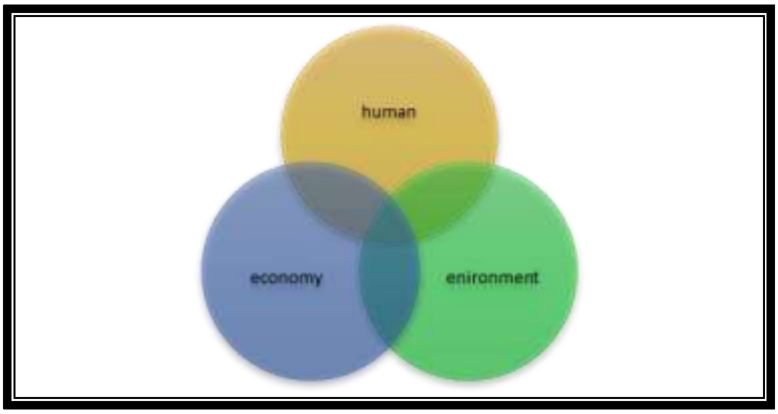

Figure 1: Sustainable development

There are three pillars of sustainable development, which is an environmentalist view (Keleş and Hamamcı 1993), which aims to ensure economic development with the principle of using the environment and natural resources in a rational way without causing waste, taking into account the rights and benefits of future generations: Economy, Human and Environment.

Water efficiency; Not more water is required for the work to be done in the most important factor in water efficiency (Capar, 2018). The aim is to ensure the efficiency of the existing water with a planned and programmed study before it is used. Contrary to popular belief, cutting unnecessary expenditures on the use of water, which is a scarce resource, both prevents water from being wasted and ensures sustainability in the use of water. Other issues that will ensure the efficiency of water is to turn to alternative water sources. Rainwater harvesting, use of gray water, prevention of leakages in water, pricing of water are important factors that will ensure efficient use of water.

Water saving is reducing the use of water, unlike water efficiency. Water efficiency targets waste reduction, while water saving limits use.

Water cycle; The liquid in the oceans evaporates with the sun's heat, moves upwards in vapor form, and this movement continues until it condenses into droplets of water that form clouds of steam. If droplets form at sufficient height, they crystallize as ice particles. As their weight increases, they fall back to earth like rain, snow and hail. Some of the water returning to the earth is re-entered into the oceans, while some remain glacial in cold areas, while others flow in the soil as streams and rivers, mixing with groundwater and evaporating into the atmosphere again. (Harvey, 2017). This process returns to where it began and follows a continuous circular movement. Urbanization, concretization and the deterioration of this cycle with the climate change it brings destabilize the natural flow and will result in increased melting of glaciers and the reduction of fresh water.

Neoliberalism and interdependency; International studies that predict that the efficiency will increase with the privatization of water resources and therefore the sustainability of water will be achieved for effective use continue to be effective all over the world. Neoliberal policies and new public administration (management) (Övgün 2009) based on the logic of managing the state as a business, and the transfer of water resources management to multinational companies cannot provide efficiency and productivity. It has been revealed that the elimination of the problems in the environment will be possible not only with the initiative of a single country, but also by acting together, the continuation of production and development.

When we look at the state of water in the world and in Turkey. It is stated that the total amount of water in the world is 1.4 billion m^3. 97% of the total amount of water is found in the seas and oceans as salt water. The remaining 3% is fresh water, and 90% of this 3% is in glaciers and underground. 10% are fresh water found in rivers and lakes (Capar, 2018). Developing countries and economically rapidly developing countries are increasing the need for consumption at the same rate. The pressures of climate change, the pressures on water caused by human activities, have been an indication that water resources will be inadequate to meet future needs. The scarcity of water resources and the fact that they continue to be consumed rapidly, while at the same time polluting limits access to clean and quality water, and threatens the sustainability of water resources by rapidly depleting them. Since 1950, the amount of water per capita in developed countries has decreased by 50%, while in developing countries it has decreased by 80% (Kınacı, 2017).

Domestic water use rate %8 in the world; However, this rate varies in developed and developing countries. Domestic water consumption increases from 8% in countries that have not yet completed their development to 11% in developed countries. The average values obtained from OECD sources for domestic water use for 1984 are given as 145 liters/person/day in Germany, 125 liters/person/day in France and 193 liters/person/day in Sweden. (Deniz, 2012)

Agricultural Water Consumption; It is seen that the consumption rate of agricultural water in the world is in the %70s. (Kodal and Ahi, 2018) The sector that consumes the most water is agriculture. Achieving efficiency in agricultural water use compared to other sectors will be an important factor in ensuring the sustainability of water use. While the amount of water used in the agricultural sector in developed countries is 30%, in developing countries it is up to 82% (Cakmak, 2018). The decrease in water resources due to the effect of climate change will affect the agricultural sector the most, and in this case, it will adversely affect the underdeveloped countries both economically and in terms of access to water (Cakmak, 2008). The rate of industrial water use in the world is 22%.

The annual precipitation average in Turkey is 574 mm/m^2. The surface area of our country is 779.500 km^2. Our annual precipitation amount is 450 billion m^3. Usable surface water is 97 billion m^3 of groundwater, annual drawable water amount is 15 billion m^3 and total net usable water amount is 112 billion m^3. (Anonymous, 2020). Turkey's use rate of usable water supply is 39%. Domestic water use in Turkey is expected to be 13% in 2017 and 16% in 2023. In 2013, 74% of total water use occurs in irrigation water. Especially the open irrigation channels and the incorrect irrigation cause the most dewatering. More than half of the water that exists in our country is mixed into the water cycle with evaporation. The rate of irrigation water is expected to fall to 64% in 2023 (Anonymous 2017)

Industrial water use is 22% in the world, 5.9% in developed countries and 13% in Turkey. Industrial water use is expected to be 20% in 2030, up from 11% in 1990 and 11% in 2004 (Anonymous 2017).

Economy policy of water The rapid development of the economy accelerated the urbanization process, damaged natural ecology, which led to an increase in the area and frequency of internal disasters (Sun, 2020). Water, which is an essential source of life, the reality of globalization that occurs for the sake of ensuring the circulation of capital after the structural crises entered by capitalism, as well as the rapid urbanization that occurs for the revival of the economy, the inability to control the rate of population growth, the occurrence of environmental pollution, the formation of heat islands with rapid urbanization, the destruction of forests and urban development have accelerated climate change, and with climate change, drought and unavoidable floods have accelerated. as a result of its formation, it has become a scarce resource and reaching the water has become a race between countries.

International cooperation is also needed to promote water efficiency and support treatment technologies in developing countries. Sustainable development goals have also emerged in this context.

Water and sanitation, "providing reliable and affordable drinking water and adequate sewage services for everyone". “improving water use efficiency by using integrated water resources management" protection of water-related ecosystems by taking measures to reduce water pollution"(Anonymous, 2019).

Climate actions, Climate change policies in Turkey are directly in the 8th century for the first time. He was part of the Development Plan, 9. The Development Plan aims to prepare a climate change strategy and a greenhouse gas reduction action plan. 10. Development Plan aims to contribute to the fight against global climate change according to the principles of common but differentiated responsibilities and relative capabilities, especially within the scope of emission reduction and compliance (Anonymous 2019)

Pricing of water; The rapid increase in the population in the world, the decrease in usable water, the decrease in water resources due to climate change due to global warming, the pricing of water is an important factor in order to ensure efficiency in the use of water and to develop alternative methods in water resources. The opportunity cost of water arises in times of drought when it is most needed (Muslu, 2015). Private property is out of question as it is an indispensable and non-substitutable resource. In this context, the pricing of water should be in order to ensure efficiency and sustainability in the use of water, rather than seeing it as a commodity.

CONCLUSION

Sustainable use of water is a must in order to ensure the continuation of life and to ensure economic, social and environmental sustainability. In order to ensure efficiency in water use, first of all, it is necessary to determine water use on the basis of sectors, to detect water loss leaks and to turn to alternative water sources in order to ensure water efficiency.

Domestic water use includes different parameters for each city. In order to save domestic water, first of all, detailed information on water use in residences is required. The use of gray water, harvesting of rainwater, prevention of loss and leakage in drinking water, changing irrigation methods in irrigation water, preventing evaporation are one of the factors in ensuring water efficiency.

It is necessary to increase forests, reduce the established hydroelectric power plants, and leave life water if there is a great need. Ensuring effective communication between institutions, raising awareness of stakeholders and ensuring cooperation, prevention of pollution at the source, and widespread use of the polluter pays principle will increase the efficiency of water. The Sustainable Development Goals can only be achieved with a strong commitment to global partnership and cooperation. The interdependence of countries on each other is increasing day by day.

By adopting an integrated water management strategy in agricultural water use, first of all, farmer awareness should be raised and optimum plant pattern should be created. In water use, instead of flood irrigation with open gutters, an irrigation system should be established by taking into account the climatic conditions and precipitation. Water use should be priced proportionally and new methods should be sought by saving water. The reuse of wastewater, which is widely used on a global scale, as irrigation water will be one of the most important components in water efficiency. Pressurized irrigation methods should be implemented with modern irrigation methods. Optimum benefit from water should be ensured by preventing evaporation.

Rapid urbanization, rapid population growth, consumption economy, which is the result of neoliberalism and capitalism, while increasing economic welfare, paradoxically disrupts the natural balance. This situation reduces the sustainability of natural resources and even drags them towards a situation that will disappear.

Efficient use of water should be made a state policy and implemented. Turkey is one of the many countries in the world suffering from water stress. From this point of view, raising public awareness and increasing awareness and sensitivity through inter-institutional cooperation and solidarity are the most important elements. First of all, central and local governments, which are related to water, should take steps to ensure efficiency in water consumption in cooperation after ensuring the division of labor. Efficient use of water should be ensured by protecting water on a basin basis. Physical and administrative losses in the distribution of mains water should be addressed and minimizing it will be an important step in terms of eliminating waste of resources both in economic terms.

Although the pricing of water has made water, which is a natural resource, a commodity, it will be one of the important factors that will lead the public to savings in water use. It is necessary to enact legislation regarding the collection of rain water in buildings, to treat and use gray water, and to establish standards for implementation. Choosing energy-saving faucets in the use of faucets in sinks is important.

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INVESTIGATION OF THE SUSTAINABILITY OF SOIL PROPERTIES OF OLIVE ORCHARDS IN DIFFERENT LAND CONDITIONS OF THE SEMIARID MEDITERRANEAN AGROECOSYSTEM

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ABSTRACT

Olive is an important product for Turkey located in the Mediterranean basin for centuries. It is particularly cultivated in rainfed conditions with a certain slope created by the topographic structures where often mechanization is difficult to implement. Recent years in the country, the subsidies, the importance of olive oil in health and use of mechanization in flat lands led to the expansion of olive towards to the valleys by shifting to narrow tree-spaced as new plantations to improve efficiency per unit area. Meanwhile, it is thought that there is a deterioration in soil with the mechanization. For this reason, soil properties were examined for two consecutive years under unirrigated olive trees in both sloping and flat land orchards, named no-tillage sustainable-sloping-SSO and tillage conventional-flat-CFO in Çanakkale (NW Turkey), where olives are grown intensively. Results showed that the soil physical properties deteriorate by tillage practices in CFO and reach over threshold level in many soil depths, especially in tillage depth, that may affect the nutrient mobility. High bulk density of 1.70 gr cm^{-3} and penetration resistance of 2 MPa were found in CFO, on the contrary, hydraulic conductivity was optimal level. Essential macro and micro nutrients have been found to be generally insufficient for both orchards, despite annual fertilization in CFO. The ratio of C:N found that there is no difference between SSO and CFO due to the fact that they have been under similar cultural practices for long-term since establishing, except for the last few years including the experimental years. This indicates that the necessary importance should be given in order to continue under both slope and flat lands with the current sustainable olive cultivation by improving the cultural practices such as native vegetation due to importance income source in countryside of the region and contribution to the environmental ecosystem.

Keywords: Olive orchards, Sustainable cultivation practices, Mechanization.

INTRODUCTION

Olive is good adapting to harsh environmental conditions, and is often cultivated in marginal land with shallow and poor fertility soils. In this regard, it has been cultivated for centuries under relatively poor precipitation conditions due to having the ability to better tolerate water stress. It covers a productive area of almost 11 million hectares in the world (FaoState, 2019), more than 90% of the area is located in countries of the Mediterranean basin on steep slope areas. Yield is usually low in such areas which particularly restrict nutrient absorption (Karyotis et al., 2014). Therefore, the olive cultivation including natural cover crop or spontaneous vegetation can be an appropriate practice by improving the structure of the soil. Thus, sustainable cultivation practices with low environmental impacts using native vegetation are required to improve the soil properties, especially on steep slopes. These practices with no-tillage decreasing soil disturbance allows residues to stay on the soil surface and increasing the rate of water infiltration. In contrast, tillage may result in soil structure degradation and cause erosion processes (Gómez et al., 2009). Residues can also protect the water content of the soil and encourage microbial activity to keep soil organic matter that positively affects soil properties (Aranda et al., 2011). The physical properties of the soil have a strong effect on the availability of nutrients which are highly dependent on soil quality and climate conditions. Therefore, for the growth and yield of the olive tree, it can be increased by providing the required quantity of nutrients with the cultural practices.

Olive has been cultivated for centuries as scattered trees on sloping areas of Çanakkale region located in north-west Turkey. Many of the olive trees in the country are grown in this region, particularly on steep slopes surrounding the foothills of Ida Mountain National Park. The olive characterizing old trees with large canopy and low yield and typically cultivated on slopes, are involving low-intensity cultural practices and inputs, but a high labour input, particularly for harvesting. On the other hand, in the last decades, the introduction of mechanization in the region gave an important number of farmers the opportunity of cultivating flat areas as well in low slopes to start new plantations or shifted from traditional with wide row-spaced to narrow row-spaced. The main reason explaining this change was subsidies, as well as high gain from good quality product, and the high value of olive oil. For this reason, during two study years, it has aimed to carry out the effects of management practices on the soil physical and chemical properties for olive orchards in sloping and flat lands under semiarid environment in north-west Tukey.

MATERIALS AND METHODS

The study are conducted out in two olive orchards which are located at an average elevation 300 m above sea level, in the southern coastal areas of the Çanakkale region within the borders of Ida Mountain National Park near the site of ancient Troy and North Aegean Sea (north-west Turkey), about 70 km southwest of Dardanelles (*Çanakkale Bogazi*) (39°60′81″N, 26°39′58″E). There are both sloping and flat areas in the natural structure in the region. Olive is among the main agricultural occupations in both areas, but fruits and cereals is other growing crops in flat areas. The climate is typical semi-arid Mediterranean with cold-rainy winters and long hot-dry summers, where plants experience drought stress, usually between June and September. According to the data of Turkish State Methodology Service, the average annual temperature is 15.1 °C (long term data of 1968-2019) (Table 1). The highest and lowest monthly temperatures are 25.1 °C and 6.2 °C recorded in July and January, respectively. The average annual rainfall is 553 mm. The actual highest amount falls in late autumn and early winter, from September to March, which corresponds to 90% of the total rainfall, and the other maximum occurs in the spring months, from April to June, and corresponds to 10% of the total (Table 1).

The study was carried out in sloping and flat productive commercial olive orchards with quite similar soil characteristics in 2017-2018 and 2018-2019 growing seasons. The orchards of the study were established in 1970 and 2000 with trees of the cultivar Ayvalık. Since 2009, tillage operations and fertilizer applications have been carried out in the conventional-flat orchard which is shifted from wide-row trees to narrow-row. The sustainable-sloping orchard, often with ancient trees, has been cultivated for almost 50 years with no-chemical fertilizer, pesticide and tillage. In addition, slopes that are too steep and not safe for tractor work or travel in all land vehicles should be avoided. Therefore, the orchard is described as no-tillage soils, in which the spontaneous vegetative is maintained under control as green manure by using herbicide. In addition, sometimes grazing is a common traditional practice to control weeds in this type orchard, especially among the region farmers. On the other hand, the CFO has been cultivated traditionally using tillage practices to reduce water competition between olive trees and weeds. It is tilled by two tillage passes per year as first and second tillage practices to a depth of 10 to 15 cm from late autumn immediately following the harvest to early spring of next year or early summer in order to clean the soil from weeds. First tillage performed at a depth of 15 cm with the 2.7 m-rotavator mounted on a tractor in the late autumn after the first autumn rainfall events following harvest at the optimum soil water content. Secondary tillage was carried out two times at a depth of 10 cm, in the first half of April and at the end of May, immediately after the late winter rainfall, with the cultivator to control weeds and destroy capillary evaporation. In the same orchard, chemical fertilizer was applied by hand as broadcasting to areas close to the rows of trees at the 70-9-42 (N, P, K) kg per hectare during the spring rainy season. The trees of the study under both orchards are lightly pruned for each of the year in quite early spring using hand tools to improve lighting inside of the canopy, and shortening of the high branches. Pruned thin branches in the sloping orchard were shredded by using a portable hand-operate tool and left on the soil surface in the inter-tree row area as mulch (around 600 kg ha^{-1} $year^{-1}$ as dry matter), while they were removed (around 800 kg ha^{-1} $year^{-1}$ as dry matter) from the conventional-flat orchard for more efficient tillage operations (Özpınar, 2020).

In the years before the establishment of the study, soil collected from the topsoil depth (0-30 cm) of two orchards in November 2017. According to the USDA classification, the soil texture in the conventional-flat is silty-loam which is a somewhat poorly drained, while the sustainable-sloping has a sandy-loam. The soil organic matter is relatively low (<2%) in two orchards, despite the high content of the organic matter of the fertilizer used in conventional-flat. The soil is a pH 7.17 and 6.64, $CaCO_3$ 7.47% and 2.80%, extractable P 8.52 and 2.62, extractable K 166 mg kg^{-1} and 227 mg kg^{-1}, respectively, in conventional-flat and sustainable-sloping orchards. The pH tolerance of olives is quite large, ranging from about 5.0 to 8.0, ideally it would be adjusted to approximately 6.5, but soils are slightly alkaline. EC values for each orchard are lower than 3.0 dS m^{-1} which upper level for olive growing. Nine pre-selected trees with similar canopy sizes were used for each orchard harvest in second year of the experiment, and then they were harvested when 50% of the fruit started to turn from green to purple. Fruits were harvested from each tree in November in sloping and flat orchards using a wooden stick and a knapsack portable shaker-machine, respectively, to drop them into the sheets on the floor. Thereafter, the fruits were weighed separately per tree, and then expressed per hectare for each orchard. Yield reached a value of 1820 and 5544 kg fresh fruit per hectare for sloping and flat orchard, respectively (Özpınar, 2020).

Table 1. Mean monthly and long-term temperature and rainfall of the olive orchard region from October 2017 to December 2019 and the long term period

Year	Month	POSR (mm)	LTOR (mm)	POST (°C)	LTOT (°C)
2017	Oct	139.3	54.3	16	16.0
	Nov	70.0	86.8	12.8	11.9
	Dec	2.6	11.7	6.3	8.4
2018	Jan	98.9	90.8	5.6	6.2
	Feb	45.9	71.5	10.9	6.6
	Mar	47.4	67.7	10.6	8.4
	Apr	19.1	47.6	15.6	12.6
	May	35.7	32	17.6	17.6
	Jun	16.5	21.8	24.0	22.4
	Jul	0.0	12.1	26.2	25.1
	Aug	0.0	7.0	26.1	24.9
	Sep	2.7	23.0	21.0	20.8
	Oct	6.8	54.3	15.8	16.0
	Nov	118.1	86.8	10.8	11.9
	Dec	15.7	11.7	3.1	8.4
2019	Jan	143.9	90.8	3.1	6.2
	Feb	36.3	71.5	7.8	6.6
	Mar	28.3	67.7	10.2	8.4
	Apr	15.5	47.6	13.1	12.6
	May	18.9	32	17.9	17.6
	Jun	3.8	21.8	23.6	22.4
	Jul	2.2	12.1	25.6	25.1
	Aug	1.8	7.0	25.1	24.9
	Sep	210.8	23.0	21.1	20.8
	Oct	54.4	54.3	14.1	16.0
	Nov	60.2	86.8	10.3	11.9
	Dec	99.7	11.7	9.5	8.4

POSR, period of study rainfall; LTOR, long-term rainfall (60-year); POST, period of study temperature; LTOT, long-term temperature (60-year).

Three rows were determined in each of the orchards at the beginning of the study following the harvest. In both orchards, the superficial vegetation was cut with a sickle their roots remained in situ and they were gently removed from soil surface to keep bare before starting measurement and sampling. In the second year of study, soil measurements and samples were made at the end of the rainy season (before starting fertilizer application in March and June) at a distance of approximately 3.0 m from the tree rows. Penetration resistance measured to a depth of 80 cm at 10 cm intervals using a digital penetrometer (Model 06.15 Eijkelkamp, The Netherlands). Measurements were made always outside the tree canopy area in transects between two trees with three replications being 30 records per orchard at each soil depth. Then, measurements were averaged for soil depths of 0-10 cm, 11-20 cm, 21-30 cm, 31-40 cm, 41-50 cm, 51-60 cm, 61-70 cm and 71-80 cm.

Soil bulk density was sampled with the undistributed core method (Eijkelkamp sampler 50 mm (diameter) x 51 mm (height) at the same time as penetration resistance. Samples were taken from the topsoil to 80 cm with 10 cm intervals on both orchards. It was determined as the mass (weight) of dry soil per given unit volume from core samples per volume of oven-dried undisturbed soil (Ozpinar et al. (2018). The same cores were used to determine soil water content (gravimetric water content, GWC) by weight of water in relation to the total weight of the soil, placing it in an oven at 105 ^{o}C for 24-hour. The total porosity (TP) was estimated using the equation. in (Ozpinar et al. (2018). The saturated hydraulic conductivity (K_{sat}) was measured in the days following penetration resistance and was continued for a few days. K_{sat} was recorded by using a constant head well permeameter (Model 2800K1 Guelph Permeameter, USA) and calculated using Richards' analysis equation. The readings were made for soil depths of 15 and 25 cm with at least three-repetition for only flat orchard in the inter three-tree row, being nine repetitions. Three composite soil samples were collected from both orchards with the same rows of penetration resistance and bulk density measurements at the 0-30 cm depth of two-year study. Soil samples were oven-dried at 40 ^{o}C, disaggregated and then sieved through a 2 mm mesh. They were analysed for nutrients by means of the inductively coupled plasma atomic emission spectroscopy (ICP/OES, Optima 8000, Perkin Elmer) and reduction method. Nitrogen were estimated by elemental analysis using a Leco CHN (Euro-EA3000, EuroVector, Milan, Italy) elemental analyser and P by Olsen procedure. Soil organic carbon (SOC) was determined using Walkley-Black method. Soil organic matter (SOM) was derived from the SOC values by the relation SOM=1.724 SOC. Total nitrogen was determined with the Kjeldahl method and the C:N ratio calculated by dividing the SOC (%) into the total nitrogen (%).

RESULTS AND DISCUSSION

Bulk density of the soil

In second year of the study, bulk density was found higher in the conventional-flat orchard soils than in the sustainable-sloping through all soil depths (Table 2). It was found around 1.57 gr cm^{-3} at 0 and 20 cm, which is known as topsoil in flat orchard, and increased even more in deeper depths, and then continued to increase gradually up to 70 cm. However, it was even higher at soil depth between 20 and 70 cm, and reached values such as 1.68 and 1.70 gr cm^{-3}, respectively, exceeding 1.60 gr cm^{-3}, which is considered to be the threshold value that adversely affects plant root growth (Taylor et al., 1966), even if some reports the threshold values range from 1.45 to 1.60 g cm^{-3} for a healthy root development and nutrient uptake, particularly in fine-textured soils. In the same orchard, in contrast to other soil depths, the lowest bulk density was found to be 1.53 g cm^{-3} at 70-80 cm, the reason for the low value at this depth was that cultural practices (tillage, fertilization, harvesting) were not affected by more than in the soil layer above this depth. On the contrary to flat orchard, bulk density in the sloping orchard was found slightly low through all soil depths (Table 2) and it not exceed the 1.22 gr cm^{-3}, except of 0-10 cm with 1.28 gr cm^{-3}, which may be due to no-field traffic with the farm equipment. The higher bulk density at 0-10 cm may be the result of the soil being chewed by walking on the surface during sheep grazing, especially in rainy periods. The gravimetric soil water content varied between 33 and 42% (Table 2) in the flat orchard whose soils were silty-loamy. The corresponding soil water content values for sloping orchard were lower, varying between 16 and 21%, and the reason for this low values may be due to the soil texture of the sandy-loam.

Table 2. Soil bulk density, soil total porosity and gravimetric soil water content at the different depths of soil in both olive orchards of the second year of study

	Depth (cm)	Bulk density (gr cm^{-3})	total porosity (%)	Water content (%)
Conventional-flat	0-10	1.57±0.01(0.93)	40.80±0.55(1.35)	41.84±6.12(14.62)
	10-20	1.58±0.05 (2.92)	40.35±1.74(4.32)	34.65±0.09(0.26)
	20-30	1.68±0.05(2.83)	36.77±1.79(4.86)	32.92±1.62(4.94)
	30-40	1.70±0.05(2.74)	35.83±1.76(4.91)	36.56±4.18(11.43)
	40-50	1.68±0.04(2.31)	36.75±1.46(3.98)	41.09±4.53(11.01)
	50-60	1.64±0.02(1.42)	38.26±0.88(2.29)	37.25±2.36(6.34)
	60-70	1.60±0.17(10.70)	39.59±6.46(16.32)	40.36±4.42(10.95)
	70-80	1.53±0.21(13.65)	42.24±7.88(18.66)	39.29±3.00(7.63)
Sustainable-sloping	0-10	1.28±0.01(0.18)	56.07±0.09(0.15)	20.60±0.07(0.33)
	10-20	1.13±0.06 (5.07)	57.43±2.16(3.76)	21.04±1.16(5.49)
	20-30	1.11±0.03(2.82)	58.27±0.18(2.02)	20.09±1.47(7.30)
	30-40	1.14±0.02(1.81)	56.85±0.78(1.37)	18.74±0.12(0.66)
	40-50	1.22±0.09(7.63)	54.08±3.50(6.48)	19.23±0.86(4.45)
	50-60	1.15±0.03(2.20)	56.55±0.96(1.69)	17.07±0.66(3.84)
	60-70	1.09±0.03(2.79)	59.00±0.14(1.94)	16.32±0.92(5.67)
	70-80	1.15±0.03(2.82)	56.43±1.23(2.18)	17.81±1.30(7.28)

Values are means±standard deviations of soil samples, and coefficient of variation as a percentage in parentheses.

An excess or deficient water during the active period of the olive tree may affect growth, yield, quality of oil, especially in the study area, water stress can occur in the period corresponding to summer and early autumn. Total porosity as a result of the low bulk density in sloping orchard was higher and varied between 54 and 59% in regardless of soil depths, conversely, it was lower in flat orchard, with a value varying between 35 and 42%. The total porosity values in both orchards remained above level reported by Glinński and Stepniewski (1985) who indicate that a total porosity of ≤40% often represent critical limits of soil aeration. They also pointed out that in sandy and sand-loamy soils with low content of fine material, the critical porosity is generally higher than 10% due to lack of stable and continuous macro pores.

Penetration resistance of the soil

In flat olive orchard, penetration resistance showed a progressive increase up to tillage depth of the 30 cm soil depth recording 2 MPa and then continued in the same way up to the depth of 50 cm (Table 3), but in soils deeper than 50 cm, it decreased slightly up to 80 cm.

On the other hand, penetration resistance in all soil depths of the sloping orchard did not exceed 2 MPa, which negatively affects plant root growth (Taylor et al., 1966), when the highest penetration resistance was measured between 28 and 50 cm, varying 1.61 to 1.77 MPa. In addition, the penetration resistance of the sloping orchard in all soil depths was found to be lower those in the flat orchard (Table 3). In both orchard, the decrease in deeper than 50 cm can be interpreted as the soil that not affected by the effects leading from cultural practices, especially in flat orchard soils or maintains in its natural structure as with sloping orchard soils. On the other hand, Glinński and Stepniewski (1985) indicate that penetration resistance of 2.5 MPa often represent critical limits of soil aeration and root growth.

Table 3. Soil penetration resistance (MPa) of conventional-flat and sustainable-sloping olive orchards at the different depths of soil in the second year of the study

Depth (cm)	Sustainable-sloping	Conventional-flat
4	0.67±0.18(28.96)	0.65±0.17(24.20)
8	1.03±0.25(23.05)	1.49±0.12(7.77)
12	1.31±0.52(39.01)	1.55±0.09(5.87)
16	1.49±.57(38.17)	1.54±0.11(7.23)
20	1.59±0.54(33.02)	1.45±0.35(24.80)
24	1.56±0.34(21.36)	1.60±0.47(29.62)
28	1.82±0.44(23.11)	1.81±0.47(10.87)
32	1.74±0.12(7.07)	2.00±0.22(10.87)
36	1.87±0.11(5.86)	2.09±0.31(14.67)
40	1.62±0.19(11.48)	2.06±0.27(13.34)
44	1.73±0.11(6.18)	2.03±0.25(12.31)
48	1.73±0.12(6.70)	2.05±0.25(12.07)
52	1.77±0.06(3.58)	2.05±0.22(10.62)
56	1.68±0.19(11.51)	1.98±0.26(13.00)
60	1.05±0.47(46.60)	1.86±0.33(17.62)
64	1.05±0.50(46.89)	1.80±0.42(23.43)
68	1.15±0.48(41.90)	1.68±0.40(23.40)
72	0.71±0.04(6.15)	1.82±0.33(18.39)
74	0.77±0.07(7.85)	1.76±0.33(18.97)
80	0.77±0.05(10.12)	1.72±0.18(10.37)

Values are means±standard deviations of soil measurements, and coefficient of variation as a percentage in parentheses.

Soil saturated hydraulic conductivity

K_{sat} was measured in only conventional-flat orchard soils of the second study year to evaluate the difference between 15 cm and 25 cm soil depths. K_{sat} was higher in 15 cm by 44.31±10.59 mm h^{-1} (mean±standart deviation) than in 25 cm by 29.49±19.69 mm h^{-1}, setting the former in the medium K_{sat} class in comparison to the conductivity classification of FAO (Allen et al., 1998), which gave conductivity classes and their respective values are low with 8.0-20.0, medium with 20.0-80.0 and high with 80.0-125.0 mm h^{-1}. K_{sat} were generally ideal for the both soil depths which have the values above to the very low limit of the ideal class (e.g. $18<K_{sat}<180$ mm h^{-1}) suggested by Di Prima et al. (2018). The high K_{sat} in 15 cm depth were probably due to the recent shallow tillage operations that might be have increased porosity of the surface soil. Nevertheless, K_{sat} measurements performed to evaluate the vertically confined water flux showed a reduction from 15 cm to 25 cm soil depth by 14.82 mm h^{-1}, confirming the presence of a plough depth, as also pronounced by the reduction of porosity from 40.35% to 35.83% below the 20 cm (Table 2). K_{sat} values also showed inversely relationship with the soil bulk density (Table 2) and PR (Table 2) were in correlation with the soil structural variables such as bulk density, organic matter than the textural (clay, silt, sand) (Di Prima et al., 2018). Palese et al. (2014) found that an increase of 50% in soil organic matter results in increasing hydraulic conductivity from 0.5 to almost 7 mm h^{-1}

Soil nutrients

N is an essential nutrient and frequently applied in mineral fertilizer for perennial plants. N fertilization for olive in optimal quantities results in more vigorous vegetative growth, proliferation of shoots that increase the potential yield of the following year. In the first year of the study, N was found 1108.80 and 952.50 mg kg^{-1} in CFO and SSO, respectively, and corresponding values for the second year were 1287.50 and 1056.50 mg kg^{-1} (Table 4). The values in both orchards are highly lower than the average level observed by Karyotis et al. (2014). This shows that there is no relationship between organic matter and N. On the contrary, Karyotis et al. (2014) reported a close relationship between the two, and showed the increase in organic matter directly increased N. Hence, N difference in both orchards within the scope of the study is thought to be due to mineral fertilization. For example, high N in CTO may have been the result of fertilization in comparing to the SSO. The reason for low N in the SSO may also be the result of soil conditions (slope, shallow soil depth, erosion) and fertility (degree of soil nutrients mobility, organic matter content of soil depths). The amount of P in both years was considerably increased and varied between 9.94 and 11.78 mg kg^{-1} in regardless of the orchards (Table 4), in fact, it is highly low 2.62 mg kg^{-1} in SSO, conversely higher 8.52 mg kg^{-1} in CFO. High P may be attributed to the results of cultural practices during the study years for both CFO and SSO; for example, vegetation and pruning material left on the soil surface in SSO. On other hand, the seasonal fertilization and other cultural practices (Özpınar, 2020), which are carried out on time and regularly, can improve the quality of the soil in CFO. In the first year, the highest CV of P for CFO was recorded and this may be attributed mainly to regularly annual phosphate fertilization, but the increment was higher in CFO than in SSO that might be due to the presence of very high content in one sample (e.g. 11.92 P mg kg^{-1}). Considering the P in both orchards and all soil depths in Table 4, it was found to be within normal ranges (8-25 mg kg^{-1}) for both study years (Karyotis et al., 2014) when others reported the optimal level as 20 mg kg^{-1} (Recald, 1975). Karyotis et al. (2014) found higher P of 23 mg kg^{-1} for olive in Greece than in this study, while others recorded P of 16 mg kg^{-1} within medium range in Eygpt (Shaaban et al., 2016). According to reporting of Jug and Vesel (2015), the optimal value of P varies 13-25 mg kg^{-1} and they recorded lower P than the optimal level in many locations of olive soils in Slovenian. Karyotis et al. (2014) concluded that it needs the fertilizer application, although P requirement in olive is not as high as N and K. K is another essential element for olive and its deficiency is relatively common, especially under dry or rainfed conditions because low soil moisture restricts the spread of the K trough the soil. In contrast, it becomes more available to the olives under conditions of water availability (Zipori et al., 2015). They highlighted that the main nutritional disorder in the olive can be caused by the lack of K, especially in the soils under rainfed or non-irrigated lands. Therefore, regular K application to eliminate K-deficiency is recommended with annual amounts of 50-100 gr $tree^{-1}$ or 300 kg ha^{-1} at the optimal soil moisture content. From the first year of the study, K ranged from 7.33 mg kg^{-1} in very low of SSO to 9.02 mg kg^{-1} in low level of CFO, when corresponding values for the second year were 8.53 and 7.72 mg kg^{-1} (Table 4). K of both years remained below the threshold level, while sufficient value is between 140-370 mg kg^{-1} pointed out by Sumner and Miller (1996). Pošćić et al. (2018) reported similar K, which varies between 6 and 10 mg kg^{-1} under different olive growing conditions, while Jug and Vesel (2015) found higher K in rainfed karstic soils than in the soils of the study. In addition, higher bulk density in CFO, especially in deep soil (30-40 cm, 1.70 g cm^{-3}) (Table 2), may be due to decreasing K with compared to SSO (1.24 g cm^{-3}) which were consistent with Lipiec and Stepniewski (1995). They recommended that the K-uptake commonly decreases by increasing the compaction of soil. Compacted soils have K-fixing (Brady and Weil, 2002) and may restrict root uptake during the growth period. Deficiency of K in the soils of both CFO and SSO may be the lowest threshold K which is directly proportional to pH. The another reason that it may be responsible with no-K fertilization and dry-conditions throughout growing season, especially under SSO. Similar to K, Mg was very low in CFO and SSO (Table 4) compared with sufficient value of 160-480 mg kg^{-1} (Summer and Miller, 1996). Ca ranged from 8.61 to 9.29 mg kg^{-1} in regardless of years and orchards, and it was quite below the threshold level reported by Sumner and Miller (1996). Pošćić et al. (2018) was found similar Ca of 9 mg kg^{-1} for olive trees grown in limestone soils in the island of Brač (Croatia). They indicated that Ca-deficiency are expected in acidic soils, in contrast, lime soils with excess Ca reduce prevents the intake of micro elements such as Mg, Fe and Zn, even K. Under saline conditions, K and Na opposition is common (Pošćić et al., 2018). Therefore, K uptake decreases when environmental Na concentration increases. Na recorded below 2 mg kg^{-1} in both orchards of both study years, which are lower than threshold level of 20 mg kg^{-1} (El-Fouly et al., 2014).

The olive tree requires small amounts of micro nutrients (Zn, B, Cu, Mn, Fe, Ni) for the growth and fruiting and they vary according to soil lime and alkaline (Buttafuoco et al., 2016). Zn was higher in CFO of 86.19 ng kg^{-1} than in SSO of 77.67 ng kg^{-1} in the first year, on the contrary, it was higher in SSO of 103.60 mg kg^{-1} than in CFO of 94.85 mg kg^{-1} in the second year (Table 5). Zn was found to be in the tolerable levels in agreement with Buttafuoco et al. (2016) who recorded 83.30 ng kg^{-1} under rainfed Mediterranean environments. Considering the Zn values of the two study years, there is no relationship between pH and Zn availability. On the contrary, Sahaaban et al. (2016) recorded a negative correlation between pH and Zn. They highlighted that Zn tends to be less available at 7.50 pH or above and light prone soils.

Table 4 Macro nutrients (mg kg^{-1}) from the topsoil (0-30 cm) of conventional-flat (CFO)and sustainable-sloping orchards (SSO) in the first and second year of the study

Nutrient	First year		Second year	
	CFO	SSO	CFO	SSO
N	1108.8±119.2(10.75)	952.5±47.5(4.99)	1287.5±158.5(12.31)	1056.5±88.5(8.38)
P	11.78±0.12(1.01)	11.77±0.03(0.25)	11.33±0.03(0.31)	9.94±0.15(1.52)
K	9.02±0.09(0.95)	7.33±0.02(0.34)	7.72±0.07(0.86)	8.53±0.03(0.32)
Mg	3.43±0.03(0.83)	3.2±0.03(1.04)	3.35±0.02(0.49)	3.08±0.01(0.30)
Ca	9.29±0.04(0.41)	9.23±0.08(0.91)	8.67±0.02(0.27)	8.61±0.01(0.08)
Na	1.71±0.01(0.69)	1.24±0.02(1.51)	1.82±0.01(0.62)	1.4±0.04(0.28)

Values are means±standard deviations of soil measurements, and coefficient of variation as a percentage in parentheses.

On the other hand, Karyotis et al. (2014) highlighted that the deficiency of Zn was observed generally in calcareous soils. The soil of both study years and orchards shows that Zn is at normal levels (Table 5). B plays an important role in growth behaviour and productivity of trees by formation of cell wall, elongation of pollen tube and participation of the metabolism and transport of sugars. The deficiency of B usually is lower in olives spread in areas with slopping and shallow (Tsadilas et al., 1994). They pointed out that the amount of B with a value higher than 0.33 mg kg^{-1} of soil was found sufficient for the olive tree (Tsadilas et al., 1994). Considering threshold values, the B in the study in regardless of the orchards and years was found to be very low ranging from 24.70 to 28.19 ng kg^{-1} as compared to sufficient level pointed out by Tsadilas et al. (1994). The low B in the soil of both orchards is due to no-boron fertilizer application, therefore, whose deficiency may be reduced through boron application as a fertilizer in borax or boric acid to the soil or forage. On the other hand, others recorded very high B of 12.99 mg kg^{-1} in a southern Italy in a rainfed olive orchard (Buttafuoco et al., 2016). Cu plays a key role in many physiological processes of plants by influencing the metabolism of nitrogen and carbohydrates. It was varied from 39.45 ng kg^{-1} in SSO of the first year to 46.93 ng kg^{-1} in CFO of the second year and lower than others with 19.31 mg kg^{-1} (Buttafuoco et al., 2016). They pointed out that Cu deficiency is generally occurred in coarse texture and extremely calcareous soils, therefore, sandy SSO soils had lower Cu than loamy-sandy CFO soils (Table 4). The results of the study showed that the amount of soil Cu is lower than that is required by the olive trees by 0.20 mg kg^{-1} or above, and it was highly lower than the permissible ranges of Cu≤100 mg kg^{-1} (El-Fouly et al., 2014). Low Cu in the studied soils may be due to no-containing Cu application used in olive cultivation of two orchards because it is known that agricultural activities may prove to be have effect on the accumulation of copper in the soils. Fe supplies chlorophyll synthesis while Mn is necessary in the photosynthesis process. Tolerable contents of Fe and Mn based on information from Buttafuoco et al. (2016) were Fe≤50000 mg kg^{-1} and Mn≤3000 mg kg^{-1}. According to those critical ranges, Fe and Mn values were found to be very low in both olive orchards (Table 5). Ni is 151.30 and 179.50 ng kg^{-1} in CFO and 150.40 and 159.20 ng kg^{-1} in SSO in the first and second years (Table 5), respectively, and all the values Ni were lower than the normal ranges of Ni≤50 mg kg^{-1}. All micro nutrients are found slightly high in CFO compared with SSO. On the other hand, most researcher concluded that micronutrients are low in olive orchards which are grown on calcareous soils, with a pH higher than 7.0 (Marschner, 2011). According to the critical ranges of Pb and Co (≤100 and Co≤50 mg kg^{-1}, respectively), the value of those were found to be within normal ranges, Cr and Cd (≤100 and ≤3 mg kg^{-1}, respectively) were higher than the permissible ranges in the soils.

Table 5 Micro nutrients from the topsoil (0-30 cm) of conventional-flat (CFO)and sustainable-sloping (SSO) orchards in the first and second year of the study

Nutrient	First year		Second year	
	CFO	SSO	CFO	SSO
Zn (ng kg^{-1})	86.19±0.39(0.45)	77.67±0.10(0.13)	94.85±0.58(0.61)	103.60±0.31(0.29)
B (ng kg^{-1})	28.19±0.04(0.12)	24.70±0.28(1.13)	27.69±0.23(0.82)	26.39±0.133(0.50)
Cu (ng kg^{-1})	40.32±0.27(0.67)	()39.45±0.21(0.53)	46.93±0.25(0.53)	40.66±0.322(0.79)
Mn (ng kg^{-1})	929.8±3.19(0.34)	924.20±4.91(0.53)	929.70±6.67(0.72)	817.40±2.85(0.35)
Fe (ng kg^{-1})	327.9±1.27(0.39)	301.40±1.40(0.46)	311.60±1.44(0.46)	285.00±2.63(0.92)
Ni (ng kg^{-1})	151.3±0.71(0.47)	150.40±0.35(0.23)	179.50±0.32(0.18)	159.20±4.13(0.74)
Pb (mg kg^{-1})	30.62±1.41(4.61)	31.43±1.12(0.65)	32.48±0.26(0.80)	29.53±0.68(2.31)
Co (mg kg^{-1})	25.01±0.16(0.64)	24.91±0.31(1.25)	26.27±0.05(0.20)	23.21±0.16(0.70)
Cd (mg kg^{-1})	9.95±0.10(1.01)	9.82±0.04(0.42)	10.70±0.01(0.14)	10.11±0.03(0.28)
Cr (mg kg^{-1})	160.8±0.31(0.19)	135.00±0.53(0.39)	166.50±0.78(0.78)	242.10±3.99(1.65)

Values are means±standard deviations of soil measurements, and coefficient of variation as a percentage in parentheses.

Soil carbon and nitrogen ratio

Soil organic carbon and total nitrogen play an important role in terms of sustainable soil quality, plant cultivation and environmental effects. They affected by many factors, such as climatic conditions (temperature, moisture), soil properties (texture, pH), and land use and management practices (Lozano-García et al., 2017). At the same time, soil C:N ratio is a sensitive indicator of soil quality and shows how much nitrogen will be mineralized compared to the carbon dioxide gas released by microorganisms. On the other hand, it has been reported (Gómez et al., 2009) that the ratio between carbon and nitrogen in agricultural soils generally varies between 8 and 17, and this ratio is an important productivity index for determining soil quality. The ratio also has an important effect on the soil accumulation of plant nutrients and pH, especially the elements such as P, Mg, Na and Mn increased their usefulness to the plant. In this study, soil organic carbon was found relatively high in flat orchard soils through the soil depths, especially at 0-40 cm, which may be attributed to using mineral fertilizer (Table 6). Meanwhile, the sloping orchard soils presented relatively low values at the same depths. In contrast, several previous studies have shown that tillage in olive orchards caused the loss of soil organic carbon because of the large rate of mineralization that results from crushed soil aggregates, especially in the Mediterranean conditions (Gómez et al., 2009). Similar to organic carbon, total nitrogen also exhibited slightly differences in the top soil areas in terms of olive orchard. The highest total nitrogen values were recorded in flat orchard soils, especially at 0-10 cm soil depth, because of nitrogen fertilizing, on the contrary, it shows slightly lower in sloping. On the other hand, total nitrogen increased with depth under the soils of flat orchard while sloping orchard showed higher nitrogen in the soil between 20-50 cm. Considering both orchards, C:N ratio was generally considered to be over optimal range for agricultural soils, varying from 23.15 to 33.67 in regardless of orchard and soil depths (Table 6), while the ratio with 25 or less is sufficient for olive tree (Gómez et al., 2009). Gómez et al. (2009) stated that the ratio above 30 causes immobilization of inorganic nitrogen by decomposing microbial biomass and plants suffer from nitrogen-deficiency. The ratio of C:N found that there is no difference between sloping and flat orchard soils while the result of both soils ratio and its variation are greatly depended on gains (input of soil organic carbon and total nitrogen) and losses. The reason for the lack of difference in carbon, nitrogen and the ratio of both between the two orchards may have been due to the fact that they have been under similar cultural practices for long-term since establishing, except for the last few years during the experimental period. In contrast, others found that the cultivation with no-tillage can increase soil C:N ratio as compared to the use of traditional plough tillage (Lozano-García et al., 2017) because of maintenance of plant residues at the soil surface, which may have beneficial impacts on soil fertility and increase the carbon and nitrogen ratio of the soil. Additionally, they also indicated that tillage disrupted soil environment and caused loss of soil carbon and nitrogen. Similar results were reported by Gómez et al. (2009) who found around 26 the ratio of carbon and nitrogen in topsoil of conventional tillage, they found the ratio even lower with a value of 21 in no-tillage orchard.

Table 6. Soil organic carbon, total nitrogen and C:N ratio of the conventional-flat (CFO) and the sustainable-sloping (SSO) olive orchards in the second year of study

Depth	N (%)		Depth	C (%)		Depth	C:N	
(cm)	SSO	CFO	(cm)	SSO	CFO	(cm)	SSO	CFO
0-10	10	14	0-10	281	326	0-10	28.1	23.29
10-20	12	9	10-20	297	303	10-20	24.8	33.67
20-30	11	10	20-30	308	335	20-30	28.0	33.50
30-40	13	10	30-40	319	279	30-40	24.5	27.90
40-50	12	12	40-50	305	335	40-50	25.4	27.92
50-60	10	13	50-60	299	301	50-60	29.9	23.15
60-70	10	13	60-70	299	333	60-70	29.9	25.62
70-80	11	11	70-80	326	333	70-80	29.6	30.27

CONCLUSIONS

In study orchards receiving different cultural practices, the nutrients are also recorded slightly different level. For example, N was higher in flat orchard than in sloping, but it was below threshold level in both. P was found to be sufficient, within the normal ranges, but not for K, which remained below the threshold level, and even a slight K-deficiency may have been observed in both orchards. Similarly, all other remaining micro and macro nutrients were found to be below acceptable limit values, except Zn and B, which are in tolerable levels, despite no-boron and zinc fertilizer application. Tillage of flat orchard has been shown to increase bulk density and penetration resistance, especially in tillage depth, both of them have increased above the limit values for plant root growth, in contrast, they were low in sloping orchard, might be attributed to no-receiving any machinery traffic.

In the last decades in the study region, the trees in the wide row-spaced orchards are gradually removed and replaced by new densely cultivated orchards, particularly in flat and fertile soils, where mechanization can be easily implemented due to concerns of higher yield in the unit area and the current regulations such as subsidies for diesel, seedling, fertilizer, etc. This situation, which is seen as a good regulation for the region farmers, is also expected to be an important source of income when it continues to cultivate with appropriate agricultural practices, including mechanization and without soil organic matter depletion and structure degradation. On the other hand, in rainfed hilly-steep sloping orchard with typically involved low density trees with low productivity on marginal soils, the use of cover crop or native vegetation can play a multifunctional role including the reducing of raindrop-impact-induced erosion, the providing autumn-winter rainwater storage in soil, the improving soil structure, the excluding competition for the use of resources with trees, the regulating of pests, weed control in the winter period.

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FORTIFIED CAKE WITH POMEGRANATE AND ORANGE PEELS POWDER AS A FUNCTIONAL PRODUCT

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ABSTRACT
Fruits peels as a by-product of the juice processing industry contains a range of valuable bioactive and nutritional compounds. It's application as a food supplement leads to enhance the nutritional value, health, physicochemical and sensory properties of final products. The present study aims to evaluate physicochemical and sensory properties of sponge cake supplemented with pomegranate and orange peels powders (PEG and PEO) as partially substituted of flour (5 %) during 14 days of storage at diffrent temperature. The prepared cakes show an enhancement of texture properties, specific volume, moisture and color parameters compared to sample control. In addition, the sensory evaluation reveals that the substitution with 5% (PEG+PEO) improve the appreciation of cake sensory characteristics: color, odor, taste and aftertaste. The peroxide value in cakes stored at room temperatures for 14 days of storage under room temperature decreased with increasing fruits peels powder levels. The results also show the sponge cakes with PEG and PEO supplementation had good antioxidant activity compared with the control. This can suggest that the shelf-life of these cakes could be extended as a consequence.

Keywords: Cake, Pomegranate Peel, Orange Peel, Polyphenols, Oxidation, Antioxidant Activity

INTRODUCTION

Cake is one of the most popular bakery products consumed nearly by all levels of society. According to Gustavsson et al. (2011), about 30% of cereal products, are lost each year. A longer shelf life can reduce these losses as well as the economic and environmental impacts of the distribution logistics.

Lipid oxidation is a major factor affecting the sensory properties and shelf life of sponge cakes. Several studies have been investigated to improve their quality. The utilization of by-products of fruits, especially pomegranate and orange, as potential source of natural antioxidants has become a trend as of late and many studies has evaluated their effects on food properties (Çam et al., 2013, Ismail et al., 2014, Hanıeh et al., 2015, Rani et al., 2021, Terzioğlu et al., 2021).

Therefore, the present study aims to evaluate physicochemical and sensory properties of sponge cake supplemented with pomegranate and orange peels powders. The effects of this replacement on lipid oxidation and antioxidant activity were also investigated during storage at different temperature.

MATERIAL AND METHOD

Pomegranate and orange were manually selected, washed, and peeled. Then, the material was oven-dried at 40 °C for 48 h, ground in a mill, and reduced to a fine powder (Φ=200µm). The obtained pomegranate and orange peels powders (PEG and PEO, respectively) were stored in a refrigerator until analyses.

Wheat flour, fresh whole eggs, sunflower oil, sugar, whole milk powder and baking powder were purchased from the local market.

The functional properties of flours have concerned the determination of the oil-adsorption capacity (OAC) and Water holding capacity (WHC) (Femenia et al.,1997, Sudha et al., 2007)

The formula of sponge cake was adopted from the work of Lee et al. (2008) with little modifications. The 4 different formulations of cakes samples were presented in Table 1. Sponge cakes were allowed to cool at room temperature. They were carefully taken out of the pans; placed in plastic boxes and stored at three different temperature (30; 45 and -18 °C). Peroxide value (Wu et al., 2013) and total antioxidant activity (Uçar and Hayta, 2018) were evaluated weekly for 14 days of storage.

Table 1. Cake formulation

#	Ingredients (%)	Control	C-PEG	C-PEO	C-(PEG+PEO)
1	Cake flour	36	34.2	34.2	34.2
2	PEG	0	1.8	1.8	0.9
3	PEO	0	0	0	0.9
4	Egg	4	4	4	4
5	Sugar	22	22	22	22
6	Milk powder	2	2	2	2
7	Water	14	14	14	14
8	Sunflower Oil	20	20	20	20
9	Baking powder	2	2	2	2

Cake quality attributes included: moisture (AACC, 2000) and specific volume (Alshimaa, 2012). Crumb and crust colors were measured using a Minolta Chroma Meter CR-300. Texture profile analysis was performed using at texture analyzer (Perten) according to the work of Uçar and Hayta (2018) with some modifications.

Sensory evaluation was performed by untrained panelists. The panelists were asked to evaluate each cake for appearance, crust color, odor, crumb texture, taste, aftertaste and overall acceptability. A 7-point scale was used where 7 “Like extremely” and 1 “Reject”.

All experiments were repeated three times with duplicate samples. Data were processed with the Statistical Analysis System for a one-way analysis of variance and Duncan’s multiple range test was used to determine whether significant difference ($P< 0.05$) existed between mean values.

RESULTS AND DISCUSSION

Results of WHC and OAC determination are summarized in Table 2. The WHC of the tested fruit peels powders are about 39.86 and 20.51 for PEG and PEO, respectively. A difference in fiber content could explain this difference in WHC values. According to Boulos et al. (2000), fibers have a high water retention capacity representing the hydrophilic constituents.

For the Oil Absorption Capacity, no significant difference was observed. These results are agreement with those obtained by Thebaudin et al. (1997). Non-lipid constituents such as proteins and carbohydrates are responsible for oil retention of these powders (Aditya et al., 2015).

Table 2. Functional properties of pomegranate and orange peels powder compared to refined wheat flour as control

#	Samples	WHC(g water/g dw)	OAC(g oil/g dw)
1	PEG	39.86 ± 0.01^{c}	37.32 ± 0.01^{b}
2	PEO	20.51 ± 0.01^{b}	37.52 ± 0.01^{b}
3	Refined white flour	1.00 ± 0.002^{a}	2.05 ± 0.09^{a}

Each value is the mean of 3 independent trials ± S.D. Data sharing different letter are significantly different at $p < 0.05$.

Cake characteristics with added pomegranate and orange peels powder are shown in Table 3. The physical properties of the different cake samples were measured after 24 hours after baking. The moisture content of cakes ranged between 17.20 and 21.60 % (Table 3) and it increases with the addition of fruit peels powder. The highest value has been observed for the cake prepared with 5% PEG. Whereas, this increase is not significant for cakes prepared with 5% of the powder mixture (PEG+PEO).

The results of the specific volume show a significant increase related to the addition of fruit peel powders. Quiles et al. (2018) indicate that the increase of specific volume may be due to the replacement of flour with cellulose, which has been reported to weaken the gluten matrix responsible for retaining gases in baked foods.

Color measurements were expressed by Hunter L, a, and b values corresponding to lightness, redness, and yellowness, respectively. All data were conducted on crust and crumb of cake samples (Table 3). The crust color of samples was affected by the replacement of cake flour with fruit peel powders. In general, as pomegranate powder added the crust color become darker. The crust of the control was lighter and more yellow than any of the other cakes followed by the sample incorporated with PEO (5%).

For crumb color, the replacement of the flour with 5% of PEG induce a variation in “L” and “a” parameters indicating that a darker, redder and less yellow crumb was obtained compare to the control. It was observed that cakes elaborated with orange peel powder have colors characteristics the most similar to the control. The color change of baked cakes might be related to the fact that fruits peels pigments and polyphenols compounds underwent oxidation reaction, and sucrose also participated in caramelization during baking (Goranova et al., 2019).

Table 3. Physical characteristics of sponge cakes prepared with pomegranate and orange peels powder partially substitution for cake flour.

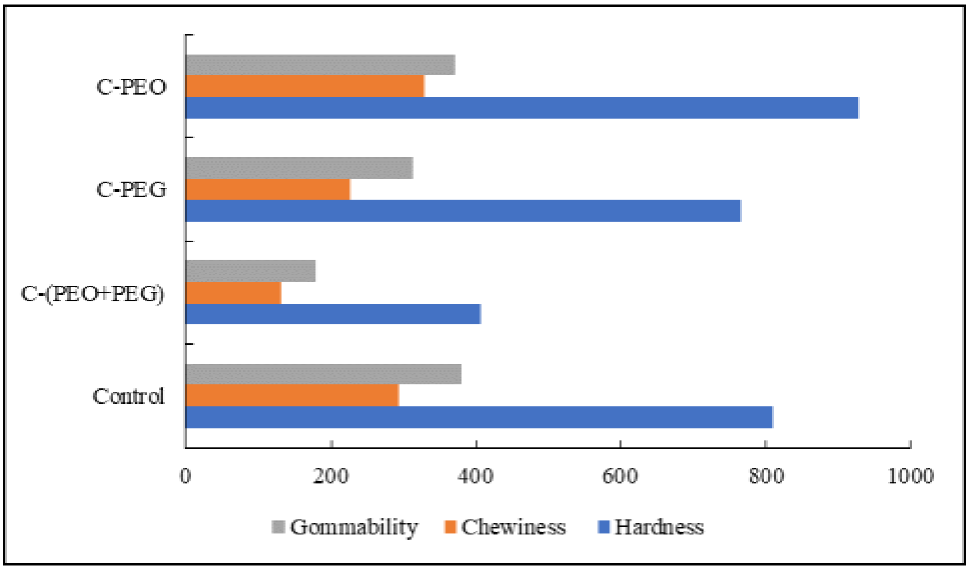
#	Samples	Control	C-PEG	C-PEO	C-(PEG+PEO)
1	Moisture (%)	17.20± 1.50^a	21.60± 1.00^b	20.56± 0.90^b	19.86± 1.00^a
2	Specific Volume (g/cm^3)	0.65± 0.03^a	0.88± 0.10^b	0.78 ± 0.08^b	0.79± 0.10^b
3	Crust color				
	L	49.98 ± 0.01^d	46.21± 0.04^a	48.52± 0.02^c	47.97± 0.03^b
	a	15.79 ± 0.03^c	12.55 ± 0.08^b	15.83 ± 0.10^c	11.92 ± 0.07^a
	b	42.92 ± 0.05^d	33.51 ± 0.13^b	40.97 ± 0.02^c	32.55 ± 0.09^a
4	Crum color				
	L	69.16 ± 0.04^d	47.93± 0.06^a	67.37± 0.06^c	54.67± 0.06^b
	a	-1.68± 0.05^a	5.85± 0.18^c	1.66± 0.04^b	5.77± 0.03^c
	b	28.35± 0.03^c	25.61± 0.22^a	28.30± 0.06^c	26.46± 0.05^b

Each value is the mean of 3 independent trials ± S.D. Data sharing different letter are significantly different at $p < 0.05$.

Figure 1 illustrates the TPA results of cakes examined in the 24 h after baking. Hardness is the ability of the sample to resist deformation due to stress, a significant decrease in hardness was noted for the sample supplemented with 5% (PEO+ PEG).

The results of cohesiviness show a slight decrease for the cakes enriched with fruits peel powder compared to the control cake. This indicates that the cakes formulated with the peel powders have a capacity to resist before the deformation teeth. A similar trend was also observed in cheonnyuncho powder added to cake (Kim et al., 2012).

The addition of PEG and PEO in the cakes have significantly increased adhesiveness and resilience compared to the control cake. No notable differences in gummability and chewiness were observed for the studied samples.



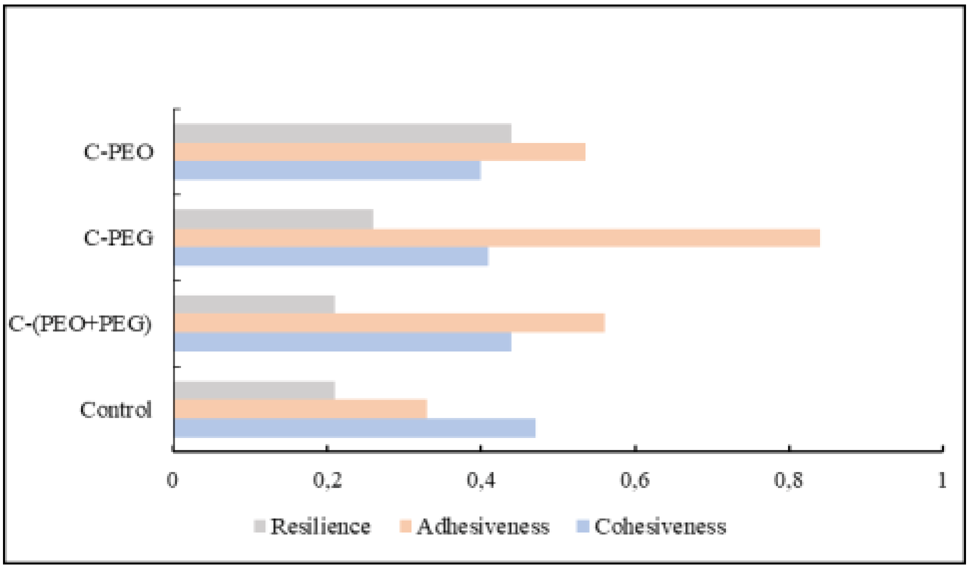


Figure 1. TPA profile of the sponge cakes

The results of the sensory evaluation given in Figure 2 showed that there were significant differences for the liking scores of all sensorial characteristics (appearance, crust color, odor, taste, aftertaste, crumb texture and over acceptability) between control and sponge cakes elaborated with PEG and PEO. The addition of PEG (2.5 or 5%) decrease the color appreciation scores. This suggests that dark color induce a consumer depreciation of the cake. However, the substitution of 5%(PEG+PEO) improve the appreciation of the odor, taste and aftertaste. An et al. (2016) suggests that the addition of pomegranate bark powder reduced the undesirable taste of eggs. The cake prepared with 5% PEO have the most appreciate crust color and crumb texture. In addition, the overall acceptability of cake ranged between 5.1 and 6.2 indicating that cakes prepared with fruit peels powders are acceptable. These scores are in agreement with previous studies (Lu et al., 2010, Mau et al., 2017).

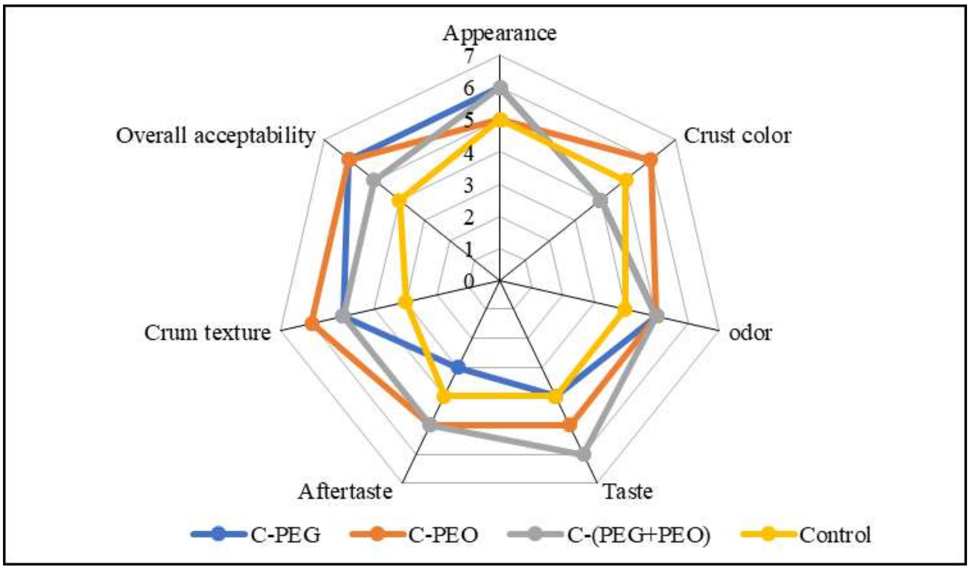


Figure 2. Sensory evaluation of sponge cakes prepared with pomegranate and orange peel powders replacement for cake flour

According to Table 6 the total antioxidant activity on the free radical DPPH of the control cake is lower than that of cakes prepared with the fruit peel powders. This implies that the polyphenolic compounds could contribute to the radical scavenging activity. The cake prepared with 5% PEG have the highest percent of inhibition during storage at different temperatures.

Table 4. The percent DPPH radicals Inhibition (%)

Storage Temperature	Storage time (day)	C-PEG	C-PEO	C-(PEG+PEO)	Control
	J0	67.00±0.001	49.40±0.001	37.40±0.001	30.00±0.001
30°C	J7	50.00±0.001	45.64±0.001	28.89±0.001	25.00±0.001
	J14	47.75±0.001	43.50±0.001	28.00±0.001	21.75±0.001
45°C	J7	44.00±0.001	40.00±0.001	25.00±0.001	17.80±0.001
	J14	40.80±0.001	35.50±0.001	22.60±0.001	15.20±0.001
-18°C	J7	60.00±0.001	48.40±0.001	34.60±0.001	27.75±0.001
	J14	50.40±0.001	47.75±0.001	31.75±0.001	20.33±0.001

Chang et al. (2006) investigated the effect of storage temperature on phenolics stability in hawthorn fruits and found that phenolic compounds were stable at 4°C, but they were unstable at temperature above 40°C. In addition, phenolic stability was reported to decrease at 4°C, 23°C and 40°C after 6 months of storage in hawthorn drink. An Other study reported on green tea extract (Lu et al., 2010) showed an increase of antioxidant activity of cakes prepared with extract. Uçar and Hayta (2018) was identified that after 14 day of storage the antioxidant activity of all cake samples decreased.

The peroxide value (PV) was employed for determining the formation of lipid oxidation products during storage of the cakes. The effects of fruit peel powders on changes in PV of lipids are shown in Table 5. During the storage period, the PV was evidently higher in control samples than those of supplemented samples. This result indicated that lipid oxidation in sponge cakes could be inhibited by the use of pomegranate and orange peel powders, probably due to its antioxidant activity. This result is consistent with pomegranate and orange peels having high antioxidative and radical-scavenging activity (Kandylis and Kokkinomagoulos, 2020, Rani et al., 2020). In general, PV values were less than 20 Meq/Kg. Indeed, the food product having over 20 Meq/Kg will be considered rancid and unacceptable to consume (Alshimaa 2012).

Table 5. Peroxide value (Meq/Kg) of sponge cakes during storage at different temperature

Storage Tempeture	Storage time (day)	C-PEG	C-PEO	C-(PEG+PEO)	Control
	J0	1.50±0.001	2.00±0.001	1.86±0.001	3.01±0.001
30°C	J7	2.00±0.001	2.93±0.001	2.06±0.001	3.60±0.001
	J14	3.00±0.001	3.50±0.001	2.50±0.001	5.00±0.001
45°C	J7	7.50±0.001	9.01±0.001	5.00±0.001	10.00±0.001
	J14	9.00±0.001	10.00±0.001	6.55±0.001	13.00±0.001
-18°C	J7	1.75±0.001	2.70±0.001	1.80±0.001	3.88±0.001
	J14	2.00±0.001	3.02±0.001	2.10±0.001	4.00±0.001

CONCLUSIONS

The result of the present work revealed that fruits peel such as pomegranate and orange can be used in bakery products to improve functional, physical and sensorial properties. Moreover, the studied peel powders can extend the shelf life of sponge cakes by retarding the lipid oxidation. Although sponge cakes are exposed to high temperatures during storage, cake samples are able to maintain DPPH activity. The findings of this research implied that fruits peel in powdered form can be considered as functional ingredients to provide functional improvements in bakery products.

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EVALUATION OF SOILWAT MODEL FOR SOIL PHYSICAL PROPERTIES IN THE DIFFERENT LAND USE

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Abstract

In sustainable soil management, it is necessary to determine the physical properties of the soil and to provide ideal conditions. Aeration, root development, water, and nutrient movements are closely related to soil physical properties. In this study, the estimation accuracy of the SOILWAT model (version 6.1.52) in the Soil-Plant-Air-Water (SPAW) program developed by the USDA Agricultural Research Service was calculated for different land-use types (dry farming, irrigated farming, pasture) in the territory of Isparta-Güneykent town. Observed and predicted values of saturation (ST), field capacity (FC), wilting point (WP), available water content (AWC), and bulk density (BD) were compared by using sand, clay, organic matter, EC, and penetration resistance values of the soils. In the study, it was determined that there were no significant changes in the predictive accuracy of the model according to different land-use situations. The mean absolute error (MAE) values for the examined properties were determined as 4.93%, 5.27%, 3.83%, 3.53% and 0.13 g cm^{-3}, respectively. The R^2 values obtained in the comparison of the linear relationship between the actual and predicted values of the field capacity and wilting point properties were found to be 0.54 and 0.68, and the R^2 values for the other properties were obtained quite low. The lowest mean absolute percent error (MAPE) was obtained in the bulk density estimation (9.74%). The highest error rate was determined with 27.26 % in the available water content of the soils. The RMSE values obtained as a result of the estimation of the field capacity and wilting point of the soils were found as 6.23% and 4.51 %. As a result of the study, it has been revealed that the SOILWAT model can be used successfully in the prediction of ST, FC, WP, and BD properties of soils under different land use, and the error rate in the estimation of AWC is higher than other properties.

Keywords: SPAW, moisture constants, soil physical properties

1.Introduction

Optimum efficiency needs to ensure the appropriate air and water balance, as well as the presence of plant nutrients in the soil in sufficient quantities, For plants to grow in an ideal environment. Ensuring this balance is closely related to the physical properties of the soil. Soil physical properties are quality indicators that directly and indirectly affect plant yield and yield components (Şenol et al., 2020). The physical properties of soils such as water content, air-filled porosity, temperature, and penetration resistance directly affect plant growth, while other properties such as bulk density, texture, aggregate stability, and pore size distribution affect indirectly (Letey, 1958). The productivity ability of the soil, which is a plant growth environment, is closely related not only to its nutrient content but also to its physical properties. In addition to the necessity of irrigation, it is also a necessity to know the amount of water to be applied for optimum efficiency in plant production. Therefore, it is very important to determine the field capacity and wilting point constants of the soils. Accurate estimation of soil moisture status and scheduling of irrigation will have an important place in drought management. To evaluate how much of the water given in irrigation programming will wet how many cm of the soil or how much will move away from the root zone, it is necessary to know the amount of soil moisture held at certain tensions.

Determining some soil properties is time-consuming, difficult, and costly. In recent years, prediction model studies on soil properties have attracted the attention of researchers. Pedotransfer functions (PTFs) are defined as mathematical models created for the prediction of soil properties measured by laborious, time-consuming, and expensive methods, usually using easily measured soil properties (McBratney et al. 2002; Pachepsky and Van Genuchten, 2011). Many parametric and point models have been developed to predict soil physical properties (Alaboz and Işıldar, 2019). One of them is the Soil-Plant-Air-Water (SPAW) Soil Water Characteristics Program, the SOILWAT model has been used for many years. The Soil and Water Assessment tool (SOILWAT) is a modeling software package for analyzing water, soil, agriculture, and nutrient interactions in watershed modeling. A soil-water (SOILWAT) model that can simulate soil structure, soil hydrological properties helps provide an important dataset to better understand our soils for better soil management. These data are highly effective requirements by decision-makers aiming to achieve optimum results. In the SOILWAT model, predictions are made through the equations developed by Saxton and Rawls, (2006).

It has been stated that the bulk density of sandy soils can be successfully estimated with the SOILWAT model, while the predictive power of other features is weak (Aliku and Oshunsanya, 2016). In the estimation of Saturated hydraulic conductivity with the SOILWAT model, R^{2}' was determined as 0.92 and RMSE was determined as 0.03, and the estimation accuracy was found to be high (As et al, 2019). Different estimation accuracies were obtained in studies in which the SPAW model was evaluated. The validity of the models created is not successful for every soil feature. More accurate estimations are made especially in soil properties, which are similar to the data set that created the model (Alaboz and Işıldar, 2019). For this reason, after examining the suitability of the program and model according to regions and different soil groups, it becomes necessary to use it.

This study; It is aimed to evaluate the estimation of some soil physical properties with the SPAW model of soils in different land-use types (dry farming, irrigated farming, and pasture) in Güneykent town of Isparta province.

2. Material and Method

2.1. Studying area

The study area is located within the borders of Güneykent Town of Gönen District of Isparta Province in the Western Mediterranean Region. Güneykent town is located in the west of the district, 20 km away from the Gönen district center. Its location is between the coordinates X: 265733-277030, Y: 4198561-4212827 (UTM WGS 1984 Zone 36N). According to Corine (2018) land classification, the land use within the boundaries of the study area is forest and semi-natural areas 85.21% (8415.49 ha), agricultural areas 13.89% (1371.88 ha), water bodies 0.29% (28.86 ha), and artificial areas 0.61% (It covers an area of 59.83 ha). Isparta oil rose cultivation is widely practiced in the district. Fruit and vegetables are grown in irrigated lands. Dry farming is practiced in areas where irrigation is not possible. Sampling was made from 47 points with different textures in the study area (Figure 1). The land uses of the sampling points are dry farming (16 points), irrigated farming (18 points), and pasture (13 points).

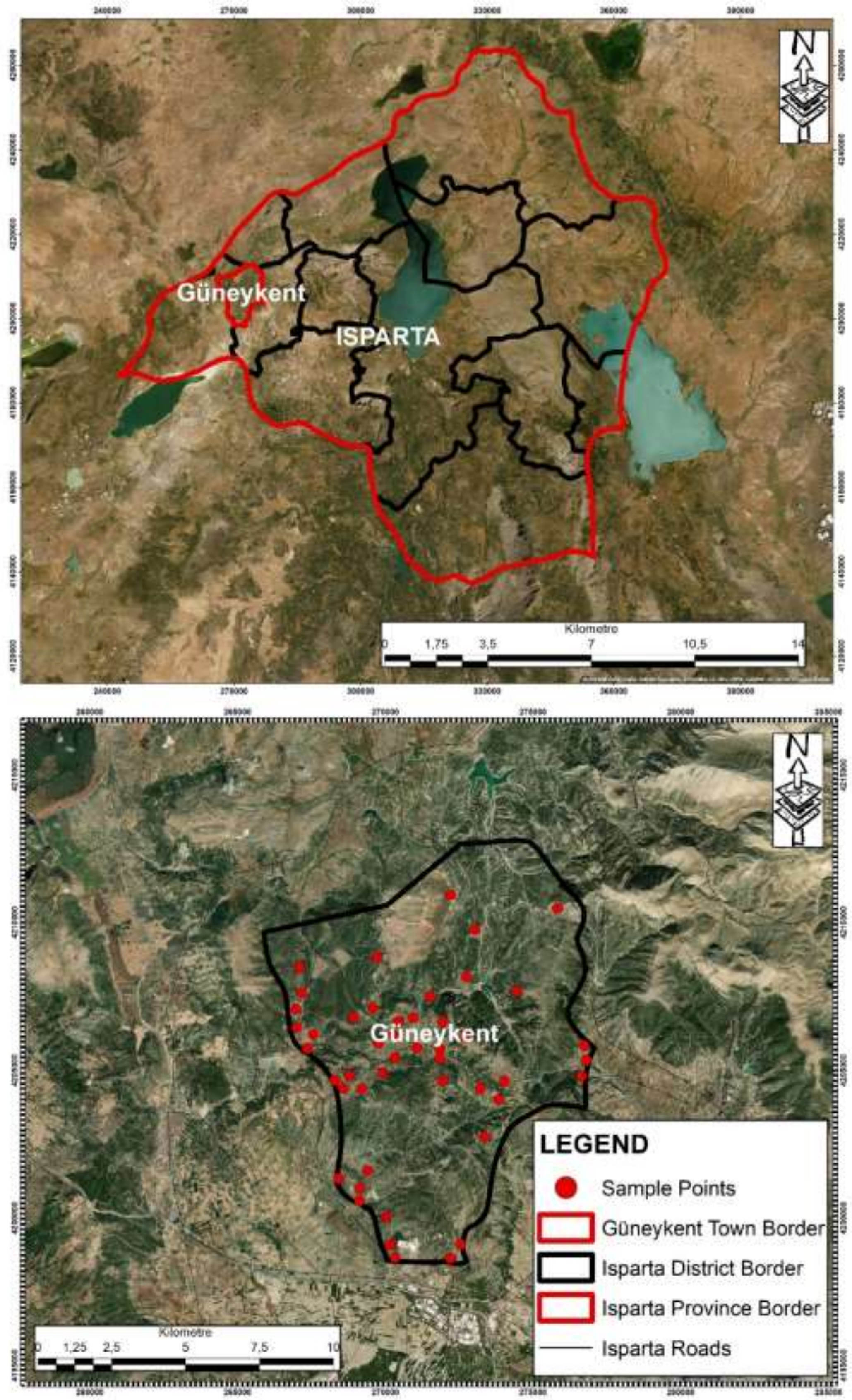


Figure 1. Study area

2.2. SPAW model

Soil-Plant-Air-Water (SPAW), Soil Water Characteristic Program (SOILWAT model) is a predictive system programmed for a graphical computer model to provide easy application and quick solutions in hydrological analysis (Saxton and Rawls, 2006). The estimation equations used for the SOILWAT model are a comprehensive laboratory data set obtained from the USDA/NRCS National Soil Characterization database (Soil Survey Staff, 2004). The data were initially estimated based on the sand, silt, and clay contents and organic matter contents of soils to estimate the soil water content, bulk density at 33 and 1500 kPa tensions (USDA-SCS, 1982). Then, according to Saxton and Rawls (2006), regression equations were developed for retained moisture at tensions of 1500, 33, 0 kPa, and air intake value. While air intake values are estimated using the exponential form of Campbell's equation (Rawls et al, 1992), Saturated moisture (θs) values are estimated according to 2.65 g cm^{-3} particle density value and bulk density values (Saxton and Rawls, 2006). Finally, the moisture tension equations were developed by Rawls (1998) conductivity equations. Organic matter, gravel, and salinity effects were also included in the model (Figure 2) (Saxton and Rawls, 2006).

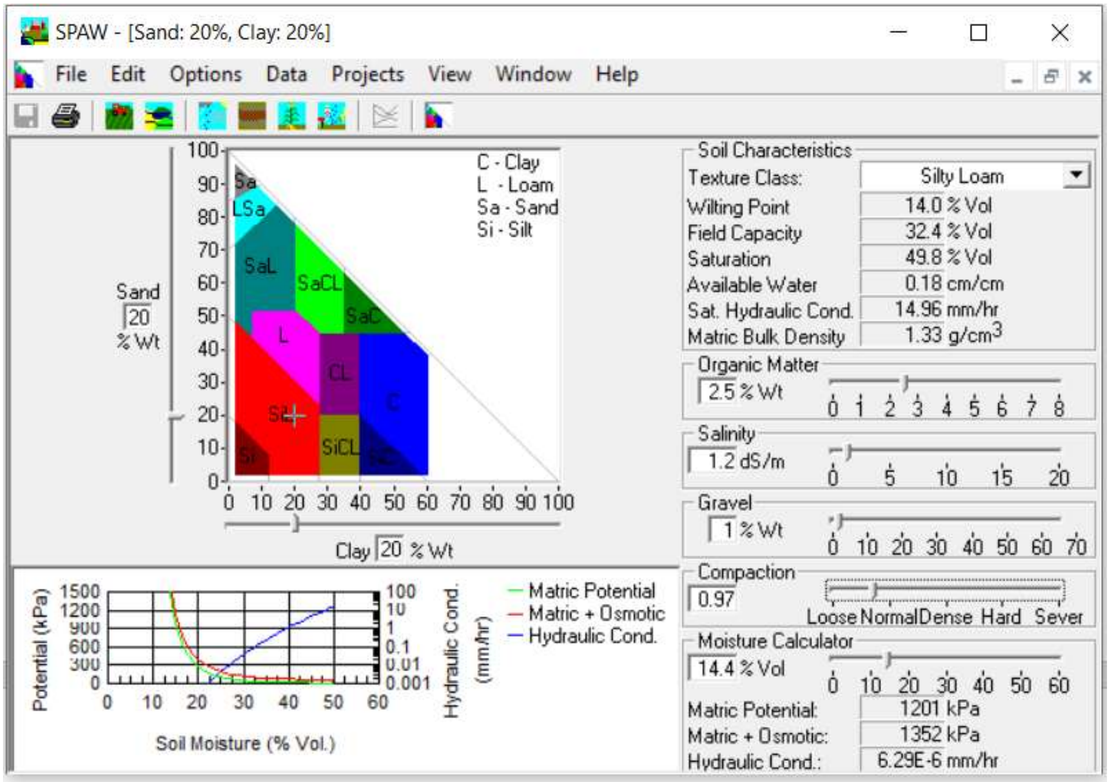


Figure 2. SOILWAT assesment tool

2.3. Soil analyzes

Within the scope of the study, pH and EC were determined according to Kacar (2016) and US Salinity Laboratory Staf (1954). The organic matter content of the soils was found using the modified Walkey-Black method and % $CaCO_3$, Scheibler calcimeter method (Kacar, 2016). Mechanical analysis was determined by hydrometer method (Demiralay, 1993), penetration resistance was determined using a digital penetrologer (1 cm^2 cone tip). The bulk density was determined by making gravel corrections in undisturbed soil samples of 100 cm^{-3} volume. Soil water characteristics; in undisturbed soil samples, starting from saturation, the 0.33 and 15 bar moisture contents were determined in a pressure plate (Klute, 1986).

2.4. Testing the accuracy of the model

The root means square error (RMSE), mean absolute error (MAE), and mean absolute percentage error (MAPE) parameters were used to examine the relationships between predicted and observed values. Estimates were determined using the following formulas (Eq. 1, 2, 3).

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|Zi - Z| \text{ (Eq. 1)}$$

$$\text{RMSE} = \sqrt{\frac{\sum (\text{Zi-Z})^2}{\text{n}}} \text{(Eq. 2)}$$

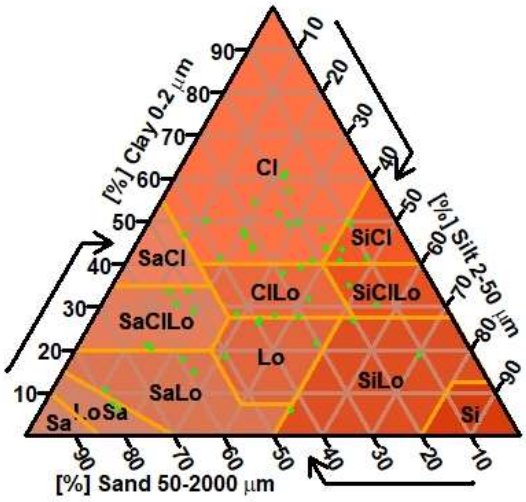
$$\text{MAPE} = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{Zi-Z}{Z}\right| * 100 \quad \text{(Eq. 3)}$$

Zi: predicted value, Z: observed value, n: number of observations.

TUKEY, one of the multiple comparison tests, was used. IBM SPSS 23 was used in statistical approaches.

3. Results and Discussion

Descriptive statistics of soil properties in the study area are given in Table 1. The sand (Sa), silt (Si), and clay (CI) contents of the soils were determined in the range of 9.37-78.4%, 9.03-70.41%, 5.69-61.18%, respectively. Depending on the ratios in the textural fractions, the texture classes of the soils showed a distribution as in Figure 3. Soils have texture classes of clay, clay loam, loam, sandy clay loam, silty clay, silty loam, sandy loam, silty clay loam, and loamy sand. According to Hazelton and Murphy (2016), the organic material (OM) contents of the soils were very low (0.1%) and high (3.985%), and salt contents (EC) were between 39.2-1236µs cm^{-1}. There is no salinity problem in the soils. According to Schoeneberger et al.(2012), penetration resistance (PR) values were found to be between moderate (0.46 MPa) and high (2.35 MPa). The lowest bulk density (BD) is 1.05 g cm^{-3}. The water retention of the soils at the saturation (ST) level varied between 28.39-60.36%. Soil properties that affect penetration resistance are frequently cited as texture, structure, porosity, water content, cementing agents, and compaction (Grunwald et al., 2001). Stewart and Hartge (1995) stated that compaction affects plant root development and distribution, and also changes the water and air balance by causing a decrease in the amount of pores. Field capacity (FC) and wilting point(WP) varied between 14.79-50.115% and 6.72-35.69%, respectively, depending on the differences in soil properties. The available water content (AWC) varies between 4.88% and 26.10% depending on the soil texture and other factors.



Sa:Sand, Lo:Loam, Si: Silt, CI:clay
Figure 3. Soil texture triangle of the soils

When the coefficient of variation (CV) of soil properties was examined, the lowest CV value was determined in the BD feature. The narrow range of change in BD resulted in a low CV. The BD values showed a change of 11.63% compared to the mean. According to Wilding (1985), CV values were classified as low (< 15%), moderate (< 35%) and high (> 35%).

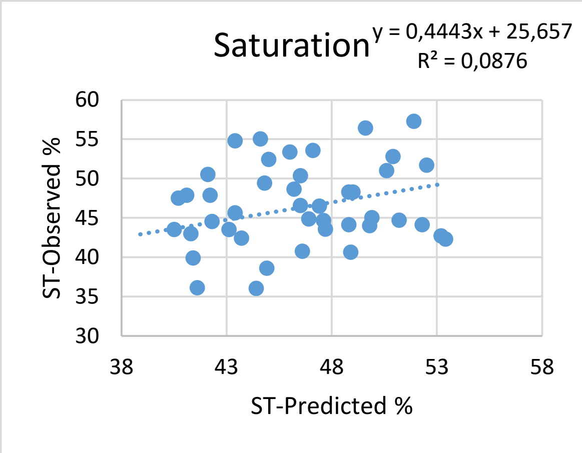
Table 1. Descriptive statistics of soil properties

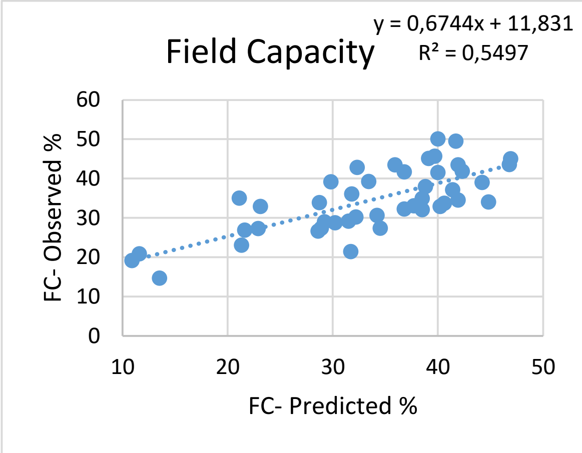
Variable	Mean	StDev	CoefVar	Minimum	Maximum	Skewness	Kurtosis
CI -%	34.8	14.55	41.8	5.69	61.18	-0.25	-0.7
Si-%	30.22	13.86	45.86	9.03	70.41	0.63	0.05
Sa-%	34.97	18.95	54.19	9.37	78.4	0.76	-0.22
OM -%	1.41	0.893	62.91	0.10	3.98	0.84	0.42
EC-µs cm^{-1}	237.1	205.6	86.72	39.2	1236	2.88	11.42
PR-MPa	1.13	0.37	33.28	0.46	2.35	1.00	1.76
BD-g cm^{-3}.	1.40	0.16	11.63	1.05	1.89	0.27	0.71
ST-%	46.82	6.18	13.21	28.39	60.36	-0.27	0.71
FC-%	34.36	7.99	23.26	14.79	50.15	-0.16	-0.23
WP- %	20.73	7.29	35.17	6.72	35.69	0.16	-0.47
AWC-%	13.62	4.43	32.58	4.88	26.10	0.80	0.93

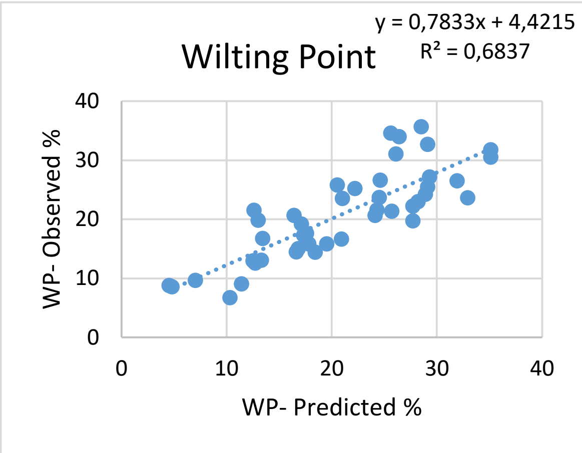
CI: clay, Si: silt, Sa: Sand, OM: organic matter, EC: electrical conductivity, PR: penetration resistance, BD: bulk density, ST: saturation moisture content, FC: field capacity, WP: wilting point, AWC: available water content

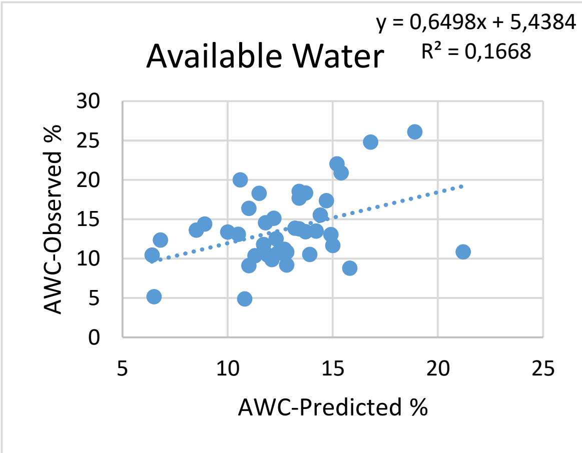
Soil properties FC, AWC, and WP have medium coefficients of variation and other soil properties have high coefficients of variation. Significant deviations from the mean were determined since the properties examined showed great variability depending on the textural fractions. This resulted in a high CV value. Rawls et al., (1982) stated that FC and WP are highly variable according to the textural fractions, while FC can be determined between 1.8-16.4% in sandy texture and 32.6-46.6% in clay texture. At the wilting point, these values were determined as 0.7-5.9% and 20.8-33.6%. Although the field capacity varies significantly depending on the texture, organic matter, and structure (Karahan et al., 2014), the variety and amount of clay minerals are more effective on the change in wilting point. (Lal and Shukla, 2004). In addition, with compaction, the field capacity contents of the soils may vary depending on the change in the pore structure (Negiş et al., 2020). The skewness and kurtosis coefficients being close to 0 indicates a normal distribution. Negative skewness indicates left skewness, positive skewness indicates right skewness. While CI, ST, and FC were skewed to the left and the other properties were skewed to the right, the EC contents of the soils were the property that showed the furthest distribution from the normal. Obtaining the highest value for the coefficient of variation in EC is a reason for its abnormal distribution. Soil properties varied from sandy texture to heavy clay texture. The variability in the structure causes changes in the adhesion of ions and salt in the soil. In addition, the different land-use conditions of the study area samples also led to variability in soil properties.Within the scope of the SOILWAT model, the sand, clay, organic matter, EC, and gravelly conditions of the sampling points were used. The linear relationship between the Saturation, FC, WP, AWC, and BD values determined by the analysis of the soils and the estimated values are given in Figure 4. The R^2 values showing the linear relationship between the observed and predicted values of the soils were determined as 0.0876, 0.5497, 0.6837, 0.1668, 0.0545 for Saturation, FC, WP, AWC, and BD, respectively. The highest R^2 values were determined for wilting point and field capacity. The wilting point can be predicted with an accuracy of 68% and field capacity with an accuracy of 55%. R^2's of the distributions of actual and predicted values of saturation, AWC, and BD values were determined to be low. The R^2 values obtained in the equations vary depending on the linear relationship. However, the linear equation R^2 values obtained were found to be low, since the errors in the estimations did not always show a negative or positive change. As et al(2019) determined the R^2 value as 0.02 in the estimation of volumetric moisture content with SOILWAT.

The RMSE, MAE, and MAPE values obtained for the evaluation of the SOILWAT model are given in Table 2. The low level of RMSE, MAE, and MAPE values indicates that the model prediction accuracy is high. The lowest RMSE and MAE values were determined in bulk density and the highest in field capacity estimation. The fact that the error in BD is low compared to other features is due to the fact that the BD value is lower than other features and the variation range is narrow. The error in the saturated state estimation was determined as ± 4.9% on average. The mean error was ± 5.27 %, 3.83 %, 3.53 %, 0.13 gr cm^{-3} in FC, WP, AWC, and BD respectively. According to Lewis (1982), the model with a MAPE value has been classified as below 10% is “very good”, between 10-20% “good”, between 20-50% “acceptable” and 50% “wrong and faulty”. The MAPE value was highest in AWC (27.269%) and lowest in BD (9.749%). With the SOILWAT model, the BD estimation was determined as “very good”, ST, FC, WP as “good” and AWC as acceptable. AWC is calculated from the FC and WP properties of soils. AWC's error was found to be higher than the other two features. When calculating AWC, the MAPE value of the AWC estimation is higher as a result of the combination of the error caused by the two features. The lower determination of the RMSE and MAE values of AWC is entirely due to the values in the data set.









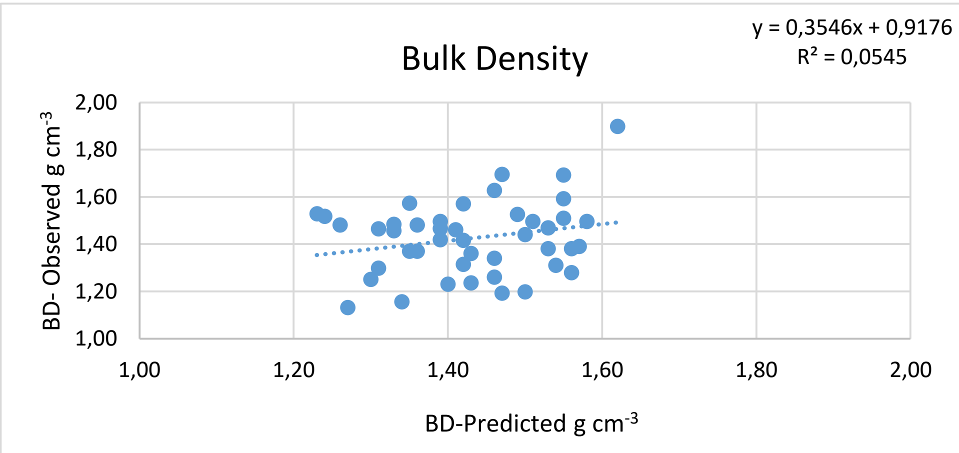


Figure 4. Distribution of observed and predicted values of soil properties

The MAPE value provides a clearer explanation in evaluating the predictive power of different features. While RMSE and MAE depend on the dataset, MAPE reveals the % error. Gijsman et al. (2002) reported that the performance of SOILWAT was not effective for every soil group. As et al. (2019) found that BD was significantly estimated for Sandy loam and sandy clay loam soils among the observed and simulated values with SOILWAT (RMSE-0.033 gr cm^{-3}). It is thought that the sources of error in the water retention rates of the soils may also be due to the clay type differences. Although the clay content is high, it has been revealed by the studies that there are differences in the water holding capacity of the soils depending on the clay type (Tunçay et al., 2020).

Table 2. Evaluation of SOILWAT model prediction accuracy

	RMSE	**MAE**	**MAPE (%)**
ST (%)	5.914	4.934	11.011
FC (%)	6.235	5.274	16.093
WP (%)	4.517	3.838	19.594
AWC (%)	4.352	3.533	27.269
BD (gr cm^{-3})	0.162	0.137	9.749

ST: Saturation moisture content, FC: field capacity, WP: wilting point, AWC: available water content, BD: bulk density

The results of MAPE values obtained according to different land-use types are given in Figure 5. The differences between the predicted values based on land use were not found to be statistically significant (P>0.05). The estimation accuracy obtained for all features did not change significantly from the values specified in Figure 5, where the general averages are present. Depending on the change in land use, dynamic characteristics usually change. However, the model takes into account textural fractions, one of the stable genetic traits. Variations in textural fractions are not expected depending on land use.

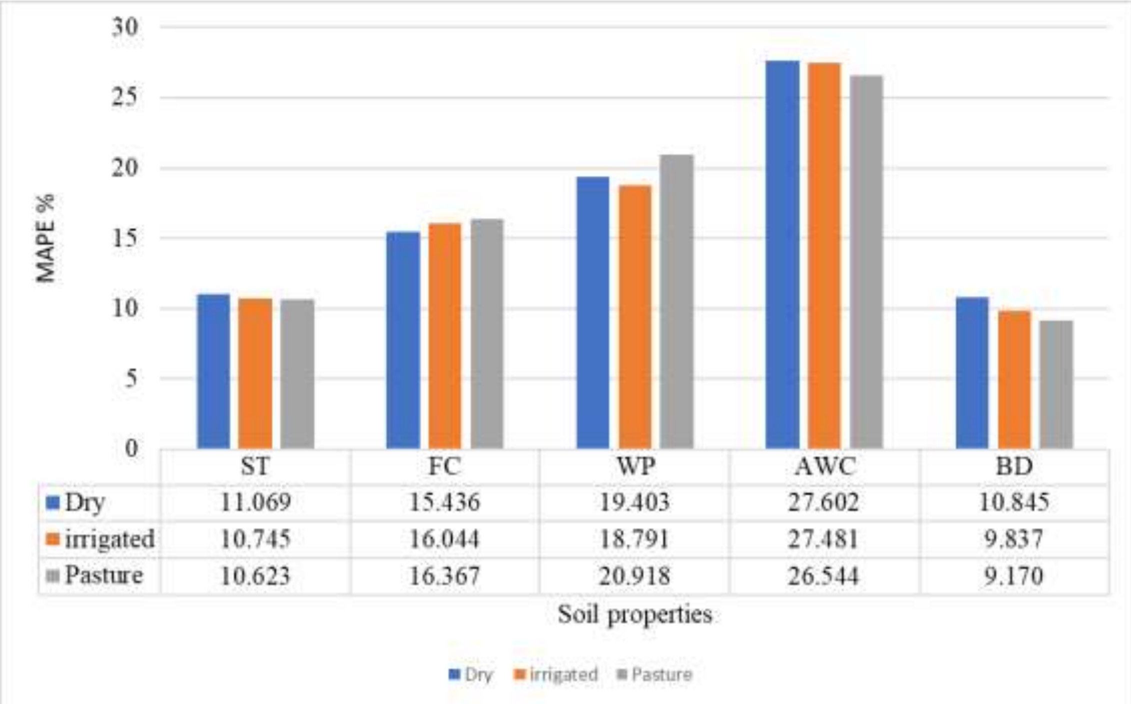


Figure 5. SOILWAT model prediction accuracy (MAPE %) for different land use

4. Conclusion

This study, it is aimed to investigate the usability of the SOILWAT model for soils in different land-use types. According to the results obtained, ST, FC, WP, and BD properties can be estimated reliably using the SOILWAT model, while the error rate in the estimation of AWC has been determined at higher levels than the others. In addition, there was no significant difference in the accuracy of the estimations due to different land uses. This study has demonstrated that the SOILWAT model can be used successfully in defining the general physical properties of the study areas by using the textural fractions of the soils, OM, penetration resistance, and EC contents.

Agriculture is widely practiced in the study area. With the increase in closed irrigation systems, it is important to determine the amount of irrigation required for drip irrigation and the irrigation time. In areas where irrigation infrastructure has been completed, drip irrigation projects are being prepared. It has been revealed that the SOILWAT program can be used in these projects for the town. This study is important in programming the irrigation amount and irrigation time in the region. The use of the SOILWAT model in determining soil physical parameters is a reference for producers and users in public institutions.

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EVALUATION OF THE CONTENT OF PHENOLIC COMPOUNDS AND ANTIOXIDANT POTENTIAL OF WHITE MULBERRY FRUITS AND PEKMEZ

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ABSTRACT

Mulberry fruits and pekmez are known for their protective, or therapeutic effect to human health. Pekmez is considered as energy source food containing concentrated nutrients, also in Albania is known as for many years it has been produced by traditional method. The study was conducted to evaluate the content of phenolic compounds and antioxidant potential of white mulberry fruits collected in Tirana region, and its product pekmez. Pekmez control sample was prepared without clarifier agents and no sugar, which was compared with other pekmez samples preparedwith the aid of various clarifier agents (2% w/w): bentonite, gelatin, calcium carbonate, and samples with sugar added. The total phenolic content, flavonoids and antioxidant potential were evaluated for whole fresh fruit, juice, pomace and pekmez, and the study of influence of various clarifier agents on them. Based on results mulberry fruit had the total polyphenolic content 285.41 mg gallic acid equivalent/100 g of sample in fresh weight (f.w.), and was found 46.97% lower content in juice, whereas in pomace and pekmez was found a greater content (55.32 % and 41.23 %) compared to fresh fruit. Pekmez samples prepared with the aid of clarifier agents resulted to have a greater amount of phenolic content (0.27-30.00 %) compared to control samples. Total flavonoids content resulted 20.67 mg catechin equivalents/ 100 g f.w., juice had 69.55 % lower content, whereas pomace and pekmez had 44.06 % and 56.49 % greater content, also pekmez samples prepared with the aid of clarifier agents resulted to have a greater amount of flavonoids (10.18-87.19%) compared to control samples. Antioxidant activity evaluated with ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay resulted from 158.06 to 170.66 mg ascorbic acid equivalent/ 100 g f.w. in pekmez samples, whereas the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay resulted from 29.30 to 43.31 % of inhibition in pekmez samples. Based on study findings may be concluded that clarifier agents contributed positively, as a higher content of phenolic compounds and antioxidant potential resulted, and was in the order: control< sugared< bentonite< gelatin< calcium carbonate, where among clarifier agents calcium carbonate had a higher influence, which may be recommended to be utilized as beneficial for the pekmez production, and for its commercial importance.

Keywords: white mulberry, pekmez, clarifier agents, phenolic compounds, antioxidant potential

INTRODUCTION

White mulberry (*Morus alba* L.) belongs to the Morus genus of Moraceae family, and is native to Asia, which has been highly adaptable and well grown in Albania. Mulberry is dispersed extensively in diverse climatic and environmental circumstances ranging from tropical to temperate. Amongst all the species of genus Morus as *Morus alba* is a dominant species (Ercisli and Orhan, 2007).

The presence of valuable constituents in mulberry fruits makes the plant suitable to be utilized as a functional food useful to human health in addition to its basic nutritional function (Kadam, 2019). *Morus alba* has abundant phytochemicals, including phenolic acids, flavonoids, flavonols, anthocyanins, macronutrients, vitamins, minerals, and volatile aromatic compounds (Chen et al., 2021). Mulberry fruits are famous for their mouth-watering taste that makes it suitable to con-sume either in fresh or as an ingredient in value-added products, for culinary uses, and processed product forms such as juices, syrups, liquors, molasses, jams, wines, natural dyes, beverages, or dehydrated fruits (Gundogdu et al., 2011).

Pekmez is one of the popular and food products produced by concentration of fruit juice of mulberry. It is rich in carbohydrates, minerals, some vitamins and antioxidants, and are energy storage and food source (Badem, 2018).

The aim of this study was to evaluate the content of total phenolic content, flavonoids and antioxidant potential of white mulberry (*Morus alba* L.) fruits collected in Tirana region for whole fresh fruit, juice, pomace and pekmez samples prepared with the aid of various clarifier agents (2% w/w): bentonite, gelatin, calcium carbonate, and with sugar added. The generated data of this study may serve for fruit processors, consumers and researchers.

MATERIAL AND METHOD

Mulberry fruits (*Morus alba*) were randomly collected in May-June 2021 in Tirana, Albania, and were transported immediately to the laboratory for further analysation and processing to pekmez. For preparation of pekmez samples was followed the flow diagram as in Figure 1.

Prior processing a pre-selection was done based on fruits maturity, shape, size, and color. Than fruits were washed and cleaned and a part of them was mixed with sugar (~8% w/w), which served for pekmez with added sugar preparation, and remained fruits were crushed, pressed to obtain fruit juice and pomace. Filtration of extracted juice was made with through muslin cloths, and clarifier agents 2% (w/w) bentonite, gelatin, calcium carbonate were mixed with filtered juice for clarification. Then the upper phase was collected, filtered again through a muslin cloths and transferred in an open pan for boiling, and during boiling, any foam produced was removed. This process last until the desired consistency of pekmez was obtained (min. 65-70ºBrix). After pekmez samples were cooled, were packed and stored.

Samples were coded depend on the steps during processing or clarifier agent used, or sugar added: F=fresh fruit, JF= fruit juice, P/C= pomace from fresh fruit, PS=pomace from fruit with sugar, JB= juice with bentonite, JG= juice with gelatin, JCa= juice with calcium carbonate, and their respective precipitates P/B, P/G, P/Ca, C=pekmez with no added sugar or clarifier agent which served as control, S=pekmez with sugar, B=pekmez with betonite, G=pekmez with gelatin, and Ca=pekmez with calcium carbonate

Extracts were prepared according Velioglu, et al., (1998), with modification. 1 g of sample (fresh fruit, juice, pomace and pekmez) was weighted in a test tube, adding 10 ml of 60% aqueous methanol, then the mixture was vortexed for five minutes, kept for 15 minutes at a temperature of 20 °C under ultrasounds action, after were centrifuged at 3500 rpm for 15 min and supernatants were collected, and analysed for total phenolic, flavonoid content and antioxidant potential.

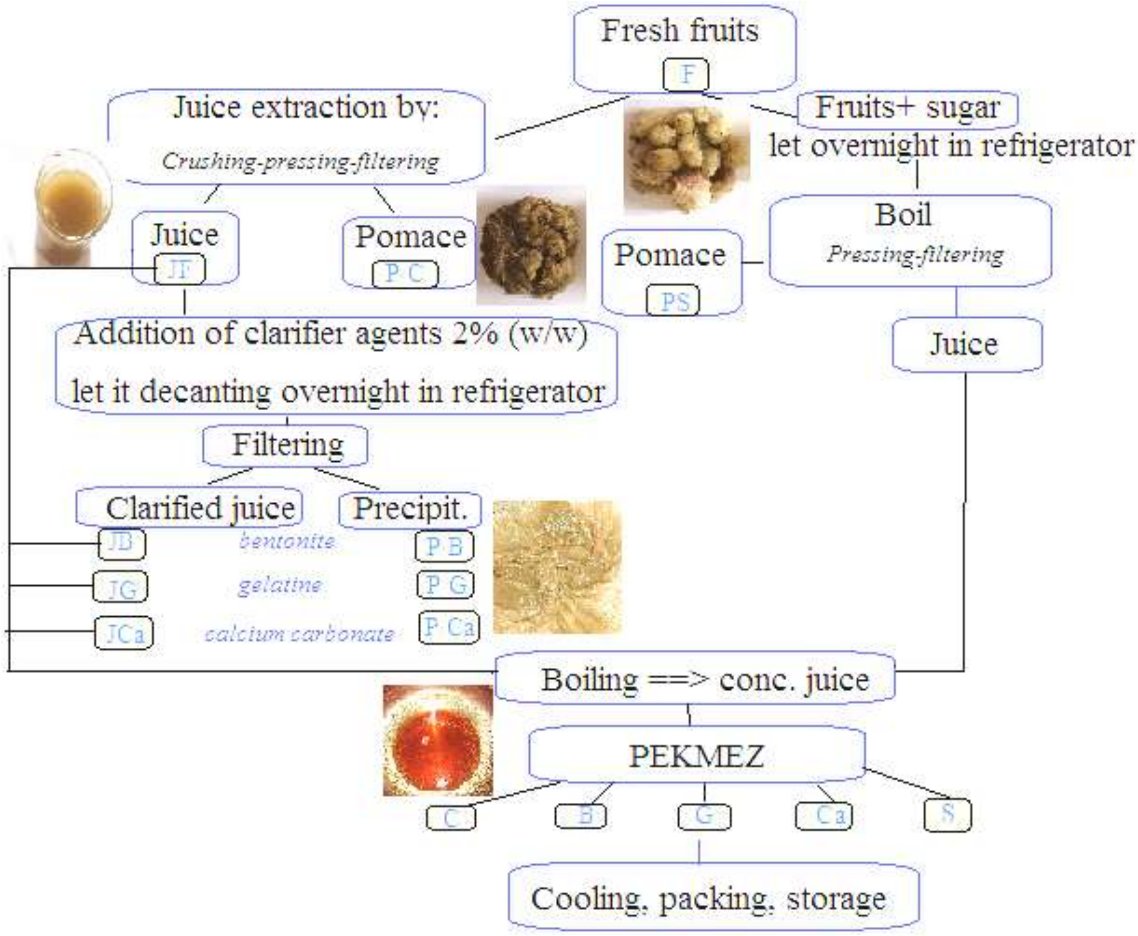

Figure 1. Flow diagram for pekmez production

Total phenolic content of mulberry fruits, juice, pomace and pekmez was determined using Folin–Ciocalteu's reagent, according to Singleton & Rossi (1965), with modification. An aliquot (500 µL) of sample was mixed with FC reagent (Folin/water; 1:8). After that, 1 mL of sodium carbonate 7.5% (w/v) was added to the former mixture and kept for 30 min at room temperature in the dark place. The solution was mixed completely and the absorbance level was measured spectrophotometrically versus prepared reagent blank at 765 nm using UV/Vis spectrophotometer Libra S22. A standard curve of the gallic acid (50 – 600 mg/L) was constructed ($y = 0.0027x - 0.0623$), and the results for TP were expressed as mg gallic acid equivalent (GAE)/100 g FW of sample.

Total flavonoid content was determined using aluminum chloride colorimetric assay, as described by Abu Bakar, et al., (2009) with modification. An aliquot (500 µL) of sample was added to 10 mL volumetric flask containing 5 mL distilled water, followed by addition of 0.3

mL 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $AlCl_3$ solution was added and allowed to stand for another 6 min before 2.0 mL of 1 M NaOH was added, and the total volume was made up to 10 mL with distilled water. The solution was mixed completely and the absorbance level was measured spectrophotometrically versus prepared reagent blank at 510 nm using UV/Vis spectrophotometer Libra S22. A standard curve of the catechin was constructed (y=0.0036x+0.0007) and the results for TF were expressed as mg catechin equivalent (CE)/100 g FW.

Antioxidant activity was determined using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay according to Re et al., (1999). ABTS and potassium persulfate mixture was kept in the dark at room temperature for 16 h before use. For the analysis, the stock solution was diluted in aqueous methanol 80% (v/v) until the absorption at 734 nm reached 0.7 ± 0.02. An aliquot (25µl) of sample was mixed with 975 µl of ABTS reagent. A standard curve of the ascorbic acid was constructed (y =-0.1641x + 0.7139) and the results were expressed as mg ascorbic acid equivalents (AAE)/100 g FW.

The capacity of the prepared extracts to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH·) was carried out as described Yıldırım et al., (2001). 0.1 mM DPPH radical solution in methanol was prepared, and then this solution was added to 280µL extracts of samples, till a final volume of 2mL. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. Antioxidant activity was given as percent of inhibition, which was calculated with the equation:

$$Inhibition\% = \left(\frac{Abs_{Control} - Abs_{sample}}{Abs_{Control}}\right) * 100$$

The analyzes were performed at least in three replications, and were calculated as Mean values, ± standard deviation.

RESULTS AND DISCUSSION

Based on the study results mulberry fruit has the total polyphenolic content 285.41 mg gallic acid equivalent/100 g of sample in fresh weight (f.w.), and was found a lower content in juice (46.97%), whereas in pomace and pekmez was found a greater content (55.32 % and 41.23 %) compared to F samples (Figure 2). Juices with added clarifier agents resulted to have greater amounts of polyphenols compared to JF in amount 15.79% (JB), 19.92% (JG), and 22.26% (JCa).

Samples with sugar added resulted to have slightly lower amount of phenolic content in pomace as in PS was found 7.71 % lower compared to PC sample (377.22 mg GAE/100 g f.w.), whereas in precipitates of clarified juices were found greater amounts 6.86 % (PB), 7.14% (PG), and 8.18% (PCa) compared to PC sample.

In pekmez with sugar added was found 1.91 % (S) lower compared to C (434.08 mg GAE/ 100 g f.w.), whereas pekmez samples prepared with the aid of clarifier agents resulted to have a slightly greater amount of phenolic content 2.08% (B), 5.53% (G), and 17.16% (Ca) compared to C sample.

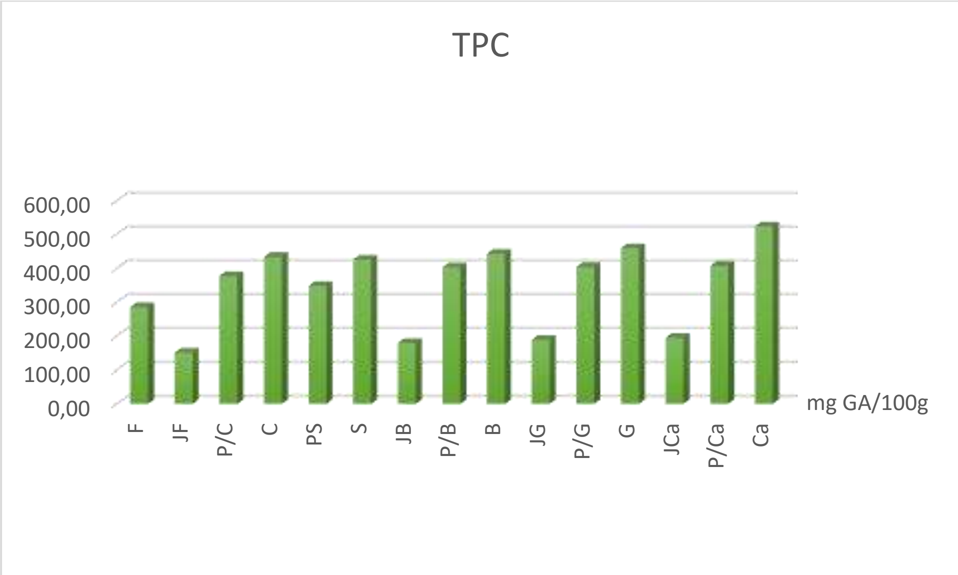

Figure 2. Total phenolic content in mulberry fruit, juice, pomace and pekmez samples

Results revealed that clarifier agents contributed positively, as a higher content of phenolic content was determined, and generally was followed the order for pekmez samples: sugared< control< bentonite< gelatin< calcium carbonate.

Total flavonoids content resulted in mulberry fruit 20.67 mg catechin equivalent/100 g of sample in fresh weight (f.w.), and was found a lower content in juice (69.55%), whereas in pomace and pekmez was found a greater content (44.06 % and 56.49 %) compared to F sample (Figure 3). Juices with added clarifier agents resulted to have greater amounts of flavonoids compared to JF 39.51% (JB), 41.14% (JG), and 48.73% (JCa).

Samples with sugar added resulted to have greater amounts of flavonoids content in pomace as in PS sample was found 59.3% greater compared to PC (29.78 mg CE/100 g f.w.), whereas in precipitates of clarified juices were found lower amounts 39.54% (PB), 23.8% (PG), and 17.37% (PCa) compared to PC sample.

In pekmez was found greater flavonoids in S sample (87.16 %) compared to C sample (32.35 mg CE/100 g f.w.), whereas pekmez samples prepared with the aid of clarifier agents resulted to have a slightly greater amount of flavonoids content 9.22% (B), 18.12% (G), and 26.22% (Ca) compared to C sample.

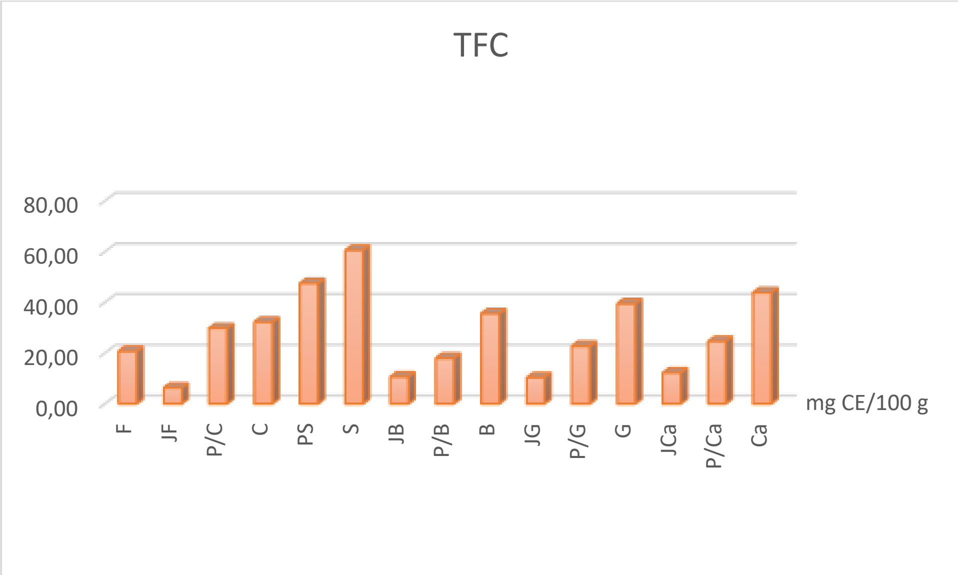

Figure 3. Total flavonoid content in mulberry fruit, juice, pomace and pekmez samples

Results revealed that clarifier agents contributed positively, as a higher content of flavonoids content was found, and generally was followed the order: control< bentonite< gelatin< calcium carbonate< sugared samples.

Antioxidant activity evaluated with ABTS assay resulted from 158.06 to 170.66 mg ascorbic acid equivalent/ 100 g f.w. of samples (Figure 4). Mulberry fruit had antioxidant activity 61.73 mg AAE/100 g f.w., and in juice was found to be lower (30.84%), whereas in pomace and pekmez was found to have a greater antioxidant activity (47.44% and 61.6 %) compared to fresh fruit. Juices with clarifier agents resulted with greater antioxidant activity compared to JF, 18.10% (JB), 20.90 % (JG), and 21.84% (JCa).

Samples with sugar added resulted to have lower antioxidant activity in pomace as PS was found 29.55% lower compared to PC (117.44 mg GAE/100 g f.w.), also precipitates from clarified juices had slightly lower antioxidant activity 2.46% (PB), 10.24% (PG), and 17.09% (PCa) compared to PC sample.

In pekmez with added sugar (S) was found 1.67 % lower compared to C (434.08 mg GAE/ 100 g f.w.), whereas pekmez samples prepared with the aid of clarifier agents resulted to have a slightly greater antioxidant activity 2.59% (B), 5.65% (G), and 5.81% (Ca) compared to C samples.

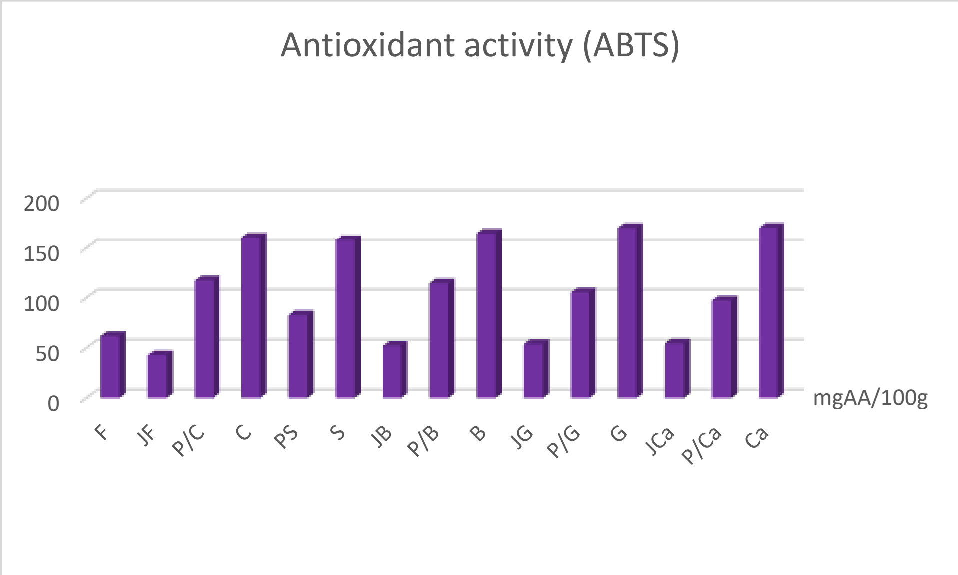

Figure 4. Antioxidant activity (with ABTS assay) of mulberry fruit, juice, pomace and pekmez samples

Antioxidant activity evaluated with DPPH assay expressed as % of inhibition resulted from 29.30 to 43.31 % of samples (Figure 5).

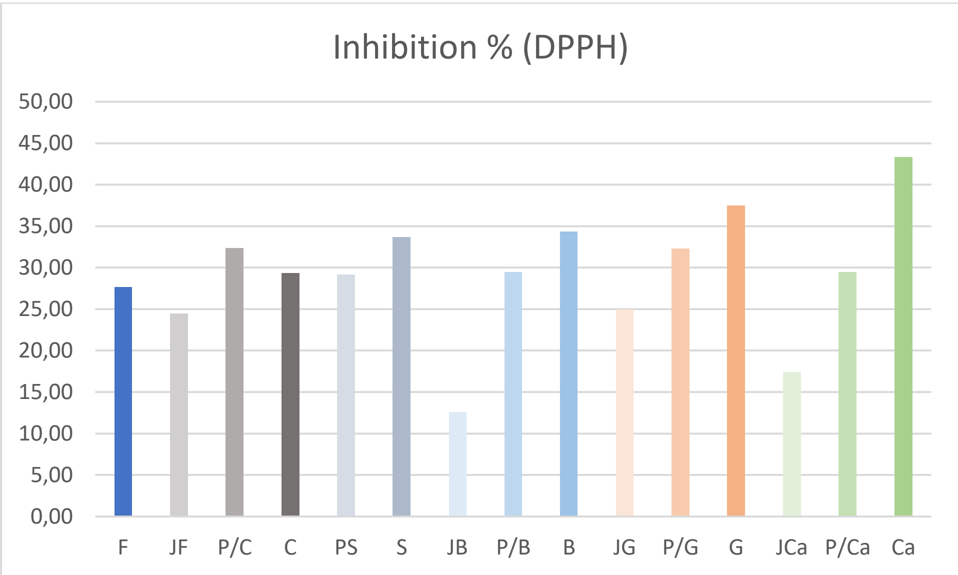

Figure 5. Antioxidant activity (with DPPH assay) of mulberry fruit, juice, pomace and pekmez samples

Antioxidant activity evaluated with ABTS and DPPH assays correlate well with each other and with determined phenolic content. Based on above results is notable that clarifier agents have a positive influence following the order: control< sugared< bentonite< gelatin< calcium carbonate.

CONCLUSIONS

This study clearly shows the potential of mulberry and pekmez to human health, as good sources of polyphenols 151.35-524.01 mg GAE/100 g f.w., flavonoids 6.29-60.54 mg CE/100 g f.w., antioxidant activity 42.69-170.66 mg AAE/100 g f.w. or 29.30-43.31 % of inhibition.

Based on results mulberry fruit had the total polyphenolic content 285.41 mg gallic acid equivalent/100 g of sample in fresh weight (f.w.), and was found 46.97% lower content in juice, whereas in pomace and pekmez was found a greater content (55.32 % and 41.23 %) compared to fresh fruit. Pekmez samples prepared with the aid of clarifier agents resulted to have a greater amount of phenolic content (0.27-30.00 %) compared to control samples. Total flavonoids content resulted 20.67 mg catechin equivalents/ 100 g f.w., juice had 69.55 % lower content, whereas pomace and pekmez had 44.06 % and 56.49 % greater content, also pekmez samples prepared with the aid of clarifier agents resulted to have a greater amount of flavonoids (10.18-87.19%) compared to control samples.

Based on study findings may be concluded that phenolic content, flavonoid content and antioxidant activity were found lower in juice, and higher in pomace compared to whole fresh mulberry fruit. In pekmez these substances were concentrated making pekmez as good source of natural antioxidant compounds.

Furthermore, clarifier agents contributed positively, as a higher content of phenolic content, flavonoid content and antioxidant activity were found and generally was followed the order: control< sugared< bentonite< gelatin< calcium carbonate. Among clarifier agents calcium carbonate was noted to affect phenolic content and antioxidant activity positively, as greater amount were determined in samples with added calcium carbonate, which may be recommended to be utilized as beneficial for the pekmez production.

The generated data of this study may serve for fruit processors, consumers and researchers.

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HYDROLOGICAL DROUGHT ANALYSIS AND DROUGHT HYDROLOGY

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ABSTRACT

Drought is a natural phenomenon that adversely affects land and resource generation systems as a result of rainfall falling significantly below normal levels, leading to serious hydrological imbalances. Low flow can be defined as flow of water in a stream during prolonged dry weather is the decrease of flow in a stream and low flow is indicative of the minimum expected flow level given usage and environmental needs. Drought and low flow are interrelated. But while low flows can be observed during dry periods, not every low flow is indicative of drought. Within the scope of this research, a sample drought analysis study was conducted with Standardized Precipitation Index (SPI) using the R program. Turkey is located in the Mediterranean Basin, where the negative effects of climate change are observed. and shows middle-arid climate. It is necessary to evaluate low flow analysis supported by drought analysis in order to use water with maximum efficiency in wet periods and in periods when rivers may dry up or when water is reduced. Therefore, in this study, it was concluded that low flow analysis in our country, which is expected to encounter more drought disasters in the future, should be evaluated by supporting with drought analysis.

Keywords: Low Flow, Drought, Low Flow Analysis, Hydrological Drought Analysis

INTRODUCTION

Low flow is a state of decreasing flow in a stream, usually observed in summer, and is a hydrological process that changes randomly every year. Low flow is indicative of the minimum expected current level given usage and environmental needs. Drought, on the other hand, occurs as a result of the decrease in flows over a period of time, which is below the required values. The concepts of drought and low flow are related to each other, and detection and interpretation of low flows is as important as the detection of drought in terms of efficient use of water. Not all low flows are indicative of drought. however, drought can be observed as a result of long-term occurrence of low flows during a certain period. Therefore, how important it is to use water with maximum efficiency in wet periods, it should be investigated how to achieve maximum efficiency with less water in periods when rivers can dry up or water decreases. In addition, the water that can be supplied from the stream and the water that should be given to the stream for life should be determined and for this purpose, low flow analysis should be evaluated by supporting them with drought analysis.

Flow rates that can be drawn from streams for various purposes in dry periods depend on low flows. Low flow studies are important in terms of taking measures that can reduce the negative effects of dry periods.

Low flow analysis studies can be used in the following subjects; In water resources and water quality management studies, in determining management strategies, in regional analysis of surface water resources, in the design of water supply facilities within confidence limits, calculation of the minimum amount of water that should be released downstream in water collection structures, reliable energy calculations in hydroelectric power plants, in the design of water storage and in determining the appropriate discharge point for waste water treatment facility (Köken, 2009).

Most of the low flows are supplied by groundwater recharge. Low flows are sustained by drainage of saturated the surface of the soil rather than deep groundwater. For low flows to be sustained, the draining aquifer must be seasonally refilled with sufficient flow. In addition, the water table must be shallow, and the size and hydraulic characteristics of the aquifer must be sufficient to sustain flows during the dry season.

The low flow regime in a river can vary depending on how large the aquifer is, what the aquifer's hydrological characteristics are, the amount of recharge, the evapotranspiration of the basin, the distribution of vegetation, topographic characteristics, the structure of the ground, its seepage characteristics, climate and geology.

Precipitation, evaporation and icing are climatological factors and the most important of these is precipitation, since water is the main source of income. Vegetation, soil permeability and slope play an important role role in the infiltration of precipitation into groundwater. In karstic basins, the mixing of precipitation with groundwater takes a short time, while in some basins this may take much longer. Generally, the relationship between evaporation and low flow is inversely proportional if there is sufficient moisture in the ground. In addition, when the stream freezes, It can be observed that the low flow rate decreseases during cryogenic temperature

The hydrogeological conditions depend on some factors such as the type of soil, the type of vegetation, the presence of lakes and swamps, and the accumulating capacity of aquifers. In addition, these hydrogeological factors play a decisive role in the rate of decrease of low flows and these factors determine the recession curve. Low flow values are high in sandy-gravelous units with high permeability and porosity. Streams in different types of unconsolidated sedimentary rocks have low yields during the low flow period. On the contrary, streams in metamorphosed sedimentary and igneous rocks show very high flow values compared to their basin sizes.

Since the topography of the basin is effective on precipitation, it also affects the amount of low flows. Swamp and lake surface area will increase the amount of evaporation and will cause a decrease in low flows. In areas with high slopes, on the other hand, since the amount of infiltration will decrease, reductions in groundwater and low flow values will be observed. The ratio of the total length of the river channels in the basin to the basin area is an indicator of the ability of precipitation to reach the river, and as this ratio increases, low flows also increase.

Human influences also have an impact on low flows. The increase in the amount of urbanization will increase the impermeable areas and cause a decrease in low flows. Excessive use of irrigation water, especially in dry years, will further reduce low flows. Low flow levels are also affected by dams keeping the stream under control. In addition, activities such as hydroelectric power plants, water transfers, transportation activities on rivers, treatment and forestation are also effective on low flows.

Low flow analysis can also be performed with two different analysis methods, hydrological and statistical, and daily flow data is needed for these analysis. Flow duration curves are drawn with the average lowest flow values obtained by using n-day flow rates within the scope of hydrological analysis. The value corresponding to 90%, 95% and 99% of the observation time is determined. Statistical low flow analysis, on the other hand, is to determine the appropriate probability distributions by performing frequency analysis of n-day flow values.

Flow duration curves can also be used in the design phase of engineering structures and to determine the flow rate in a stream at a certain percentage of time. It is one of the most illustrative tools in showing the full range of river discharges, from low flows to flood events.

Values below 50% of the time (such as Q70, Q90, Q95, Q99) are used as low flow indices, and Q90 and Q95 flows are frequently used flows. Q95 is used in low flow studies in the UK. Also the ratio Q50/Q90 or Q20/Q90 is a measure of the variability of low flows (Bayazıt and Önöz, 2008).

Low flow studies and applications are not common in our country yet. Therefore, there is no generally accepted low flow analysis method. For this reason, determination of low flow analysis methods suitable for our country will allow water management to be more sustainable.

Drought is generally divided into four different types: meteorological, agricultural, hydrological and socioeconomic drought. There is no sharp transition between these four different types of drought. Generally, the drought situation occurs first with meteorological drought due to the decrease in precipitation. However, not every meteorological drought provides continuity as a hydrological drought or agricultural drought. In general, the deviation of precipitation from the long-term average is called meteorological drought. Meteorological drought detection may vary from region to region. For example, meteorological drought can be expressed as 2 years or more without precipitation in some parts of Saudi Arabia, 6 days or more without rain in Bali, and a total annual precipitation of less than 180 mm in Libya. (Kadıoğlu, 2008).

Agricultural drought is the situation where the water required for the growth and development of the plant is not enough in the root zone. Agricultural drought occurs when there is not enough moisture in the soil for the plant, especially during the growth period, when the plant is most sensitive to water (Wilhite and Glantz, 1985). Agricultural drought poses a great danger, especially for countries that make agricultural production based on precipitation. Unlike other types of drought, even if the rainfall is insufficient, if the soil moisture needed by the plant can be given by irrigation, the effects of agricultural drought cannot be mentioned. However, in our country, it is impossible to produce crops only depending on precipitation, except for the Eastern Black Sea Region, and irrigation water is essential in crop production.

Hydrological drought, on the other hand, is a type of drought that is observed due to a decrease in ground and surface waters. Depending on the decrease in precipitation, it may occur depending on the effect of the geological and hydrogeological characteristics of the region after the decrease in the presence of water in the hydrological system.

With the decrease in precipitation, the inability to provide irrigation water and the decrease in soil moisture, the plant cannot receive the water necessary for growth and yield decreases accordingly. With the decrease in productivity, cost increases, purchasing power decreases, insufficient and quality food cannot be consumed and accordingly deaths can be observed in case of famine. In addition, diseases and deaths can be observed due to the lack of access to water and the occurrence of hygiene problems. Socio-economic drought can be defined as the indicator of the impact of the effects observed as a result of meteorological, hydrological and agricultural drought on the social, economic and supply-demand balance.

Sustainable and efficient use of water resources is becoming increasingly important today, where the effects of drought and climate change are being felt more and more. In dry periods when there is limited water availability, the protection of water resources and maximum efficiency are related to how well drought management can be done. For this purpose, it is necessary to analyze the drought well and to interpret these analyses correctly in the basin conditions.

Drought analysis can be done by means of drought indices and indicators developed according to meteorological, agricultural and hydrological drought types. Indicators are variables that show signs of stress or deficiency due to meteorological, hydrological, agricultural and socio-economic drought. In other words, it is the conversion of a raw measurement data into information that can be used in decision-making processes by passing through statistical processes. Precipitation, temperature, runoff, evaporation, soil moisture etc. data are examples of indicators.

An index is a statistical tool or value that summarizes a group of data into a single base number. SPI, SRI, PDSI, RDI are examples of indices. In the index calculations, indicators such as precipitation, soil moisture, evaporation, flow data can be used according to the characteristics of the indices. Evaluation and interpretation of indices and indicators are important scientific tools in terms of deciding on the management of water resources.

MATERIAL AND METHOD

Within the scope of this study, SPI drought index sample analysis, which is the most widely used worldwide, was performed using the R program. Seydişehir MGM station monthly precipitation data were used for SPI analysis.

Standardized Precipitation Index (SPI):

The Standardized Precipitation Index (SPI) was developed by McKee et al. at Colorado State University in 1993. The purpose of SPI is drought monitoring. The input parameter is precipitation. It is used in the detection of seasonal drought. SPI analysis should be obtained with standard normal distribution precipitation sequences. In general, the precipitation series that are suitable for normal distribution are first converted to the gamma probability distribution function and then normalized standard precipitation series are obtained with the inverse standard normal distribution function. Finally, a standardized precipitation index with a mean of zero and a variance of one is obtained.

The standard precipitation index is calculated by the formula in (1.1). SPI is obtained by subtracting the mean precipitation (X_j) from the current precipitation (X_i) and dividing this value by the standard deviation. The obtained SPI value is classified according to the values given in Table 1.

$$SPI = \frac{X_i - X_j}{\sigma} \tag{1.1}$$

Table 1 Standardized Precipitation Index (SPI) Drought/Humidity Classification (McKee et al., 1993)

Values of SPI	SPI category
≥2	Extremely wet
1.5–1.99	Severely wet
1–1.49	Moderately wet
-0.99 to +0.99	Normal
-1.49 to -1	Moderately drought
-1.99 to -1.5	Severely drought
≤-2	Extremely drought

SPI can be calculated with calculation steps such as 1, 3, 6, 9, 12 and 24 months. 1-month and 3-month SPI analysis are short-term drought analysis and are used for meteorological drought detection. The 6-month and 9-month SPI analysis are medium-term drought analysis and are used to detect agricultural drought. Generally, 12-month and 24-month SPI analysis are used as hydrological drought indicators. The strengths of SPI are that it is calculated on multiple time scales, 1-month and 3-month SPI values can be used as an early warning, and can be compared because it consists of standardized values. The weakness of SPI is that the calculation system relies only on precipitation data.

Calculation Steps for SPI Analysis:

1-) The "Standardized Precipitation Index User Guide" prepared by the World Meteorological Organization in 2012 was used as a source for SPI calculations. Today, SPI can be calculated through many different programs. There are 2 different programs developed by The National Drought Mitigation Center (NDMC) and spi_sl_6.exe program is widely used. In addition, the SPI generator program developed by The National Drought Mitigation Center and its user manual can be obtained free of charge from http://drought.unl.edu/droughtmonitoring/SPI/SPIProgram.aspx. In this study, the R program was used for SPI analysis because it is easy to use and available free of charge.

2-) The points to be considered in order to prepare the precipitation data in accordance with the analysis are listed below.

- The data should be set in three columns, respectively year, month and measurement. Each measurement should be placed one below the other in turn.
- The R program can accept data in many formats. Generally, documents with .txt extension can be used in drought analysis calculations.
- Turkish characters should not be used in file names and file content.
- While creating the files of the data, a dot must be used as a separator. Commas should be replaced with periods.
- While preparing the data, it is necessary to choose the appropriate definitions for the parameters shown below (Beguería and Serrano, 2013).

In example files as	In function defined as	Explanation of the parameter	In
YEAR	**-**	year of data	
MONTH or mon	**-**	month of data	
PRCP	**PRCP**	monthly precipitation totals	mm
TMAX	**TMAX**	monthly mean daily maximum temperature	ºC
TMIN	**TMIN**	monthly mean daily minimum temperature	ºC
TMED	**TMED**	monthly mean temperature	ºC
AWND	**AWND**	monthly mean wind speed	km/h*
TSUN	**tsun**	monthly mean sun hours	h
ACSH	**CC**	monthly mean cloud cover	%
RH	**RH**	monthly mean relative humidity	%
U2	**U2**	monthly mean daily wind speeds at 2 m height	m/s
-	**lat**	latitude – parallels	decimal º
Z	**Z**	elevation	m
Rs	**Rs**	monthly mean daily incoming solar radiation	MJ m^2/d
-	**ed**	monthly mean actual vapour pressure at 2 m height	kPa
-	**Tdew**	monthly mean daily dewpoint temperature	ºC
-	**Ra**	monthly mean daily external radiation	MJ m^2/d

*1 km/h = 0.27777 m/s

3-) After installing the R program and R-studio, the SPI calculation package is downloaded by typing >install.packages (SPEI) on the command screen in order to be able to calculate SPI. Then the prepared .txt extension data is added from the “Import Dataset” and

"From Text(base)" tabs. The spix<-spi(file_name$prcp,X) command is used to calculate X-month SPI. For example, spi12<-spi(file_name$prcp,12) can be used to calculate 12 months SPI.

<u>For graphing the analysis results;</u>

plot(spi(ts(file_name$prcp,freq=12,start=c(start_year,month)),X,ref.start=c(start_year,month), ref.end=c(end_year,month)), main ="Chart_Title") command can be used. It is possible to calculate and graph other steps for SPI analysis with the same commands, and it will be sufficient to enter the month value to be calculated instead of just X.

4-) Finally, analysis results can be interpreted by using the limit values specified in Table 1.

5-) In this study, SPI analysis were performed using the R program SPEI package. With the help of this package, drought indices such as SRI (Standardized Runflow Index), SGI (Standardized Groundwater Index), SRSI(Standardized Reservoir Supply Index), which include standardization processes, can be calculated with similar calculation steps. Interpretation of 12 and 24-month SPI, SRI, SGI and SRSI index analysis results on the same graph is a guide for the interpretation of hydrological drought.

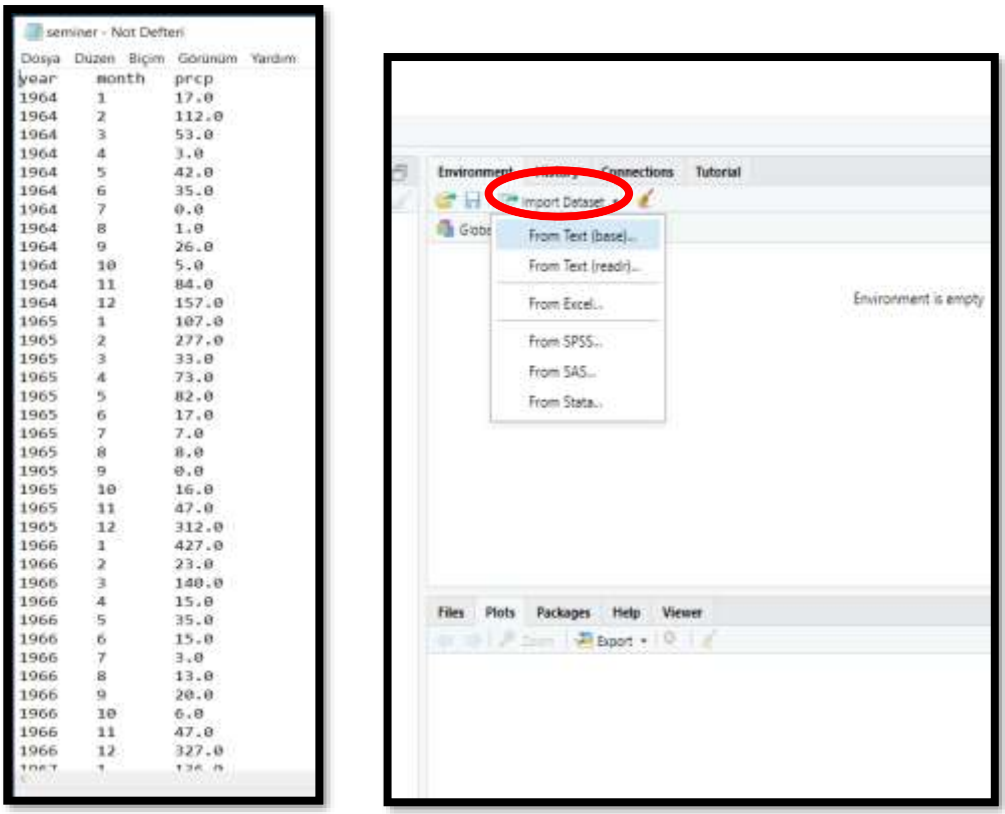


Figure 1 Adding Data to R Program

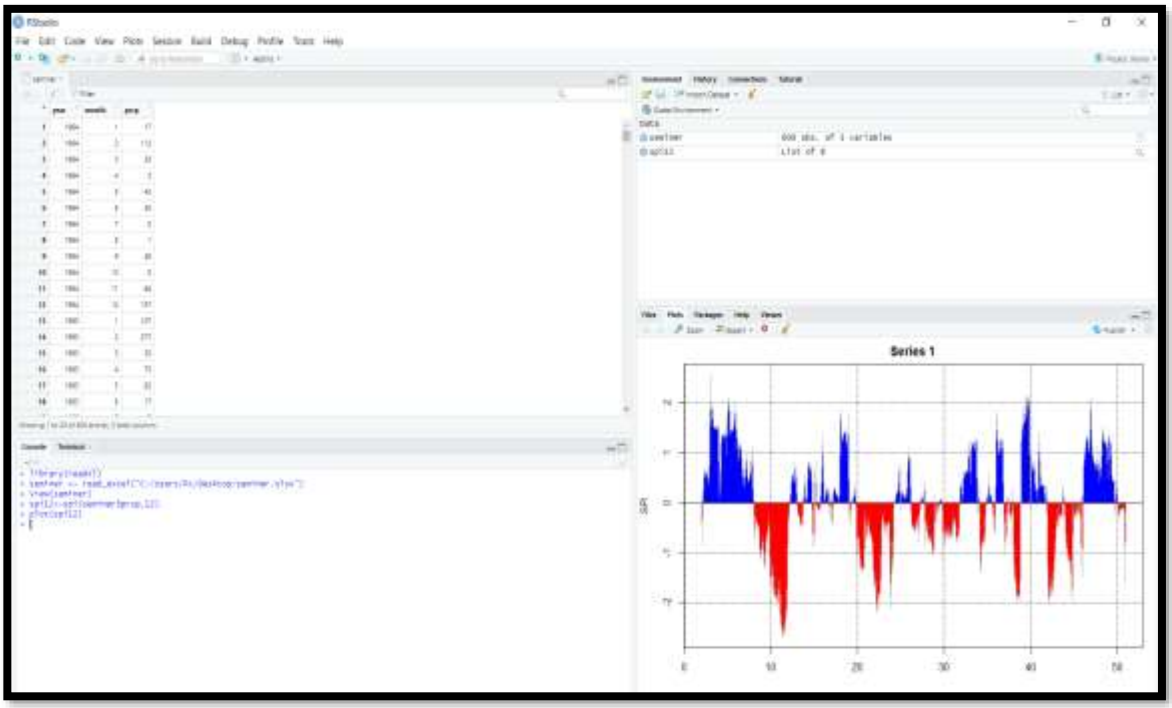
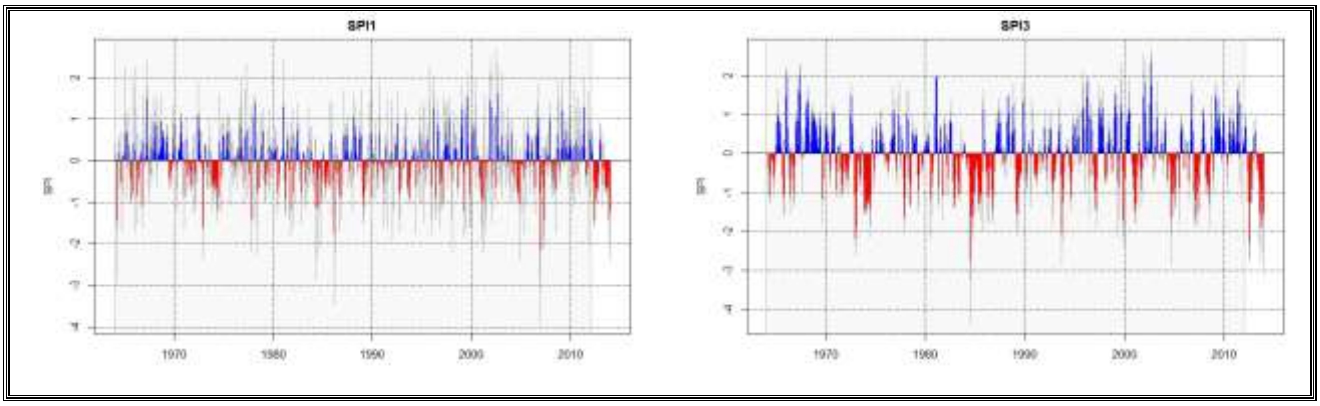


Figure 2 Obtaining SPI-12 Analysis Using Sample Data

Figure 3 Commands Used for SPI Analysis with Sample Data



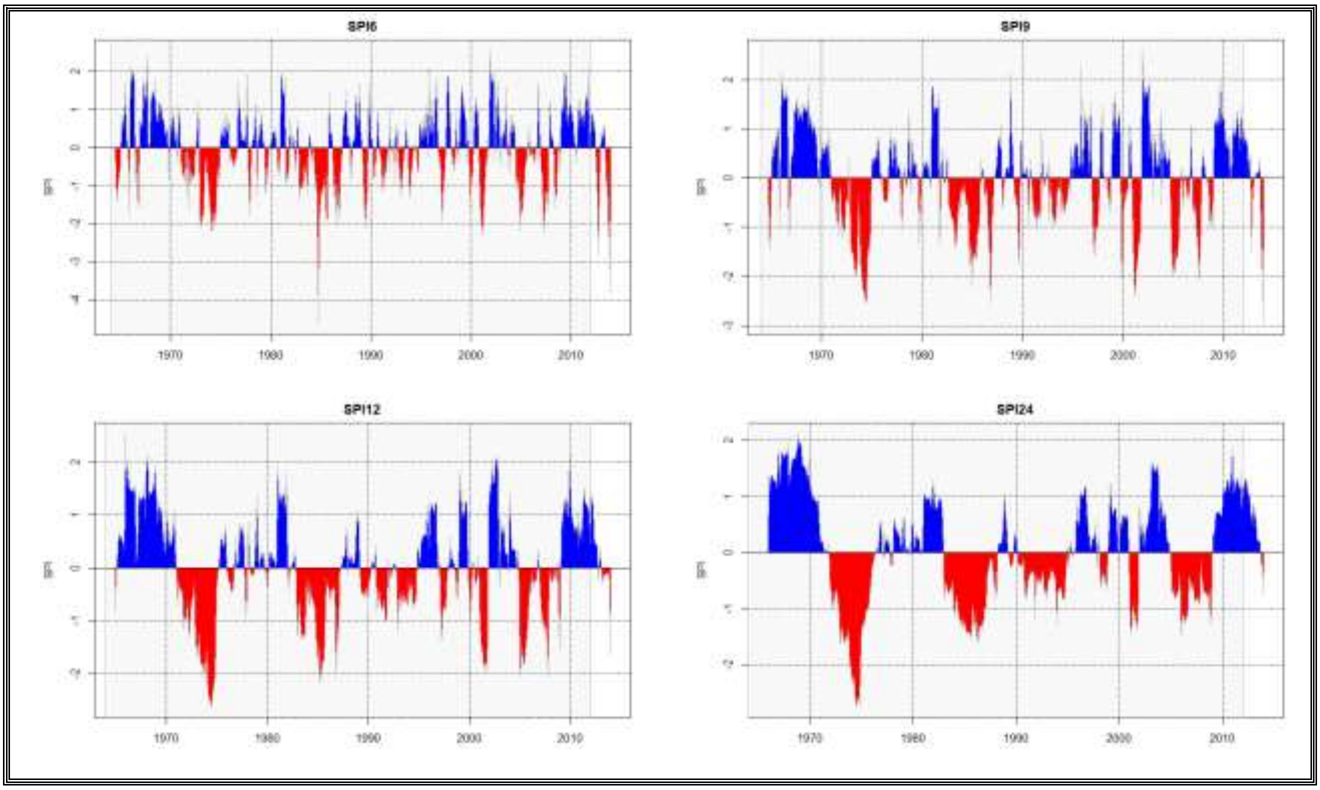


Figure 4 SPI Analysis Result Graphs

RESULTS AND DISCUSSION

Analysis results can be interpreted by using the limit values specified in Table 1. When the sample analysis results were examined, it was determined that the years 1973-1975 were moderately-extremely dry, 1984-1986 moderate-severe dry, 2001 severely dry, and 2006-2008 moderate-severe dry.

In addition, when the 1, 3, 6, 9, 12 and 24-month SPI analysis in Figure 4 are examined, the 24-month SPI analysis shows clearer ranges than the others. This shows us that when the 1- and 3-month SPI analysis are examined, meteorological drought is observed more frequently, and if the meteorological drought is persistent, agricultural and hydrological droughts may occur.

CONCLUSIONS

Drought is a reality of our country due to its location. Drought disaster has been observed many times in our country in the past, and it is expected to be observed more in the future with the effect of climate change. Therefore, the analysis and interpretation of drought and low flows are essential for obtaining maximum efficiency from water and managing water. Within the scope of this study, a sample drought analysis study was carried out with the Standardized Precipitation Index (SPI), which is the most widely used in the literature, using the R program and the calculation steps are shown.

Low flow studies and applications are not common in our country yet. Therefore, there is no generally accepted low flow analysis method. For this reason, determination of low flow analysis methods suitable for this country will allow water management to be more sustainable. As a result of the research, it was concluded that low flow analysis should be evaluated by supporting them with drought analysis.

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THE QUALITY CHARACTERISTICS OF DRIED RED CAPIA PEPPER

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ABSTRACT

Convective drying (CVD) at 50, 60 and 70 ^{o}C was applied to obtain dried red capia peppers. The effects of CVD on color values (*L**, *a**, *b**), rehydration capacity, and selected chemical properties including dry matter, pH and titratable acidity of seven dried red capia peppers were compared. In addition, the effects of the cutting types (ring and cube) on the final quality of pepper samples were determined at room temperature. The samples dried at 60 ^{o}C and cut in ring forms (6-R samples) resulted with high-quality dried peppers. 6-R samples showed the highest dry matter, pH, titratable acidity and rehydration capacity (87.06%, 5.69, 807.15 mg citric acid/100 g, 6.72%, respectively). The closest *L**, *a**, *b** values to the fresh pepper samples were achived for 6-R samples (*L***:* 29.87, *a***:* 12.83, and *b***:* 17.03). In overall, dried red capia peppers in ring forms at 60 ^{o}C can be used to acquire a high-quality food material along with an enhanced color, physical and chemical properties.

Keywords: Color, Convective drying, Red capia pepper, Rehydration capacity, Titratable acidity

INTRODUCTION

Pepper belongs to the Capsicum genus of the Solanaceae family and grows in the United States, South America, Peru, Bolivia, Costa Rica, Mexico and almost all Southern European countries (Kumari, 2012). In addition to being rich in vitamins (especially A and C), minerals, phenolic components and carotene, it also has antioxidant properties (Karaağaç and Balkaya, 2010). Capia pepper is a type of pepper with a long conical structure, meaty, red color and a sweet flavor and has been used as "paste" and "oiliness" for years (Demirel et al., 2012). Although Capia pepper is consumed fresh and dried, it is also used in the food industry in the production of canned, tomato paste, pickled peppers, frozen food and sauce. In addition to these, it has been reported that it is also used in the production of antibiotic raw materials, feed materials and dyes (Hekimoğlu and Altındeğer, 2009; Akgün, 2010). As many fruits and vegetables, peppers can also be preserved for a long time by drying process. Drying is one of the oldest methods known for the preservation of food. The purpose of drying is to remove free water from the product, and to store the products for a long time. So, the crops can be available in areas besides the harvest season. In addition, the volume and weight of dried products are reduced, making packaging and transportation easier with a low-cost. The most popular method of drying food is the convective drying method (Michalska et al. 2016). However, this method causes deterioration of taste, color and nutritional compounds in products (Calin-Sanchez et al. 2014). In this sense, the importance of the temperature applied during the drying process is understood and it is wondered how these degrees will have an effect on the quality of the foods. This research, for this reason, has the primarily aim of contributing to the works of the use of convective drying on capia pepper quality. Specifically, the influence of the drying temperature and cutting type on color values (L^*, a^*, b^*), rehydration capacity, and selected chemical properties including dry matter, pH and titratable acidity of dried red capia peppers were compared.

MATERIAL AND METHOD

Sample preparation

The red capia peppers were obtained at a local market in Iğdır, Turkey and kept at 4 ± 0.5 ºC until the experiments. They were washed to clean the dust, chemical residuals and attached dirt. After removing the excess water from the surface of pepper samples by paper towel, the peppers were cut in two different forms (ring and cube) with a sharp knife. The beginning moisture ratio of the pepper slices was defined as 83.8 ± 0.54 % by drying at 105 ± 5 °C before reaching the stable weight via forced air convective oven.

Drying process

Convective drying was applied in a lab convective oven (Arçelik KMF 833I, Turkey) following the method proposed by İzli (2018). The ring and cube shaped capia pepper samples were located in a thin layer. Air velocity was well-set at 1 m/s with air temperatures of 50, 60, 70 °C for the drying process. The drying process was continued until 12% moisture content. The experiments were conducted with three replications. A short definition of the treatments is tabulated in Table 1.

Table 1. Treatments used in the study

Sample names	Treatments
Control	Fresh sample, no treatment
5-R	Capia peppers dried at 50 ^{O}C in ring forms
5-C	Capia peppers dried at 50 ^{O}C in cube forms
6-R	Capia peppers dried at 60 ^{O}C in ring forms
6-C	Capia peppers dried at 60 ^{O}C in cube forms
7-R	Capia peppers dried at 70 ^{O}C in ring forms
7-C	Capia peppers dried at 70 ^{O}C in cube forms

Color measurement

The color changes of fresh and dried capia peppers were analyzed by a Konica Minolta (CR400, Japan) that is assembled with illuminant D 65 and 8 mm measuring scope in the CIE L^* a^* b^* color scale. Color parameters were described in a 3-dimensional L^*, a^*, and b^* color space, where L^* shows the lightness/darkness of the capia peppers, a^* demonstrates the redness/greenness, and b^* displays the yellowness/blueness (Yildiz, 2021).

Rehydration Capacity

The rehydration ratio (RR) of the dried capia pepper slices was determined according to Cemeroğlu (2009) by weighing ~10 g of CVD pepper samples in distilled water (100 mL) at room temperature for 24 hours. Subsequent to soaking, the extra water was taken off and the capia pepper samples were weighed. The calculation of RR of the pepper samples were determined as below:

Rehydration ratio (%) = $m_3 / (m_1 - m_2)$
m_1: weight of water (fresh capia pepper), g
m_2: weight of water (dried capia pepper), g
m_3: weight of water (following rehydration), g

Dry matter

Homogenized capia pepper samples (5 g) were weighted in disposable aluminum dishes and dried at 105 ± 5 °C until reaching the stable weight via forced air convective oven (Cemeroğlu, 2009).

pH

Fresh samples put into blender directly and dried pepper samples were added with some water. The samples were homogenized, centrifuged and the clear supernatant was obtained. pH of the samples was measured using an Accumet Research AR15 pH meter (Fisher Scientific, USA) (Cemeroğlu, 2009).

Titratable acidity

Titratable acidity was determined following the procedure stated by Cemeroğlu (2009). While the fresh samples put into blender directly, dried pepper samples were added with some water. The samples were homogenized, centrifuged and the clear supernatant was obtained. Then, the samples were titrated with 0.1 N NaOH to the end point of 8.2. Titratable acidity was calculated by taking account of NaOH used.

Statistical data analysis

A factorial experiment (3 × 2: temperature and cutting type) with a randomized complete design was used to analyze the effect of three different temperature levels (50, 60, and 70°C) and two different cutting types (ring and cube). Three replications for each treatment were used for all measurements, unless otherwise stated. Statistical analyses were managed using a randomized plots factorial experimental design. The results were analyzed using the

JMP (Version 7.0, SAS Institute Inc., Cary, NC, USA). Differences among the mean values were obtained by Fisher's least significant difference (LSD) test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The color changes of fresh and dried capia pepper samples are tabulated in Table 2. It was observed that the L^* value of all the dried samples decreased significantly compared to the fresh capia pepper ($p < 0.05$). When compared with the pepper samples dried in cube forms, the samples dried in ring forms showed significantly higher L^* values (Table 2). While the highest L^* value (the closest to the fresh pepper sample) was found for the samples dried at 60 °C in ring forms (29.87 ± 0.63) and cube forms (28.75 ± 0.45), the lowest L^* value was determined for the samples dried at 70 ºC (Table 2). The lower lightness value means darker appearance of the convective-dried pepper slices at 70 ºC might be because of the non-enzymatic browning (Maskan, 2000). On the contrary to the L^* values, a significant increase in a^* values were observed for all dried samples compared to the fresh pepper samples. Among the dried pepper samples, the highest a^* value was observed for the samples dried at 70 ºC whereas the lowest a^* value was found for the samples dried at 60 ºC (Table 2). In brief, the pepper samples dried at 60 °C in ring forms showed the highest L^* value, and lowest a^* value. The preferred colors are those closest to the original color of fresh pepper samples. The capia pepper having higher lightness value can be evaluated as more favourable and marketable products with respect to color quality (Ergunes, and Tarhan, 2006). From this point of view, drying at 60 °C in ring forms was suitable since it resulted with a lighter product color compared to other drying temperatures (50 and 70 ºC) and closest color values to the fresh pepper sample.

Table 2. Color values of convective dried ring and cube shaped capia peppers

Color values	L^*	a^*	b^*
Control (Fresh sample)	38.55 ± 0.43^{a}	10.21 ± 0.98^{f}	18.12 ± 0.13^{b}
5-R	24.31 ± 0.12^{c}	19.24 ± 0.15^{d}	19.15 ± 0.34^{a}
5-C	22.26 ± 0.78^{cd}	23.45 ± 0.07^{b}	15.67 ± 0.72^{e}
6-R	29.87 ± 0.63^{b}	12.83 ± 0.16^{e}	17.03 ± 0.69^{c}
6-C	28.75 ± 0.45^{b}	12.74 ± 0.65^{e}	16.88 ± 0.18^{cd}
7-R	19.88 ± 0.03^{d}	21.18 ± 0.81^{c}	13.08 ± 0.04^{f}
7-C	15.63 ± 0.19^{e}	25.44 ± 0.27^{a}	15.54 ± 0.71^{e}

$^{a\text{-}f}$: Means superscript with different alphabets in the same column differ significantly ($p < 0.05$).

Dry matter, pH, titratable acidity and rehydration capacity of convective dried ring and cube shaped capia peppers are demonstrated in Table 3. As shown in Table 3, dry matter of convective-dried pepper samples in ring forms at 60 ºC (87.06%) were significantly higher compared to the fresh capia pepper and other dried pepper samples. On the other hand, the lowest dry matter was obtained for the pepper samples dried at 70 ºC (Table 3). All dried pepper samples no matter if the samples dried in ring or cube forms or dried at 50, 60, and 70 ºC showed a significant increase on dry matter compared to the fresh pepper samples. In addition, the pepper samples dried in ring forms showed a higher dry matter compared to the samples dried in cube forms. Dried pepper samples showed a significant decrease for pH, and a significant increase for titratable acidity compared to the fresh capia peppers (Table 3). As shown in Table 3, rehydration capacity convective-dried pepper samples in ring forms at 60 ^oC were significantly ($p < 0.05$) higher than fresh capia pepper samples, showing that dried pepper samples in ring forms at 60 ^oC were easier to recover by rehydration. The possible reason was that better porous structure and higher cell membranes permeability were formed in the dried pepper samples in ring forms at 60 ^oC. From the findings, it is obvious that the water gain is more noticeable in dried pepper samples at 60 ^oC (6.72 ± 0.11% and 5.99 ± 0.02% in ring and cube forms, respectively) compared to the other capia peppers dried at 50 and 70 ^oC (Table 3). Similar results were observed in the study of İzli and Yildiz (2021). They've observed significantly higher rehydration ratio for the convective-dried quince samples at 60 ^oC compared the samples dried at 50 ^oC and 70 ^oC. In addition, each and every dried capia peppers in ring forms have better rehydration capacity compared to the convective dried capia peppers in ring forms.

Table 3. Dry matter, pH, titratable acidity and rehydration capacity of convective dried ring and cube shaped capia peppers

Samples	Dry matter (%)	pH	Titratable acidity (mg citric acid /100 g)	Rehydration Capacity (%)
Control	16.28 ± 0.96^{c}	5.78 ± 0.01^{a}	471.83 ± 0.65^{g}	0.18 ± 0.01^{e}
5-R	79.19 ± 0.64^{ab}	5.58 ± 0.02^{c}	656.37 ± 0.71^{d}	5.12 ± 0.07^{c}
5-C	77.34 ± 0.47^{ab}	5.54 ± 0.04^{c}	839.13 ± 0.48^{a}	5.05 ± 0.23^{c}
6-R	87.06 ± 0.34^{a}	5.69 ± 0.03^{b}	807.15 ± 0.67^{b}	6.72 ± 0.11^{a}
6-C	84.37 ± 0.65^{a}	5.66 ± 0.01^{b}	711.29 ± 0.94^{c}	5.99 ± 0.02^{b}
7-R	74.91 ± 0.03^{b}	5.63 ± 0.07^{bc}	565.65 ± 0.12^{e}	4.64 ± 0.07^{d}
7-C	73.15 ± 0.67^{b}	5.62 ± 0.02^{bc}	514.75 ± 0.73^{f}	4.58 ± 0.01^{d}

$^{a-g}$: Means superscript with different alphabets in the same column differ significantly ($p < 0.05$).

CONCLUSIONS

The convective drying of capia peppers at 50, 60, and 70 ^oC in ring and cube forms was investigated. A significant development in the physical and chemical properties of ring-shaped convective-dried pepper slices at 60 ^oC was accomplished. A practical implication is that drying of capia pepper slices with an optimum condition by drying temperature and cutting type can be used to get a high-quality product with an improved color and better physical and chemical properties.

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EFFECTS OF SOME ESSENTIAL OILS (CITRUS LIMON AND CITRUS SINENSIS) AGAINST APHIS FABAE SCOPOLI (HEMIPTERA: APHIDIDAE)

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ABSTRACT

Aphids can cause significant economic losses in agricultural productions because they have the ability of fast reproduction. In other hand, they are vector of several plant diseases and viruses. Therefore, in the scope of biological control studies, it is important to reveal the effects of some essential oils against aphids. In this study, essential oils of *Citrus sinensis* and *Citrus limon* were studied insecticidal effects on black bean aphid *Aphis fabae* Scopoli (Hemiptera: Aphididae). The lethal and sublethal effects of essential oils of *C. sinensis* and *C. limon* on one day old adults of *A. fabae* were investigated under laboratory conditions. The trials were evaluated at the different concentrations (0.5, 1, 2, 4, 6, 8, 10, 12 µL/L), for 24 h after treatments. After this process, lethal concentrations (LC_{50}, LC_{90}) of EOs were calculated according to the obtained data. Life table parameters of new emerged aphid surveyed at sub-lethal concentrations (LC_{40}, LC_{30}) of EOs and these parameters were calculated by Euler-Lotka equation. According to the results, the mortality rate of EOs increased with the increasing of concentration. Lethal concentrations (LC_{50}, LC_{90}) of *C. sinensis* were calculated as 4.79 and 11.86 µL/L, respectively. Lethal concentrations (LC_{50}, LC_{90}) of *C. limon* were calculated as 3.86 and 10.46 µL/L, respectively. Sub-lethal concentrations (LC_{40}, LC_{30}) of EOs caused decrease in adult longevity, fertility of surviving aphids and intrinsic rate of increase. It has been concluded that the essential oils can be used in the control of these pests.

Keywords: *Aphis fabae*, essential oils, lethal effects, *Citrus sinensis*, *Citrus limon*

INTRODUCTION

Considering the agricultural production around the world, it has an important share in the development of countries and people and its importance is increasing day by day (Tunçer and Günay, 2017). Depending on the increase in agricultural production, there is an increase in the problems caused by diseases and pests. In the fight against such problems, chemicals are mostly preferred and a new chemical is introduced every day. However, pests and disease causing organisms also develop resistance to new chemicals, and as a result, manufacturers increase the use of chemicals. In addition to the resistance problem, alternative methods have been sought and developed due to the negative effects of these products on the environment and human health (Lacey et al, 2001). One of the methods applied is biological control. Although there is continuity in this method, it does not create environmental pollution when applied, and does not harm human and wildlife (Uygun et al, 2010).

Aphids cause fumagine by promoting the development of saprophytic fungi due to the honeyed substance they secrete, as well as direct sucking damage on the plant. At the same time, it causes significant damage to plants by being a vector for plant virus diseases (Von Dohlen et al., 2006; Will and Vilcinskas, 2015; Boissot et al, 2016; Kloth et al, 2017; Stevens and Lacomme, 2017; Helvacıoğlu and Akşit, 2020; Satar, 2020). The broad bean aphid *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) is a small, black-colored species. (Kennedy et al, 1962). Its hosts include more than 200 wild plants, as well as vegetables, sugar beet, broad beans, beans, potatoes, sunflowers and tomatoes. (Barnea et al, 2005; Fericean et al, 2012).

The most commonly used method in the fight against aphids is chemical control as in the control of many pests. As a result of intensive use of synthetic insecticides, aphids have developed a strong resistance to insecticides (Elbert et al., 2008; Sial et al., 2019). As a result of excessive use of insecticides, the environment and human health are adversely affected, the natural balance is disturbed, and residue problems occur in the products (Grdıša and Gršıć, 2013; Gill and Garg, 2014, Rother, 2018). Due to the negative effects of pesticides used against agricultural pests on the environment and human health, it has been sought for organisms with a shorter decomposition time compared to them, undesirable to be killed, and plant-derived compounds with less negative impact on the environment (Arnason et al., 1989; Feng and Isman, 1995; Wewetzer, 1995; Hedin et al., 1997; Momen et al., 1997; Liao et al., 2017; Kunbhar et al., 2018). Botanical insecticides attract attention as a good alternative to synthetic insecticides due to the bioactive chemical compounds in the plants (Isman, 2000; Kim et al., 2003b; Govindarajan et al., 2016; Khan et al., 2017; Sammour et al., 2018). Plant extracts and essential oils have emerged as alternatives to other synthetic insecticides for pest control. These are natural insecticides produced from plants and containing a number of bioactive chemicals (Isman, 2000; Govindarajan et al., 2016; Sammour et al., 2018). Many of the volatile compounds found in plants are rapidly decomposed in nature and do not accumulate in the environment like other chemicals, so they are preferred in biological control (Arnason et al., 1989; Hedin et al., 1997; Regnault-Roger et al., 2012). These compounds pose a low risk to non-target organisms (predators and parasites) and are mostly non-toxic to mammals (Scott et al., 2003). Considering the studies, many studies on the insecticidal activities of essential oils in plants and their components stand out (Regnault-Roger et al., 1993; Regnault-Roger and Hamraoui, 1995; Golob et al., 1999; Weaver and Subramanyam, 2000; Kéita et al., 2001; Lee et al., 2001; Papachristos and Stamopoulos, 2002; Kim et al., 2003a; Isman and Miresmailli, 2011; Ntalli and Menkissoglu-Spiroudi, 2011; Miresmailli and Isman, 2014; Regnault-Roger et al., 2012; Pavela and Benelli, 2016; Chaubey, 2019; Feng et al., 2020; Gaur and Kumar, 2020; Sayed et al., 2021). Herbal extracts or oils obtained from plants have different advantages over pests when compared to traditional insecticides. These secondary metabolites act on physiological or behavioral adaptations in the target organism and contain many components with mechanisms that slow down the evolution of insects in these parts (Isman, 2006). In this study, lethal and sub lethal effects of Essential oils obtained from two different plants (*Citrus limon* and *Citrus sinensis*) on *Aphis fabae* were determined.

MATERIAL AND METHOD

Essential oils

The essential oils (*Citrus limon* and *Citrus sinensis*) used in this study were commercially obtained from Botalife®.

Production of plants for aphids

The broad bean (*Vicia faba*) plant used in the experiments was grown in plastic containers (200 ml) containing 1:1 soil: peat. The production was carried out in a climate room with 25±1 °C, 60±5% proportional humidity and 16:8 (light: dark) lighting conditions in Yozgat Bozok University, Faculty of Agriculture.

Mass production of aphids

The last period nymph and/or *A. fabae* individuals were transferred on the pod plants that reached the height (15 cm) and the number of leaves (6 pieces) to be used in the experiments and they were reproduced in separate environments. The initial population of aphids infested with clean plants was obtained from the mass production in Yozgat Bozok University, Faculty of Agriculture, and Department of Plant Protection. In order to prevent the aphids taken into production from mixing with each other, they were placed in cages of 50x50x50 cm, covered with tulle. To ensure the continuity of mass production, aged and decaying plants were replaced with clean plants at weekly intervals. Production of aphids was carried out in a climate room with 25±1 °C, 60±5% proportional humidity, 16:8 (light: dark) lighting conditions.

Contact insecticidal activity

The lethal effects of different doses (0.5, 1, 2, 4, 6, 8, 10, 12 µL/L) of applied vegetable oils (*Citrus limon* and *Citrus sinensis*) on *Aphis fabae* were determined at this stage. Tween20 2% was used to dissolve the oils used in the trials. Petri dishes with 9 cm diameter filter paper were used in the experiments. The prepared doses were absorbed into filter papers as 1 ml in each petri dish. One-day-old nymphs were transferred onto these papers with the help of a fine sable brush and contacted (tarsal, ventral and labial contact) with the dose left on the paper. Then, a bean leaf was left in the petri dish to feed the aphids. At this stage, 10 petri dishes were used for each dose and 10 aphids were used for each petri dish. For the control application, Tween20 2% was used. Live and dead individuals were recorded 24 hours after the trials were set up and the effects of the oils were determined. All of the experiments were carried out in the climate room in Yozgat Bozok University, Faculty of Agriculture, with 25±1 °C, 60±5% proportional humidity 16:8 (light: dark) lighting conditions.

Abbott's formula was used to determine mortality rates over living and dead individuals and the percentage of mortality rates was calculated (Abbott, 1925). Analysis of variance (ANOVA) was applied to the obtained results. If the difference between the means was statistically significant, the level of this significance was determined according to the TUKEY multiple comparison test. Lethal concentrations of vegetable oils (LC_{30}, LC_{40}, LC_{50} and LC_{90})

were determined using the mortality rates obtained at this stage of the study. PROBIT analysis was used to determine these concentrations.

$$\text{Percent Effect} = \left(\frac{\text{Number of live individuals in control} - \text{Number of live individuals in the application}}{\text{Number of live individuals in control}}\right) \text{X } 100$$

Estimating life table parameters

At this stage, the effects of LC_{30} and LC_{40} concentrations of vegetable oils on *Aphis fabae* were determined. As in the previous stage, the prepared doses were impregnated with the filter papers in the petri dishes and contact of 1-day-old individuals was ensured. Then, cotton was left on the bottom of the filter paper so that the leaves would not fade and it was wetted. Then, bean leaves were placed to feed the aphids. The petri dish was opened on top of the petri dish to ventilate and covered with tulle. Then, the daily developments of the individuals were followed; the newborns were recorded and removed from the environment. The counts continued until the aphids died. In this part of the trials, 50 individuals were used separately for each age and dose. These experiments were carried out in a climate room with 25±1 °C, 60±5% proportional humidity and 16:8 (light:dark) lighting conditions. The data that obtained from the experiments were recorded to determine the development of age-related life tables for each temperature that used. Life table parameters of the predator were calculated by using RmStat-3 software (Özgökçe and Karaca, 2010) according to Euler-Lotka equation (Birch, 1948) and were evaluated separately. Tukey multiple comparison test was used for comparison of the periods with Minitab (ver. 16) at the significant difference $p<0.05$ level. To calculate the parameters, several equations were used which were:

Age-related survival rate (l_x), Fertility rate (m_x) (Birch, 1948);

Reproductive value (V_x) $V_x = \frac{\sum_{y=x} (e^{r_m \cdot y}.\, l_y.\, m_y)}{l_x.e^{-r_m \cdot x}}$ (Imura, 1987);

Net Production Rate (R_0) $R_0 = \sum l_x.\, m_x$ (Birch, 1948);

Intrinsic Rate of Increase (r_m) $\sum e^{(-r_m \cdot x)}\, l_x.\, m_x = 1$ (Birch, 1948);

Mean Generation Time (T_0) $T_o = \frac{\ln R_0}{r_m}$ (Birch, 1948);

Total Productivity Rate (GRR) $GRR = \sum m_x$ (Birch, 1948);

Daily maximum reproductive value (λ) $\lambda = e^{r_m}$ (Birch, 1948);

Doubling time (T_2) $T_2 = \frac{\ln 2}{r_m}$ (Kairo and Murphy, 1995).

RESULTS AND DISCUSSION

The lethal effects of different doses (0.5, 1, 2, 4, 6, 8, 10, 12 µL/L) of vegetable oils (*C. limon* and *C. sinensis*) applied on *Aphis fabae* were determined in this study. According to the data obtained, it was observed that *C. limon* was more effective than *C. sinensis*. When aphids were exposed to different doses of *C. limon*, the effect at the highest dose (12 µL/L) was 94.74%, while the effect at the lowest dose (0.5 µL/L) was calculated as 29.48%. When *A.*

fabae was exposed to different doses of *C. sinensis*, the effect at the highest dose (12 µL/L) was 89.25%, while the effect at the lowest dose (0.5 µL/L) was 22.58% (Figure 1).

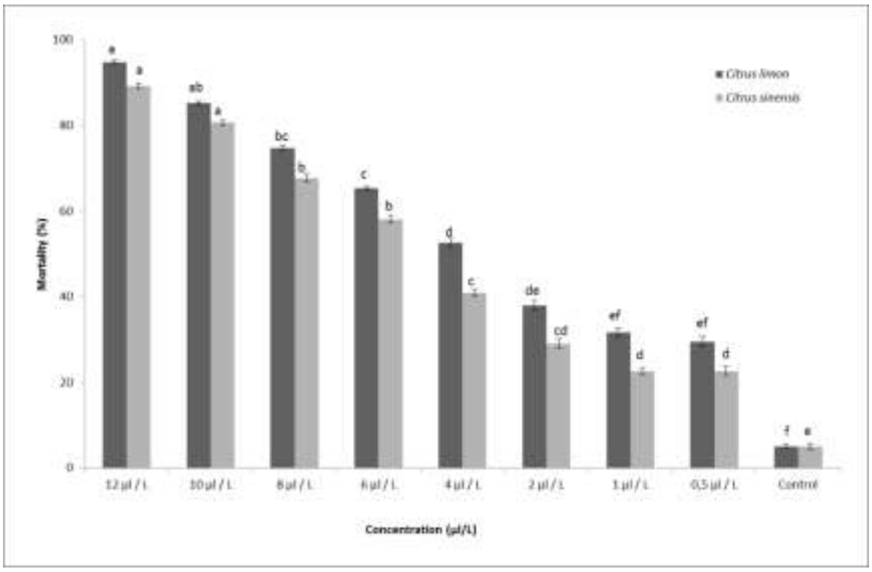

Figure 1. Mortality percentage of *A. fabae* exposed to different concentrations of different essential oils (*Citrus limon* and *Citrus sinensis*) for 24 h. Means above columns followed by different letters were significantly different according to Tukey.

Lethal concentrations of essential oils (LC_{30}, LC_{40}, LC_{50} and LC_{90}) were determined using the mortality rates obtained at this stage of the study. The obtained values are given in Table 1.

Table 1. Toxicity of different essential oils on *Aphis fabae* after 24 h

Essential oils	LC_{30} (µl/L)	LC_{40} (µl/L)	LC_{50} (µl/L)	LC_{90} (µl/L)	Slope ±SE	X^2(df)
Citrus limon	1.16 (0.49-1.72)	2.56 (2.01-3.05)	3.86 (3.38-4.33)	10.46 (9.61-11.52)	1.268±0.118	25.30 (7)
Citrus sinensis	1.90 (1.24-2.47)	3.40 (2.85-3.90)	4.79 (4.30-5.30)	11.86 (10.90-13.08)	1.264±0.118	13.54 (7)

At this stage, the effects of LC_{30} and LC_{40} concentrations of vegetable oils on *Aphis fabae* were determined. The effects of the administered doses on the life table parameters of *A. fabae* have been demonstrated. Accordingly, when the application doses were compared with the control, it was observed that lx, mx and Vx values decreased in the applied doses. (Figure 2).

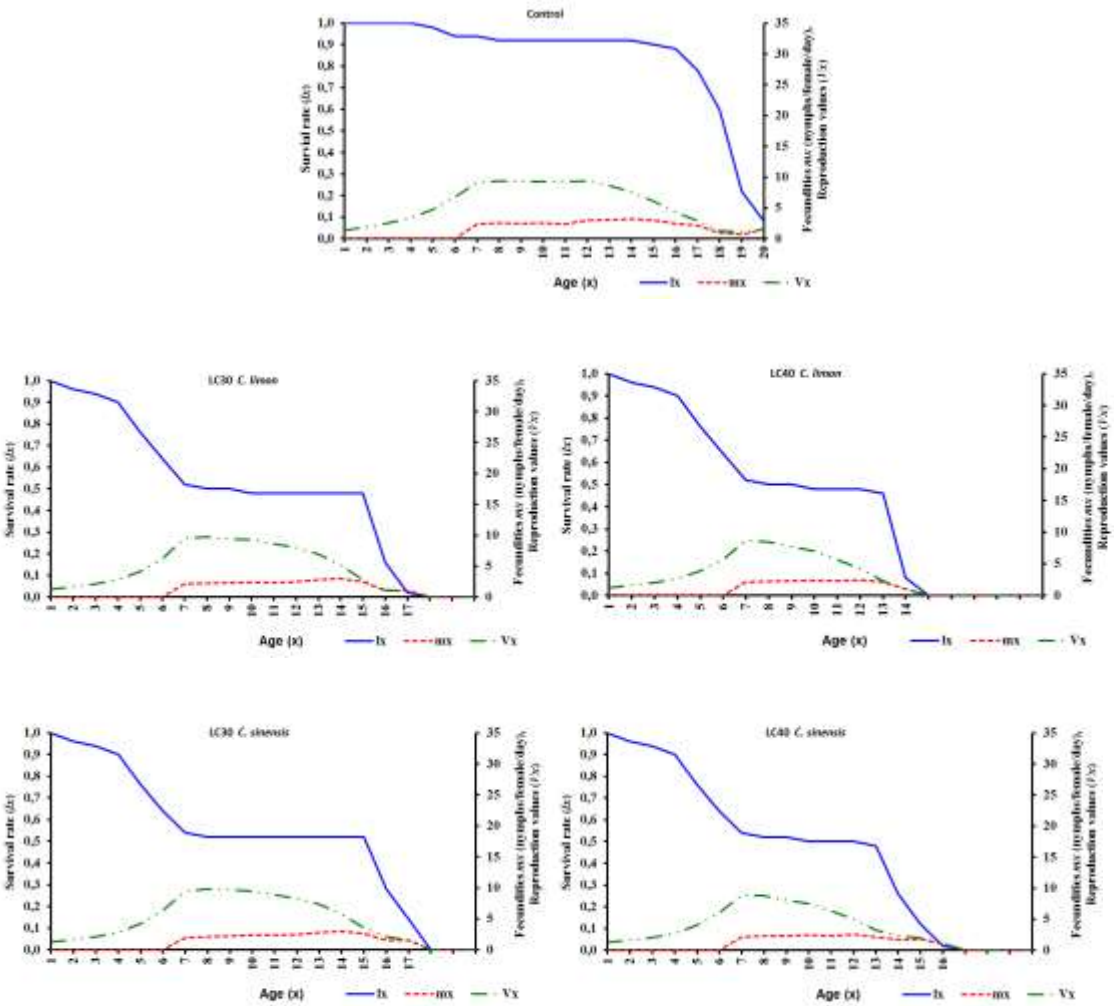

Figure 2. Survival rate, fecundity and reproductive value of *Aphis fabae* under influence of different concentrations of essential oils (*Citrus limon* and *Citrus sinensis*)

According to the results obtained, it was determined that Intrinsic rate of increase (r_m), Net reproductive rate (R_0) and Total reproductive rate (GRR) decreased at the applied doses when compared with the control. Accordingly, the intrinsic rate of increase was calculated as 0.308 nymphs/female/day in the control application. The same parameter was calculated as 0.229, 0.216, 0.233 and 0.224 nymphs/female/day at different doses (LC_{30} and LC_{40}) of oils (*C. limon* and *C. sinensis*), respectively. While the net reproductive rate was calculated as 27.249 nymphs/female in the control application, the values occurring at different doses of the oils were found to be 10.975, 7.875, 12.084 and 8.909 nymphs/female, respectively. A similar situation exists for the total reproduction rate. While this value was high in the control application, it decreased at other concentrations. Other parameters in the life table are also given in Table 2.

Table 2. Life table parameters of *Aphis fabae* under influence of different concentrations of essential oils (*Citrus limon* and *Citrus sinensis*)

Parameters	Control	LC_{30} *C. limon*	LC_{40} *C. limon*	LC_{30} *C. sinensis*	LC_{40} *C. sinensis*
Intrinsic rate of increase, r_m	0.308	0.229	0.216	0.233	0.224
Net production rate, R_o	27.249	10.975	7.875	12.084	8.909
Mean generation time, T_o	10.748	10.458	9.574	10.681	9.776
Total production rate, ***GRR***	32.322	24.121	16.960	25.003	20.716
Doubling time, T_2	2.254	3.026	3.216	2.971	3.098
Finite rate of increase, λ	1.360	1.257	1.241	1.263	1.251
N	50	50	50	50	50

It is seen that studies have increased in recent years to investigate the effects of plant-based pesticides on pests. The most emphasized groups in these studies are the families Meliaceae, Rutaceae, Asteraceae, Labiateae, Piperaceae, and Annonaceae (Schoonhoven, 1982; Jacobson, 1989; Isman, 1995; Durmuşoğlu et al, 2011; Sayeda & El-Mogy, 2011; Balcı et al, 2020). Azadirachtin, which is commercially available today, is obtained from the Neem tree (*Azadirachta indica*, Meliaceae) and is used as a feeding and growth inhibitor against many harmful insects. (Isman, 1997; Kısmalı and Madanlar, 1988; Castagnoli et al., 2005; Charleston et al., 2006; Seljåsen and Meadow, 2006; Göçmen et al., 2007; Duso et al., 2008; Uçak et al., 2014; Sayeda and El-Mogy, 2011; Schneider et al., 2017). In addition, it is known that trans-anethole, a phenylpropanoid obtained from *Pimpinella anisum,* is effective on many harmful species from Coleoptera, Hymenoptera and Lepidoptera (Saraç and Tunç, 1995 a; b; Ho et al., 1997a; b; Kelm et al., 1997). Although the effects of bio pesticides used in the fight against agricultural pests are not fully known, it is seen that they have different effects on them. For this reason, the dose, concentration and application frequency of the bio insecticides used are very important (Bakkali et al., 2008). In addition, when the studies conducted in recent years are examined, the effects of different vegetable oils on aphids or other pests have been investigated and it has been observed that they are effective on pests, although they vary from species to species (Górski and Tomczak, 2010; Yazdgerdian et al., 2015; Górski et al., 2016; Albouchi et al., 2018; Benelli et al., 2018; Czerniewicz et al., 2018; Behi et al., 2019; Ravan et al., 2019). When the results we obtained were evaluated, similar results were obtained.

CONCLUSIONS

When the results obtained in this study were examined, it was seen that the oils obtained from *Citrus limon* and *Citrus sinensis* were effective on the broad bean aphid *Aphis fabae*. According to this, it was concluded that the vegetable oils used in controlling both this pest and the similar pest group can be beneficial. However, it would be more accurate to determine the effects against pests by investigating both the doses and the contents of vegetable oils.

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A STUDY ON THE DETERMINATION OF THE YIELD AND PLANT CHARACTERISTICS OF SOME DRY BEAN (*Phaseolus vulgaris* L.) GENOTYPES UNDER CANKIRI CONDITIONS

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ABSTRACT

The aim of this study is to determine the seed yield performances of advanced dry bean lines and registered varieties under Cankiri conditions with some plant characteristics. Research; It was carried out at CAKU Uluyazı Campus in 2015. The field experiment was carried out in a randomized block design with 4 replications. The plant material was a total of 7 dry bean genotypes that were resistant to high temperature and drought, provided from the Black Sea Agricultural Research Institute. According to the genotypes in the examined features; plant height, number of main branches per plant, number of seeds per pod and harvest index showed significant changes ($p<0.01$). While the number of pods per plant, total yield and 1000 seed weight showed significant ($p<0.05$) changes, the changes in first pod height, seed yield per plant and seed yield were insignificant. Seed yield varied between 153.61-198.61 kg/da, and F5.Ç.224 line gave the highest yield, although it was not statistically significant.

Keywords: Bean, genotype, yield.

INTRODUCTION

The increasing population all over the world also increases the need of people for foodstuffs. In order for people to have a balanced diet, they should take protein in certain proportions in addition to carbohydrates in their daily diets. The global climate change seen in the world in recent years has made it important to develop new techniques, technologies and materials that will increase production in the agricultural field. In our world where agricultural areas have reached their final limits; In order to meet the food needs of people, it is a necessity to increase the unit area yield in plant production. For this, it is very important to develop varieties/varieties that best respond to new technologies, inputs and methods used in agricultural production. Due to the high cost of animal origin proteins, people, especially in developing countries, meet a significant part of their protein needs from plants (Azkan, 1999). Bean seeds have an important place in nutrition with 22% protein, 57.8% carbohydrates, 1.6% fat, 4% cellulose, 3.6% ash and rich mineral and vitamin contents (Sepetoglu, 1994).

Data, dry bean production area and production amount in Turkey are 102.986 ha, 279.518 tons, respectively in 2020 (TUIK, 2021). Beans are a temperate climate plant. It requires an average temperature of 10-32°C during the growing season. High temperatures adversely affect

pollination and fertilization in bean flowers, flowers and pods fall (Sehirali, 1988; Akdag, 2001).
In Cankiri, summers are hot and dry, and winters are cold and harsh. The average temperature in the province is 11.1 °C and the total precipitation is 402 mm. The hottest months in the central district are July (42.4 ºC) and August (41.8 ºC) (Anonymous, 2016).
The main purpose of this study is; The aim of this study is to determine some vegetative characteristics of some advanced bean lines developed from parents that are drought and high temperature stress tolerant/tolerant, and some vegetative characteristics of registered cultivars under Cankiri conditions.

MATERIALS AND METHODS

Field trials of the study were carried out in 2015 vegetation period in ÇAKÜ Uluyazı Campus. Plant material was obtained from the Black Sea Agricultural Research Institute (KTAE).

Table.1 Some information about the genotypes used in the study.

Genotype	**Breeding level (line/registered variety)**	**Institution**
Weighing		Abroad
Matterhorn		Abroad
TB 117	Ust-Ant1xbarbun-4sa-0sa	KTAE
TB 155	W.KidneyxZülbiye-3sa-1sa-0sa	KTAE
TB.224	W.KidneyxCardinal-4sa-0sa	KTAE
F5.Ç.153	Pinto2xCranberry-13sa-1sa-0sa	KTAE
Zülbiye	Registered variety	KTAE

Average temperatures are lower than the long-term averages in April (8.5 °C), June (18.4 °C) and July (23.1 °C) and higher in May (16.1 °C), August (23.2 °C) and September (21.2 °C). Total precipitation amounts were above the long-term averages except for April and September. In May and June it was 97.5 and 91.7 mm, respectively. Relative humidity values are higher than the long-term averages in June (71.6%) and August (55.1%), and close to long years in other months. Experimental area soil is slightly alkaline (pH 7.62), poor in organic matter, available potassium amount (260.49 kg/da) is sufficient, phosphorus (1.83 kg/da) is very low. Unsalted and lime content (13.78%) is moderate.
The field experiment was arranged with 4 replications in the Random Blocks Trial design and the sowing was done manually on 21.05.2015. Each genotype was planted in 4 rows at a frequency of 0.50 m x 0.10 m x 5 m. In the plots at the beginning and end of the block, one more row was planted as an edge effect. Before planting, diammonium phosphate (DAP) fertilizer was given to each plot at 15 kg/da (2.7 kg N/da, 6.9 kg P2O5/da). Irrigation (Drip irrigation) and maintenance were done regularly. Harvesting was done by hand as genotypes matured. Observations were made in the remaining area, leaving 50 cm from the beginning of each plot and the outermost row at the beginning and end of the block as the edge effect. In the study, according to Ozcelik (1993) and Akcin (1988); plant height, first pod height, number of main branches per plant, number of pods per plant, number of pods per pod, seed yield per plant, harvest index, seed yield, total biological yield and 1000 seed weight were investigated. The results were subjected to analysis of variance in accordance with the trial design. The Least

Significant Difference (LSD) test was applied to the differences between the means (Düzgüneş et al., 1987).

RESULTS AND DISCUSSION

In the bean genotype grown in Cankiri conditions, according to the genotypes; plant height, number of main branches per plant, number of seeds per pod and harvest index characteristics showed significant changes ($p<0.01$). While the number of pods per plant, total yield and 1000 seed weight showed significant ($p<0.05$) changes, the changes in first pod height, seed yield per plant and seed yield were found to be insignificant (Table 2).

According to Table 2, the general average plant height of the genotypes was 34.13 cm, and it varied between 28,40-41,77 cm according to the genotypes. The highest value was determined in Weighing variety, and the lowest in F5.Ç.224 line. Although genotypes did not have a statistically significant effect on first pod height, genotype averages were between 10.15-1.12 cm. The genotype averages of the number of main branches in the plant varied between 3.50 and 4.15 numbers per plant, statistically TB 155, Zülbiye and F5.Ç.224 were in the first class and Matterhorn was in the lowest class.
The number of pods per plant of the 7 genotypes examined varied between 20.28-25.58 numbers per plant, with an average of 22.65 numbers per plant. While Zülbiye gave the highest value, Weighing and Matterhorn varieties were the genotypes that gave the lowest value. While the number of seeds per pod of dry bean genotypes was determined between 4.05-5.40 numbers per pod, the general average was determined as 4.74 numbers per pod. Weighing and TB 117 genotypes were statistically the highest and Zülbiye was in the last class statistically in F5.Ç.153 and F5.Ç.224. The number of seeds per pod of seven bean genotypes varied between 4.05-5.40 numbers per pod, with an average of 4.74 numbers per pod. Of the genotypes, Weighing and TB 117 constituted the highest class, and Zülbiye, F5.Ç.153 and F5.Ç.224 the last class. Although the genotype factor did not have a statistically significant effect on the changes in seed yield in the plant, the genotype averages ranged from 28.38 (Matterhorn) to 33.17 (F5.Ç.224) g, with an average of 30.60 g (Table 2).
While the average harvest index of the genotypes was between 22.16% (Zülbiye) – 37.14% (TB 117), the overall average was 30.97%. Although the effect of the genotype factor on the changes in seed yield was not statistically significant, the genotype averages changed between 153.61-198.61 kg/da, and the F5.Ç.224 line gave the highest yield. The total yields of bean genotypes varied between 608.54-779.58 kg/da, with an average of 674.67 kg/da. While the Zülbiye variety (779.58 kg/da) was in the top class, all other genotypes were statistically in the same class. F5.Ç.153 line (608.54 kg/da) is the genotype giving the lowest value. In this study, 1000 seed weight values obtained for genotypes were between 306.25 (Matterhorn)–440.00 (F5.Ç.224) and the average was 369.29 g (Table 2).

Table 2. Plant height (cm), first pod height (cm), number of main branches per plant (number/plant), number of pods per plant (number/plant), number of seeds per pod (number/plant), seed yield per plant (g/plant), harvest index (%), seed yield (kg/da), total yield (kg/da), 1000 seed weights of F values, genotype averages and LSD groups of seven bean genotypes grown in Cankiri conditions.

Averages						
#	**Genotypes**	**PH**	**FPH**	**NMBP**	**NPP**	**NSP**
1	Weighing	41.77 a†	10.15	3.97 ab	20.28 c	5.40 a
2	Matterhorn	30.19 d	10.23	3.50 d	20.60 c	5.05 ab
3	TB 117	31.87 bc	11.12	3.62 cd	22.30 bc	5.39 a
4	TB 155	40.92 ab	10.22	4.15 a	22.33 bc	4.92 b
5	F5.Ç.224	28.40 d	10.17	4.05 a	24.58 ab	4.20 c
6	F5.Ç.153	29.70 d	10.77	3.75 bc	22.88 abc	4.18 c
7	Zülbiye	36.05 bc	10.75	4.13 a	25.58 a	4.05 c
Averages		**34.13**	**10.48**	**3.88**	**22.65**	**4.74**
F values		**8.127****	**1.374 n.s.**	**9.248 ****	**2.959***	**20.301 ****
CV(%)		**11.29**	**6.30**	**4.38**	**9.91**	**5.50**
Averages						
#	**Genotypes**	**SYP**	**HI**	**SY**	**TBY**	**TSW**
1	Weighing	28.75	31.92 c	182.78	723.87 ab	343.7 bcd
2	Matterhorn	28.38	35.43 abc	153.61	647.21 bc	306.2 d
3	TB 117	31.23	37.14 abc	171.67	646.47 bc	325.0 cd
4	TB 155	30.57	27.62 bc	184.45	631.13 bc	352.5 bcd
5	F5.Ç.224	33.17	30.62 ab	198.61	685.87 abc	440.0 a
6	F5.Ç.153	29.20	31.93 a	172.50	608.54 c	405.0 abc
7	Zülbiye	32.92	22.16 d	186.39	779.58 a	412.5 ab
Averages		**30.60**	**30.97**	**178.57**	**674.67**	**369.29**
F values		**0.465 n.s.**	**7.822 ****	**0.745 n.s.**	**3.129***	**3.021 ***
CV(%)		**18.63**	**11.49**	**18.52**	**10.00**	**15.58**

†: There is no statistical difference between the values indicated with the same letter.
n.s..,*, **: Not significant, P<0.05 and P<0.01, respectively.
PH: Plant height (cm), FPH: First pod height (cm), NMBP: Number of main branches per plant (number/plant), NPP: Number of pods per plant (number/plant), NSP: Number of seeds per pod (number/pod) , SYP: Seed yield per plant (g/plant), HI: Harvest index (%), SY: Seed yield (kg/da), TBY: Total biological yield (kg/da), TSW:1000 seed weight.

Among the characters examined in the study, plant height, number of main branches per plant, number of seeds per pod and harvest index were very important (p<0.01) and the number of pods per plant (p<0.05) showed significant changes (Table 2). First pod height and seed yield per plant did not show any statistically significant difference according to genotypes. These findings are partially compatible with literature reports. The main reason for our findings, which are inconsistent with the literature, may be the different genotype and environment in which the study was conducted. The genotypes constituting the vegetative material in our study are advanced lines developed from parent material or their hybridization in a breeding program established to develop a variety or varieties resistant to high temperature and dry periods. In addition, during the field trials, the average temperature values in July (23.1°C) and August (23.2°C) are close and similar to the long-term values (23.2-22.6 °C). These values are around the average values that the beans want to see during the flowering period. Again, in our study, the genotypes may have revealed their genetic potential because they were grown under water-free conditions. It has been reported in various literatures that the differences between the findings determined for the traits may be due to genotype and environment (Sehirali, 1980; Lyman, 1983; Azkan and Yurur, 1987; Ozcelik and Gulumser, 1988; Acosta and Shibata, 1989; Bozoglu, 1995; Schneider et al. 1997; Duzdemir and Akdag, 2001; Peksen, 2005; Duzdemir, 2009; Babagil et al. 2011).

Some literature values related to single plant characteristics in bean in different studies can be summarized as follows: Plant height is 24.55-164.00 cm (Ciftci and Sehirali, 1984; Bozoglu and Gulumser, 2000; Duzdemir and Akdag, 2001; Peksen 2005; Babagil et al., 2011).); first pod height 9.9-23.9 cm (Bozoglu and Gulumser, 2000; Duzdemir and Akdag, 2001); The number of main branches per plant is 1.27-9.00 number per plant (Ozcelik and Gulumser, 1988; Peksen, 2005; Babagil et al. 2011); the number of pods per plant is 8.3-38.3 pieces/plant (Azkan and Yurur, 1987; Ozcelik and Gulumser, 1988; Duzdemir and Akdağ, 2001; Peksen, 2005; Babagil et al., 2011); 3.24-6.06 pieces/pod of the number of seeds per pod; seed yield per plant varied between 4.56-27.40 g/plant (Akdag and Sahin, 1994; Duzdemir and Akdag, 2001; Peksen, 2005). The above-mentioned literature reports we obtained as a result of our research are similar.

The fact that the harvest index characteristic of our study showed a significant change depending on the genotypes is compatible with the findings of Ozcelik and Gulumser (1988) and Duzdemir and Akdag (2001) that this character exhibits variation according to the genotype. In the previous studies, the harvest index values in beans varied between 23.85 and 46.04% (Ozcelik and Gulumser, 1988; Duzdemir and Akdag, 2001). The harvest index values between 22.16% and 37.14% obtained for bean genotypes in our study are compatible with the literature.

In this study, the effect of genotypes on the changes in total yield and 1000 seed weight was significant ($p<0.05$) (Table 2). Duzdemir and Akdag (2001) and Bozoglu and Gulumser (2000) stated that the total yield in beans varies according to genotypes and varies between 296.9-694.6 kg/da. The total yield values (between 608.54 and 779.58 kg/da) obtained for the 7 genotypes in our study are slightly above the literature reports. The genotypes in the study developed strong green parts due to the effect of their genetic structures and because they did not experience any water restriction stress during the growing period. This was reflected in the total yield values of the plant and the plants reached high values. It is a vegetal character whose weight of 1000 seeds is determined mainly by the genotype in bean (Ozcelik and Gulumser, 1988; Akdag and Sahin, 1994; Duzdemir and Akdag, 2001). In our study, statistically significant differences were found between the 1000 seed weights of the genotypes. The weight of 1000 seeds in beans varied between 236.2 and 627.8 g in some studies (Ozcelik and Gulumser, 1988; Akdag and Sahin, 1994; Duzdemir and Akdag, 2001). The values obtained for 1000 seed weight in our experiment are compatible with the literature.

Seed yield is a quantitative character under the influence of many genetic factors. Genotype varies depending on the environment and environment x genotype interactions (Sehirali, 1980; Lyman, 1983; Acosta and Shibata, 1989; Bozoglu, 1995; Schneider et al. 1997). In our study, the highest seed yield was found in F.5.Ç.224 line with 198.61 kg/da, and the lowest in Matterhorn variety with 153.61 kg/da. The lack of statistical significance in terms of seed yield may be due to the fact that there are consanguineous relationships between the genotypes in the experiment. The cultivars in our experiment are of dwarf plant type. These lines have been studied in multiple environments for high temperature and drought stresses.

CONCLUSION

The traits considered in this study varied according to genotypes. In terms of seed yield, advanced lines developed by crossing from the varieties in the experiment gave similar results with these varieties in terms of performance. It was concluded that these lines, which were obtained from a breeding program with the aim of developing new cultivars/varieties tolerant to high temperature and drought, can be recommended for Cankiri and similar hot and arid ecologies.

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THE EFFECT OF STORAGE ON THE TEXTURE QUALITY OF HAZELNUT CHIPS PRODUCED IN DIFFERENT FORMULATIONS

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ABSTRACT

In recent years, changing consumption habits with the trend of healthy eating have made it necessary to produce products with different tastes and varieties. Accordingly, post-harvest processing of hazelnuts has been emphasized and it has been brought to the market after processing into different products. As an alternative product in the snack type food industry, which has recently become widespread and widely consumed chips, with the project number TAGEM / HSGGYAD / 15 / A05 / P03 / 94 supported by TAGEM and carried out in the Hazelnut Research Institute Directorate between 2015-2018. The production possibilities of the hazelnut chips considered were investigated. It is aimed to develop a healthier and more nutritious product as an alternative to the existing chips with low nutritional properties. In the study carried out for this purpose; First of all, the usability and usage rates of hazelnut flour together with wheat flour in the production of chips were determined. Chips were produced by adding spices from hazelnut flour and wheat flour blends in determined proportions, packed under nitrogen gas, and stored at 3 different storage temperatures (4° C, 20° C and 30° C) for 12 months. During the storage period of hazelnut chips, texture analysis was carried out at 2-month intervals, and the hardness value (N) and average number of cracks were determined. Depending on the increase in the amount of hazelnut flour use, it was determined that there was a decrease in the hardness ratio and an increase in the number of cracks in hazelnut chips. In the texture analyzes made during the storage period of the chips, the highest hardness value (3.08 N) at the beginning of storage was found in the chips formed with 50% hazelnut flour and 50% wheat flour, and the lowest hardness value (1.87 N) was 70% hazelnut flour 30% wheat. It was determined in the chips produced with the formulation formed with flour. Considering the storage temperatures and storage times, an increase was detected at the end of storage compared to the beginning of storage, regardless of the formulation, in the hardness values, while fluctuations were observed in the crack number values of the chips. While the chips formulation made with 50% hazelnut flour and 50% wheat flour stood out in terms of hardness value, the formulation formed with 70% hazelnut flour and 30% wheat flour came to the fore when the cracking numbers of the chips were taken into consideration. As a result of the study, it has been determined that storage at 4℃ stands out in terms of hardness value and breakage number during 12 months of storage.

Keywords: hazelnut, chips, new product, fatty acidity, storage.

INTRODUCTION

Throughout history, the most important problem faced by people has been food supply and nutrition. For this reason, people process and evaluate many foods in various ways and transform them into forms they can use. Today, ready to serve foods have an important place in human nutrition due to the changing and developing living conditions. As a result, a wide variety of ready-to-eat foods are produced with technological developments. The appeal of ready to serve foods encourages the consumption of these products. Chips have become indispensable products especially in the eating habits of children and young people and are widely consumed by this group as a snack food.

In recent years, changing consumption habits with the trend of healthy eating have made it necessary to produce products with different tastes and varieties. In this direction, it has come to the fore that hazelnut should be included in the market after being processed into different products. Uncovering new usage areas of hazelnut is also gaining great importance. Due to the unique taste, aroma and nutritional properties of hazelnut, hazelnut can be added to many food products, especially as a functional compound (Shaidi and Alasalvar, 2004).

In this study, it was aimed to investigate the possibilities of using hazelnut flour in the production of chips and to develop a new product from hazelnut, which has limited consumption, so that the chips, which have become indispensable products in the eating habits of children and young people, are consumed as healthier products. With the results obtained, it is possible that hazelnut chips, which is a new product, can be included in the food market as a product with high added value and contribute to the country's economy.

MATERIAL AND METHOD

Hazelnut flour obtained from Çakıldak hazelnut cultivar grown in Hazelnut Research Institute was used in the study. Flour blends mixed with each other at different rates were prepared for 3 different dough formulations. The flour ratios of the blends of hazelnut flour and wheat flour are given in **Table 1**. Apart from hazelnut flour, wheat flour and spices (table salt, red pepper (*Capsicum annuum* L.), garlic powder (*Allium sativum* L.) and onion powder (*Allium cepa* L.)) required for the production of chips were obtained from local markets.

Table 1. Flour ratios of the blends of hazelnut flour and wheat flour

Formulations	Hazelnut Flour (%)	wheat flour (%)
B_1*	50	50
B_2*	60	40
B_3*	70	30

* Formulations expressing abbreviations used in the project on schedule.

In the preparation of the chips, the flour and spice mixtures were mixed with water in the determined ratio in a laboratory type mixer at high speed for 60 seconds until dough is formed. The chips dough was rolled out to be 1 mm thick with the help of a dough rolling machine and shaped with a triangular mold. Chips 8 min at 210°C. has been baked. Chips are packed with nitrogen gas in the packaging machine. The produced chips were stored at 3 different temperatures (4°C, 20°C and 30°C) for 12 months.

Texture measurements on chips were made with the Brookfield CT3 Texture Analyzer device (TA-TPB). The texture values of the chips are given as the average "Hardness" (N) and the average "Number of Breaks". The experiment was carried out according to the randomized plot design with 3 replications. Analysis of variance was performed to test the results of the examined traits. Multiple comparison test was performed for those with significant difference.

RESULTS AND DISCUSSION

In the texture analyzes performed in 2-month periods during the storage period, the highest hardness value (3.08 N) at the beginning of storage was determined in the chips produced with the B_1 formulation, and the lowest hardness value (1.87 N) in the chips produced with the B_3 formulation. Depending on the increase in the amount of hazelnut flour use, hardness ratio was decreased. In the hardness determination made during the storage period, regardless of the formulation and storage temperature, the hardness value of the chips increased until the end of the 8th month, while a decrease in the hardness value occurred from the 10th month. At the end of the storage period, the highest hardness value (3.23 N) was found in chips produced with B_1 formulation and stored at 4°C, and the lowest hardness value (1.63 N) in chips produced with B_3 formulation and stored at 30°C. It was observed that the hardness values increased depending on time at the end of storage compared to the beginning of storage. In order to determine the importance of the difference between the hardness values of the chips, the multiple comparison test was applied to the sources of variance that were found to be important. The results are given in **Table 2.**

Table 2. Variance analysis test and multiple comparison test results for hardness value

Sources	**DF**	**Sum of Squares**	**Mean Square**	**F**
Formulation	2	48,61054	24,305	2240,827*
Storage Temperature	2	2,65804	1,329	122,5290*
Storage Period	6	348,46663	58,078	5354,487*
Formulation X Storage Temperature	4	0,54834	0,137	12,6385*
Formulation X Storage Period	12	1,1687e-30	0,001	0,0001
Storage Temperature X Storage Period	12	0,00030	0,001	0,0023
Formulation X Storage Temperature X Storage Period	24	0,00061	0,001	0,0023
Error	126	1,36667	0,011	
Total	188	401,65112	2,136	

	Variance of Sources	**means ±Std Error**
Formulation	$\mathbf{B_1}$	$4{,}49^{\mathbf{A}}$ ±0,013
	$\mathbf{B_2}$	$3{,}66^{\mathbf{B}}$±0,013
	$\mathbf{B_3}$	$3{,}27^{\mathbf{C}}$±0,013
Storage Temperature	**4°C**	$3{,}95^{\mathbf{A}}$±0,013
	20°C	$3{,}81^{\mathbf{B}}$±0,013

	30°C	$3{,}66^{C}$±0,013
Storage Period	**0**	$2{,}21^{G}$±0,020
	2	$2{,}95^{E}$±0,020
	4	$3{,}70^{D}$±0,020
	6	$5{,}56^{B}$±0,020
	8	$5{,}91^{A}$±0,020
	10	$3{,}95^{C}$±0,020
	12	$2{,}36^{F}$±0,020
Formulation X Storage Temperature	**B_1, 4°C**	$4{,}68^{A}$±0,022
	B_1, 20°C	$4{,}48^{B}$±0,022
	B_1, 30°C	$4{,}30^{C}$±0,022
	B_2, 4°C	$3{,}71^{D}$±0,022
	B_2, 20°C	$3{,}67^{D}$±0,022
	B_2, 30°C	$3{,}60^{E}$±0,022
	B_3, 4°C	$3{,}47^{F}$±0,022
	B_3, 20°C	$3{,}27^{G}$±0,022
	B_3, 30°C	$3{,}08^{H}$±0,022

*****It is significant at the p<0.01 level. Values shown with different letters in the sorting column are statistically different at the P <0.01 level.

The lowest hardness value (3.27 N) was determined in the chips produced with the B_3 formulation. It was determined that the hardness values of the chips decreased depending on the increase in the storage temperature. When the data were evaluated in terms of storage temperature, the lowest hardness value (3.66 N) was determined in the chips stored at 30°C. While there was an increase in the hardness values of all chips during the first 8 months of storage, there was a decrease in the hardness values in the 10th and 12th months of storage. However, it was observed that the values at the end of storage were higher than the hardness values at the beginning of storage. When the hardness values of the chips were examined in terms of the "Formulation X Storage Temperature" interaction, the highest hardness value (4.68 N) was determined in the chips produced with the B_1 formulation and stored at 4°C.

Kayacier and Singh (2003) investigated the effects of mono and diglycerides on the moisture content and textural properties of chips during storage. They stated that while the moisture content of the control chips increased linearly, there was no change in the chips containing emulsifier, and the hardness and brittleness of the chips increased with the addition of these additives. Kita et al. (2007) reported that potato chips were fried in 8 different oils and 3 different temperatures (150, 170 and 190 °C), and the hardness of the chips decreased with the increase in frying temperature. The results obtained in the same study showed that frying temperature was effective on the texture properties of potato chips. Demir (2015); In his study, which aimed to produce biscuits with whole wheat flour substitution at different rates, he reported that the hardness value of the biscuits increased with the whole wheat flour substitution. The situation detected in the studies conducted and the situations we detected in our study are similar.

At the beginning of storage, the highest breaking number (100.00) was determined in the chips produced with the B_3 formulation, and the lowest breaking number (56.68) was determined in the chips produced with the B_1 formulation. It was determined that the number of cracks in chips increased due to the increase in the amount of hazelnut flour use. It was observed that there were fluctuations in the crack number values of the chips in the determination of the breaking number during the storage period. While the number of cracks in the chips increased until the end of the 8th month during the storage period, there was a decrease in the number of breakages from the 10th month. In order to evaluate the texture changes of the chips during storage, variance analysis was performed on the number of breakage data. Multiple comparison test was applied to the significant sources of variance. The results are given in **Table 3.**

Table 3. Variance analysis test and multiple comparison test results for breaking number

Sources	**DF**	**Sum of Squares**	**Mean Square**	**F**
Formulation	2	33521,843	16760,922	904,9737*
Storage Temperature	2	5539,447	2769,724	149,5459*
Storage Period	6	15312,262	2552,044	137,7927*
Formulation X Storage Temperature	4	2150,835	537,709	29,0326*
Formulation X Storage Period	12	35,503	2,959	0,1597
Storage Temperature X Storage Period	12	7,542	0,629	0,0339
Formulation X Storage Temperature X Storage Period	24	104,964	4,374	0,2361
Error	126	2333,633	18,521	
Total	188	59006,030	313,862	

	Variance of Sources	**means ±Std Error**
Formulation	**B_1**	$66,57^{C}$ ±0,54
	B_2	$78,63^{B}$±0,54
	B_3	$98,85^{A}$±0,54
Storage Temperature	**4°C**	$87,64^{A}$±0,54

	20°C	81,97$^{\mathbf{B}}$±0,54
	30°C	74,43$^{\mathbf{C}}$±0,54
Storage Period	**0**	72,50$^{\mathbf{E}}$±0,83
	2	76,95$^{\mathbf{D}}$±0,83
	4	82,90$^{\mathbf{C}}$±0,83
	6	88,45$^{\mathbf{B}}$±0,83
	8	95,00$^{\mathbf{A}}$±0,83
	10	86,54$^{\mathbf{B}}$±0,83
	12	67,09$^{\mathbf{F}}$±0,83
Formulation X Storage Temperature	**B_1, 4°C**	67,79$^{\mathbf{F}}$±0,94
	B_1, 20°C	66,32$^{\mathbf{F}}$±0,94
	B_1, 30°C	65,59$^{\mathbf{F}}$±0,94
	B_2, 4°C	86,29$^{\mathbf{C}}$±0,94
	B_2, 20°C	79,09$^{\mathbf{D}}$±0,94
	B_2, 30°C	70,51$^{\mathbf{E}}$±0,94
	B_3, 4°C	108,85$^{\mathbf{A}}$±0,94
	B_3, 20°C	100,51$^{\mathbf{B}}$±0,94
	B_3, 30°C	87,18$^{\mathbf{C}}$±0,94

*****It is significant at the p<0.01 level. Values shown with different letters in the sorting column are statistically different at the P <0.01 level.

There was an increase in the number of breakage values of all chips during the first 8 months of storage. There was a decrease in the number of breakage values in the 10th and 12th months of storage. When the breaking number values of the chips were examined in terms of "Formulation X Storage Temperature" interaction, the highest breaking number value (108.85) was determined in the chips produced with the B_3 formulation and stored at 4°C.

It was determined that the number of cracks in chips increased due to the increase in the amount of hazelnut flour use. In the study conducted by Göncü (2011) it was stated that there was a decrease in the force (kg) value depending on the increase in the baking time. Quintero et al. (1999) in a study investigating the textural properties of baked tortilla chips made from sorghum and rice flour, reported that tortilla chips made from 20% sorghum or rice flour created a lower force field than the control samples.

CONCLUSIONS

The main importance of this issue is to increase the internal and external consumption of hazelnut with the processing of hazelnut into chips, which is limited in consumption as a snack, as well as to ensure the consumption of chips, which have become indispensable products in the eating habits of children and young people, as healthier products. In addition, with the development of hazelnut chips, it will be possible to take place in the food market as a product with high added value, and in the future, small and medium-sized local enterprises will be able to produce hazelnut chips, increase the product diversity of our food industry and contribute to the country's economy.

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DISTRIBUTION OF NOSEMA SP (CAUSATIVE AGENT OF NOSEMOSIS IN HONEY BEES) IN ALGERIA

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Abstract

The bee is an indispensable part of the environmental balance in the world as a pollinator of many species. It also has other interests including the production of honey, propolis, royal jelly and wax. Unfortunately, this species is threatened by several factors, the most important of which is the presence of pathogens that cause pathologies in the colonies. One of the most dangerous conditions is nosemosis. The interest of this present work is to assess the health situation of bee colonies in some regions of central Algeria (Boumerdès, Bouira and Tizi ouzou). The comparison of the prevalence shows that the apiaries located in the Boumerdès area have the highest rate of infestation (21%). This high prevalence of nosemosis in this area is linked to particular climatic conditions such as the presence of high humidity and a long cold period. Symptoms have been detected in a few apiaries but no correlation exists between the presence of the signs and the rate of contamination.

Key words : Bee - Nosemosis - Center of Algeria - prevalence - climate.

Introduction

The bee is a social insect with a very important role in pollination and in agriculture. A third of the food consumed in the world is linked to the pollinating activity of bees (Gallai et al. 2009). For several years, a noticeable decrease in honey bee populations has been reported in many countries by beekeepers and scientists. Several risk factor hypotheses have been put forward to try to explain this phenomenon (VanEngelsdorp et al., 2008; Guzman-Novoa et al., 2010; Currie et al., 2010; Fries, 2010)

In Algeria, many cases of bee colony mortality have been observed since 2007. The presence of pathogens in these colonies as well as their health status are the main causes of this lethality (Adjlane, 2009). Varroasis is the most well-known pathology in Algeria. However, the reader has very little information on other diseases, especially nosemosis. Indeed, few studies have been carried out in Algeria to determine the prevalence of this disease in bee colonies. This apparent gap justifies the present work. The causative agent of nosemosis is Nosema sp., A single-celled microorganism that infects the epithelium of the mesenteron wall of the worker bee (Faucon, 2005). Nosema forms resistant spores which remain viable for long periods of time.

Materials and Methods

Place and period of work:

The work was carried out on 21 apiaries. At the level of each apiary, sampling is carried out on 5% of the hives. Samples are taken in 2019 at the end of winter and at the beginning of spring in 3 agricultural areas: Boumerdès, Bouira and Tizi Ouzou with 7 apiaries in each region.

Bee sampling method

Worker bees of undetermined age are collected from the 4th or 6th frame of the brood of colonies of the race *Apis mellifera intermissa*. After sampling, the bees are kept in boxes containing 95 ° ethanol and at a temperature of -20 ° C until the day of the analyzes.

Figure 1: Location of the study area.

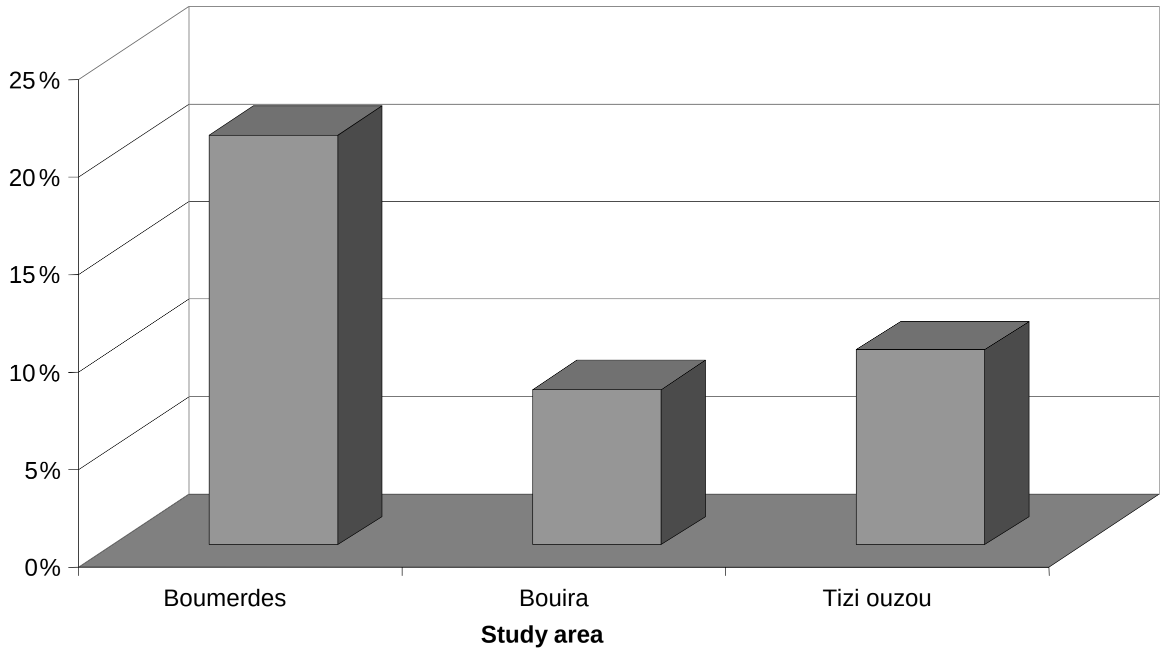
Method of statistical analysis of the results

The data obtained are analyzed with Statistica version 5.0 software following the analysis of variance (ANOVA) process.

Results and Discussion

Upon observation with the light microscope, Nosema spores appear transparent with a very distinct dark outline, measuring between 5 and 7 µm long and 3 to 4 µm wide. The comparison of the percentage of contamination between the three zones studied shows that the apiaries located in the zone of Boumerdès record the highest rate of contamination (21%) (Figure 2). This rate is higher than those noted in the other two zones, those of Bouira (08%) and Tizi ouzou (10%). Analysis of variance shows a highly significant difference in the prevalence results between the areas studied. Analysis of the climatic data mentioned for the studied region shows that the Boumerdès area corresponds to a prolonged winter and high humidity. There appears to be a relationship between the increased prevalence and particular climatic conditions such as high humidity and long cold spell. Indeed, it is generally accepted that Nosema sp. is prevalent in cold areas and the severity of infection is limited to areas with long winters (Moeller, 1978). Bailey (1981) indicates that the causes which favor the development of this pathology are mainly linked during long winters to the prolonged confinement of the bee inside the hive, which favors an active dissemination of Nosema. According to Barbançon and L'Hostis (2007). Research on Nosema also indicates that the infestation reaches its highest rate during the wet seasons of the year (Fries, 1988; Huang et al. 2007). In Mediterranean regions where humidity is high in summer, infection is also high during this same period (Martin-Hernandez et al. 2007). The maximum infestation is recorded in spring and the lowest rate in summer (Higes et al. 2006).

On a worldwide scale, several research works have focused on the distribution of nosemosis within bee colonies, particularly in Europe and Spain (Higes et al., 2008), in the North-West of Turkey. (Aydin et al., 2006), France (Chauzat et al., 2007), Denmark, Finland, Germany, Greece, Hungary, Italy and Serbia (Klee et al., 2007) and Asia among others in Taiwan
(Huang et al., 2007). Research on Nosema indicates that the infestation reaches its highest rate during the wet seasons of the year (Fries, 1988; Huang et al., 2007). Specifically, in research conducted in Turkey, 23.8% of 168 colonies studied are infected with Nosema during the spring period (Aydin et al., 2006). In Mediterranean regions where humidity is high in summer, infection is also high during this same period (Martin-Hernandez et al., 2007).



Contamination rate (%)

Figure 2: histogram of the percentage of contamination by nosemosis in the 3 areas.

Table 1 : Search for a significant difference by an ANOVA between the rate of prevalence of nosemosis between the areas studied

Source of variations	Sum of squares	Degree of freedom	Mean of squares	*F*	Probability	*Valeur critique pour F*
Between Groups	686	2	343	25,9411765	0,000	3,55455715
Within groups	238	18	13,2222222			
Total	924	20				

In Iran, infection of bee colonies with Nosema reaches its highest level in the spring (Lotfi et al., 2009). There is a time lag in the Mediterranean Basin since in Spain the maximum infestation is recorded in spring and the lowest rate in summer (Higes et al., 2006).

Recently, Copley and Jabaji (2012) reported through a study done in 2009 and 2010 in Canada that the Nosema apis infestation rate is 29% with seasonal peaks detected in spring and fall. A typical Nosema infection results in a low level of spores, even undetectable during the summer (Fluton, 2007). Mention should be made of the low level of infection in the fall, followed by a slow increase at first in winter, then rapid in spring when brood feeding and cleaning intensify as the temperature rises in the colony. . In addition to the climatic conditions favorable to the increase in the prevalence of nosemosis, other factors may explain this heavy infestation of the colonies, such as the honeydew produced by Homoptera and brought back to the hives which leaves residues in the intestine of each bee in winter, which favors the development of Nosema (Faucon, 1992).

The results of the presence of symptoms in the colonies studied are presented in (Fig 3) we observed in the field that the area of Tizi ouzou did not show symptoms (0%), so nosemosis can go unnoticed for a long time when the infestation is low because there are no observable symptoms. Nosema sp is called the silent killer, due to its insidious development (Aurière, 2001). Bees die, most often in the wild, which causes progressive depopulation which may go unnoticed by the beekeeper (Dottin, 1986). Note that the analysis of variance shows a highly significant difference in the presence of symptoms between the study regions.
On the other hand, the agricultural study zone of Boumerdès presented 5% and that of Bouira (1%). So only a microscopic examination can provide a definite laboratory diagnosis that really indicates the presence of Nosema in bee colonies..

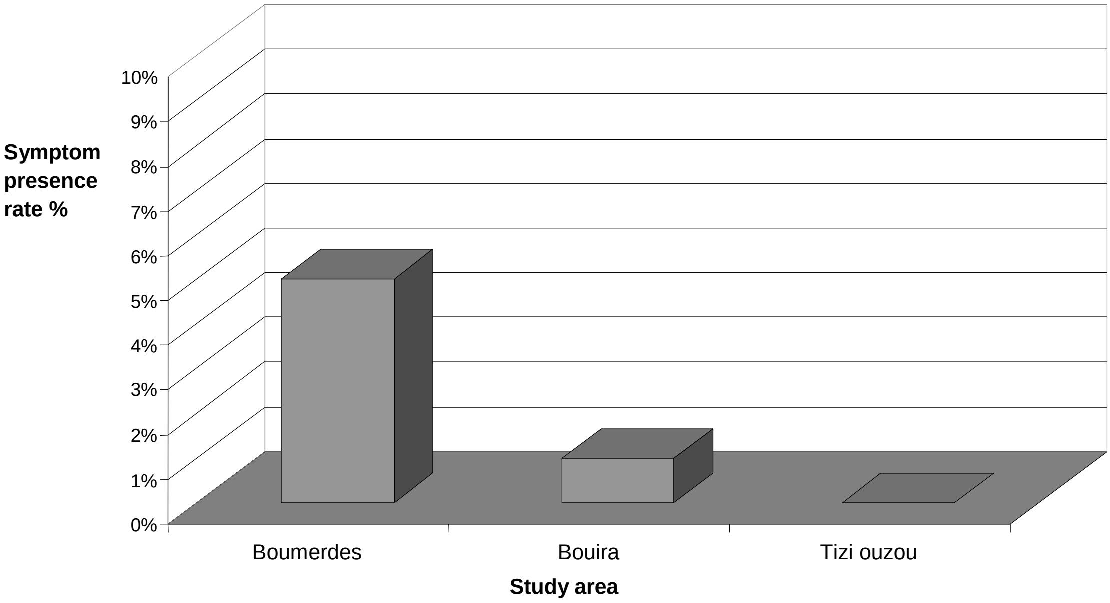


Figure 3: Symptom presence rate in the areas studied

Tableau 2: Recherche de différence significative par une ANOVA entre le taux de présence des symptômes de la nosémose entre les zones étudiées

Source of variations	Sum of squares	Degree of freedom	Mean of squares	*F*	Probability	*Valeur critique pour F*
Between Groups	98	2	49	11,025	0,00074822	3,55455715
Within groups	80	18	4,44444444			
Total	178	20				

A typical Nosema infection results in low or even undetectable spore levels during summer (Fluttom, 2007). In addition to the climatic conditions favorable to the increase in the prevalence of nosemosis, other factors may explain this heavy infestation of the colonies, such as the honeydew produced by Homoptera and brought back to the hives which leaves residues in the intestines of the colonies. bees in winter, which favors the development of Nosema (Faucon, 1992). The heavy infestation of the colonies may be due to the susceptibility of certain breeds of bees. This is the case for the Italian and Caucasian races (Faucon, 1992). Yet according to Malone and Stevanovic (1999) there is no difference between the Italian race and the black race in the face of Nosema infection. No study has yet been carried out on the susceptibility of the two local bee breeds, Tellian and Saharan, to nosemosis. A colony heavily parasitized by Varroa destructor constitutes a favorable field for the development of nosemosis (Orantes Bernejo & Garcia Fernandez, 1997; Barbançon & L'Hostis, 2007; Colin et al, 2007).

In addition, poor farm hygiene resulting from prolonged use over several years of the same frames is another source of disease development (Fries, 1988). Finally, the low presence in the hive of pollen, a source of proteins, also favors the development of the disease (Fries, 1993).

Heavy infection of bee colonies with nosemosis causes a drop in honey production. According to Hucorne (2002) a 50% drop in honey production is observed in a hive where the Nosema infestation rate is 5 to 25%. This reduction reaches 80% in an infested hive between 30 and 50%. Indeed, Nosema sp. reduces the lifespan of workers by more than half (Kleinschmidt and Fergusson, 1989). This fungus disrupts the physiology of the bee by reducing the development of the hypopharyngeal glands (Dottin, 1986), by causing atrophy of the ovaries and by reducing the development of the wax glands (Faucon, 1992). It also causes a rapid decrease in the proteolytic capacities of the bee (Malone and Gatehouse., 1998). The impact of this infection is considered to be equal to or greater than the losses caused by all other diseases combined, including the more easily diagnosed brood diseases (Mussen et al. 1975).
In recent years, the parasite Nosema ceranae, which was then only known from the Asian species Apis cerana, appeared in Apis mellifera for the first time noted in 2005 (Huang et al., 2007). The disease caused by Nosema ceranae is now called nosemosis type C and is considered to be one of the major threats to bee colonies. Mayack and Nuag (2009) hypothesize that the presence of Nosema in the colony is one of the explanations for the absence of dead bees around hives affected by CCD (Colony Collapse Disorder). Unlike Nosema apis, Higes et al. (2009) have shown that factors such as access to fresh pollen, of various origins, or the variation of humidity and temperature conditions throughout the year do not seem to influence the development of the parasite within beehives.

Conclusion

The results of our study show that this pathology is present in all the areas studied, with a difference in the rate of infection. The fact that the Boumerdès area is characterized by infestations by Nosema sp. significantly higher than in other regions shows the important influence of climatic factors in particular high humidity following heavy rainfall and lengthening of the winter. Thus, humidity and low temperatures favor the development of nosemosis.

The observation of the symptoms of the pathology in the apiary does not in any way constitute proof of the presence or absence of Nosema. Indeed, the results obtained show the presence of Nosema spores without any presence of typical symptoms. Probably, these signs of the disease appear when the rate of presence of the spores in the bees is very high. Hence the need to plan in the future work to study a possible correlation between the rate of the presence of spores and the onset of symptoms.

Early detection of the disease is essential to avoid colony loss. It can help prevent the infection from spreading to healthy bee colonies. When colonies are affected by this disease, production becomes a secondary problem due to the need to urgently treat the infected colony to save it. It would be useful to take 4 samples per year, ie 1 per season. This precaution could help beekeepers determine the trend of infection with Nosema sp. and to evaluate the effectiveness of control strategies for this disease. It is also necessary to determine the occurrence of the two species, Nosema apis and Nosema ceranae using the tools of molecular biology.

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THE INFLUENCE OF THE COMPOUND FEED SUPPLEMENTATION WITH FISHMEAL ON RAISING PERFORMANCE DURING THE AGE 1 - 42 DAYS OF BROWN JUMBO QUAILS

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ABSTRACT

In order to establish the effect of supplementation the compound feed with fishmeal on the raising performance of youth breeding brown Jumbo quail (especially on consumption of the compound feeds) in the period 1 - 42 days of age, an experiment was established on a total number of 300 quail chicks divided into two groups (150 chicks/ group). At the age of 42 days the average weight of the chickens in the experimental group was 245.55 g / head, while in the chickens of the control group it was 225.75 g / head. The average daily gain during the period 0 - 6 weeks of growth was 236.00 g / head in the experimental group and 215.90 g / head in the control group. The total consumption of compound feeds was 1150.50 g / head in the chickens from the experimental group compared to 1351.45 g / head in the control group. The specific consumption of compound feed was lower by 32.17 %, of 3.959 g / g gain in the experimental group, while in the of the control group it was of 5,837 g c.f / g gain. Research has shown that the growth performance of the group that received compound feed with fishmeal supplement was superior to that of the control group.

Keywords: quail, meat, growth, compound feed, fish meal

INTRODUCTION

Quail breeding for meat production occupies an important place in the poultry farming branch and contributes to the diversification of poultry meat production (Genchev A. et al., 2008). For human nutrition the biggest problems are the provision of protein, especially those of animal origin, which is why scientists predict to develop new sources of animal protein (Pană C.O., 2000). Raising quails for meat production is a real alternative of raising other animals as a source of animal protein (Faitarone et al., 2005). And it represents an activity with good development perspectives considering the very high productivity of these birds and the short period of growth so as far as can be obtained large quantities of meat of special quality in a very short time (40 - 50 days of growth). We must also mention that it is about a meat that comes from adult birds, because maturity in quails occurs around the age of 30 days after hatching. Feeding is one of the key factors in growing the quails as well as in their reproductive activity. The purpose of the present research was to establish the effect of supplementation with fishmeal the compound feed administered to chickens raised for breeding.

It was taken into account that in general the combined feed administered to the quail youth contains fishmeal only in the 1st phase of growth (period 0-3 weeks), not in the finishing phase, because meat can have taste and smell of fish. It should also be noted that the use of fishmeal leads to improved growth performance in raised quail young for breeding purposes and their carcases are not marketed for meat. Elena Popescu-Miclosanu (2007) mentions that the need for protein and energy in quail chickens is very high (24-26% CP and 2800 - 3000 kcal EM/kg c. f. in the first three weeks of growing, 20 % CP and 2600 kcal EM/kg c.f. in weeks 4-6) and the specific compound feeds consumption of 3.5 - 4 kg/ kg gain. Bălășescu M. (1980) mentions also that the need for quail protein is very high.

Other authors have also shown that in order to achieve optimal production performance, quails need energy and protein very high (Altine et al., 2016). Stoica et al. (2001) mention that the protein in fishmeal has a high biological value due to its high amino acid content, primarily methionine and lysine, but also threonine, tryptophan and cystine. The same authors mention that fishmeal is rich in calcium and potassium phosphate which gives it an appreciable mineral value and also in vitamins A, B, D, K, especially in vitamin B 12.

MATERIAL AND METHOD

The experiment was performed on a total of 300 breeding chickens of brown jumbo meat quail divided into two equal batches (150 chicks / batch). At the age of one day were formed two batches, experimental and control, then measurements were determined (live weight at 1 day, 7, 14, 21, 35 and 42 days, as well as the combined feed consumption for these ages) by random for each batch separately. Quail chicks of both groups were raised on permanent bedding soil. Watering and feeding were made ad libitum.

The control group received a combined feed without fishmeal, while the experimental group received a combined feed supplemented with 4% fishmeal in both the first (0-3 weeks) and second phase (4-6 weeks). Combined feed and fishmeal were purchased from a company specialized in the production and sale of compound feed (S.C. Agromar S.R.L., Balotești, Romania). The nutritional values of the combined feeds administered to the control group and the experimental group are shown in Table 1.

Table 1: The nutritional values of the combined feed recipes administered to the two batches

Nutritional parameters	Control group.		The experimental group	
	Phase I (0 - 3 weeks)	Phase II (4-6 weeks)	Phase I (0 - 3 weeks), 4 % fishmeal	Phase II (4-6 weeks), 4 % fishmeal
Metabolisable Energy (kcal/kg c.f.)	2820	2950	2831.52	2961.521
Crude Protein (%)	26.00	21.00	28.56	23.56
Methionine (%)	0.62	0.56	0.82	0.86
Lysine (%)	1.50	1.16	1.70	1.36
Crude Fibre (%)	4.26	4.31	4.26	4.31
Calcium (%)	0.94	0.79	0.94	0.79
Phosphorus (%)	0.76	0.65	0.76	0.65

In the structure of the recipes of combined feeds entered wheat, corn, soybean meal, calcium carbonate, phosphate monocalcium, fishmeal, vitamin - mineral premix and salt.

The nutritional value of the fishmeal used in the experiment determined by the manufacturer was as follows: 2880 kcal / kg, 64% crude protein, 10% crude fat, 5 % methionine and 5 % lysine. It should be noted that the fish meal was analysed by the manufacturer from the bacteriological point of view for salmonella and pathogenic microflora and tested negatively.

The environmental conditions in which the experiment took place were within the limits provided by the specialized literature.

The testing of the differences between the averages was performed with the help of the Student test.

RESULTS AND DISCUSSIONS

1. The evolution of the live weight in quail chicks from the two groups during 1-42 days

The evolution of live weight in quail chicks from the two groups in the period 1 - 42 days is presented in tab. 2 and fig. 1.

Table 2 : Evolution of the average live weight of quail chicks from the two groups during 1-42 days

Age (days)	Control group	Experimental group
1 day	9.35 ± 0.55 ns	9.55 ± 0.67 ns
7 days	27.50 ± 1.33***	34.55 ± 0.98***
14 days	52.25 ± 1.76***	62.54 ± 1.55***
21 days	120.75 ± 2.56 ***	135.00 ± 2.33***
28 days	163.55 ± 2.89***	180.00 ± 3.15 ***
35 days	202.45* ± 4.05 ***	220.50 ± 3.85***
42 days	225.25 ± 3.95***	245.55 ± 4.23***

Note: between values noted ns - the differences are insignificant;

between values noted*** - the differences are very significant

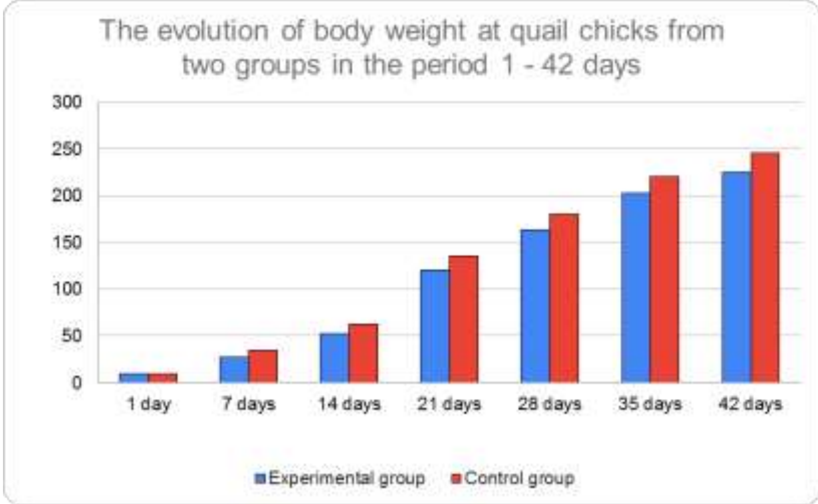

Fig. 1 – The evolution of body weight at quails in the period of 1 – 42 days of age

At the age of one day the average weight was approximately the same in the case of both groups, the differences being insignificant. At the age of 7 days, the average weight was 34.55 g / head in chickens from the experimental group, while in chickens in the control group it was 20.40% lower (27.50 g / head), the difference between the two groups being statistically very significant.

At 14 days of age, the mean weight of the chickens in the experimental group was 62.54 g/head, while in the chicks in the control group it was with 18.56 % lower (52.55 g/head), the difference between the two lots being very significant. At the age of 21 days, the average live weight was with 10.55 % higher in the chickens from the experimental group (135.00 g/head) compared to that of the chicks in the control group (120.75 g/head), the difference between the two groups being very significant.

At the age of 28 days, the average weight was 9.13 % higher in quails from the experimental group (180 g/head) compared to that of chicks in the control group (163.55 g/head), the difference between the two groups being very significant. At the age of 35 days, the mean weight was higher in the chickens from the experimental group (220.50 g/head) by 8.19 % compared to that recorded in the chicks from the control group (202.45 g/head), the difference between the two lots being very significant.

At 42 days of age, the average weight was 8.27 % higher in quails in the experimental group (245.55 g/head) compared to that recorded in chicks in the control group (225.25 g/head), the difference between the two groups being very significant.

In a study conducted in South Africa (Fezile Mbhele et al., 2019) on several batches of Jumbo meat quails, the authors determined on several analysed batches average live weights at the age of 42 days between 207.30 g and 223.60 g/head. Another nutrition experiment conducted on a youth quail Jumbo flock during 3-6 weeks of growth in South Africa (Mveleli M., 2020) mentions average live weights at the age of 42 days between 234.20 g and 247.60 g/head.

In an experiment conducted in Brazil (Dirceu N.G.J. et al., 2017) on a flock of meat quails was determined the average live weight at the age of 14 days of 72.56 g /head, at the age of 21 days of 127.87 g, at the age of 35 days of 211.07 g and at the age of 42 days of 233.80 g /head.

In an experiment conducted in Romania (Elena Popescu-Miclosanu et al., 2006) on two batches of quail chickens from two populations, mixed and meat, on quail chickens from the batch of meat population was obtained a live weight at the age of 42 days of 244.93 g/head, an average daily increase of 6.10 g/head/day and a specific consumption of 3.47 kg c.f. /kg gain.

2. Evolution of the average weekly and daily weight gain in quail chicks in the two groups during the period of 1-6 weeks of growth

The evolution of the average weekly and daily weight gain in quail chicks in the two groups during the period of 1-6 weeks of growth is presented in the tab. 3 and fig. 2.

Table 3: Average weekly and daily weight gain in quail chicks in the two analysed groups during the 1-6 weeks of growth (g/head)

Age (weeks)	Control group		Experimental group	
	Weekly	Daily	Weekly	Daily
1	18.15	2.59	25.00	3.57
2	24.75	3.54	28.00	4.00
3	68.50	9.78	72.50	10.36
4	42.80	6.11	45.00	6.43
5	38.90	5.56	40.50	5.79
6	22.80	3.26	25.05	3.58

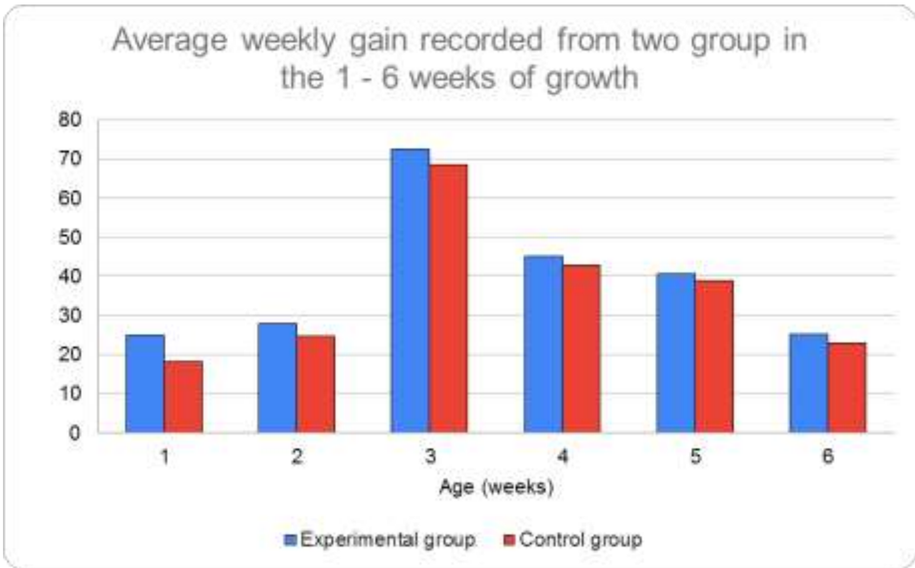

Fig. 2 – Average weekly gain recorded in the quails of 1 – 6 weeks of age

Thus, in the chickens from the experimental group, the average growth rate (tab.3) was 25 g/head/week (3.57 g/day) in the first week, of 28.00 g/head/week (4.00 g/head/day) the second week of growth, of 72.50 g/head/week (10.36 g/head/day) in the third week of growth, of 45 g/head/week (6.43 g/head/day) in the fourth week, of 40.50 g/week (5.79 g/head/day) in the 5th week of growth and of 25.05 g/week (3.58 g/head/day) in the sixth week of growth.

At quail chicks in the control group, the average gain was 18.15 g/week (2.59 g/day) in the first week of growth, of 24.75 g/week (3.54 g/day) in the second week, of 68.50 g/week (9.78 g/day) in the third week, of 42.80 g/week (6.11 g/day) in the fourth week of growth, of 38.90 g/week (5.56 g/day) in the fifth week and of 22.80 g/week (3.26 g/day) in the sixth week of growth.

As can be seen, the peak of growth increase in both lots was recorded in the third week of growth, the weekly growth increase being with 4.13 % higher in chicks in the experimental group compared to chicks in the control group, the differences between the two groups being not statistically insured.

Mveleli M. et al. (South Africa, 2020) recorded in their experiment a total increase for the period of 3-6 weeks of growth between 161.70 g and 171.90 g/head. In the present experiment, the total growth increase in the period 3-6 weeks was 173 g in the control group and of 183.05 g for the experimental group.

3. **Average daily and weekly consumption of compound feeds in quail chickens from the two groups during the period 1- 6 weeks of growth**

Table 4: Average daily and weekly consumption of compound feed in the two groups during the 1-6 weeks of growth

Age (weeks)	Control group		Experimental group	
	Weekly (g /week)	Daily (g/head)	Weekly (g /week)	Daily (g/head)
1	35.70	5.10*	23.80	3.40*
2	175.75	25.11*	140.20	20.03*
3	235.00	33.52*	205.00	29.29*
4	285.00	40.71*	255.00	36.43*
5	305.00	43.57*	260.00	37.14*
6	315.00	45.00*	266.00	38.00*
Average consumption	-	32.16 ± 6.19*	-	27.38 ± 5.54
Total	1351.45	-	1150.50	-

In the first week of growth the average daily consumption of compound feed (tab.4) was 5.10 g/day (35.70 g /week) in the control group and of 3.40 g/day (23.80 g/week) in chickens from the experimental group, with 33.33% lower in the experimental group compared to the control group, the differences being significant. In the second week, the average consumption of compound feed was of 25.11 g/head (175.75 g/week) at the control group and of 20.03 g/head (140.20 g/week) in the experimental group, with 20.23% lower in the experimental group, the differences being significant.

In the third week, the average daily consumption of compound feeds was of 33.52 g/head (235.00 g/week) in the control group and of 29.29 g/head (205 g/week) in the experimental group, with 12.61 % lower in the experimental group, the differences being significant. In the fourth week, the average daily consumption of compound feeds was of 40.71 g /head (285 g/week) in the control group and of 36.43 g/head (255.00 g/week) in the experimental group, with 10.51 % lower in the experimental group, the differences being significant.

In the 5th week of growth the average daily consumption of compound feeds was of 43.57 g/head (305 g/week) in the control group and of 37.14 g/head (260 g/week) in the experimental group, with 14.76 % lower in the experimental group, the differences being significant. In the sixth week of growth, the average daily consumption of compound feed was of 45 g/head (315 g/week) in the control group and of 38.00 g/head (266 g/week) in the experimental group, with 15.55 % lower in the experimental group, the differences being significant.

The total consumption of compound feeds during the 1-6 weeks of growth was of 1351.45 g/head in the control group and of 1150.50 g/head, with14.87 % lower in the experimental group. A study conducted in Brazil (Dirceu N.G.J. et al., 2017) mentions a combined feed consumption of 165.32 g/head in the period 1-14 days, of 304.09 g/head in the period 1 - 21 days, of 704.07 g/head in the period 1 - 35 days and 908.11 g/head in the period 1- 42 days.

4. **Specific consumption of compound feeds in quail chickens in the two groups during the period of 1-6 weeks of growth**

Table 5: Specific weekly and daily feed consumption in quail chickens from the two groups during the period of 1-6 weeks of growth (g c.f. /g gain)

Age (weeks)	Control group		Experimental group	
	Weekly	Daily	Weekly	Daily
1	1.967	0.281	0.952	0.136*
2	7.158	1.023	5.007	0.715*
3	3.430	0.490	2.828	0.404*
4	6.650	0.950	5.667	0.810*
5	7.840	1.120	6.420	0.917*
6	13.810	1.973	10.260	1.517*
Total	40.855	5.837	31.490	3.959

In the first week of growth, the specific consumption of compound feed (tab. 5) was 1.967 g/week (0.281 g/day) in the chicks in the control group and of 0.952 g/week (0.136 g/day) in the experimental group. The specific consumption was by 51.60 % lower in the experimental group, the differences being significant.

In the second week of growth, the specific consumption of compound feed was 7.158 g/week (1.023 g/day) in the chicks in the control group and of 5.007 g/week (0.715 g/day) in chickens in the experimental group. The specific consumption was with 30.05 % lower in the experimental group, the differences being significant.

In the third week of growth, the specific consumption of compound feed was 3.430 g/week (0.490 g/day) in the control group and of 2.828 g/week (0.404 g/day) in the experimental group. The specific consumption was with 17.55 % lower in the experimental group, the differences being significant. In the fourth week of growth, the specific consumption of compound feed was 6.650 g/week (0.950 g/day) in the control group and of 5.667 g/week (0.810 g/day) in the experimental group, with 14.78 % lower in the experimental group, the differences being significant.

In the fifth week of growth, the specific consumption of compound feed was 7.840 g/week (1.120 g/day) in the control group and of 6.420 g/week (0.917 g/day) in the experimental group, with 18.11 % lower in the experimental group, the differences being significant. In the sixth week of growth, the specific consumption of compound feed was 13.810 g/week (1.973 g/day) in the control group and of 10.620 g/week (1.517 g/day) in the experimental group, The specific consumption was with 23.10 % lower in the experimental group, the differences being significant.

The cumulative specific consumption of compound feeds during the 1-6 weeks of growth was 40.855 g c.f. /g gain (5.837 g c.f. /g gain/day) in the control group and of 31.490 g c.f./g gain/week (3.959 g c.f./g gain/day) in the experimental group. The specific consumption was with 22.91 % lower in the experimental group.

6. Average specific consumption of compound feeds in quail chickens from the analysed batches during the 1-6 weeks of rearing period (g c.f. /g live weight)

The specific consumption reported to live weight in the first week of growth (tab.6) was 1.298 g c.f./g body weight (b.w.) in the control group (0.185 g/day) and of 0.688 g c.f./ g b.w. (0.098 g/day) in the experimental group. In the second week of growth, the specific consumption was 3.364 g c.f./g b.w. (0.480 g/day) in the control group and of 2.241 g c.f./g b.w. (0.320 g/day) in the experimental group. In the third week, the specific consumption was 1.946 g c.f./g b.w. (0.278 g/day) in the control group and of 1.518 g c.f./ g b.w. (0.217 g/day) in the experimental group. In the fourth week, the specific consumption was 1.742 g c.f./g.b.w. (0.249 g/day) in the control group and of 1.417 g c.f./g b.w. (0.202 g/day) in the experimental group.

In the fifth week, the specific consumption was 1.507 g c.f./g b.w. (0.215 g/day) in the control group and of 1.180 g c.f./g.b.w. (0.169 g/day) in the experimental group. In the sixth week of growth, the specific consumption was 1.398 g c.f./g.b.w. (0.200 g/day) in the control group and of 1.083 g c.f./g b.w. (0.155 g/day) in the experimental group. The average specific consumption relative to live weight during the 1-6 weeks of growth was with 27.79 % lower in the experimental group (1.875 g c.f./g b.w.) compared with the control group (1.354 g c.f./g b.w.).

The study conducted in Brazil (Dirceu N.G.J. et al., 2017) mentions an average specific consumption in relation to live weight of 2.290 g c.f./g b.w. for the period of 1-2 weeks of growth (in the present study: 2.331 g c.f./g b.w. in the control group and 1.465 g c.f./ g b.w. in the experimental group), of 2.380 g c.f./g b.w. for the first three weeks of growth (2.203 g c.f./g b.w. in the control group and 1.482 g c.f./g b.w. in the experimental group), of 3.180 g c.f../g b.w. for the period of 1-5 weeks of growth (1.582 g c.f./g b.w. in the control group and 1.409 g c.f./g b.w. in the experimental group) and of 3.880 g c.f./g b.w. for the period of 1-6 weeks of growth (1.875 g c.f./g.b.w. in the control group and 1.354 g c.f./g b.w. in the experimental group).

Table 6: Average weekly and daily specific consumption of compound feeds in quail chicks from the two analysed groups during the period of 1-6 weeks of growth (g c.f./g body weight)

Age (weeks)	Control group		Experimental group	
	Weekly	Daily	Weekly	Daily
1	1.298	0.185	0.688	0.098
2	3.364	0.480	2.241	0.320
3	1.946	0.278	1.518	0.217
4	1.742	0.249	1.417	0.202
5	1.507	0.215	1.180	0.169
6	1.398	0.200	1.083	0.169
Average specific consumption 1-6 weeks	1.875	0.267	1.354	0.194

CONCLUSSIONS

The average live weight in the experimental group was higher with 20.40 % at the age of 7 days, with 18.56 % at the age of 14 days, with 10.55 % at the age of 21 days, with 9.13% at the age of 28 days, with 8.19 % at the age of 35 days and with 8.27 % at the age of 42 days compared to that recorded in the control group.

The larger differences in live weights recorded in the experimental group show the positive effect induced by the supplementation of the compound feeds with fishmeal, especially in the first 3-4 weeks of growth. Throughout the period studied (0 - 42 days) the average daily weight gain was higher with 13.70 % in the experimental group compared to the control group. The average daily consumption of compound feeds for the entire study period was with 14.86 % higher in the experimental group compared to the control group. The specific weekly consumption of compound feeds for the entire study period was lower with 22.91 % in the experimental group compared to the control group.

Given the superior growth performance recorded in the experimental group, we can recommend supplementing the recipes of combined feeds administered to quail youth both in the first phase of growth (0-3 weeks), as well as in the second phase of growth (4-6 weeks). Administration is recommended especially for breeding flocks of chickens for which both the nutritional requirements and growth performances are superior to the flocks raised for production (meat, eggs or mixed). One aspect that remains to be studied is the percentage by which the combined fodder with fishmeal can be supplemented from economic and productive point of view.

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ADDITION OF NATURAL TANNINS DURING FERMENTATION TO STABILIZE THE COLOR OF RED WINE FROM VAR. "*SHESH I ZI*".

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ABSTRACT
Wine is a complex alcoholic beverage, containing many components that affect the quality of the wine. Phenols are the main ingredients widely known in wine as ingredients with a substantial impact on the quality of red wine. Among the phenolic compounds, tannins are of special importance in red wine. In Albania, one of the most widely used grape varieties for the production of red wine is var. "Shesh i Zi". The wines produced from this variety are characterized by having a low alcoholic strength (~ 12% vol.) and storage for more than three years is not recommended. During aging, the wine is characterized by loss of color. Therefore, this study took into account the evaluation of the addition of natural tannic compounds (stems and oak chips) during fermentation and its impact on the preservation of color in aging.
In this study, 100 kg of grapes of var. "Shesh i Zi" from the Tirana area were used in three harvests (2017-2018-2019). Three vinification schemes were used (SLC - classic, SLCH - oak chips and SLF - stems). The evaluation of the polyphenolic components (total polyphenols, total tannins, total anthocyanins, color parameters) was carried out by spectrophotometric methods.
From the results obtained, it was observed that the wines produced in the presence of the stems had a significant impact on the total anthocyanins content and the color parameters.

Keywords: alcoholic fermentation, total tannins, total anthocyanins, color of wine, var. *"Shesh i zi"*

INTRODUCTION

Red wines, which can vary in taste and color, are prepared by crushing and fermenting, dark-colored grapes. Alcoholic fermentation in wine is a biological process carried out by the yeast. Maceration is a physico-chemical process that mainly allows the extraction of a number of important phenolic compounds from wine, which gives it its typical color and structure (Morata et al., 2019).

During the production of red wine by the traditional method, the grape skins are in contact with the grape juice during alcoholic fermentation. What is worth mentioning is that grape skins, especially in red varieties, are rich in phenolic ingredients like anthocyanins and tannins. Some authors have stated that only 50% of these compounds are transferred to the wine during fermentation (Gutiérrez-Escobar et al., 2021). This phenomenon is closely related to the limited permeability of cell walls and cytoplasmic membranes (Pinelo et al., 2006). For this reason, usually for wine production, a controlled fermentation process is followed in order to extract the right polyphenols from the solid parts of the grapes (anthocyanins, tannins and aroma-related ingredients) (Ruiz-Rodríguez et al., 2021), because these polyphenols have a considerable impact on sensory properties (color, flavor, astringency, and bitterness) and on the aging behavior of red wine (Maza et al., 2019). Anthocyanins are easily soluble in water due to their polarity, while tannins are more easily soluble in must in a certain amount of alcohol content (Jackson. 2008). The quality of red wines depends on the presence / absence and quantity of polyphenols and is highly determined by the composition of phenolic compounds (Pasqual et al., 2015).

In Albania, one of the most popular varieties for the production of red wine is the grape variety "Shesh i Zi", autochthonous of the Tirana area (Kukali et al., 2017). This variety is characterized by a high intensity of color in the grape berry. During winemaking, the main problem that has always accompanied this variety is the loss of color after alcoholic fermentation. The main purpose of this study was to achieve the evaluation of the best procedure for the production of high quality wines from the grape variety " Shesh i Zi ', cultivated in the area of Tirana. For this, two new fermentation schemes were built, with stems and oak chips, to assess their impact on improving and preserving the color in the wine produced by this variety. To identify this effect, total polyphenolic content, total anthocyanins, total tannins, and color parameters were analyzed.

MATERIALS AND METHODS

To carry out this study, the variety (Vitis vinifera L.) "Shesh i Zi", from the Tirana area, was taken into account. These samples, after being collected in optimal ripening conditions, were transported to the Food Research Center of the Faculty of Food and Biotechnology.

This study was conducted for three consecutive years, following the same fermentation schemes each year. Thus, for each year the quantity of grapes obtained was 100 kg and was divided into three parts, where three different fermentation experiments were followed. In the first experiment, classical fermentation was followed, which would serve as a control test, and the wine produced was marked with the SLC code (for the first year); 2SLC (for the second year); 3SLC (for the third year of study). In the second experiment, fermentation was followed in the presence of oak chips, and the wine produced was marked with the code SLCH (for the first year); 2SLCH (for the second year); 3SLCH (for the third year of study), while the third experiment was performed with the presence of the stems (50% of the mass of the stems of experiment 3) and the wine produced was marked with the SLF code (for the first year); 2SLF (for the second year); 3SLF (for the third year of study). All three experiments, over three years, were treated with the same dose of 5g / hl SO_2 and inoculated with market yeasts at a dose of 20 g/hl, fermentation was followed at a controlled temperature at 14-18ºC for 10 days.

Analytical determinations

Physico-chemical analyzes of wine include: volatile acidity, total acidity, pH and alcoholic degree (Compendium of International Methods of Analysis – OIV.), total polyphenols (Cetó et al., 2012) content of total tannins (Porter et al., 1985), total anthocyanins (Puissant and Leon, 1967) and color parameters according to Glories (1984). The analyzes were carried out with a minimum of three repetitions and all the results were statistically analyzed using factor analysis of variance (ANOVA). The differentiations were considered significant with $p < 0.05$.

RESULTS AND DISCUSSIONS

During the fermentation process, a number of biochemical processes take place, which affect the extraction of the responsible components of aroma and taste in wines (Añón et al., 2014). Based on various bibliographies, the study was carried out to observe the extraction of polyphenols, tannins, anthocyanins, and color parameters in red wine produced from the grape variety "Shesh i Zi" with three different fermentation schemes.

Physico-chemical parameters of wine

Wine samples were initially analyzed for basic parameters such as: total and volatile acidity, pH as well as alcoholic degrees.

Table 1, presents the results of the analysis of physico-chemical parameters of the wine produced. As can be seen from the table, the indicators are within the quality standards for all three experimental fermentation tests during the three years of study.

Table 1. Quality parameters of the wine produced by *var. 'Shesh i zi'*

	Volatile acidity (mg/L)	Total acidity (mg/L)	pH	Alcoholic degree (% vol.)
SLC	0.55 ± 0.05	6.3 ± 0.0	3.00 ± 0.00	13.47 ± 0.03
SLCH	0.28 ± 0.02	6.2±0.03	3.03±0.01	12.43 ± 0.00
SLF	0.48 ± 0.01	6.4 ±0.06	3.20±0.04	12.43 ± 0.01
2SLC	0.78 ± 0.05	6.1±0.06	2.85±0.00	12.01 ± 0.01
2SLCH	0.24 ± 0.00	5.8±0.1	2.92±0.00	12.87 ± 0.01
2SLF	0.42± 0.03	6.1 ± 0.07	2.91 ± 0.00	12.78 ± 0.02
3SLC	0.92 ± 0.03	7.9 ± 0.12	2.96 ± 0.00	17.19 ± 0.03
3SLCH	0.80 ± 0.02	7.9 ±0.06	2.93 ± 0.00	16.55 ± 0.03
3SLF	0.80 ± 0.02	7.8±0.07	3.42 ± 0.00	16.65 ± 0.03

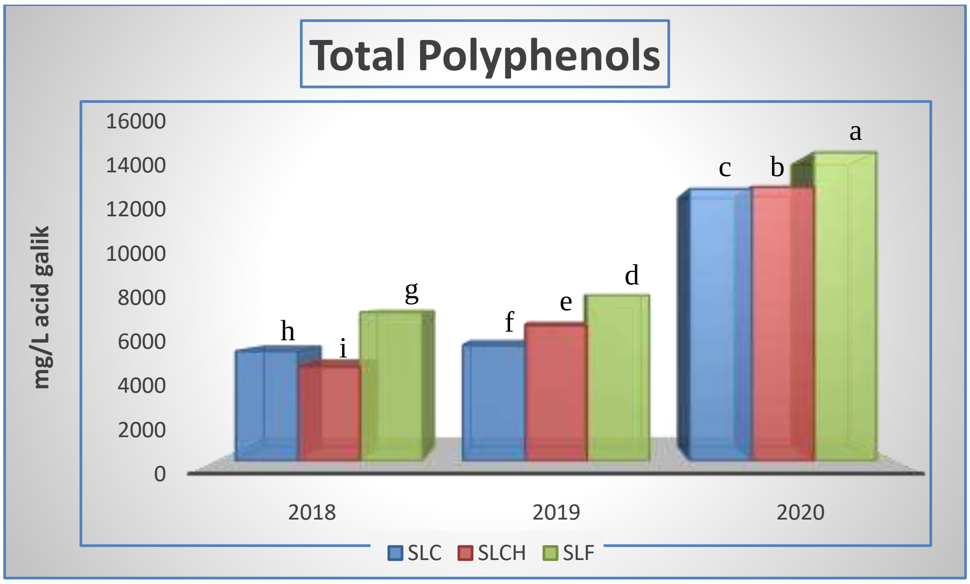
a – Mean ± SD

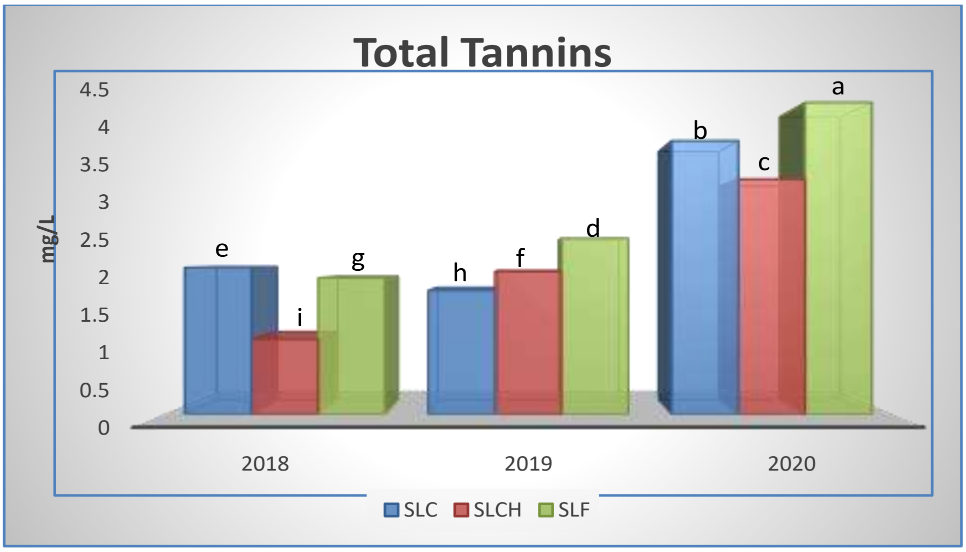
Total Polyphenols, total Tannins and total Anthocyanins contents

Polyphenols are important components which are found in significant quantities in wine, their content is influenced by many factors such as: genetic (Fang et al., 2008), agronomic (Bekara et al., 2017) and technological (Olejar et al., 2015) etc. Therefore, we can say that the application of different fermentation schemes in wine, directly affects the extraction of these ingredients in wine (Olejar et al., 2016).

In addition, tannins and anthocyanins have an important role in the wine process, they affect its characteristics. Anthocyanins are chemical compounds found in the skin of grapes. During the aging of the wine, they form insoluble aggregates in the wine and precipitate, causing the wine to lose its color (He et al., 2012). The addition of oenological tannins is intended to keep anthocyanins bound in the wine thus preventing their precipitation.

In our study the results obtained regarding the content of total polyphenols, total tannins and total anthocyanins are presented in the following graphs.


Total Polyphenols
mg/L acid galik
16000
14000
12000
10000
8000
6000
4000
2000
0
h
i
g
f
e
d
c
b
a
2018
2019
2020
SLC
SLCH
SLF


Total Tannins
mg/L
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
e
i
g
h
f
d
b
c
a
2018
2019
2020
SLC
SLCH
SLF

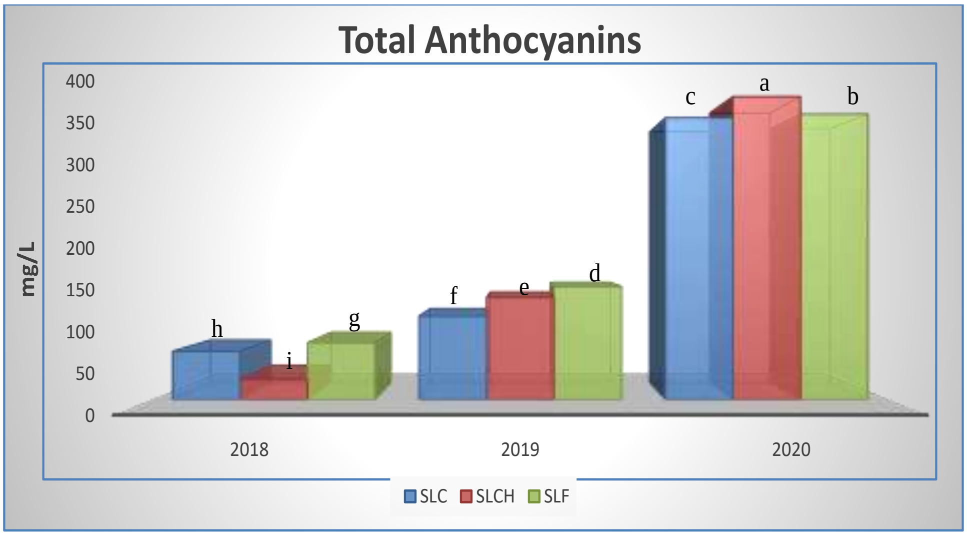

Total Anthocyanins
mg/L
400
350
300
250
200
150
100
50
0
h
i
g
f
e
d
c
a
b
2018
2019
2020
SLC
SLCH
SLF

Figure 1. Evaluation of the impact of fermentation schemes on the content of Total Polyphenols (expressed as mg L^{-1} of gallic acid), Total Tannins (expressed as mg L^{-1} of catechin) and Total Anthocyanins (expressed as mg L^{-1} of malvidin-3-glucoside), in wines of variety ”Shesh i Zi” during aging (Tukey Test, P ≤ 0.05).

As can be seen in the graphs, the content of polyphenolic compounds in wines fermented in the presence of stems, results to be higher, compared to wines produced by classical fermentation and in the presence of oak chips in the three years (2018 - 2019 - 2020). This explains that the presence of stems in fermentation has a positive influence on the quality of the wine. According to Blackford (Blackford et al., 2021), stems are rich in polyphenols and their application in fermentation stimulates the high content of total polyphenols in wine.

A high content of total anthocyanins is also observed in fermented wines with stems. The stems, according to Suriano et al., 2015, are believed to affect anthocyanin absorption by reducing their content in wine. In fact, the presence of stems makes the cap less compact during fermentation (Del Llaudy et al., 2008), but the tannins extracted from the stems protect anthocyanins from oxidation (Casquete et al., 2021) maintaining deep red color of the wine produced from the variety " Shesh i Zi " even during maturation, which is also the objective of this study.

From the statistical analysis performed (Anova factorial), it was noticed that the content of total anthocyanins of wine produced by fermentation with the stems represents a significant difference of $P < 0.05$ compared to the wine produced by fermentation with oak chips and the classic one during the period of aging.

Color parameters

Color intensity is an important parameter and is related to the evaluation of red wine oxidation as a result of some changes that occur during the fermentation and storage of this product.

The results of the color evaluation of wines produced from the variety "Shesh i Zi" with three different fermentation schemes are presented in Table 2.

Table 2. Performance of wine color evaluation during fermentation and aging

Fermentation	Vintage	Hue of color	Index of color	Color Intensity
SLC	2018 2019 2020	0.803^{b}	1.25^{b}	18.48^{a}
SLCH	2018 2019 2020	0.90^{a}	1.21^{c}	15.75^{b}
SLF	2018 2019 2020	0.80^{c}	1.29^{a}	12.4^{c}

a, b and c – Homogeneous groups according to Tukey's test for unequal sample size. The tools within each column of modified letters are significantly different using Tukey's test (P≤0.05).

As can be seen in this table, the color index values during the aging period are higher in wines produced by fermentation with stems. The highest average color index value is distinguished in SLF wine (1.29), compared to SLC control wine (1.25) and SLCH wine (1.21).

In terms of color intensity, the control sample showed higher values in this parameter, regardless of storage time.

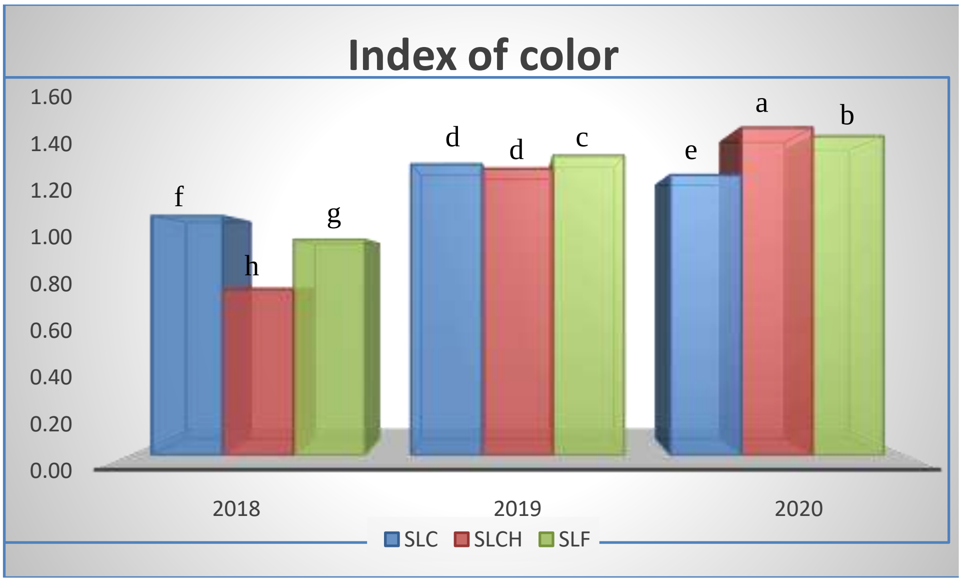


Figure 2. Evaluation of color index in wines produced with three fermentation schemes, during the aging period (Tukey Test, $P \leq 0.05$).

Fermentation with stems and oak chips according to the statistical analysis Anova and Tukey Test for color index has shown that it has a significant impact $P \leq 0.05$ (Figure 2). The treatment and the year of aging indicate the formation of homogeneous groups. From these results it is noticed that in the third year of storage we have a slight decrease of the color index.

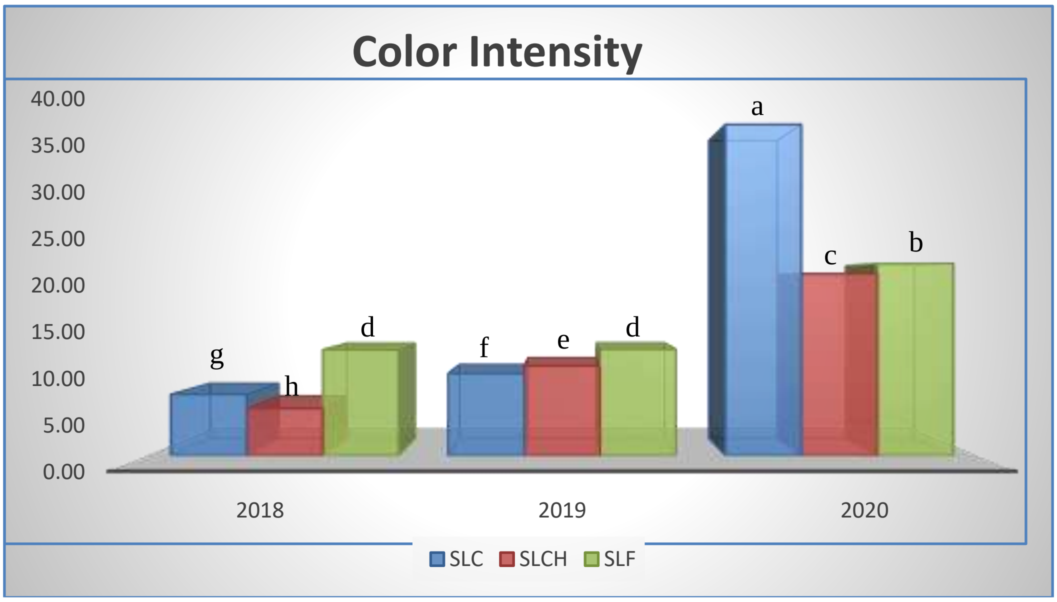
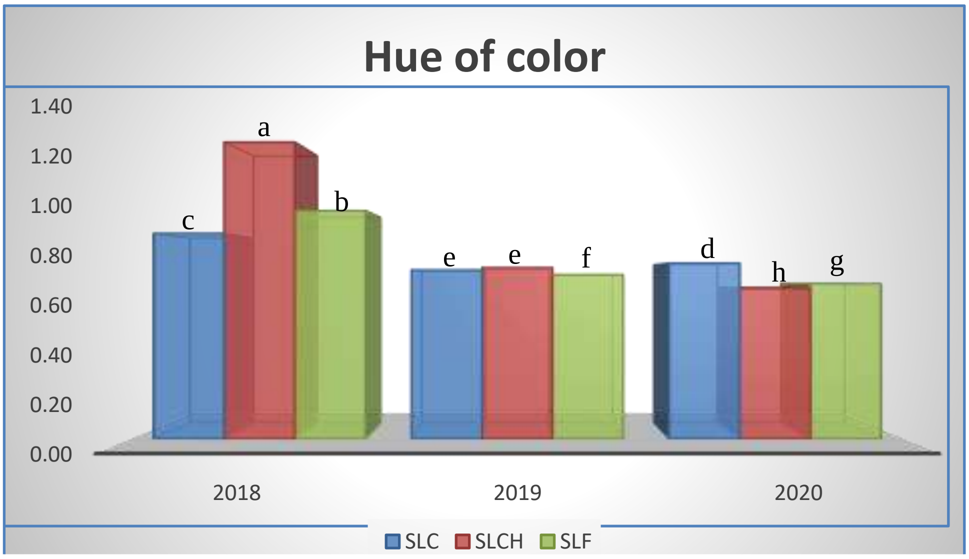


Figura 3. Evaluation of color intensity in wines produced with three fermentation schemes, during the aging period (Tukey Test, $P \leq 0.05$).

According to the statistical analysis used for this study, it is observed that the color intensity decreases during the aging period. At the beginning, the control sample presents significantly higher values and then these values decrease. While wines produced by fermentation with stems do not show a decrease in color intensity. From the statistical results it is noticed that the wines produced in 2018 have higher values of hue of color than the wines produced in 2019 and 2020. This difference may come as a result of climatic conditions.



Figur 4. Evaluation of hue of color in wines produced with three fermentation schemes, during the aging period (Tukey Test, $P \leq 0.05$).

CONCLUSIONS

From this study, we conclude that the addition of stems and chips during alcoholic fermentation to stabilize the red color of wines does not affect their quality.

The amount of total polyphenols in wine produced with three different fermentations schemes did not show significant differences during the aging.

Fermentation with stems showed that it produces wines with higher amounts of total anthocyanins compared to wines produced with chips during fermentation and classical fermentation.

The development of the fermentation of the red wine of the variety " Shesh i Zi " in the presence of the stems favors the conservation of its color

The presence of oak chips in fermentation stimulates an increase in the amount of tannins, but these tannins are not sufficiently bound to anthocyanins, producing wines with less color intensity compared to fermentation with stems and classical one.

From this study, we conclude that wines produced with the presence of stems increase the bond between tannins and anthocyanins while preserving the color of wines more than when fermentation is performed with chips or even in classical fermentation.

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EVALUATION OF THE PHYSICAL-CHEMICAL CHARACTERISTICS OF THE APPLE VARIETIES FOR THE PRODUCTION OF APPLE - CIDERS

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ABSTRACT

The apple is one of the main fruits of consumption in Albania. This consumption is mainly focused on fresh apple and its unfermented juice. In recent years, there has been a great demand for fermented apple juice (cider). Apple cider production is an incredibly complex process that involves a variety of biochemical reactions. The quality of the cider is influenced by many factors such as apple varieties, yeast strains, fermentation conditions, production process, and treatments during aging, etc. The purpose of this study is to determine the appropriate varieties grown in Albania for cider production. The apple varieties used in this study are varieties grown in the Korça area, in Albania. In this study, six varieties of apple were taken, in which the physical-chemical characteristics suitable for the production of cider were evaluated. From the results obtained, only two varieties of apples were selected that comply the requirements for cider production (Jonagold and Annagold). The technological scheme used in this study is the classic fermentation of apples. The evaluation of the quality parameters of the cider (volatile and total acidity, pH, SO2 and dry extract) and the polyphenolic components (total polyphenols, total flavonoids and color parameter) were carried out according to the standard methods of the OIV and spectrophotometric methods.

The results obtained from this study showed that the quality parameters for the two selected varieties were within the limits established according to the EEC standard, 2008. The cider produced by the Jonagold variety presented higher values in its polyphenolic content compared to the cider produced by var. Annagold. These preliminary results will be accompanied by new studies taking into account the combination of other varieties for the production of a higher quality cider.

Keywords: Apple variety, apple cider, physical parameters, chemical parameters, quality parameters, phenolic compounds.

INTRODUCTION

In recent years there has been an increasing trend in the production and processing of agricultural products. Among these products we mention in particular the cultivation and processing of apples. Apple juice is the raw material for various fermented beverages, such as. Cider. Fermentation of apple cider is an incredibly complex process involving several biochemical reactions. In general, the quality of the must is influenced by many factors (Trude et al., 2020).

Albania is a country famous for producing and selling various varieties, especially apples. The output of these fruits in our country has reached 105,900 tons (Insat, 2017), and Korça is a leader in export, and fruit production is an important contributor to the development of agriculture. A sector in Albania that accounts for about 20% of total agricultural production. Apples are one of the main fruits produced and consumed in Albania.

Apples are considerably consumed in all nations at some stage in the world, being very famous due to their preferred taste, juiciness, color, texture and dietary contribution. Additionally, they have got a high-quality preservation capacity, they'll be to be had year-round in markets, at quite low prices and they'll be visible as a healthy food (Bars-Cortina et al., 20018, Bars-Cortina et al., 2020, Mohebbi et al., 2020, Bílková et al., 2020). Besides being consumed fresh, apples moreover can be converted into many particular forms of apple products, consistent with the processing technology used (Li et al., 2020). Some of these apple products encompass juices (Pruksasri et al., 2020, Sauceda-Gálvez et al., 2021), dehydrated (Kidón and; Grabowska, 2021, Feng et al., 2021, Cruz et al., 2021, Guiné et la., 2014), canned ([Li et al., 2020, Doiás et al., 2006) or purées (Lan et al., 2020, Lan et al., 2020). Additionally, particular apple products are acquired via fermentation processes, together with probiotic fermented apple juices (Peng et al., 2020, Roberts et al., 2018, Chen et al., 2019) and cider (Sousa et al., 2020, Lobo et al., 2021, Cusano et al., 2020), or fermented products acquired from apple pomace generated as business employer by-products (Bortolini et al., 2020, Madrera et al., 2017, Jin et al., 2019, Zheng et al., 2020).

Apple fruit is one of the principal end result of intake in Albania. This intake is specifically centered on clean apple fruit in addition to its unfermented juice. Also, apple manufacturing in our country in latest years has been in growing order. This order is because of the favorable weather for its cultivation, however additionally way to purchaser demands, however additionally their possibilities for brand new merchandise that may be presented from apple fruit. One of the products we can factor out is cider. The apple kinds which might be used for wine production need to have specific characteristics, simply so the final wine product has the right high-quality and bouquet. Based on the ones requirements, this check emerge as submitted, which has as purpose the selection of cultivars intended for the producing of apple cider.

The goal of this study, it is far the assessment of high-satisfactory parameters of *Malus Domestica* (apple) cultivar for cider manufacturing in Albania. Determination of chemical indicators of regionally grown apple cultivars, cider excellent parameters and polyphenolic compound of the final product.

This study affords facts and hints that can be beneficial in guiding cider manufacturing strategies from domestically grown apple fruit.

MATERIAL AND METHOD

For the realization of this study were taken into consideration 5 (five) apple cultivars of Korça area (Pink lady, Grand Smith, Jonagold, Annagold and Renet). These samples after being placed in optimal baking conditions, were transported to the Food Research Center of the Biotechnology Food Faculty.

Immediately after arriving on the laboratory, those samples underwent physical-chemical analyzes, together with pH, total acidity, sugar content, ash content and polyphenolic content, the effects of which guided us in the choice of cultivars for cider production.

There were two cultivars that were used for cider production, Jonagold (S1) and Anagold (S2). For cider production the classic scheme of wine production was followed.

The experimental checks had been dealt with a dose of 5g/hl SO2 and positioned in cold maceration for 24h at 5°C. After a cold maceration of each vintage become pressing. The acquired juice become brought 2 g/hl of pectolytic enzyme for higher rendering and left for static decantation (48h at 5°C). Subsequent withdrawal of the juice from the lees and inoculation of 20 g/hl of S. beans BC yeast and fermented at managing temperature of 14–18ºCPhysicochemical analysis of fruit

1. Physical and chemical parameter of apple fruit

1.1.The determination of acidity of apple fruit and cider.

This parameter is performed according to the AOAC method (1990), and is based on the titration of acid-based fruit juice in the presence of phenolphthalein as an indicator.

$$\text{Total acidity (gr/L)} = 0.67 \times n/Gr$$

1.2.Determination of pH.

The pH value of fresh fruit is measured immediately after sampling (AOAC method ref. 981.12). Place the pH-meter electrode, prepared for measurement, in the sample fluid for analysis and read its value. The results are expressed in pH values at a temperature of 22ºC that must be accurate to 0.01 units of pH.

1.3.Determination of Ash content

Twenty grams of almost dry material were weighed into a tared platinum dish and dried in an electric oven at 100° C. for 24 hours and again weighed to determine the solids in the partly dry sample. It was next charred over in a muffle furnace at 700-750° C. The ashing was continued until practically all of the carbon was destroyed; the ash was cooled in a desiccator and weighed rapidly. The weight thus obtained was designated as "crude ash".

1.4.Determination of total soluble solid (TSS)

The juice was extracted using domestic juice extractor at ambient temperature (32±1 °C) and was filtered through muslin cloth. The TSS of the filtered juice was measured thrice using a hand held digital refractometer.

1.5.Determination of total polyphenol content (TPC) using Folin-Ciocalteu (FC) method.

Total polyphenol content (TPC) of fresh fruit and cider was determined spectrophotometrically according to a slightly modified FC method (Singelton & Rossi Jr. 1965; Singh et al. 2007).

The measurements were compared to a standard curve of prepared gallic acid solutions (25–500 mg/l) and expressed as mg of gallic acid equivalents (GAE) per 1 l ± SD of apple juice. All measurements were performed in triplicate.

2. Determination of quality parameter of apple cider

Free and Total Sulfur Dioxide the method according to OIV (Method OIV-MA-AS323-04B, 377/2009) was used for the determination of free and total sulfur dioxide, determination of total acidity according to OIV (Method OIV-MA-AS313-01 Oeno Oeno 551/2015), pH value was determinate according to OIV-MA-AS313-15 (Oeno 438-2011), alcoholic degree was determinate according to methods of Becchetti, 1999, total dry matter was determinate according to OIV-MA-AS2-03B (Oeno 465/2012).

3. Determination of total flavonoid contents (TF).

The total flavonoid content was determined by spectrophotometric method (Zhishen et al., 1999). This method based on the formation of complex flavonoid-aluminum. At 510 nm was measured the absorbance. The concentration of the total flavonoid compounds in the wines was expressed as catechin equivalent (mg/L). The samples were analyzed in triplicate.

4. Determination of color intensity.

Chromatic characteristics of the wines were determined by spectrophotometric method. The color intensity was calculated as the absorbance measured at 420 nm. The color intensity assessment refers to the numerical value determination of the white wine chromatic characteristics (Becchetti, 1999).

RESULTS AND DISCUSSION

1. Physical parameters of apple cultivars for cider production

Apples supposed for the manufacturing of alcoholic liquids with an alcohol content material among 1.2% and 8.5% (low-alcohol cider might also additionally have much less than 1.2%) should go through a partial or entire fermentation of the juice, without or with the addition of sugar, water or flavorings (ECFA, 2018).

According to the European Association of Apple and Fruit Wines (ECFA), cider apples are categorized into 4 huge categories: sour, bitter sour, bitter sweet and sweet. The fundamental standards for classifying apples are their acidity, which offers the astringent aroma (Włodarska et al., 2017), phenolic compounds, which provide the bitter taste (Laaksonenet et al., 2017) and the sugar content, which determines the alcoholic awareness of must. Table 1 indicates the classification of a few varieties of apples grown in the the country, with average values of sugar content, titratable acidity and total phenolic content.

Table 1. Classification of cider apples in terms of chemical content of some parameters such as TSS (rixBrix), titratable acidity (AT), pH, ash content and total polyphenols.

Cultivars	TSS (ºBrix)	Titration acidity (% v/v)	pH	Ash (%)	Total Polyphenols mg/L acid gallic
Pink lady	10.0[a] ± 0.02	0.55 ± 0.02	3.92 ± 0.19	0.16 ±0.00	137.86 ± 6.5
Grand Smith	11.4 ± 0.02	0.74 ± 0.03	3.30 ± 0.01	0.26 ±0.01	253.34 ± 5.7
Jonagold	15.2 ± 0.01	0.71 ± 0.02	3.22 ± 0.02	0.98 ± 0.03	377.37 ± 5.9
Anna Gold	13.0 ± 0.00	0.6 ± 0.01	4.14 ± 0.03	0.44 ± 0.01	326.27 ± 3.6
Renet	12.2 ± 0.01	0.85 ± 0.03	4.39 ±0.00	0.09 ± 0.01	328.31 ± 2.2.

a- Mean and SD ±

The chemical composition, respectively, the sugar content, in apple juice determines a few sensory and dietary properties of the final product (Ye et al., 2014). Sugar content material is an important factor, specially while identifying to combine apple juice types to achieve a selected cider variety. From Table 1 it is determined that the chosen cultivars gift different average sugar contents, however, the Jonagold and Anna cultivars provided the highest values of 15.2 and 13.0 ºBrix respectively. Organic acids are important components of apple cider, as they significantly have an effect on its sensory profile (Ye et al., 2014). Some of those components are a part of polyphenolic compounds, the table indicates that the average quantity of total polyphenols of the studied types varies from 137.86 to 377.37 mg/L of Gallic acid, wherein the Jonagold cultivar confirmed the highest values on this parameter.

Cider manufacturers need to attention on apple juice traits to optimize cider quality and aroma, together with decrease pH, better titratable acidity, and excessive total polyphenol content (Cline et al., 2021). Table 1. gives the pH values of apple juice for the cultivars selected on this study.

Apple varieties have special chemical traits (Table 1), which have an effect on the sensory profile of the final product. Some of the strategies used to enhance those traits are mixing, which may be accomplished in lots of levels of the cider manufacturing technique. This technique is composed of blending numerous varieties of apples or juices and its goal is to adjust acidity, bitterness, tightness, sweetness, alcoholic concentration, color and aromas. Mixing is a key thing in keeping the consistency and exceptional of the cider utilized by big producers on a business scale (Buglass et al., 2011). The precise varieties used for cider manufacturing range from area to area. In this study, it changed into located that the Anna Gold and Jonagold cultivars are of the cultivars to evolve to the traits of cider product.

2. Physical and chemical characteristics of cider

There isn't many definitive research on the connection among unique varieties of apples and the volatile composition of cider, however the chemical composition of apples varies consistent with the variety, which confirms the have an impact on of the apple variety at the aroma and taste of the final product. Regarding the non-volatile compounds, further to the fluctuations in the concentration of sugars and acidity, the primary variations among the fruit varieties go away their mark at the phenolic content (Table 2). The degree of maturity of the fruit additionally has an excellent effect at the aromatic profile of the cider (Rosend et al., 2019).

In this study have been analyzed a number of the parameters which have an effect on the quality and organoleptic properties of apple cider. Table 2 indicates the mean values of titratable acidity, pH and alcoholic degree of cider produced from only the 2 decided on cultivars. An important quality parameter for cider is the ethanol concentration. This is a key issue affecting the quality, sensory properties and consistency of beverages (Lachowicz et al., 2019). The alcohol content withinside the ought to acquire from the alcoholic fermentation of apples must be 1.2-8.5% vol. (Witt and Śmiechowska, 2015). The ethanol concentration in all of the cider ought to examine on this have a look at became withinside the variety of 7.02-7.9% vol.

Table 2. Quality parameters (total acidity, pH and alcoholic degree) of cider produced from two apple cultivars

Cider	Total acidity (g/L tartaric acid)	pH	Alcoholic grade (% vol)	Volatile acidity (g/L acetic acid)	Total SO_2 mg/L	Total extract g/L
S1[b]	4.82 ± 0.19[a]	3.23 ± 0.0	7.79 ± 0.2	0.42 ± 0.01[a]	263.87 ± 1.04	12.44 ± 0.0
S2	5.02 ± 0.13	3.23 ± 0.0	7.03 ± 0.3	0.35 ± 0.0	231.47 ± 3.9.	17.01 ± 0.7

a- Mean ± SD, b - S1 (Jonagold cider), S2 (Anna gold cider)

The total acidity of the must should be between 3.5 g and 7 g of malic acid per liter (CEE, 2008). The initial acidity of apple juice before fermentation was 0.7% v / v. All samples analyzed had a high overall acidity. The volatile acidity of the must should be less than 0.9 g/L, calculated as acetic acid (CEE, 2008). From the results obtained from both cider samples analyzed, the average values of total acidity were presented, within the limits set according to the CEE 2008 standard.

The content of the general extract is directly related to the content of ethyl alcohol and residual sugars. Usually, a higher concentration of ethanol suggests less sugars remaining after fermentation and a lower total extract (Ye et al., 2014). The value of this parameter in the analyzed cider was in the range of 0.42g/L - 0.35 g/L (Table 2).

Table 2 shows that the total extract content was highest in the cedar produced from the Annagold cultivar. These values correspond to the alcohol content, where a higher concentration of the extract indicates lower cider strength. Higher total extract content than in control samples is likely to be associated with glycerol synthesis. Glycerin is an important component of cider and wines, affecting their sugar-free extract and determining the full aroma of beverages. Mean values of total SO_2 are within the set limits (CEE, 2008).

3. Phenolic compounds of cider.

Polyphenols are an important group of bioactive compounds because they have a strong impact on the quality of food products. These compounds are also powerful antioxidants with a wide range of biological activities (Alonso-Salces et al., 2004; Guyot et al., 2003). Particular attention is paid to the content and profile of polyphenols when choosing the apple variety used for cider production, as they affect the color and astringency, which make up sensory perceptions (Khanizadeh et al., 2008). In studies conducted by Riekstina-Dolge et al., 2012, the total polyphenol content in sweet apples averaged 27.78 mg/L - 92.32 mg/L. The results obtained of the polyphenol content in this study are presented in Figure 5.3, the content of these components varies from 95.7 - 101.3 mg/L. The amount of polyphenolic components depends to a large extent on the fermentation methods used, on the addition of mineral elements, on the time of apple harvest, as well as on the technology and conditions for the preparation of cider (Tarko et al., 2018).

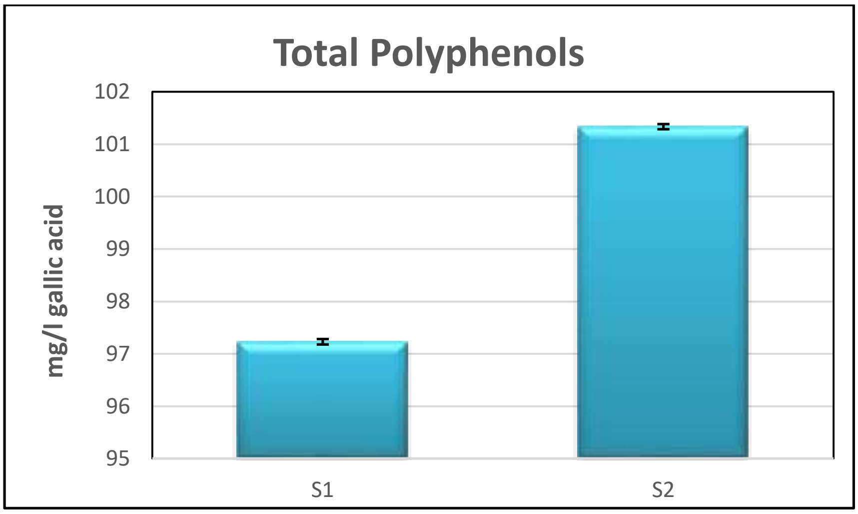

Figure 1. Content of total cider polyphenols produced by Jonagold and Annagold cultivars obtained in the study.

Figures 2 and 3 show the average values of total flavonoids and color intensity. From the results obtained it was observed that the cider produced by the Jonagold cultivar showed higher values for both components.

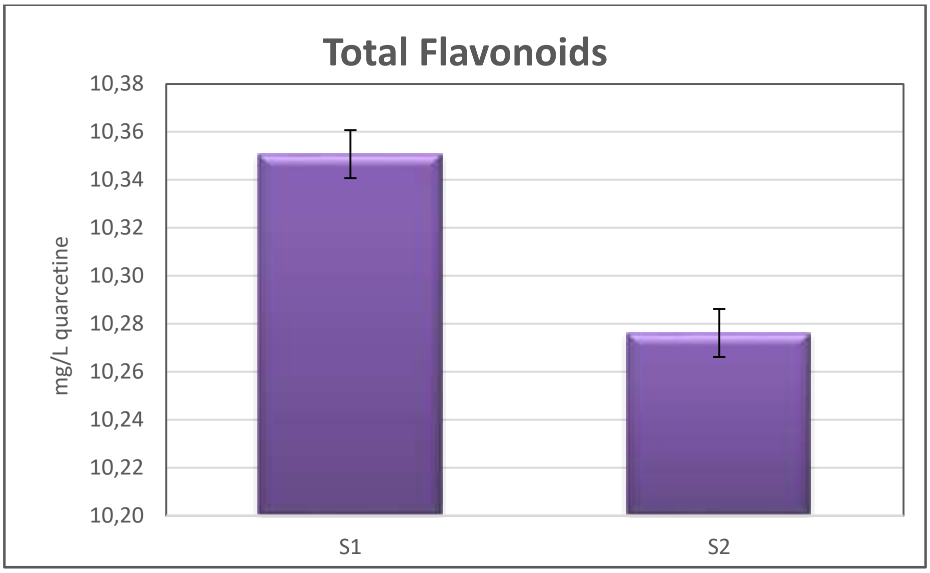

Figure 2. Content of the total flavonoids of Jonagold and Annagold cider

As can be seen from the figures the cultivar that has high flavonoid content produces cider with more intense color as these components are responsible for the yellow color.

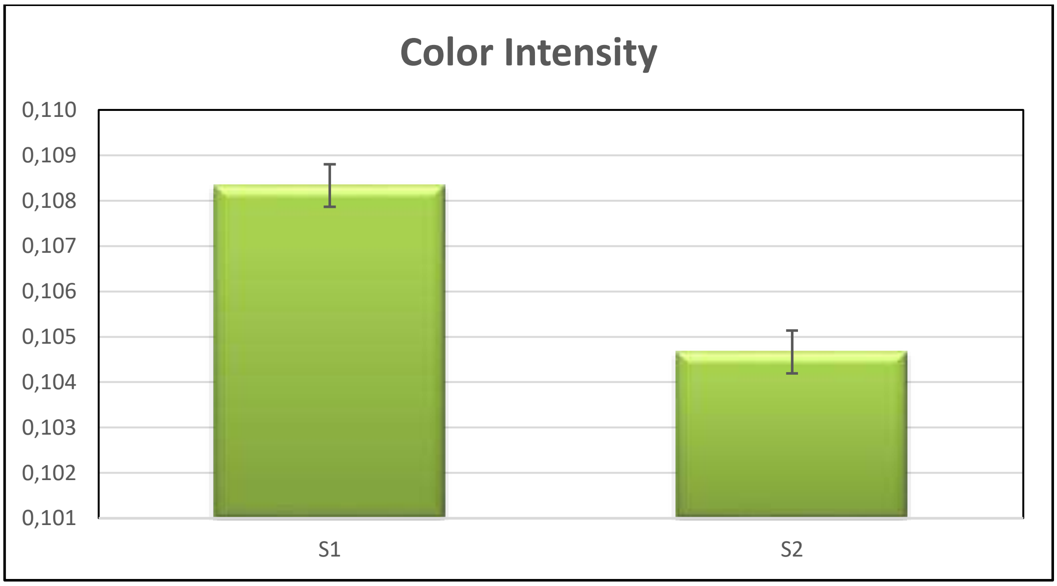

Figure 3. Jonagold and Annagold cider color intensity content

CONCLUSIONS

Based on the results obtained for the parameters analyzed in this study we can conclude that:
The apple cultivars studied (Pink lady, Grand Smith Jonagold, Anna gold and Renet) present good values of their physico-chemical characteristics.
Based on quality indicators, the cultivars which showed values of a high level of sugars, polyphenols, minerals, total acidity, etc., were the cultivars Jonagold and Annagold, which were selected for cider production.

From the results obtained from the analysis of cider we came to the conclusion that these cultivars are suitable for cider production.
Anna Anna gold cultivar is distinguished for slightly higher values of total polyphenols in cider, compared to Jonagold, while in terms of flavonoid content it results in lower values than S2.
By comparing the results of the two cultivars, Jonagold is presented as a cultivar with high processing values and suitable

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NUTRITIONAL ENRICHMENT OF SESAME SEED BY Aspergillus niger SOLID-STATE FERMENTATION

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ABSTRACT

The aim of the study was to investigate the effect of solid-state fermentation on the nutritional composition of sesame seed. *Aspergillus niger* strains (ATCC 200344, ATCC 200345, ATCC 201572, and ATCC 52172) were used as the microbial inoculants in this study. Sesame seed was fermented by the mixture of *A. niger* strains at 30 °C for seven days. The crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of unfermented and fermented sesame seed were determined. Solid-state fermentation increased the CP ($P<0.001$) and ash ($P<0.01$) content while decreased the EE ($P<0.05$), nitrogen-free extract + crude fiber ($P<0.001$), NDF ($P<0.001$), ADF ($P<0.01$), and ADL ($P<0.05$) content of sesame seed. The results showed that the nutritional value of sesame seed can be improved by *A. niger* solid-state fermentation.

Keywords: sesame seed, nutritional composition, solid-state fermentation, *Aspergillus niger*, fermented feed

INTRODUCTION

Sesame (*Sesamum indicum* L.) seeds are important oil seed crops in human and animal nutrition (Lee et al., 2010). It is one of the most ancient oilseed crop and cultivated in Asia and Africa for a long time (Makinde and Akinoso, 2014). Sesame seeds produced at 6.5 million tonnes worldwide (FAO, 2019). Sesame seed contains 50% lipid, %20 crude protein, and 14% carbohydrate (Bae et al., 2016). It is also a rich source of vitamin and minerals (Hajimohammadi et al., 2020).

Solid-state fermentation (SSF) refers to the development of microorganisms within moistened solid substrates without free water (Gungor and Erener, 2020). Fermentation method can be used to improve the nutritional composition of agricultural products (Altop, 2019). Hajimohammadi et al. (2020) reported that solid-state fermentation using *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* increased the crude protein, amino acid and decreased the crude fiber content of sesame seed meal. Similarly, Olude et al. (2016) stated that *Lactobacillus planetarium* improved the nutritional composition of sesame seed meal. *Aspergillus niger* is also preferred microorganism in the solid-state fermentation studies because of the growth ability on the moistened substrates (Gungor et al., 2021a). This study aimed to determine the effects of the solid-state fermentation using *A. niger* on the nutritional composition of sesame seeds.

MATERIAL AND METHOD

Solid-state fermentation was performed with 3 replicates according to Gungor et al. (2021b). *Aspergillus niger* strains (ATCC 200344, ATCC 200345, ATCC 201572, and ATCC 52172) were obtained from American Type Culture Collection (ATCC) and cultured in Potato Dextrose Agar. Sesame seed was milled to a size of 2 mm and sterilized at 121 °C for 15 min by autoclave. The nutritional salt (glucose:urea:$(NH_4)_2SO_4$:peptone: KH_2PO_4:$MgSO_4.7H_2O$ =4:2:6:1:4:1) were added to sesame seeds. After mixing, sesame seed was inoculated by the mixture of *A. niger* strains at 1 ml 10^6 spores/ml for each 100 gram sesame seed and incubated at 30 °C for 7 days. Samples were dried at room temperature approximately 30-35 °C for 6 days until samples reached 90% dry matter (DM).

Sesame seeds were analyzed for DM, ash, CP, and ether extract (EE) according to AOAC (2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) analyses were conducted according to Van Soest et al. (1991). Nitrogen-free extract+crude fiber content (NFE+CF) was calculated using following equation: NFE+CF (%)=100-(CP+EE+ash).

All experimental analyses were performed in triplicate. Data were analyzed with Student t test (SPSS 21.0 Statistics). Results were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Solid-state fermentation increased the CP ($P<0.001$) and ash ($P<0.01$) content of sesame seeds. However, *A. niger* decreased the EE ($P<0.05$), NDF ($P<0.001$), ADF ($P<0.01$), ADL ($P<0.05$) and NFE+CF ($P<0.001$) content of sesame seeds. Similar to the results of the present study, Hajimohammadi et al. (2020) reported increased CP content and decreased CF content in sesame seed meal after *L. acidophilus* and *S. cerevisiae*. However, fermentation increased the EE and decreased the ash content of sesame seed meal in the same study contrarly to the result of the present study. Olude et al. (2016) also reported that *L. plantarium* increased the CP and ash content but decreased the EE, CF and NFE content of sesame seed meal. Increase in the CP with fermentation were attributed to the mycelia and ezymes produced by *A. niger* (Raimbault, 1998).

Carbohydrates are firstly consumed as a carbon source by microorganisms (Papagianni, 2007). Dhillon et al. (2012) showed that *A. niger* can produce cellulase enzyme in solid-state fermentation. These may explain the reason for decrease in the CF+NFE, NDF, ADF and ADL content by fermentation in this study. Difference between the results of the present study can be attributed to difference between the microorganism, inculation level in the studies.

CONCLUSIONS

Solid-state fermentation improved the nutritional composition of sesame seeds. The mixture of *Aspergillus niger* strains (ATCC 200344, ATCC 200345, ATCC 201572, and ATCC 52172) can be suggested for solid-state fermentation to convert sesame seeds into a more valuable feedstuff for animal nutrition.

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SOLID-STATE FERMENTATION USING Aspergillus niger IMPROVES THE NUTRITIONAL QUALITY OF OLIVE CAKE

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ABSTRACT
The effect of solid-state fermentation on the nutritional composition of olive cake was investigated in this study. Olive cake was fermented with *A. niger* (ATCC 9142) at 30 °C for seven days. The crude protein (CP), ether extract (EE), ash, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of unfermented and fermented olive cake were determined. Fermented olive cake had lower CF ($P<0.01$), ADF ($P<0.01$), and ADL ($P<0.001$) content than unfermented olive cake. Fermentation decreased ($P<0.01$) the nitrogen-free extract but did not change the CP, EE, ash, and NDF content of the olive cake. Obtained results showed that the nutritional quality of olive cake can be improved by *A. niger* solid-state fermentation.

Keywords: olive cake, nutritional quality, solid-state fermentation, *Aspergillus niger*, fermented feed

INTRODUCTION

Olive cake is a by-product of the olive oil production, which consists of olive skin, pulp and pit (Fathy et al., 2018). The total olive cake production in the world is calculated as 2.8 million tonnes per year (Nunes et al., 2016). Olive cake can not be stored for a long time because it deteriorates quickly due to its high water and oil content. It cause a serious storage problems in olive oil factories (Brlek et al., 2012).

Solid-state fermentation (SSF) means to the development of microorganisms within moistened solid substrates without free water (Gungor and Erener, 2020). Fermentation method can be used to utilization of the agricultural by-products by improving nutritional values (Altop, 2019). Chebaibi et al. (2019) reported that *A. niger* increased the crude protein (CP) of olive cake with solid-state fermentation. The aim of the present study was to investigate the effect of *A. niger* solid-state fermentation on the nutritional composition of olive cake.

MATERIAL AND METHOD

Solid-state fermentation was performed with 3 replicates according to Gungor et al. (2021). *Aspergillus niger* strain (ATCC 9142) was obtained from American Type Culture Collection (ATCC) and cultured in Potato Dextrose Agar. Olive cake was dried at 75 °C for 48 hours and milled to a size of 2 mm. It was sterilized at 121 °C for 15 min by autoclave. The nutritional salt (glucose:urea:$(NH_4)_2SO_4$:peptone: KH_2PO_4:$MgSO_4.7H_2O$=4:2:6:1:4:1) were added to sesame seeds. After mixing, olive cake was inoculated by *A. niger* at 1 ml 10^4 spores/ml for each 100 gram olive cake and incubated at 30 °C for 7 days. Samples were dried at room temperature approximately 30-35 °C for 6 days until samples reached 90% dry matter (DM).

The DM, CP, ether extract (EE), ash, and crude fiber (CF) content of olive cake were determined according to AOAC (2000). Olive cake was analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) according to Van Soest et al. (1991).

All experimental analyses were performed in triplicate. Data were analyzed with Student t test (SPSS 21.0 Statistics). Results were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Chebaibi et al. (2019) reported increased CP in olive cake by solid-state fermentation. However, there was no any change in CP content of olive cake by fermentation process. This can be attributed to differences between *A. niger* strains and fermentation conditions between the studies.

Solid-state fermentation decreased the CF ($P<0.01$), ADF ($P<0.01$), and ADL ($P<0.001$) content of olive cake in this study. Similarly, Fathy et al. (2018) reported that *A. niger* decreased the CF, NDF, ADF, and ADL content of olive cake. Leite et al. (2016) reported that *A. niger* can produce cellulase and xylanase with the olive pomace in solid-state fermentation. Decrease in the celulotic components by solid-state fermentation can be due to enzymes that break down the structural carbohydrates of the olive cake, which is produced during fermentation process.

Microorganisms prefer carbohydrates to the other nutrients as carbon source (Papagianni, 2007). Indeed, Leite et al. (2016) reported decreased free-sugar in olive cake by *A. niger* solid-state fermentation. However, *A. niger* increased the NFE content of olive cake in this study. Similarly, Fathy et al. (2018) reported that carbohydrate content of olive cake was increased by *A. niger* solid-state fermentation.

CONCLUSIONS

The nutritional composition of olive cake was improved by *A. niger* solid-state fermentation by decreasing CF, ADF and ADL and increasing NFE content. Solid-state fermentation using *A. niger* can be suggested for making olive cake a valuable feedstuff for animal nutrition.

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PERFORMANCE EVALUATION OF AUTOMATICALLY EXTRACTED CITRUS TREES FROM LIDAR POINT CLOUD DATA: REVISITING A TRADITIONAL STRATEGY BASED ON LOCAL MAXIMA AND WATERSHED SEGMENTATION

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ABSTRACT

Information on agricultural areas, such as the type and amount of fruit trees, crown height, and stem locations, is useful for a variety of applications in agricultural studies. The main goal of this study is to assess the performance of automatically extracted citrus trees from point cloud data of a LiDAR sensor (G-LiHT) using a traditional automatic strategy based on local maxima detection followed with the watershed segmentation. In total 10 parcels of citrus trees were selected from the south of Florida, USA. First, Cloth Simulation Filter was applied to split the point data into two categories, ground and off-ground, to generate canopy height models for each citrus parcel. Treetop positions were determined using a local maxima filter, and the boundaries of trees were delineated by traditional watershed segmentation. Finally, in each parcel, different tree species other than citrus were manually queried and eliminated, and boundaries of citrus trees were finally improved by post-processing. According to the results computed, the parcels with the greatest accuracies (≈ 85%) had more regular and sequential planting characteristics and were composed of trees of similar height. However, it was observed that in parcels with high tree density, more trees were recognized than the actual number, causing the boundaries to be erroneously delineated.

Keywords: LiDAR, Citrus Tree Detection, Canopy Height Model, Local Maxima Filter, Watershed Algorithm, Automatic Detection

INTRODUCTION

One of the most important topographic elements on our planet is trees. Many applications including horticulture, forestry, ecosystem services, and agricultural activities can be made possible by determining up-to-date information about trees, e.g. cover, type, number, height and location. For those applications to produce useful outputs, accurate extraction of such information is essential.

Fruit trees are crucial horticultural crops, with tens of billions of dollars revenue every year to farmers, creating value added activities and job prospects at village level. Therefore, it is advantageous to characterize tree species that are strategically important in horticultural activities, such as citrus, in order to increase production efficiency and sustainability. However, collecting information about trees using terrestrial measurements requires a lot of time and effort. Remote sensing data has become a reliable source for estimating several key parameters of trees, both at individual and at canopy levels, due to their wide data coverage, ease of accessibility, and availability of image processing techniques (Mielcarek et al., 2018).

Light Detection And Ranging (LiDAR) provides 3D information describing the structure of the Earth's surface by combining a micropulse laser unit with a global positioning system and an inertial measurement unit (Pack et al., 2012). LiDAR enables quick and non-destructive measurements of vegetation's 3D structure. Because of LiDAR signals' ability to penetrate tree canopies, such data is currently being employed in a large variety of applications, involving terrestrial, airborne and spaceborne LiDAR data (e.g. Hyyppä et al., 2001; Koch et al., 2006; Dong et al., 2020; Campbell, 2021).

Goddard's LiDAR, Hyperspectral and Thermal Imager (G-LiHT) is a lightweight and portable aerial imaging technology that maps the composition, structure, and function of terrestrial ecosystems at the same time. It covers LiDAR, hyper-spectral and thermal sensors that can collect data for global applications in a low-cost manner (Cook et al., 2013). The G-LiHT system has two different lasers, the airborne laser scanner (Riegl VQ-480) and profiling laser scanner (Riegl LD321-A40), operating at near- and mid-infrared wavelengths of 905 and 1550 nm, respectively (Cook and Corp, 2012). A high precision direct position (0.1m CEP positioning) and attitude (0.1° heading) measurements are collected by GPS-INS (RT-4041) instrument, which is directly attached to the airborne laser scanning unit. RiPROCESS software is used to manage, process, analyze, and visualize data obtained with the ALS system. RiPROCESS receives raw laser scanner data and pre-processed GPS-INS data, adds calibration information, converts scan data to geographic coordinates, and produces point cloud data (Cook et al., 2013). So far, a number of studies have been carried out benefiting from the data collected by G-LiHT system (e.g. Hernandez-Serna, 2019; Margiotta, 2020; Cessna, 2020)

Quite a few approaches for extracting trees from LiDAR point clouds have been developed so far (see review paper by Zhen et al., 2016). These approaches mostly rely on the fact that tree tops are the highest point in the observed region to identify local maxima in the data that represent distinct trees. Thereafter, data are segmented to generate tree representations, for example, using region growing. Watershed transform is another method used during the segmentation, both used for images and elevation models, providing good results (Millikan et al., 2019; Yang et al., 2020). Nevertheless, the accuracy of findings derived from these approaches has been shown to be influenced by the under- or over-detection of number of trees. As a result, due to the difficulties in identifying local maxima, different methods have also been proposed (e.g. Ozdarici-Ok, 2015; Ok and Ozdarici-Ok, 2018a; Ok and Ozdarici-Ok, 2018b).

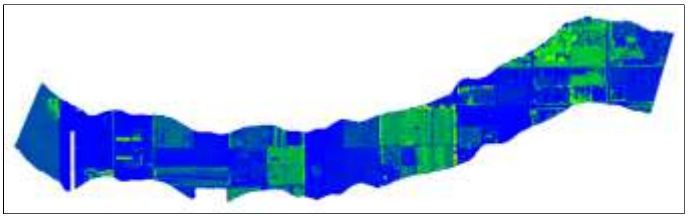
In this paper, we use a traditional automatic strategy based on local maxima detection and watershed segmentation to evaluate the performance of automatically extracted citrus trees from LiDAR point cloud data. Florida State LiDAR point cloud data collected by G-LiHT aerial imaging system by the United States National Aeronautics and Space Agency Goddard Space Flight Center were utilized. A total of 10 citrus parcel data were obtained by determining the intersecting areas of citrus parcels and LiDAR point cloud data. Ground and above-ground point cloud data were separated for all parcel data obtained, and above-ground point cloud data were exploited. For each parcel, a canopy height model (CHM) was created, and a local maxima filter was used to determine treetop positions, and marker-controlled watershed segmentation was used to define tree boundaries. Finally, different tree species other than citrus were manually queried and eliminated in each parcel, and citrus tree boundaries were improved through post-processing.

The rest of this paper is organized as follows. Section 2 contains information on the study area, datasets, and reference data. Section 3 details our method. Section 4 reports evaluation strategy and discusses the results. Section 5 contains the concluding remarks.

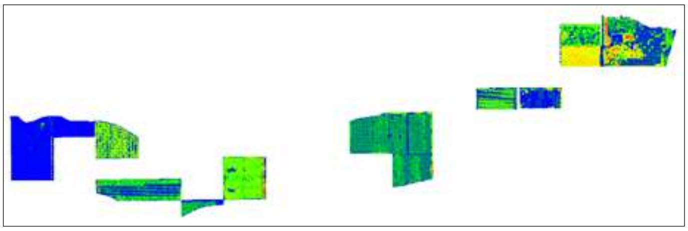
STUDY AREA AND DATASET

The majority of citrus produced in the United States is grown in California, with the southern parts of the Florida peninsula as a close second. With approximately 74 million citrus trees, Florida produces more than 40% of the citrus for the United States, including oranges, grapefruit, and specialty fruit such as temple oranges, tangerines, and tangelos (USDA, 2020). Besides, Florida is the world's second largest producer of orange juice, and the state is the world's top producer of grapefruit (Court et al., 2020). Our study area (Fig. 1a) belongs to the Miami/Homestead area in the southern part of Florida, USA. Although it is an agricultural land with a relatively flat topography, there are also privately owned houses in the area.

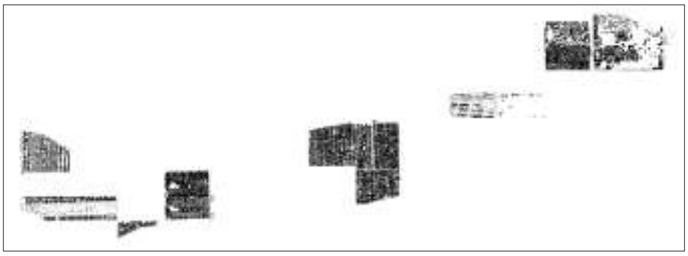
(a)



(b)



(c)



(d)

Figure 1. (a) The selected study area in southern part of Florida (center geographic coordinates: 25.476768° N, 80.547986° W), (b) the LiDAR data strip (green and blue colors represent the highest and lowest elevation, respectively), (c) 10 parcels of citrus trees, and (d) manually prepared reference data.

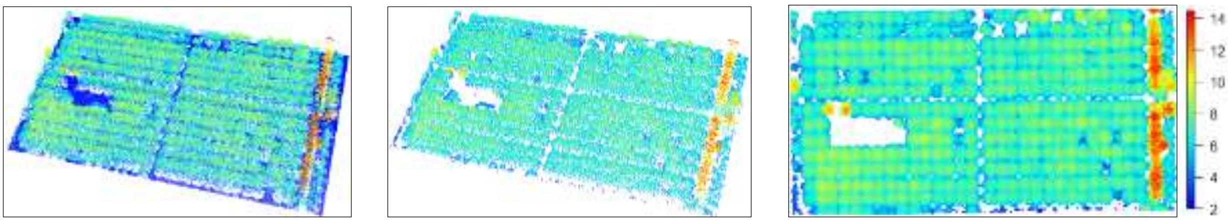
LiDAR point cloud data for the study area were acquired in March, 2017 by G-LiHT airborne imaging system. The data includes a flight plan with a 30% swath overlap, a nominal altitude (AGL) of 335 meters, and a nominal velocity of 110-150 knots. The entire point cloud data consists of a total of ≈ 14.7 million points (Fig. 1b). From the study area, we identified 10 sample parcels for our testing (Fig. 1c), and a variety of citrus orchards with mixed features and ages are represented by the selected parcels. The LiDAR data was used to manually create the reference data (in total 6,719 citrus trees), which consists of the borders of individual citrus trees (Fig. 1d).

METHODOLOGY

To construct canopy height models for each citrus parcel, the Cloth Simulation Filter (Zhang et al., 2016) was used to divide the point data into two categories: ground and off-ground. A local maximum filter was used to determine treetop positions, and marker-controlled watershed segmentation was used to delineate tree borders. Finally, tree species other than citrus were manually examined and removed in each parcel, and citrus tree borders were finally improved through post-processing. We utilized an R package designed for LiDAR based forestry applications for the processing of airborne datasets (Roussel et al., 2020; Roussel and Auty, 2021).

Identifying Above Ground Points and Generating CHMs

LiDAR returns may originate from above-ground objects, e.g. the tops of buildings, cars, tree canopies, powerlines, and other objects. Therefore, first, above ground points are extracted using the Cloud Simulation Filter (CSF) filter (Zhang et al., 2016) using the CloudCompare software. In that method, input LiDAR point cloud (Fig. 2a) is inverted, and then a rigid cloth is utilized to cover the inverted surface. The positions of the cloth nodes may be identified by studying the interactions between the cloth nodes and the associated LiDAR points, resulting in an approximation of the ground surface. Finally, by comparing the original LiDAR data with the created surface, the above-ground points may be retrieved from the LiDAR point cloud (Fig. 2b). According to our experiments, we preferred a fine cloth resolution (i.e. 0.4) to represent the terrain, and other default parameters to run the approach (i.e. max. number of iterations and classification threshold are set as 500, and 0.5, respectively). Finally, canopy height model (Fig. 2c) is extracted after manually removing other man-made objects, e.g. privately owned houses and huts. For that purpose, a canopy grid having 30 cm pixel size is generated, and the *pitfree* algorithm that is based on a series of classical triangulation calculations is carried out (Khosravipour et al. 2014).



(a) (b) (c)

Figure 2. (a) LiDAR point cloud of Parcel #5, (b) above-ground points extracted after CSF filtering, and (c) CHM of Parcel #5 generated using above-ground points in (b). The units in the legend are in meters.

Extraction of Trees and Their Borders

The locations of tree peaks were determined using a local maximum filter (*findtrees* algorithm). The algorithm examines nearby points for a given point, determining if the processed point is the highest. This may be accomplished by using a constant window size (ws) in meters, and comparing a given point to its neighbors within a ws/2 radius circle to check if it is the highest locally. Experiments were carried out for different window sizes (Fig. 3), and thereafter, we set ws to 5 meters, because it provided the best results. Note that the filter was applied over the CHMs to speed up the process as we did not observe any significant differences between directly applying the filter over the point cloud data used to generate the CHMs.

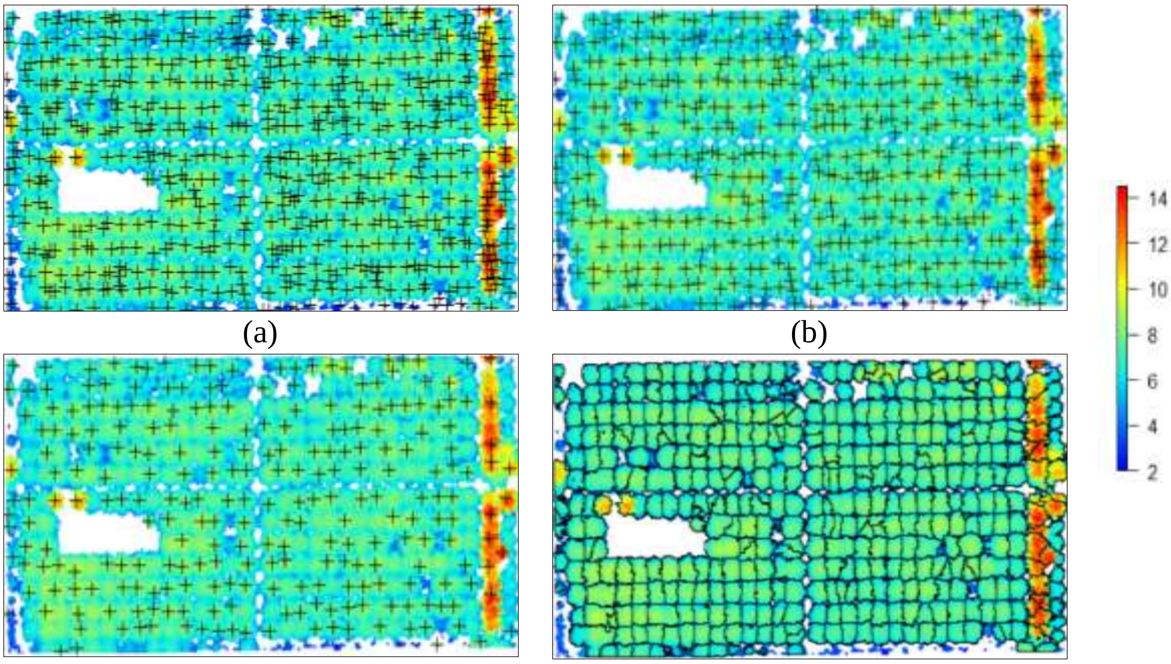


Figure 3. Detection of tree peaks (a) ws = 3*m*, (b) ws = 5*m*, and (c) ws = 8*m*. The units in the legend are in meters. The output of the watershed algorithm is shown in (d).

Object boundaries are extracted via the marker-controlled watershed transform (*mcws algorithm*) (Roussel et al., 2020; Roussel and Auty, 2021). Watershed segmentation applies mathematical morphology to detect objects, and thereafter, pixels with a 0 elevation value in the watershed transformed image are labelled as borders of objects. Note that the point positions of treetops guide segmentation at this step, and the boundaries of each tree are retrieved depending on the point locations provided. Finally, the boundaries are drawn around the previously determined treetop positions (Fig. 3d). We have to note that mature citrus trees can have heights around 8 meters, whereas non-bearing young trees (<3 years old) may have heights less than 3m. Therefore, we preferred different height thresholds for parcels with young tree plantations during the marker-controlled watershed transform. As a final post-processing, we fit a circle to each boundary region generated using QGIS, and other tree species in parcels having higher elevations than 8 meters are removed. In the last step, a binary image is created, in which the regions of trees are allocated as 1 while regions outside of tree borders are assigned as 0.

RESULTS & DISCUSSION

The final performance of the proposed approach was assessed by comparing the results of the proposed approach with the manually prepared reference data. In this study, four quality measures, Precision (*Pre*), Recall (*Rec*), F_1-score (F_1), and Accuracy (*Acc*), are used to evaluate the pixel-based performance of the proposed approach:

$$Pre = \frac{\|TP\|}{\|TP\|+\|FP\|} \quad Rec = \frac{\|TP\|}{\|TP\|+\|FN\|} \quad F_1 = \frac{2.precision.recall}{precision+recall} \quad Acc = \frac{\|TP\|+\|TN\|}{\|TP\|+\|FN\|+\|TN\|+\|FP\|}$$

where TP are true positives, FP are false positives, FN are false negatives, and TN are true negatives. Note that *Accuracy* is the ratio of correctly predicted observations (TP+TN) to total observations, and F_1-score reflects the overall performance by equally weighting *Precision* and *Recall* measures.

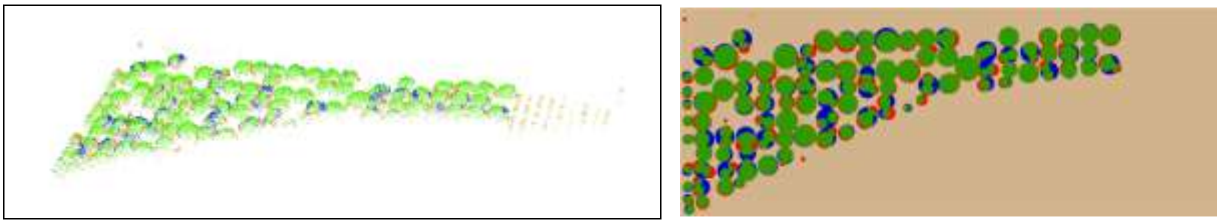
The numerical results of the methodology presented are provided in Table 1. The parcel with the best results in terms of both accuracy and F_1-score measures the parcel #3 (92.1% and 87.6%, respectively). The region has a low tree density, existing trees have almost equal height values, and mature trees outnumber young plantations, which delivers a relatively successful outcome. Individual boundaries may also be identified more clearly owing to the spaces between the trees, and therefore, borders of trees do not overlap as frequently (Fig 4a). The parcel with the highest recall performance (≈ 87%) is the parcel #5. As can be seen in Fig. 4b, this parcel is made up of citrus trees that are relatively dense, comparable in maturity and were planted in a certain order. Therefore, most of the peak positions and boundaries of the trees are estimated correctly with a final accuracy of around 84%.

Table 1. Numerical results of the methodology presented.

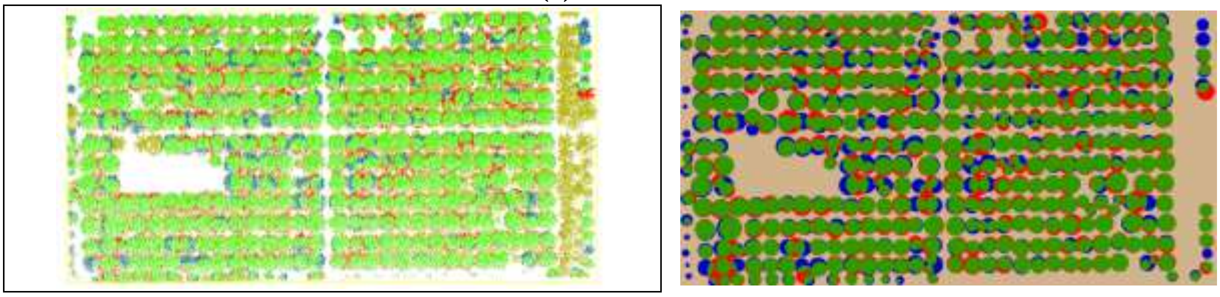
Parcel ID	# of Trees (Reference Data)	Performance (%)			
		Precision	Recall	F_1-score	Accuracy
#1	764	89.24	79.36	84.01	89.74
#2	848	88.71	65.53	75.38	89.79
#3	130	92.58	83.2	87.64	92.11
#4	382	90.07	82.9	86.33	83.82
#5	398	85.02	86.85	85.93	84.39
#6	1908	79.09	73.66	76.28	80.28
#7	1006	68.85	52.1	59.32	84.54
#8	520	83.00	73.4	77.91	89.82
#9	423	80.82	73.37	76.91	80.26
#10	340	85.95	78.81	82.23	76.38

The lowest performance of all parcels in terms of F_1-score (%59.3) is computed for parcel #7. For this case, although the citrus trees appear to be planted in a regular pattern, the poor scores might be attributed to an overabundance of young trees in the region (Fig. 4c). Besides, because of the newly planted citrus trees, some of the peak positions could not be detected, which eventually resulted in completely missing trees and their canopy coverage (i.e. recall ≈ 52%).

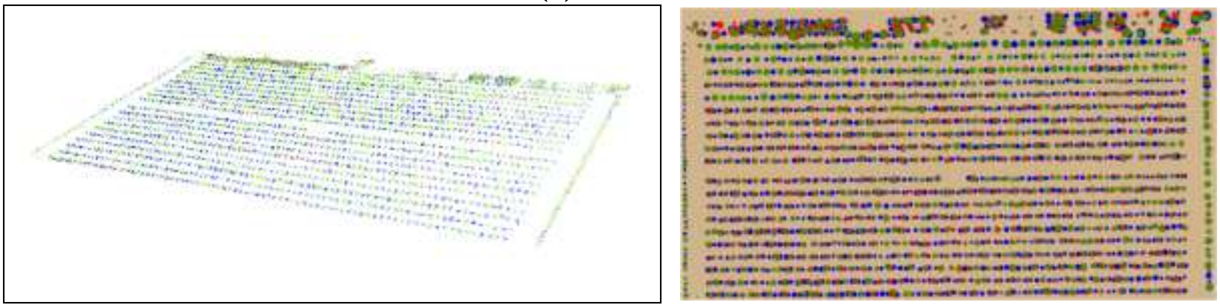
When compared to other parcels, parcel #9 has a relatively irregular topography with unevenly distributed citrus plantations. For this parcel, young and mature trees are mixed. Unfortunately, for this case, identification of the tree borders is challenging since the treetops detected suffer from over-detections due to the imperfect local maxima filtering.



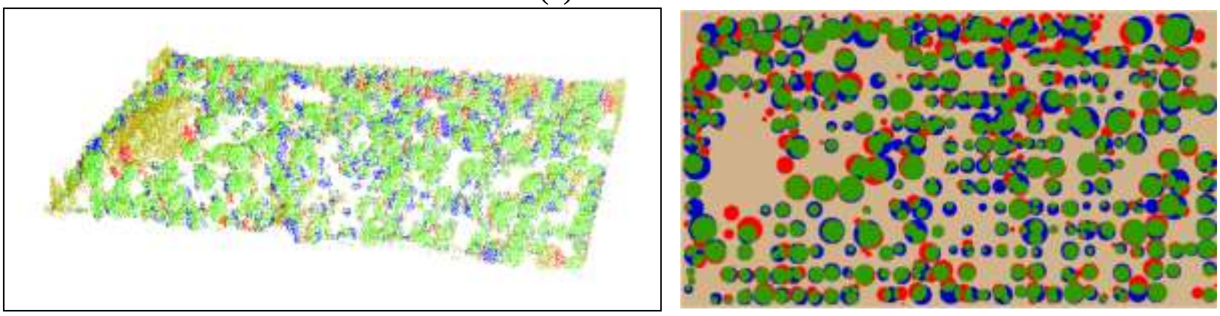
(a) Parcel #3



(b) Parcel #5



(c) Parcel #7



(d) Parcel #9

Figure 4. Visual results of the methodology presented.

6. CONCLUSION

In this study, we evaluated the performance of automatically extracted citrus trees from point cloud data of the G-LiHT LiDAR sensor using a traditional automatic strategy based on local maxima detection and watershed segmentation. A total of 10 citrus tree parcels were identified from the southern part of Florida, USA. To generate canopy height models for each citrus parcel, the Cloth Simulation Filter was used. Thereafter, a local maxima filter was applied to determine treetop positions, and traditional marker controlled watershed transform was used to define tree boundaries.

The selection of the parameters has shown to have a considerable impact on the individual tree detection outcomes. Different window widths were explored for the local maximum filter. Furthermore, the inference of tree boundaries is influenced by the treetops as we utilized marker controlled watershed segmentation. According to the results computed, the parcels with the greatest accuracies (≈ 85%) had more regular and sequential planting characteristics and were composed of trees of similar height. The peak positions of certain trees could not be detected in parcels with many non-fruit bearing young trees and with varying tree heights. In such cases, because the tops of certain trees could not be collected, their boundaries could not be determined either. Besides, it was observed that in parcels with high tree density, more trees were recognized than the actual number, causing the boundaries to be erroneously delineated.

Manually defined tree crowns were utilized as the reference data in this study, and such data may contain subjective mistakes. Furthermore, the tallest peak location of a tree may not accurately represent the trunk's location; hence, extensive fieldwork must be undertaken to validate such an output. In the future, we will carry out studies dealing with LiDAR point clouds and deep learning. In this respect, we also plan to integrate images collected by the G-LiHT system into the methodology presented.

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CHANGES IN CHLOROPHYLL CONTENT AND FRUIT QUALITY IN ALPHONSE LAVALLÉE GRAPE VARIETIES FROM VERAISON TO RIPE

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ABSTRACT

In the study, Alphonse Lavallée, one of the colorful table grape varieties that has an important place in the world and in our country, was used. The periods of change in the quality of the grains from veraison ripening (with samples taken at four different times) was determined by physical, chemical and phytochemical analyzes. At the same time, the change in the amount of chlorophyll in the leaves was investigated in this periods. In terms of physical properties, differences in cluster width, berry weight, berry width, berry size and berry hardness were found to be statistically significant. Considering the chemical changes, the differences were found to be statistically significant in terms of all properties (Sçkm, pH, Titration Acidity, Maturity Index). Considering the phytochemical changes, the differences in the values of shell anthocyanin and pulp anthocyanin were found to be statistically significant. Considering the changes in spad values in the Alphonse Lavallée grape variety from veraison to ripening, the values obtained did not make a statistical difference.

Keywords; total phenolic, berry hardness, berry weight, total anthocyanin,

INTRODUCTION

Grape, one of the most important fruits that nature offers to people; It deserves this position with its historical development, diversity and place among other fruits. Grape has not only been seen as a hunger quenching fruit throughout history, it has become an indispensable value of the society with its own products and products made from it (Anlı, 2006). Grape, which has an important place in the world and in our country in terms of production amount and cultivation area, is a food that can be consumed in all seasons with its ability to be processed both for table and in different ways. It is seen that approximately 32% of the total fresh grape production in the world is used in the table grape industry for fresh consumption. The main reason for the increasing table grape production in the 21st century is the consumption demand (Anonymous, 2013).

The fact that colored table grapes are very rich in phenolic compounds and have bioactive properties important for human health is an indication that they should be in our daily diet (Gülcü et al.,2008). Among the components of grapes, especially carbohydrates, organic acids and phenolic compounds have a special place (Çetin et al., 2012). Phenolic compounds are the most abundant group of compounds in grapes after sugars and organic acids. Phenolic compounds are the most important metabolites affecting the taste, color and aroma of grapes, as well as highly effective on nutrition and health (Kunter et al., 2013). In general, it is stated that colored grape varieties are richer in nutritional value than white varieties (Yang and Xiao, 2013). Presence of secondary metabolites in grape seed; the amount in the content, depending on the type and variety; It is shaped according to climate and soil characteristics, cultural practices and maturity stages (Ribéreau-Gayon et al., 2000). Climate Factors are effective on growth, development and maturation in grapes as in all fruit types. Grape varieties have different demands for temperature, precipitation and sunshine duration. While different cultivars can mature at the same time in different ecologies, there can be significant differences in developmental stages even between clusters on the same vine (Winkler ve ark., 1974; Smart ve ark., 1985). With maturation, some morphological and physiological changes are observed in all organs of the vine. While morphological changes mostly express physical changes, physiological changes mostly express changes in the physical structure. Both changes are closely related to the quality of the grape. There are many factors that can affect the quality of grapes and as these factors increase, quality control becomes more difficult. Quality is closely related to maturity time (Fanizza, 1982). Maturation in grapes; It is a phase that starts with the fall and continues until the harvest. With the onset of berry development and the time elapsed until maturity, the amount of dry matter, which is initially in trace amounts, increases, and the amount of general acid, which is very high, decreases (Haris et al., 1968; Calo et al., 1996). Sugar content of the grain, acid balance, aroma components are the factors that determine the quality of grapes. Apart from these components; organic acids, water-soluble dry matter ratio, phenolic substances and other components provide information about the quality of the berry content.

As in all plants, the leaf in the vine is one of the important organs that gives clues about the plant body. It reports the physiological changes that the plant creates during the vegetation period (Hendry et al., 1987). The process of the grape from fall to maturity is also very important for vine vegetation. The most important role in the photosynthesis cycle, which is the basis of plant nutrition, is chlorophyll. The chlorophyll pigment is green in color and is found in the mesophyll cells in the vine (Ağaoğlu, 1999; Çelik, 2011). It is reported that the chlorophyll density in the leaf of a plant directly affects the amount of photosynthesis (Curran et al., 1990).

Since the amount of chlorophyll in the vine leaves is affected by the environmental conditions, cultural factors and nutritional status of the vineyard plantation, this situation also directly affects the grape quality (Sucu et al., 2017). Today, as in all types of aquaculture, quality is as important as yield in viticulture. Quality, on the other hand, consists of many criteria (variety characteristics, ripening time, physical and chemical components of the berry, etc.). In this study, the period of change in quality of Alphonse Lavallée, one of the most important colored table grape varieties in the world and in our country, from fall to maturity (with samples taken at four different times) was determined by physical, chemical and phytochemical analyzes, and at the same time, the change in the amount of chlorophyll in the leaves in this period was examined. It has been tried to explain what kind of interaction there is between the data.

MATERIAL AND METHOD

Study; It was carried out in the collection vineyard of Tokat Gaziosmanpaşa University Agricultural Research and Application Center. The variety used is Alphonse Lavallée. It is known in the region that the time between the grape variety Alphonse Lavallée and the ripening period is approximately 40 days. Grape samples were harvested four times in total at 10-day intervals after veraison. Required physical analyzes of harvested grapes (cluster weight, bunch width-length characteristics, berry weight, berry width-length characteristics, berry hardness), chemical analyzes (SÇKM, pH, titration acidity, maturity ındex) and phytochemical analyzes (total phenolic, total anthocyanin, total flavanoid) horticultural laboratories were also made. Likewise, the amount of chlorophyll in the leaves was measured with the help of Chlorophyllmeter (Spad CCM-200) on the dates of the grape samples.

RESULTS AND DISCUSSION

In the Alphonse Lavallée grape variety, when the physical properties of the clusters and berry were examined from the mole to the ripening periods, the differences in the bunch width, berry weight, berry width, berry size and berry hardness were found to be statistically significant. Although the properties of cluster weight and cluster length changed, it did not make a statistical difference.

Considering the values of the width of the cluster, the highest value was 13.33 mm in the 4th period and the lowest value was 11.16 mm in the 1st period. The highest berry weight was obtained in the 4th period with 7.09 g and the lowest berry weight was obtained in the 1st period with 2.95 g from the berry properties. The highest grain width was obtained in the 4th period with 21.26 mm and the lowest 16.08 mm in the 1st period. While the highest value in grain size was 21.57 mm in the 4th period, the lowest value was 16.49 mm in the 1st period. Finally, when the grain hardness values are examined, the highest figure was obtained from the 1st period with 57.63, while the lowest figure was obtained from the 4th period with 20.00.

Table 1. Cluster weight (g), cluster width (cm), cluster length (cm), berry weight (g), berry width (mm), berry length (mm), berry hardness values in Alphonse Lavallée grape variety.

	Cluster Weight (g)	**Cluster Width (cm)**	**Cluster Length (cm)**	**Berry Weight (g)**	**Berry Width(mm)**	**Berry Length (mm)**	**Berry Hardness**
1.Period	392,40	11,16 b	18,66	2,95 c	16,08 c	16,49 b	57,63 a
2.Period	399,47	11,5 b	18,33	3,34 c	16,66 c	17,19 b	56,96 a
3.Period	391,63	12,33 a	18,66	6,54 b	20,25 b	20,86 a	54,43 a
4.Period	399,70	13,33 a	19,66	7,09 a	21,26 a	21,57 a	20,00 b

The characteristics (shape, color, width, length) of the cluster, which is the structure formed by the combination of grape grains, are also physical criteria for grapes. Parameters such as cluster weight and cluster size vary according to grape varieties. It is not only the variety that affects the cluster structure and properties, but also; Many factors such as ecological conditions, presence of buds and its condition on the shoot, cultural processes applied to the vine also come into play as influencing factors (Çelik ve ark., 1998; Çelik, 2011; Kamiloğlu ve Üstün, 2014).

In the study, changes in cluster width, grain width, grain weight and hardness were found to be significant in terms of periods. Changes in cluster characteristics did not differ statistically. In the process from mole to maturation, important changes occur mostly with the internal structure. It is an expected situation that changes in the cluster characteristics do not make a difference as much as the changes in the grain make a difference.

Considering the chemical changes in the Alphonse Lavallée grape variety from mole to ripening, the differences were found to be statistically significant in terms of all characteristics (Sçkm, pH, Titration Acidity, Maturity Index). In terms of SÇKM value, the highest value was obtained in the 4th period with a brix value of 16.06, while the lowest value was obtained in the 1st period with a brix value of 7.5. Considering the pH value, the highest figure was obtained with 4.26 in the 4th period, while the lowest figure was 3.44 in the 1st period. In terms of titration acidity, the highest value was obtained with 46, 43 in the 1st period, and the lowest value was 4.85 in the 4th period. When we look at the maturity index values of the chemical properties, the highest value was obtained with 30.33 in the 4th period, while the lowest value was obtained with 1.60 in the 1st period.

Table 2. TSS, pH, titration acidity, maturity index values of Alphonse Lavallée grape variety

	TSS	**Ph**	**Titration Acidity**	**Maturity İndex**
1.Period	7,5 c	3,44 d	46,43 a	1,60 d
2.Period	10,63 b	3,66 c	24,58 b	4,33 c
3.Period	15,53 a	4,11 b	9,87 c	10,57 b
4.Period	16,06 a	4,26 a	4,85 d	30,33 a

The sugar/acid ratio in the grape is very important, no matter how the grapes are evaluated (for table, wine, grape juice, dried). One of the factors that determine the taste and ripeness of grapes is Brix value and the other is titration acidity (Creasy and Leroy, 2009). With the ripening of grapes, pH and SÇKM in the must increase, and these two criteria appear as the most important criteria in determining the best harvest time (Fanizza, 1982; Eriş and Türkben, 1984). It has been reported that the amount of SÇKM in grapes increases in the period from mole to ripening, the total acid amount increases until the mole stage, decreases towards maturity after this stage, and gains an almost constant acceleration with the over-maturity period (Deryaoğlu, 1997; Ağaoğlu, 2002; Şen, 2008). The study shows parallelism with these sources.

Considering the phytochemical changes in Alphonse Lavallée grape variety from mole to ripening, the differences in peel anthocyanin and pulp anthocyanin values were found to be statistically significant. The values of total phenolic, peel and pulp flavonoid properties did not differ statistically. Considering the shell anthocyanin values, the highest value was obtained with 68.67 in the 4th period, while the lowest value was obtained with 1.003 in the 1st period. In terms of pulp anthocyanin, the highest value was obtained from the 4th period with 0.56, while the lowest value was obtained from the 1st period with 0.06.

Table 3. Total phenolic (ppm), skin anthocyanin (mg/g), skin flavonoid (mg/g), pulp anthocyanin (mg/g), pulp flavanoid (mg/g) values occurring throughout the periods in Alphonse Lavallée grape variety

	Total Phenolic (ppm)	**Skin Anthocyanin (mg/g)**	**Skin Flavonoid (mg/g)**	**Pulp Anthocyanin (mg/g)**	**Pulp Flavanoid (mg/g)**
1.Period	202,32	1,003 b	61,380	0,06 b	64,21
2.Period	150,40	3,58 b	61,350	0,07 b	64,05
3.Period	143,36	61,49 a	65,100	0,53 a	64,34
4.Period	141,86	68,67 a	64,38	0,56 a	64,92

Studies by various researchers suggest that phytochemical development and composition are affected by climatic conditions (abiotic factors such as light, temperature and water) and viticulture practices (Kliewer and Torres, 1972; Jackson and Lombard, 1993; Dokoozlian and Kliewer, 1996; Bergqvist, 2001). ; Spayd, 2002; Downey, 2004). In addition, one of the most important causes of phytochemical differences is shown as variety or genetic difference (Liang et al., 2011). In another study using different table and wine grape varieties, the average amount of total phenolic compounds varied as 176-738 mgl-1 in table varieties and 230-1236 mgl-1 in wine varieties (Çabuk, 2004).

When looking at the changes in spad value in the Alphonse Lavallée grape variety from mole to ripening, the differences were not found to be statistically significant.

Table 4. Values of spad changes over the periods in Alphonse Lavallée grape variety

	1. **Period**	2. **Period**	3. **Period**	4. **Period**
Spad value	35,62	34,23	35,53	32,58

Chlorophyll are pigments that must be mandatory in the conversion of light energy into chemical energy. The amount of radiation absorbed from the sun also depends on the photosynthetic amount in the leaf. Therefore, the amount of chlorophyll content is related to photosynthetic activity and primary production (Curran et al., 1990). In the study, chlorophyll content was measured with a portable chlorophyllometer. Porro et al. (2002) determined the changes in the amount of chlorophyll in grape leaves using a chlorophyll measuring instrument (SPAD). The chlorophyll change in the leaves of the Chardonnay grape variety was investigated according to the phenological stages and the positions of the leaves on the shoots. In the measurements, 35 SPAD values were determined in 35-40 days old leaves; A SPAD value of >40 was determined in 90-100 day old leaves. In a study conducted with 13 different American grape rootstocks, SPAD values varied between (30.19-20.62)

(Gargın, 2011). In another study in which drought stress was applied, the spad value in grafted and ungrafted vine saplings varied between 4.13 and 11.75 in another study (Sucu et al., 2017). In the study, the spad values taken for the purpose of measuring chlorophyll density did not differ statistically according to the periods. This situation can be associated with the interpretation that the vine leaf has reached the size and coloration it should reach in the period until I fall, and that it gains a constant momentum after this period.

CONCLUSIONS

In the study, physical, chemical, phytochemical properties and changes in chlorophyll density were investigated in the Alphonse Lavalle grape variety in 4 periods from mole to ripening. In terms of periods, grain width, grain width, grain weight, cluster width, grain weight, and hardness values were found to be significant. In terms of chemical properties, the differences in all analyzes (TSS, pH, TA, OI) were found to be statistically significant. Differences in chlorophyll density were not statistically significant.

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THE EFFECTS OF GLYCINE BETAIN APPLICATIONS ON THE PRODUCTION AND QUALITY OF NARİNCE GRAPE VARIETY

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ABSTRACT

Narince grape variety is an important wine grape variety accepted in our country and in the world. It is also because of the quality of the leaves with leaf pickyness consumption, especially in Turkey and in Europe are much in demand. In addition to the production of pickled leaves and the grape for white wine, it is a variety that is consumed intensively in the region where it is grown and in the surrounding provinces. It can find buyers at very high prices in the region for table use. Due to its use as a pickled leaf, there is a decrease in yield and quality parameters, as well as intensive sunburn in the bunches of vines, which are exposed to intense leaf breakage.

In this study, GB application was carried out on the vines from which 4 different leaf collections (collection of 4-8-12 leaves on the cluster) were made and a total of 4 harvests were made and the vine/grape yield and quality parameters were examined. In the harvested clusters; cluster stiffness (g), cluster width (cm), grain weight (g), grain hardness, grain width (mm), TSS (%), pH, titration acidity (g/l), maturity index, sunburn rate (%), leaf chlorophyll amount (spad), leaf temperature (°C) parameters were examined. And as a result, it is thought that GB applications have a protective effect against high temperature stress, as they come to the fore in cluster parameters.

Keywords: Narince, Spad, Sun Burn, Glycine betain

INTRODUCTION

Plants are under abiotic stress factors; It has been reported that osmotic preservatives begin to increase due to the reduction of stress by biosynthetic enzymes (McNeil et al., 1999). Osmotic preservatives such as glycine betaine (GB) and proline (PRO) positively affect cellular osmotic regulation in many plants under environmental stress conditions (Rhodes and Hanson, 1993). The application of said osmotic preservatives to plants externally (from leaves or from roots by mixing with irrigation water) is a fairly new practice. The natural accumulation of GB and proline in most of the plants is not at a level that can eliminate the negative effects of water loss due to many environmental stress factors. Studies with a limited number of other plants suggest that the external application of GB and proline may contribute to overcoming the negative effects of environmental stress factors in crop plants that accumulate this compound in low amounts or not at all (Mickelbart et al., 2006).

Viticulture is an agricultural branch with an economic life of 30-40 years, that is, it can continue its existence for many years. The beginning of such an economic investment begins with the supply of healthy saplings that are true to its name. Afterwards, with the cultural practices (pruning, training, fertilization, spraying, irrigation, etc.), productivity and continuity are ensured in this agricultural branch. However, abiotic stress factors, which affect or prevent the metabolism, growth and development of the plant outside the intervention of the producers (Levitt, 1980), negatively affect the yield and quality in viticulture, as in all plant production.

Turkey ranks 5th in the world in terms of vineyard area with 448 thousand hectares and 6th in world grape production with 4.2 million tons. Grapes are classified as dried, table, wine-grape grapes according to their commercial evaluation. 42% of the grapes produced in the world are used as table grapes, 7% as dried grapes and 52% as wine grapes, in Turkey 57% as table grapes, 40% as dried grapes and 2% as wine- must (OIV, 2018).

In addition to its historical and cultural heritage, Tokat is a rare Anatolian city that is known for its viticulture culture. According to 2017 data, viticulture is carried out on an area of approximately 6 thousand hectares in Tokat, which has an agricultural area of 363 310 ha (Anonymous, 2019). Narince, the local variety of the region, has been used for many years in the production of brine leaves, for table, wine and cider, and it constitutes approximately 90% of the grapes produced in the region (Cangi et al., 2005; Kılıç et al., 2007; Çelik et al., 2010).

GB applied to wine grapes at concentrations of 50, 100, 200 mM during critical periods (before spring frosts) protected the plants and yield and provided stress resistance by lowering leaf surface temperatures (Mickelbart et al., 2006).

In his study, Jalil (2017) examined the effects of GB applications on the development of vines grown under limited irrigation conditions, and when he evaluated the results in general, he concluded that foliar SW applications can be an environmentally friendly sustainable cultural practice for viticulture in areas where water is scarce.

No studies have been found in the Narince grape variety, which has commercial importance in the province of Tokat, in which the effect of abiotic environmental conditions is felt and that can provide a solution to this situation and also increase yield and quality. In our study, it was aimed to examine the effects of different leaf removal levels and GB (glycine betaine) application on yield and quality in Narince grape variety.

MATERIAL AND METHOD

This study was carried out in the production vineyard established on the land of Gaziosmanpaşa University Agricultural Application and Research Directorate. The experiment was carried out on Narince vines grafted on 1103 P rootstock in the 2020 vegetation period. Some characteristics of Narince variety are given below. Narince: It is pruned short or mixed. The fruits are medium thick crusted and white in color (Anonymous, 2002). The clusters are medium-sized, cluster-shaped, conical, winged and very densely grained. The leaves are very preferred for consumption after being processed fresh and in brine. In some vineyards in the production region, it is grown for leaves rather than fruit (Ağaoğlu et al., 1988; Çelik, 2002).

The vines to be used in the experiment were determined before the vegetation period started, and the necessary winter pruning was carried out. In the vegetation period, the clusters of the vines to be used in the experiment were followed from the grain set to the verasion period, and the applications were started immediately after verasion. In the experiment, 4 different leaf removal applications (removal of all leaves on the cluster, removal of 4 leaves from the cluster, removal of 8 leaves from the cluster and removal of 12 leaves from the cluster), after the defoliation application, the application of GB (Novastim, Agrota) processed as a commercial preparation, except for control, was carried out has been planned. The GB (Şirin, 2013) application was made once at the rate of 3% during the verasion period, after the leaf removal applications.

Harvest was done 4 times for each application, and since the Narince grape variety started to be harvested in the second week of September, weekly harvests were carried out 4 times from the first week of September. a total of 4 harvests were made and the vine/grape yield and quality parameters were examined. In the harvested clusters; cluster stiffness (g), cluster width (cm), grain weight (g), grain hardness, grain width (mm), TSS (%), pH, titration acidity (g/l), maturity index, sunburn rate (%), leaf chlorophyll amount (µmol m-2), leaf temperature (°C) parameters were examined. The research was planned according to the split plots experimental design in randomized blocks, with 3 replications and 4 vines in each replication. The data obtained as a result of the study will be subjected to analysis of variance in the randomized blocks experimental design, and the LSD test was used to compare the means.

RESULTS AND DISCUSSION

In 2020, 3 different leaf removal/collection (4, 8, 12) was carried out in the Narince vineyard of Gaziosmanpaşa University Agricultural Application and Research Center, and glycine betaine (GB) application was applied on the vine, and the clusters were harvested in 4 periods. The cluster-berry characteristics and maturation parameters of the harvested clusters were taken, and the results of the statistical analyzes are presented in the tables below. While the averages shown with a capital letter in the same row in the charts show the evaluation of applications on the basis of harvests, the averages shown with a lower case letter in the same column show the evaluation of the harvests between applications.

Looking at Table 1., it is seen that the highest cluster weight is observed in the 4th harvest GB-4 LR (538.12g) application. The lowest cluster weight was measured in the 2nd harvest Control-12 LR (207.37g) application. The longest cluster width was measured in the 4th harvest GB-4 LR (16.00 cm) application, and the shortest cluster width was measured in the 4th harvest Control-12 LR (6.83 cm) application.

Table 1. Cluster data measured at harvest in Narince grapes

	Application	**1. Harvest (01.09.2020)**	**2. Harvest (08.09.2020)**	**3. Harvest (17.09.2020)**	**1. Harvest (24.09.2020)**
Cluster weight(g)	Control-4 LR	A 392.16 a	B 232.80 c	A 393.80 a	A 329.69 bc
	Control-8 LR	B 244.55 c	A 323.22 b	B 246.18 b	A 319.61 bc
	Control-12 LR	B 315.32 b	C 207.37 c	A 414.62 a	BC 251.99 c
	GB-4 LR	B 366.32 a	B 283.69 b	B 295.67 b	B 538.12 a
	GB-8 LR	B 267.41 bc	B 289.63 b	B 284.70 b	A 369.56 b
	GB-12 LR	A 243.58 c	A 227.90 c	A 246.76 b	A 277.40 c
Cluster Width (cm)	Control-4 LR	A 14.00 a	B 8.66 bc	A 14.00 a	B 9.66 bc
	Control-8 LR	B 7.66 cd	A 9.33 bc	B 7.16 c	A 9.83 bc
	Control-12 LR	A 10.33 b	B 7.83 cd	A 10.33 b	B 6.83 e
	GB-4 LR	B 10.50 b	C 8.75 bc	C 7.66 bc	A 16.00 a
	GB-8 LR	A 9.16 bc	A 11.50 a	A 10.66 b	A 8.83 cd
	GB-12 LR	B 7.16 d	B 7.00 d	AB 7.66 c	A 9.00 cd
Cluster Lenght (cm)	Control-4 LR	A 17.76 ab	A 19.33 ab	A 18.33 abc	A 18.83 b
	Control-8 LR	C 16.33 b	B 19.83 ab	C 16.50 abc	A 21.50 a
	Control-12 LR	A 19.83 ab	A 16.16 b	A 19.66 a	A 17.00 bc
	GB-4 LR	A 21.00 a	A 21.00 a	A 20.00 a	A 17.50 bc
	GB-8 LR	A 18.00 ab	A 17.66 ab	A 14.50 c	A 17.66 bc
	GB-12 LR	A 17.83 ab	A 18.00 ab	A 15.50 bc	A 17.83 bc

In cluster length, the longest cluster length was measured in the 4th harvest Control-8 LR (21.50 cm) application, and the shortest cluster lenght was measured in the 3rd harvest GB-8 LR (14.50 cm) application.

The grain properties of the applications made are given in Table 2. Considering the grain weight, the highest grain weight was measured in the 4th harvest GB-8 LR (3.81 g), and the lowest grain weight was measured in the 1st harvest GB-4 LR (2.36 g) application. Considering the grain width, the longest grain width was measured in the 4th harvest Control-8 LR (19.90 mm) application, and the shortest grain width was measured in the 4 harvest GB-4 LR (11.09 mm) application. In grain lenght, GB-4 LR application gave the shortest and longest grain length , the highest value was measured in 1 th harvest (15.46 mm) and the lowest value was measured in the 4th harvest (11.56 mm). The highest value in grain hardness was measured in the 1st harvest Control 4-LR (29.09) application, and the lowest value in the 4th harvest GB-12 LR (18.33) application. As the number of harvests progressed, it was observed that the grain softened as the maturation continued.

Table 2. Grain data measured at harvest in Narince grapes

	Application	**1. Harvest (01.09.2020)**	**2. Harvest (08.09.2020)**	**3. Harvest (17.09.2020)**	**4. Harvest (24.09.2020)**
Grain weight(g)	Control-4 LR	A 3.11 a	A 3.10 b	A 3.07 ab	A 3.06 bc
	Control-8 LR	BC 2.77 b	A 3.78 a	AB 3.36 a	C 2.68 cd
	Control-12 LR	AB 2.81 b	B 2.59 c	AB 2.84 b	A 2.98 c
	GB-4 LR	C 2.36 c	AB 3.01 bc	BC 2.74 bc	A 3.27 b
	GB-8 LR	C 2.43 c	AB 2.98 bc	BC 3.18 ab	A 3.81 a
	GB-12 LR	C 2.68 bc	AB 3.23 b	BC 2.87 b	A 3.59 ab
Grain width (mm)	Control-4 LR	A 13.56 c	A 14.48 ab	A 14.60 ab	A 14.30 b
	Control-8 LR	A 13.79 c	A 14.98 a	A 15.15 a	A 19.90 a
	Control-12 LR	A 13.24 c	A 13.57 bc	A 14.13 ab	A 13.64 b
	GB-4 LR	A 14.41 b	A 14.15 ab	A 13.59 b	B 11.09 b
	GB-8 LR	A 15.03 ab	A 14.16 ab	A 14.01 ab	B 12.27 b
	GB-12 LR	A 15.31 a	AB 14.45b a	B 14.06 ab	C 11.11 b
Grain lenght (mm)	Control-4 LR	A 14.34 b	A 14.40 ab	A 14.41 ab	A 14.11 ab
	Control-8 LR	A 13.26 c	A 14.54 ab	A 14.59 a	A 14.25 a
	Control-12 LR	B 13.15 c	A 14.06 b	AB 13.64 c	A 13.90 b
	GB-4 LR	A 15.46 a	B 13.99 b	B 13.62 c	C 11.56 c
	GB-8 LR	A 15.07 ab	A 14.62 ab	A 14.66 a	B 13.03 bc
	GB-12 LR	A 15.41 a	A 15.13 a	B 14.08 b	C 12.22 c
Grain hardness	Control-4 LR	A 29.09 a	B 24.08 a	B 23.66 ab	C 19.25 a
	Control-8 LR	A 24.73 ab	A 20.58 a	A 25.00 ab	A 22.83 a
	Control-12 LR	A 24.25 ab	A 19.58 a	A 22.58 b	A 19.33 a
	GB-4 LR	AB 19.86 c	B 18.25 a	A 26.83 a	AB 22.50 a
	GB-8 LR	AB 21.52 b	B 17.66 a	A 23.58 ab	AB 18.91 a
	GB-12 LR	A 21.25 b	A 20.08 a	A 21.66 c	A 18.33 a

In Table 3, TSS, pH, titration acidity and maturity index, which are among the ripening criteria of grains, were examined. In terms of the amount of TSS, an increase in the amount of TSS is observed depending on the ripening as the harvest dates progress. Control 12-LR (21.46 %) application at the 4th harvest gave the highest amount of TSS. When we look at the GB applications, it is seen that there are no fluctuations as in the last harvest of the control applications, and closer values are seen. It is thought that the application after leaf breaking helps to prevent the stress that will occur in the plant. Considering the pH measurements, since the first harvest date is early in terms of the harvest value of Narince grape, it started at low values and the pH values increased with maturity. The lowest pH value was measured at the 1st harvest at the Control-12LR (4.54), and the highest pH value was measured in the Control-4LR (4.18) application. One of the factors that determine the taste and ripeness of grapes is Brix value and the other is titration acidity (Creasy and Leroy, 2009). This value decreased with maturation in titration acidity.

Table 4. TSS (%) , pH, Titration acidity (%) and maturity index (%) measured at harvest in Narince grapes

	Application	1. Harvest (01.09.2020)	2. Harvest (08.09.2020)	3. Harvest (17.09.2020)	4. Harvest (24.09.2020)
TSS (%)	Control-4 LR	C 16.80 c	B 19.83 c	A 20.13 c	A 20.80 c
	Control-8 LR	C 17.03 c	C 17.30 e	A 19.70 d	B 18.36 d
	Control-12 LR	C 18.46 a	C 18.56 d	B 19.90 cd	A 21.46 a
	GB-4 LR	C 16.13 d	A 20.75 a	B 20.46 b	A 21.26 ab
	GB-8 LR	C 15.20 e	B 20.30 b	A 21.30 a	A 21.16 b
	GB-12 LR	C 18.0 b	C 18.53 d	B 19.93 cd	A 20.80 c
pH	Control-4 LR	B 4.00 b	B 3.97 e	C 3.89 d	A 4.18 a
	Control-8 LR	A 4.54 a	A 3.96 e	A 3.97 c	A 3.96 d
	Control-12 LR	C 3.87 b	A 4.06 d	B 3.97 c	A 4.08 c
	GB-4 LR	C 3.85 b	A 4.17 c	B 4.08 a	A 4.15 b
	GB-8 LR	C 3.78 b	A 4.20 b	B 4.07 a	A 4.15 b
	GB-12 LR	C 3.76 b	B 4.23 a	B 4.06 b	A 4.12 bc
Titration acidty ((g/l)	Control-4 LR	A 11.55 cd	B 8.45 c	C 7.71 c	D 6.68 d
	Control-8 LR	A 11.04 d	B 8.05 cd	B 10.72 a	C 6.76 d
	Control-12 LR	A 10.05 d	D 6.34 f	B 9.22 b	C 7.39 b
	GB-4 LR	A 18.73 a	B 10.74 a	C 8.38 c	D 7.11 c
	GB-8 LR	A 18.57 a	B 9.30 b	C 7.86 d	C 6.86 d
	GB-12 LR	A 16.01 b	B 8.98 b	C 7.10 e	B 9.16 a
Maturiyt index(%)	Control-4 LR	D 1.44 c	C 2.34 b	B 2.61 b	A 3.13 a
	Control-8 LR	D 1.54 bc	B 2.14 c	C 1.83 d	A 2.71 c
	Control-12 LR	C 1.84 ab	A 2.58 a	B 2.15 c	A 2.90 b
	GB-4 LR	B 1.86 a	AB 2.60 a	B 2.44 b	A 3.00 ab
	GB-8 LR	D 1.82 ab	C 2.18 c	B 2.71 ab	A 3.09 a
	GB-12 LR	D 1.13 d	C 2.06 c	A 2.80 a	B 2.27 d

The highest titration acidity value was measured in the 1st harvest GB-4 LR (18.73 g/l) and the lowest value in the 2nd harvest Control-12LR (6.34 g/l)b. In a study conducted with white table grape varieties, titration acidity results of 10 varieties varied between 4.5 g/l -14 g/l (Rolle et al., 2010). The values in the study are in agreement with the literature information given. Maturity index increases with maturation and Control-4 LR (3.09%) application gave the highest maturity index value.

Considering the rate of sunburn, it is seen that the rate of sunburn increases as the harvest dates progress (Table 4.). The highest rate of sunburn was seen in the Control-12 LR (21.00 %) at the 4th harvest, and it was seen that GB applications did not have a significant effect on the rate of sunburn. When the chlorophyll density was examined (Table 5.), the highest value was the GB-8 LR value (46.71 spad) and the lowest value was the Control-8 LR (36.53 spad) application. Similarly, Bluden et al. (1997) seaweed and Mickelbart et al. (2006) reported that glycine betaine application was effective on the increase in chlorophyll level in their study on grapes. It was observed that the leaf surface temperature was lower in GB applications than in control applications. We can say that the applied chemical protects the plant against high temperature stress. Mickelbart et al. (2006) reported that glycine betaine applied at 50, 100, 200 mM concentrations to wine grapes in one of the critical periods (before the spring frosts) protects the plants and their yield and provides resistance to stress by lowering the leaf surface temperatures.

Table 4. Sunburn rate (%) measured at harvest in Narince grapes

	Application	1. Harvest (01.09.2020)	2. Harvest (08.09.2020)	3. Harvest (17.09.2020)	4. Harvest (24.09.2020)
Sunburn Rate (%)	Control-4 LR	B 13.00 ab	B 13.66 ab	B 12.33 ab	A 18.33 b
	Control-8 LR	A 13.00 ab	A 15.66 a	A 15.66 a	A 19.00 ab
	Control-12 LR	B 14.33 ab	B 16.33 a	A 19.00 ab	A 21.00 a
	GB-4 LR	B 12.33 ab	B 11.66 bc	A 16.66 ab	A 18.66 ab
	GB-8 LR	B 13.33 ab	B 14.66 ab	A 18.00 ab	A 19.33 ab
	GB-12 LR	B 15.00 a	B 15.66 a	A 20.00 ab	A 20.33 a

Table 5. Chlorophyll amount (spad) and Leaf temperature (°C) measured at harvest in Narince grapes

Application	Chlorophyll amount (spad)	Leaf temperature (°C)
Control-4 LR	45.60 a	14.52
Control-8 LR	36.53 bc	16.15
Control-12 LR	42.01 b	14.07
GB-4 LR	46.38 a	12,35
GB-8 LR	46.71 a	13.30
GB-12 LR	43.70 b	14.82

CONCLUSIONS

Narince grape variety is a variety that is consumed intensively in the region where it is grown and in the surrounding provinces, as well as being used for brine leaf production and as white wine grapes. In the region, it can find buyers at very high prices in terms of market value for table use. Due to its use as brine leaves, the vines, which are exposed to very intense defoliation, have intense sunburn on the clusters, as well as decreases in yield and quality parameters. In this study, yield and quality parameters of kaolin and GB applications applied to vines with different defoliation were examined. It has been observed that the leaf breaking and applied chemicals in accordance with the parameters examined affect the yield and quality parameters of the clusters statistically.

In the study, it is seen that leaf cuttings increase the amount of SÇKM in the control application, and the SW applications made increase it during the harvest period. We can say that this situation inhibits the stress factors of GB applications and ensures that the clusters come to harvest maturity in a healthy way.

GB applications did not appear to have any protection against sunburn compared to control applications. It is thought that the reason for this is that the applied chemical does not form a protective film layer like kaolin.

In addition, it is thought that SW applications have a protective effect against high temperature stress, as they come to the fore in some cluster parameters.

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POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY OF MEDICINAL AND AROMATIC PLANTS

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ABSTRACT

Plants have been used for medicinal purposes long before the prehistoric period. In recent years, medicinal and aromatic plants are used in areas such as nutrition, cosmetics, body care and especially the pharmaceutical industry for an increasingly healthy and sustainable life, while aromatic plants are an important raw material used to give scent and flavor in different industrial areas. Since medicinal and aromatic plants are complex mixtures containing different components, they also differ in terms of their biological effects. The importance of these plants is increasing in the field of medicine and health due to the fact that they have a versatile effect compared to their synthetically obtained active substances and that they do not have side effects. Today, as a result of increasing concerns about the safety of synthetic antioxidants, it has been determined that the use of these substances has negative effects on human health. Therefore, modern medicine and researchers have a great interest in obtaining natural antioxidants. This situation has led to the increasing use of medicinal and aromatic plants, which have great potential as a natural source of antioxidants. In recent years, consumers have shifted to natural products, and the use of herbs and spices as antioxidants has come to the fore. This study, it is aimed to discuss the polyphenol content and antioxidant capacities of important medicinal and aromatic plants such as lavender, rosemary, fennel, sage, and thyme the importance of which is better understood every day.

Keywords: Medicinal and Aromatic Plant, Phenol Compounds, Antioxidant Activity,

Introduction

The 20th century was characterized by an excessive increase in the use of synthetic (often toxic) substances in various sectors of the economy, including agriculture, the food and the pharmaceutical industry. In addition, intense harmful solvents were often used to prepare and use the necessary drugs. “Green” solutions are indispensable today due to environmental pollution, which has negative effects on human health. However, unlike the “Green Revolution” in the mid-20th century, which significantly increased the amount of synthetic pesticides and fertilizers to achieve high yields in crops produced (Kralova et al., 2021). There is now increasing interest in the widest possible use of active compounds of natural origin. Medicinal and aromatic plants and especially their essential oils have been used in folk medicine since prehistoric times (Villaverde et al., 2016; Salmeron-Manzano et al., 2020). For example, the use of medicinal plants is mentioned in the Bible. Also, descriptions of herbs can be found in a Sumerian tablet dating back to 3000 BC and in the Ebers papyrus (1500 BC). Medieval, also protected. The Herball or Generall Historie of Plantes, published in 1597 by the English botanist John Gerarde, contains about 600 medicinal plants (Gerard, 1975; Masarovicova and Kralova, 2007) Some medicinal plants such as Myrtus communis L., Ocimum basilicum L., Rosmarinus officinalis L. and *Salvia fruticosa* Mill are known to be used as ritual plants, especially in monotheistic religion (Dafni et al., 2020). Today, it is increasingly turning to herbal formations that are known to be effective against a wide repertoire of diseases and disorders in the world. More importantly, most of the medicinal aromatic plant species, which are not known to cause any significant side effects and are readily available at affordable prices, have begun to be evaluated scientifically for their possible medicinal applications and are now useful to pharmacists (Mindell, 2003). Today, we all include fruits, seeds, greens and aromatic plants in our meals. In recent years, herbal products prepared from medicinal plants have been used to strengthen immunity during the Pandemic (Covid-19) process and also in many ailments such as chronic diseases such as hypertension, diabetes, upper respiratory tract infections, psychological disorders, gastrointestinal disorders. Besides their medicinal uses, medicinal plants, natural dye, pest control, food, perfume, tea, etc. used in the fields. In many countries, different types of herbs are used to keep ants, flies, mice away from homes and offices and to escape. Now a day herbs are important sources for drug production.

In developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases, but also often have side effects. Therefore, they have developed different medicines and herbal products in different parts of the world. In general, it has been reported that MAPs and their products are used more for traditional societies such as India and China in some countries in the world. It has also started to increase gradually in developed and developing countries, especially around the Mediterranean and in Africa, Asia, Europe, Australia and North and South America. The World Health Organization has reported that more than 80% of the world's population in developing countries depends primarily on herbal medicines for their basic health needs, and it estimates that a similar percentage of the world's population may rely on plant-based medicines in the coming years. For this reason, plants with medicinal value should be sought and researches should be continued in terms of human health (Zehra et al., 2019)

As it is known, plants are the main source of compounds with natural antioxidant properties. These chemicals are generally called "secondary metabolites" and some herbal products are evaluated under this title. Plant secondary metabolites have been used to obtain useful compounds and have created a new field of biotechnology. The structure and functions of some secondary metabolites in plants were first determined in the 19th century (Oskay and Oskay, 2009; Gundesli et al., 2019). These compounds are very important compounds for the pharmaceutical industry, chemistry, cosmetics and agricultural control sectors. Studies with these compounds have accelerated and there has been great knowledge on this subject. It has been reported in studies that many plant species contain many active compounds that have an important role in the management of various chronic diseases such as cancer, diabetes, cardiovascular disorders and so on in terms of their effects on human health. In addition, many active substances of the herbal plant combine with other substances to give an effective response in the context of biological properties (Shariff, 2001; Thomson et al., 2003; Eun and Jae, 2004; Tasdemir et al., 2008; Nassar et al., 2010; Kennedy and Wightman, 2011; Teshika et al., 2018).

Many studies have shown that antioxidant enzyme activities are closely related to the production of secondary metabolites. Among the compounds of secondary metabolites, phenolic compounds play a vital role in plants as natural antioxidant compounds (Quan et al., 2016). Presence of polyphenol compounds such as flavonoids and phenol containing free radical scavenging molecules rich in antioxidant activities. Phenolics have a wide distribution in the plant kingdom and have been reported to have a wide variety of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilator actions. (Wojdylo et al., 2007; Hcini et al., 2013). Laboratory and clinical studies have shown that phenolics are effective as antioxidants, especially in the treatment of different diseases (Antibacterial, depression, headache and anxiety) (Panchawat et al., 2010; Tavassoli et al., 2011; Prusinowska and Smigielski, 2014; Chrysargyris et al., 2020). As the knowledge about medicinal and aromatic plants increased, the use of these compounds naturally increased. Essential oils, alkaloids, terpenoids, phenolic substances, tannins, vitamins, other bioactive substances with pharmacological activity in organs such as leaves, stems, bark, flowers, fruits, seeds, roots, rhizomes, bulbs and tubers (Wojdylo et al., 2007; Baydar, 2013; Faydaoglu and Surucuoglu, 2013; Gakuub et al., 2016; Zehra et al. 2019). The use of synthetic antioxidants in foods has been severely restricted in terms of both application and level. Among natural antioxidants, rosemary has been widely recognized as one of the spices with the highest antioxidant activity, along with lavender, thyme, and sage.(Lu and Fo, 2000; Kwon et al., 2002; Eun et al., 2004; Troncoso et al., 2005; Yesil-Celiktas et al., 2007; Jordán et al., 2009; Eghdami et al., 2013; Stanciu et al., 2019). For this reason, we investigated the importance of phenol compounds and antioxidant activities in the structures of several medicinal and aromatic plants (lavender, rosemary, fennel, sage and thyme), which are used both in the pharmaceutical industry (human health) and as additives to increase the properties of foods such as smell and taste.

Lavender

Lavender is a valuable essential oil-producing medicinal plant belonging to the Lamiaceae family. Several species in the genus Lavandula, with *Lavandula officinalis* L. ve *L. angustifolia* Mill. *subsp.* (Syn: *L. spica* L.) ve *Lavandula spica* Medik being the most important. These species are grown mainly for their essential oils, which are used in the cosmetic industry and perfumery in addition to aromatherapy. The most important feature of the lavender plant is the colorless or slightly yellow essential oil it contains (Kara ve Baydar, 2014; Detar et al., 2020). The essential oil ratios of these plants or the condition of their active ingredients affect their beneficial properties. Lavender contributes to human health as it contains beneficial phytochemicals. This essential oil is used especially in perfumery and cosmetics in the industry, in the food industry, in the pharmaceutical industry to give a pleasant smell to some preparations, in aromatherapy and as a drug because it has antiseptic properties, acne, asthma, bronchitis, hair loss, gynecological diseases, nervous diseases, some skin diseases, lung diseases. diseases, rheumatism, tapeworm, cough and vertigo, it has a wide range of use due to its composition and many properties (Darbre et al., 2004; Hajhashemi et al., 2003; Fakhari et al. 2005; Balyemez, 2014). Lavender phytochemicals are often found in a wide variety of phenolic compounds. Aqueous or methanolic extracts from lavender flowers or leaves also show beneficial pharmacological effects thanks to phenolic compounds. As it is known, phenol compounds are very important for human health due to their therapeutic effects and antioxidant activities. Lavender phytochemicals often contain a wide variety of phenolic compounds and antioxidant effects. Many studies have reported that lavender has significant essential oil and high levels of phenolic compounds (Sosa et al., 2005; Hamad et al., 2013; Haddouchi et al., 2021). Lavender was reported to be rich in phenolic content, special chlorogenic ellagic, gallic, p-Coumaric, Cinnamic acid by the research investigator. Lavender, in particular, has phenolic compounds such as chlorogenic acid, which dominates widely, and catechins, which may help protect against chronic conditions such as cancer, diabetes, and heart disease. This situation increases the importance of lavender for human health. Also, higher anthocyanin levels and antioxidant capacities were found (Yaldız et al., ,2015; Spridon et al., 2011; Dif et al. 2017; Hamdaoui et al., 2018; Stanciu et al., 2019; Chrysargyris et al., 2020; Détár et al., 2020; Haddouchi et a., 2021)

Rosemary

Rosmarinus officinalis L. is a medicinal plant belonging to the Lamiaceae family and popularly known as rosemary. Besides its culinary uses due to its characteristic aroma, this herb is also widely used by indigenous peoples where it grows wild (Rotblatt et al., 2000; Perrelli et al., 2018). Rosemary as an antioxidant or natural preservative in foods soap, room fragrance, deodorant, perfume and It is also used in making lotions. essential oil of rosemary or Its extracts can be used against oxidation and rancidity in meat products, fat-containing foods, oils. Many studies have been conducted on the effects of secondary metabolites of the rosemary plant. Studies have shown that rosemary is anticancer (Valdes et al., 2012), insecticide, antimicrobial (Hussain et al., 2010) and has antioxidant effects (Coban and Batır; 2010; Yosr et al., 2010; Perelli et al., 2018). In the studies, the main components of rosemary essential oil were determined as carnosol, carnosic acid, rosmanol, rosmadial, epirosmanol, isorosmanol, rosmaridiphenol, rosmariquinone and rosmarinic acid. It has been reported that rosmarinic acid is the second most common caffeic acid ester after chlorogenic acid, and that it has antioxidant activity equivalent to caffeic acid, as well as that they contain chlorogenic acid p-coumaric acid, protocatechuic acid, ferulic acid, quercetin and syringic acid. (Gerhardt et al., 1983; Papageorgiou et al. 2008; Malayoğlu, 2010; Vallverdú-Queralt et al., 2014; Yesil-Celiktas et al., 2017; Rafie et al., 2017).

Fennel

Fennel (*Foeniculum vulgare* L.) is a perennial, umbrella-like, important spice plant of the Umbelliferae (Apiaceae) family and is cultivated almost all over the world, especially in the Mediterranean regions. It is divided into two subspecies as vulgare and piperitum. The most important cultivated fennel cultivars belong to the vulgare subspecies. Sweet fennel (*Foeniculum vulgare* Mill) is a biennial medicinal herb and is native to the Mediterranean region (Barros et al., 2010; Rawson et al., 2013; Ahmed et al., 2019). The highly aromatic essential oils obtained from fennel are used as a staple ingredient in many dishes such as salads, soups, beverages, bread, pickles, pastries, herbal teas. The plant has many culinary and traditional medicinal uses. For example, corms, young shoots, leaves, flowering stems, mature inflorescences, and fully ripened and dried seeds are commonly used for home remedies and are said to be particularly useful for digestive complaints, diabetes, bronchitis, cough, diuretic, kidney stones. . Fennel is also used as an ingredient in cosmetic and pharmaceutical products (Novais et al., 2004; Carvalho, 2005; Santayana et al., 2007; El-Awadi et al., 2010;

Nassar et al., 2010; Rawson et al., 2013; Ahmed et al., 2019). Many studies have been investigated the chemical composition and antioxidant activity of the essential oil of fennel from different origins (Oktay et al., 2003; Parejo et al., 2004; Zidorn et al, 2005; Torres et al., 2011; Endalamaw and Chandravanshi, 2015; Chang et al., 2016). Phenolic and flavonoid content relative to medicinal and edible fennel, were known as excellent natural sources of antioxidants and contributed to the daily antioxidant diet. The widely known polyphenols such as caffeic acid, gallic acid, ferulic acid, syringic acid are recognized for their antioxidant properties and are known to also occur in fennel bulb phenolic compounds, including Caffeoylquinicad, Rosmarinic acid, eriodictyol-7-Orutinoside, quercetin-3-O-galactoside, and kaempferol-3-O-glucoside, also showed antioxidant activities. (Bilia, et al., 2000; Parejo et al., 2004; Conforti, et al., 2006; Faudale et al., 2008; Shahat et al., 2011).

Sage

Sage (*Salvia* L.) is a valuable medicinal and aromatic plant with around 900 taxa of the genus Salvia belonging to the Lamiaceae family, which has been widely used since ancient times and is still widely used today and is featured in the pharmacopeias of many countries around the World (Walch et al., 2011; Pereira et al., 2017). Sage leaves and essential oil are widely used in folk medicine and modern medicine for many purposes such as soothing, analgesic, antiperspirant, expectorant, relieving muscle pain, stomachic and disinfectant. In addition, medical sage is consumed as an herbal tea for throat ailments caused by colds and flu. Medicinal sage has high antimicrobial and antioxidant activity due to its richness in essential oils and phenolic substances (Başer, 2000; Baricevic and Bartol, 2000; Zeybek and Zeybek, 2002; Baydar et al., 2007). These effects of sage essential oil, which has a very strong antimicrobial and antioxidant effect, are mostly due to components such as 1,8-cineol, α-thion, β-thion and camphor, and its antioxidant effects are due to hydroxycinnamic acid derivatives (caffeic and rosmarinic acids), flavonoids (luteolin, apigenin), glycosidesand diterpenoids (carnosol, rosmadial, rosmanol, epirosmanol, and methyl carnosate) (Hinneburg et al., 2006; Durling et al., 2007; Coban and Patır, 2010; Loizzoa et al., 2010; Karatoprak et al., 2016).

Thyme

Thymus is one of the most important genera from the Lamiaceae family. Some of these species have been used for their preservative and medicinal properties and added to foods (condiments and spices) and their oils and extracts have found applications in cosmetics such as toothpastes and deodorants (De Martino et al., 2009; Ballester-Costa, et al., 2017). For centuries, sage has been one of the natural remedies used safely for almost every health problem that comes to mind. It is calming, antispasmodic, regulating hormonal disorders, and opening the brain capillaries in the treatment of all mental and nervous diseases. Digestive system diseases, liver fattening, brain and heart vessel blockages, It has been found to have a positive effect when used regularly in arteriosclerosis and narrowing, strokes, epilepsy, Parkinson's, hypertension, hormonal disorders and infertility in women, skin disorders such as acne, psoriasis, vitiligo, immune system diseases and hyperthyroid problems. Also in various countries its fresh and dried leaves are used as food.n It is known to have strong antibacterial (Rota, et al., 2008), antioxidant (Baydar et al., 2007; Sarıkurkcu et al., 2010;), antifungal (Goncalves et al., 2010) antispasmodic carminative (Dob et al., 2006), diuretic effects (Hazzit et al., 2009) and antiviral activities (Aouam et al., 2019). The common feature of thyme species is that they contain essential oils and the main components of these essential oils are thymol and carvacrol. These substances are phenolic compounds that give thyme its unique smell and antioxidant properties. The main active compounds of the sage extract are rosmarinic acid and carnosic acid. The amounts of both compounds in the raw plant depend on genetic factors and environmental conditions. These compounds constitute 78-82% of essential oils (Botsoglou et al., 2003; Sökmen et al., 2004; Rusaczonek et al., 2007; Coban and Batır, 2010; Köksal et al., 2017)

Conclusion

At the beginning of the 20th century, as a result of rapid industrialization with the start of the industrial revolution, natural resources were rapidly polluted and the ozone layer was thinned by the effect of gases released into the ozone layer, revealing the harmful effects of sun rays. With the development of the chemical industry, products such as herbicides and pesticides, solvents, petrochemical products, drugs, which are used in many fields, began to be produced and consumed. This situation has become today's serious health problem, especially by weakening the immune system and increasing the formation of free radicals in humans, increasing the formation of many diseases (such as mental diseases, cancer, rapid aging, skin diseases, heart diseases). In this direction, interest and demand for these products have increased, as foods with high antioxidant content should be consumed to slow down or stop the negative health effects caused by free radicals. Modern medicine; Despite the extraordinary developments in the pharmaceutical and chemical industry, alternative treatment methods and treatment with medicinal plants are still up-to-date, and even in recent years, they have received increasing attention in developed countries. Along with these problems, especially researchers have focused on new searches and treatment methods with no side effects. For this reason, the fact that synthetic drugs used in modern medicine in recent years have not shown the desired success in treatments and have many negative side effects has again increased the interest in traditional methods. Therefore, today, as the incompatibility of synthetic materials with humans and nature is seen, there is a transformation towards natural products in the modern world with the slogans of "green revolution" and "return to nature". In this sense, one of the product groups that gain importance is medicinal and aromatic plants. When a general evaluation is made, it is estimated that the use of medicinal and aromatic plants in the food industry will increase in this context.

It is thought that the most important reason for this increase in the food sector is the thought that natural products have advantages over synthetic ones in terms of health. In recent years, with the increase in consumers' interest in natural products and the limitation of use of artificial antioxidants due to their negative effects on health, research on the use of natural antioxidants obtained from plant sources as preservatives in foods has increased to prevent oxidative deterioration. Medicinal plants have been the focus of many studies due to their richness in natural antioxidant molecules. The interest in medicinal and aromatic plants, which are widely used in many fields such as food, spice, paint, medicine, cosmetics and perfume, continues to increase. With the widespread use of natural additives in the food sector, the interest in natural antioxidants in plants is increasing day by day. Therefore, for the study of natural antioxidants, herbs and spices have become the most important focus of research. Medicinal and aromatic plants have been used as flavor, flavor, color, aroma and preservative in foods for centuries, as well as for cosmetic purposes, dyestuffs and traditional treatment.

As a result, it is very important to scientifically investigate medicinal aromatic plants as a potential source of antimicrobial compounds. Within Lamiaceae species, examples of new antioxidants include phenolic diterpenes, phenolic carboxylic acids, biphenils, and flavonoids isolated from rosemary, sage, and thyme. Many species of the Lamiaceae family are considered of high importance because of their uses in traditional medicine. In many cases phenolic compounds have antioxidant activities more effectiveIn addition, the increasing interest in natural therapies and increasing consumer demand, due to their effectiveness and safety as well as being natural products, has brought along the necessity of more detailed studies on herbal essential oils. According to the results obtained from this research, the antioxidant properties of the essential oils of medicinal aromatic plants come from the phenolic (caffeic acid, gallic acid, ferulic acid, syringic acid, rosmaric acid.) terpenoid and diterpenoids (carnosol, rosmadial, rosmanol) components found in their structure, and these components rich plants are also used for therapeutic purposes.

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THE EFFECT OF COWPEA (Vigna unguiculata) ON HUMAN HEALTH

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ABSTRACT

Today, many diseases that threaten human health are emerging due to rapid population growth, aging of the population, and malnutrition. A healthy and balanced diet is the basic principle to prevent diseases. Improper nutrition endangers the body and mind, affects health, causes death and diseases such as heart disease, hypertension, diabetes, and atherosclerosis. One of the keys to success in a healthy diet is the consumption of fruits and vegetables rich in protein and vitamins. Legumes from herbal food products have special importance in human health. Cowpea (*Vigna unguiculata* L.) is not only a delicious vegetable but also an important legume in human and animal nutrition in terms of low fat, high protein, and carbohydrate content. *Vigna unguiculata* belongs to the Fabaceae family and is an important crop in the semiarid regions across Africa and Asia due to its tolerance for sandy soil and low rainfall. In this research, the effects of cowpea on the health of the human body were discussed. cowpeas are rich in vitamin A, which is very important for eyesight and the health of the immune system. Cowpeas increase digestion helps to remedy sleep disorders, manage diabetes and protect the heart and reduce bad cholesterol. Cowpea also helps maintain the sugar balance by allowing its carbohydrate to pass into the blood slowly. Therefore, it can be easily consumed by diabetics. Besides, cowpea which is also be referred to as "poor man's meat" is a very effective food for vegetarians to absorb the amount of protein they need because cowpea has high levels of protein in its seeds and leaves.

Keywords: Cowpea (*Vigna unguiculata* L.), human health, protein.

INTRODUCTION

One of the greatest challenges facing humanity in the 21st century is the guaranteed supply of quality and healthy food that is economically, culturally, socially, and environmentally sound for the world's rapidly growing population. In addition, due to various constraints such as scarcity of land, water, and other resources and the unpredictable consequences of global climate change, and the need for more food, a difficult challenge lies ahead. On the other hand, unhealthy nutrition and the desire for fast foods with high calories have increased the problem of obesity and the prevalence of diseases such as hypertension, diabetes, heart problems, and atherosclerosis in human societies (Zarifikhosroshahi and Ergun, 2021, Egun et al., 2021). Therefore, one of the most important solutions for food balance is the use of a variety of food products that are both important in terms of health and the possibility of cultivation and exploitation in a wide range of climatic conditions (Zarifikhosroshahi et al., 2020). Legumes from herbal food products are a valuable source in a human healthy diet. Legumes contain remarkable levels of vitamins especially the B group as well as nutritionally vital minerals, such as calcium, iron, and potassium (Rebello et al, 2014). Cowpea is a delicious vegetable and important legume in human and animal nutrition in terms of low fat, high protein, and carbohydrate content. Cowpea and common bean have a homologous nutritional profile but cowpea contains higher levels of folic acid (Table 1) and lower levels of flatulence-producing factors. Dry cowpea grains cook in less time than other beans (Ehlers and Hall, 1997). Although this precious food source is common in India and Africa, it may be less known for Asian cuisine. The nutritional value of cowpea is so high in terms of health that including this product in the diet can be very effective in preventing many diseases and even treating them. However, limited studies have been done both on phytochemical content and also breeding programs to improve these contents besides increasing yield per unit area. Therefore, this study aimed to argue on health benefits of cowpea in a comprehensive collection.

BOTANICAL CHARACTERISTICS

Cowpea (*Vigna unguiculata* L.) belongs to the Leguminosae/Fabaceae family of the Phaseoleae order, which grows in temperate regions and provides economic benefits. Although the main origin of cowpea is still a debatable issue, it is stated that the center containing the maximum diversity of cowpea cultivated today is West Africa and wild cowpeas are found in Madagascar and Africa (Steele, 1976; Timko et al., 2007). Cowpea is an herbaceous legume with a great adaptation to warm climates with sufficient rainfall and is cultivated in Africa, Latin America, Southern United States, and Southeast Asia. However, this plant also shows higher tolerance to hot and dry climatic conditions and briefly, is adaptable to a wide range of environmental conditions. Although cowpea is not widely produced in Turkey, it is one of the most produced products in different geographies of the world (Serdaroglu, 2009). However, Turkey is quite suitable for the production of different types of cowpea (Langyintuo et al. 2003, Timko et al. 2007, Kir et al. 2017) and approximately 75% of the total cowpea produced in Turkey is cultivated in the Aegean region especially in Izmir province (about 45%) (Ozcelebi et al., 2021).

The flower structure in cowpea is cleistogamous and pollination and stigma receptivity are simultaneous, therefore, is known as a highly self-pollinated plant. Flowers are characterized as a cluster of two to four flowers at the end of a peduncle with white, yellow, or violet color. Anthers tightly covered style and stigma. While flowers are open only for several hours, stigmas are receptiveness for twelve hours before another dehiscence. Depending on the genotypes, a full life cycle from germination to dry grain production lasts for 60 to 150 days or more. The seed pods are curved, cylindrical, and smooth, with 35 cm length, with unique colors, usually purple, green, or yellow. A mature seed pod becomes tan or brown in color. The leaves are smooth trifoliate with entire leaflets and are arranged alternately on the stems. Cowpea can reach over 80 cm in height and is an annual plant that lives for only one growing season before harvest.

NUTRITIONAL PROPERTIES OF COWPEA

Approximately 80-85% of fresh cowpea is water and only 15-20% comprises dry matter which contains 23–32% protein, 50–60% carbohydrate, and about 1% fat (Ibrahim et al. 2010). This content of protein is between two to four times more than other crops such as cereal and tuber crops. Therefore, it can be replaced of meat in vegetarians' and economically depressed communities' diets. Cowpea is a rich source of amino acids such as lysine and tryptophan; however, contains less methionine and cysteine amino acids compared to animal proteins. (Khalid and Elharadallou 2013, Cruz et al. 2014, Kirse and Karklina 2015, Jayathilake et al., 2018).

Similar to other legumes, cowpea contains large amounts of soluble or insoluble dietary fibers, showing that it has significant potential in establishing and maintaining a healthy gut microbiota (Ngoma et al. 2018).

The seed coat of cowpea contains a high amount of lignin which is an antioxidant compound involved in metabolic processes (Oliveira et al 2018).

In addition to its positive effects on nutrition, cowpea contains bioactive components beneficial to human health. It contains components such as various phenolic components, flavonoids that have direct and indirect effects on oxidative stress. Cowpea also contains some peptides whose effects on the cardiovascular system continue to be investigated (Ojwang et al. 2013, Quansah et al. 2013, Awika and Duodu 2017).

Cowpeas are rich sources of some B-complex vitamins like thiamin, folates, pantothenic acid, riboflavin, pyridoxine, and niacin which most work as co-factors for the enzymes in protein, carbohydrate, and fat metabolism (Table 1). The high content of folate in cowpea is about 158% of the daily required values. Cowpea is also gluten-free food required for a diet of gluten-allergy and celiac disease patients.

Table 1. Nutritive value of Cowpeas raw and mature seeds per 100 g.
(Source: USDA National Nutrient database)

Approximate Minerals (mg /100 gr)		**Approximate Vitamins (mg /100 gr)**	
Calcium	110	Vitamin C	1.5
Copper	0.000845	Thiamin	0.853
Iron	8.27	Riboflavin	0.226
Magnesium	184	Vitamin B-6	0.1
Phosphorus	424	Vitamin A	15 IU
Manganese	1.528	Folates	0.633
Potassium	1112	Pantothenic acid	1.496
Selenium	0.009	Pyridoxine	0.357
Sodium	16	Niacin	2.075
Zinc	3.37		

BENEFICIAL ASPECTS TO HUMAN HEALTH

Malnutrition and unbalance diet cause many diseases and threaten human health. Fruits and vegetables with a high amount of healthy phytochemicals play a significant role in being healthy in the treatment of diseases causing by unhealthy nutrition. Improper nutrition is harmful for the body and mind health, and causes diseases such as heart disease, diabetes, hypertension, and atherosclerosis even death. As mentioned above, cowpea has outstanding nutritional characteristics. Based on previous studies, functional ingredients in cowpea are helpful in weight loss, improve digestion and strengthen blood circulation (Trehan et al., 2015; Perera et al., 2016). Advantageous effects of cowpea are due to its containing phytochemicals, dietary fiber and low-fat content, resistant starch, beside beneficial unsaturated fatty acids (Oboh et al., 2010). Diabetics can use cowpea in their diet because it allows carbohydrates to pass into the blood slowly and maintains the sugar balance in cells (Oboh et al., 2010). Cowpea reduces the levels of blood bad cholesterol by reducing reabsorption of cholesterol-binding bile acids in the colon and also due to containing a low lysine/arginine ratio (Kanetro 2015).

Cowpea protects the heart and optimizes cardiac functioning and lowers the risk of heart diseases. The fibers in cowpeas help in increasing digestion (Frota et al., 2008; Perera et al., 2016). Cowpea contains adequate tryptophan content (Vasconcelos, 2010) which could improve the remedy of sleep disorders and can help the body relax and ease into better sleep patterns (Silber and Schmitt, 2010).

Cowpeas have antimicrobial properties. The proteins and peptides in cowpea exhibit antimicrobial, antiviral and antifungal properties and inhibitor properties for growth of fungi and insects (Alghamdi et al., 2016). Albumins and globulins are the main amino acids in the protein structure of cowpea (Tchiagam et al., 2011). These amino acids are known as metabolic and enzymatic proteins, such as protease inhibitors, lipoxygenase, and lectins. A major globulin, vignin, is a heterogeneous globulin. Vicilin has been reported to strongly join with chitosan, chitin, and fully acetylated chitin allowing this protein to bind to fungal structures and prevent the germination of fungal spores or conidia and also inhibit the growth of yeast.

Lignin is highly present in the seed coat of cowpea has pharmacological applications (Oliveira et al 2018). Lignin plays a protective role against the development of different diseases such as diabetics, obesity, thrombosis, and viral infections. In many cases, the effects of lignin could be explained by its high antioxidant capacity (Vinardell and Mitjans, 2017). The defensive effect of cowpea extract against neurodegeneration and aging disorders such as Parkinson's and Alzheimer's has been also proved in a previous study (Tripodi et al 2020). Cowpea can be a part of the anemia treatment diet regimen, especially in the developing countries due to containing high levels of copper and iron (Table 1) which both take part in the production of blood cells (Abbaspour et al, 2014; Elvehjem 1993).

PHENOLIC COMPOUNDS CONTENT and ANTIOXIDANT CAPACITY

The free radicals are the result of daily normal metabolic pathways or various external factors in the human body. These free radicals need the energy to be neutralized, otherwise, cause many degenerative diseases such as cardiovascular diseases (Zarifikhosroshahi et al 2020). Reports from epidemiological studies have proven that there is a contrary correlation between an intake of fruits and vegetables and the occurrence of degenerative diseases such as cancer, obesity, cardiovascular diseases, diabetes, etc. The results of studies suggested that these favorable effects of fruits and vegetables in daily diet are due to their antioxidant phytochemicals content that scavenges reactive oxygen species (ROS) needed for neutralization of free radicals. Among these phytochemicals, the healthy aspects of phenolic compounds in fruits and vegetable consumption are outstanding (Laura et al 2019). Cowpea contains considerable concentration of phenolic compounds including tannins, phenol acids, and flavonoids (Cai et al., 2003; Rochfort and Panozzo, 2007; Ojwang et al., 2012). The main polyphenols of cowpea are phenolic acids and flavonol glycosides. Although anthocyanins and/or flavan-3-ols are also found in some varieties of cowpea (Avanza et al 2021) a few reports studied the anthocyanins in cowpea (Jayathilake et al., 2018). Phenolic acids in cowpea are mainly hydroxybenzoic and hydroxycinnamic acid derivatives (Cai et al., 2003). While the major phenolic acid in cowpea is protocatechuic acid (Cai et al., 2003), glycosides of quercetin are the main flavonoids in cowpea (Ojwang et al.,2012), however, glycosides of kaempferol and myricetin are also usually found in cowpea (Ojwang et al., 2012; Hachibamba 2013). In the group of roanthocyanidin, catechin, and afzelechin were recognized as the main flavan-3-ol unit (Ojwang et al. 2013). Some reports have been stated that the phenolic compounds in cowpea seed have inhibitory potentials against oxidative DNA damage (Nderitu et al., 2013, Nadeem Asghar et al., 2013). The concentration of phenolic compounds is 5 and 10 times more in seed coats of cowpea comparing whole seeds. While gallic acid and protocatechuic acid are identified as the main phenolics in seed coats, p-hydroxybenzoic acid is prevalent in cotyledons. The amount of flavonoids in seed coats were about 10 times more than their content in whole seeds, however, cotyledons are almost free of flavonoids (Gutierrez-Uribe et al, 2011)

Phenolic compounds found in cowpea seeds are beneficial for humans and animals health owing to their anticancer (Gutierrez-Uribe et al, 2011; Dominguez-Perles et al., 2016; Cicero et al., 2017), anti-atherogenic (Cui et al., 2012) and antioxidant, anti-inflammatory (Mathers, 2002; Siddhuraju and Becker, 2007; Ojwang et al., 2012; Ojwang et al., 2015) properties. The antioxidants that may be found in cowpea neutralize free radicals within the body. Vitamin C and A found in cowpeas are enough to give a boost to the immune system. The antioxidant capacity and the concentration of vitamin C in cowpea were reported to increase 50–60% after germination of the seeds (Doblado et al. 2007)

Several studies also have been done on processed cowpea seeds such as cooking and flour form. It is reported that the boiling process decrease the total phenolic content and antioxidant capacity of some cowpea varieties while are not affected in other varieties Hachibamba et al. (2013). Cavalcante et al (2019) reported that the concentrations of total phenolics, flavonoids, and condensed tannins, and also antioxidant activity increased notably in cheese bread enriched by cowpea flour.

CONCLUSIONS

Cowpea is a legume with delicious taste consuming worldwide especially in Indian and African cousin. Cowpea known as poor man's meat is a rich source of protein with low calories which can be placed in the diet especially for those who are vegetarian or with obesity problems. However, cowpea contains dietary fiber, certain types of vitamins and minerals, and rich antioxidants phytochemicals are categorized as healthy and high-quality food. Owing to these precious compounds, cowpea can be used in preventing even the treatment of diseases such as heart disease, cancer, blood pressure, and bad cholesterol. Cowpea extracts also have antimicrobial effects. The positive effects of cowpea in daily consumption have been also proved on eyesight, digestion, the remedy of sleep disorders. Amino acids, protease inhibitors and peptides in cowpea have been reported to improve the lipid profile, the levels of blood glucose, and blood pressure. Although cowpea is a good source of food with high healthy aspects, there are limited studies on phytochemical profiles. Thus, many studies should be done on both the content of phytochemicals in various cultivars and also in improving these contents.

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SALINITY AND DROUGHT STRESS IN WALNUT

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ABSTRACT

Walnut (*Juglans regia*.L.) is one of the most important nut with high economical value. Plant stressors such as salinity and drought has major impact on plant development and productivity thus causing serious agricultural yield losses. Walnut needs large amount of water to reach its optimal yield and quality and many current commercial walnut cultivars are very sensitive to drought and salt stress factors. Due to the soil salinization and drought many agricultural lands becomes arid or semi-arid. Thus finding new cultivars and rootstock that shows relatively high level of tolerance to salinity and drought stress factors in different growing stage can prevent severe yield loss in walnut. Also allow us to plant walnut in semi-arid or even arid regions. In this article we will focus on the salinity and drought stress studies conducted on walnut.

Keywords: walnut, salt stress, drought stress

INTRODUCTION

Persian or English walnut (*Juglans regia*.L.) is one of the most economically important nut in terms of production. And walnut has received increasing interest in recent years due to its high nutritional and economical value (Karimi et al., 2020). Nowadays, walnut is cultivated in many countries for its nut and wood (Bernard et al., 2018). Many of the walnut cultivated areas are semi-arid or arid regions, especially in Iran and Turkey (Famula et al., 2019). Together with the global climate change drought and salt stresses becomes one of the major factor hampering the walnut production in many areas of the world (Vahdati and Lotfi, 2013).

Water is the major limiting factor that prevents plants to reach its full genetic potential (Vahdati and Lotfi, 2013). Drought stress greatly limits nut trees production by upsetting the carbon/nitrogen (C/N) balance because of reducing photosynthesis which is associated with closing of stomata and a drop in the leaf water potential (Vahdati et al., 2009). Plants developed different physiological defense mechanisms to maintain plant growth and production under drought stress condition (López-Serrano et al., 2019). Walnut is usually cultivated by grafting on rootstock thus rootstock plays an important role in defense against stress contidions. Drought-tolerant rootstock improves crop production by maintaining CO_2 assimilation and better C/N balance by partly opening stomata which allow water uptake. In addition, alleviating oxidative stress, increasing the osmoregulator accumulation, hormonal signaling and mobility of genetic components are some of the possible mechanisms in moderating drought stress by tolerant rootstocks (Lotfi et al., 2009).

Salinity stress is similar to drought stress represents physiological dryness and leads to low water potential and osmotic stress (Chen et al., 2010). Increased salt concentration can lead to ionic toxicity, osmotic stress and nutritional deficiency. Although drought and salt stress factors seems like separate stress factors, however plants face these two stress factors simultaneously. In this study, we will focus on the rootstock breeding and molecular studies related to drought and salinity stress in walnut.

ROOTSTOCK BREEDING

Selection of drought-tolerant rootstocks is a major challenge for improving walnut trees production especially in arid and semi-arid regions. Different genotypes show different response to drought stress. Previous studies on different *Juglans* species have revealed that stem xylem anatomy of *J. microcarpa* led to most resistance to drought-induced embolism as compared to *J. ailantifolia* and *J. hindsii* (Knipfer et al., 2015; Knipfer et al., 2018). Drought-tolerant study of hybrids of *J. microcarpa* × *J. regia* (RX1) and *J. hindsii* × *J. regia* (Vlach and VX211) which used as common walnut rootstocks showed that RX1 and Vlach rootstocks has better response to drought stress and were able to preserve the root biomass. Drought tolerance in RX1 and VX211 was accompanied by increasing leaf water use efficiency and leaf turgor and decreasing root system hydraulic conductivity (Knipfer et al., 2020). Liu et al. (2019) reported that *J. mandshurica* and *J. regia* cv. Jizhaomian were more tolerance to drought stress as compared to *J. nigra* which was associated with an increase of water use efficiency, better chlorophyll fluorescence and gas exchange parameters. It seems that leaf water use efficiency, hydraulic conductivity and leaf turgor are effective canopy traits to select drought-tolerant rootstocks (Liu et al., 2019; Arab et al., 2020; Knipfer et al., 2020).

Despite drought stress, studies on salinity-tolerant rootstocks are very rare. An examination of the response of *Juglans* species to salinity stress has shown that *J. hindsii* and its hybrid (Pardox) is better tolerant to salinity stress compared to Persian walnut (Caprile and Grattan, 2006). Persian walnut origin centers is located in arid and semi-arid regions, utilization of genetic diversity can be an effective strategy to achieve drought and salinity-tolerant rootstocks. However traditional breeding in walnut takes long time and effort due to its long juvenile period.

MOLECULAR STUDIES

New and advanced biotechnology techniques have accelerated the understanding of the molecular mechanisms involved in drought and salinity tolerance in walnut. Ninety-seven Persian walnut population which were diverse in terms of water use efficiency was used to study association analysis between phenotypic and genotypic traits from a large data set of SNPs. The study led to identifying some drought stress-responsive genes which were involved in ABA signaling, antioxidant responses, stomatal regulation, osmotic adjustment, transduction of environmental signals and leaf development (Arab et al., 2020).

In addition to exploiting genetic diversity, genetic transformation was successfully used to induce drought and salt tolerance in walnut. Sheikh Beig Goharrizi et al. (2016) reported that Persian walnut genetically transformed with a flavodoxin (*fld*) gene had better growth under osmotic and salinity stresses. Bolagh et al., (2021) transformed the Betaine-aldehyde dehydrogenase (BADH) gene to juglans regia cv. Chandler by Agrobacterium-meditated transformation. Then they exposed the transgenic and wild type plants to four different osmotic stress levels (0%, 2%, 4% and 8% PEG) and four different level of salinity stress (0, 50, 100 and 200 mM NaCl). As a result, transgenic plants grow vigorously and wild-type plants showed retarded level of growth even death. All of these two stress tolerance gene transforming studies were tested the *in vitro* stress tolerance level of walnut genotypes. However, *in vivo* response of the walnut genotypes to drought and salinity stress conditions and molecular mechanism of drought and salinity stress is still remained unknown.

CONCLUSIONS

Several studies have been conducted on developing salinity and drought stress-tolerant rootstocks of walnut trees which led to release of some stress-tolerant rootstocks. Nevertheless, our knowledge about physiological and molecular mechanisms involved in tolerance to salinity, drought and other abiotic stresses in walnut trees is limited. We have started a transcriptomic sequencing study in walnut related to salinity stress in walnut and try to reveal the molecular mechanism of salinity stress in walnut. Climate change and high temperature simultaneously has led to an increase of abiotic stresses, and a serious drop in walnut production. Develop walnut rootstocks and cultivars that have tolerance to salinity, drought and other abiotic stress is an urgent task.

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GENOME ANNOTATION IN PLANTS USING ENSEMBL

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ABSTRACT

Genome annotation is description detected genes or a variant on the genome and indexing their genomic coordinates. In other words, DNA annotation or genome annotation is the process of determining the positions of genes and all coding and non-coding regions in a genome and determining what these genes do. It involves gene annotation provided by Ensembl, ie the identification of transcripts across the genome. The development of markers associated with agronomic traits in plant breeding and their position, expression or function on the genome play an important role in breeding programs. Variants in the genomes have different meanings on the exon or intron regions. In this article, the genome annotation of the variants using Ensembl and the definitions according to their position will be defined.

Keywords: Genome, Ensembl, Annotation

INTRODUCTION

Genome or exome sequencing and analysis of these data are fundamental to progress in biology, from basic research to translational genomics in humans, animals, plants and all other organisms. The potential of large-scale sequencing and variant analysis is revolutionary. For all that, large-scale population sequencing in *Homo sapiens* initiatives has been initiated in Iceland (Gudbjartsson et al. 2015), the United Kingdom (NHS) and the USA (Collins et al., 2015). In other species, studies such as Genome 10 K (Koepfli et al., 2015), 1001 Arabidopsis genomes (Cao et al., 2011) and the 1000 bull genome project (Daetwyler et al., 2014) have similar goals, but these projects have different objectives and often have to carry out breeding efforts with less support than a *Homo sapiens* focused approach.

Advances in next generation sequencing technology and a current cost of approximately $1000 per human genome, the need for analyzes requiring high volumes of genomes, exomes and subsequent interpretation is increasing remarkably. However, the cost of analyzes to determine the functions of the data obtained as a result of genome and exome analyzes continues to increase significantly due to the difficulty of variant interpretation. For example, a typical diploid human genome has been reported to have approximately 3.5 million single nucleotide variants (SNPs) based on the genome reference sequence, approximately 6.5 million SNPs in maize and approximately 3 million SNPs in a minor species, pistachio. Manual review of multiple variants is impractical and costly, and there are additional challenges, such as lack of functional annotation or interpretation of multiple variants within a haplotype.

Data analysis challenges and the increasing volume of sequencing data, a robust computational tool is needed to manage the complexities of variant analysis. To facilitate this, the Ensembl Variant Effect Predictor (VEP) has been developed (Ensembl VEP, 2016).

Ensembl (http://plants.ensembl.org) is an integrative resource that provides results such as genome annotation analysis for a total of 39 sequenced plant species. Available data include genome sequence, gene models, genome annotation and polymorphic loci; for the latter, additional information is available, including population structure, individual genotypes, linkage and phenotype data for some species. Comparative data are also available, including genomic alignments and "gene trees" showing the evolutionary history of each gene family represented in the database.

VARIANT CALLING

NGS data processing becomes dependent on reliable bioinformatics tools in research areas as NGS is highly sensitive to technological errors (Medvedev et al., 2009; Horner et al., 2010). The main bioinformatics steps in processing NGS data are the alignment of their short reads to the reference genome and the detection of SNP regions. In both stages, information will be given with a short summary describing from the NGS data to vcf format, which is the genome annotation input.

Cleaning of Poor Quality Reads and Removal Adapters

Trimmomatic is usually preferred for removal of poor quality readings and/or adapter sequences. This phase is the first stage before starting data analysis. It can be visited these websites for downloading Trimmomatic. (https://biohpc.cornell.edu/lab/userguide.aspx?a=software&i=53#c, http://www.usadellab.org/cms/?page=trimmomatic).

On the other hand, quality control of clean reads can be performed using FastQC program. The installation and useness of this program is very easy. It can be downloaded this link (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). The reads can be loaded using FastQC report page and report can be interpreted. Also, it can be commanded in terminal using in the below code.

```
#Quality Control using FastQC

> fastqc my_file.fastq.gz
```

Alignment of Reads to the Reference Genome

Currently, reference genomes are available for many species. Generally, alignment to the reference genome begins with the indexing of the reference genome, after which the alignment process can be initiated. In the first step, many alignment programs create indexes to align the short reads obtained to the reference genome. Programs such as Bowtie2 (Langmead and Salzberg 2012) and SOAP (Luo et al. 2013) are the most recent Linux-based alignment programs. Indexing of the reference genome provides an advantage in terms of shorter bioinformatics analysis time. Because while indexing of short reads separately creates problems in the completion of the analyses in a longer time and the storage of the obtained data, only indexing the genome eliminates such problems and also minimizes the errors that may occur in the analysis. The basic codes of indexing and alignment of reads were given in the below. BWA arguments can be checked from (http://bio-bwa.sourceforge.net/bwa.shtml).

```
#genome indexing

> bwa index genome.fa

#alignment of reads
```

```
> bwa mem -M -t 4 \
-R /genome_index/genome.fa \
sample1reads_1.fastq.gz sample1reads_2.fastq.gz > sample1.sam
```

Data Processing After Alignment

Files with sam extension obtained during alignment are converted to bam file format for variant identification analysis using the SAMtools package (Li et al., 2009). Converting file formats with the SAMtools not only reduces the size of the output dataset but also provides stream compatibility for programs that calling of SNPs and InDels in the genome, such as GATK (Genome Analysis Toolkit package) (McKenna et al., 2010) and many other software tools.

One of the post-alignment operations is local re-alignment. Local re-alignment is designed to realign reads near an identified type of misalignment to minimize the number of mismatched sequences (Figure 1). Generally, it is due to the presence of InDels in genotype genome.

Figure 1. Re-alignment of reads using reference genome

After the local re-alignment, another process is the removal of duplicates that occur in the raw data. It is possible to remove duplicates with SAMtools and Picard programs, as well as duplicates are detected with the MarkDuplicates option of GATK program and are not taken into account during variant calling (Figure 2).

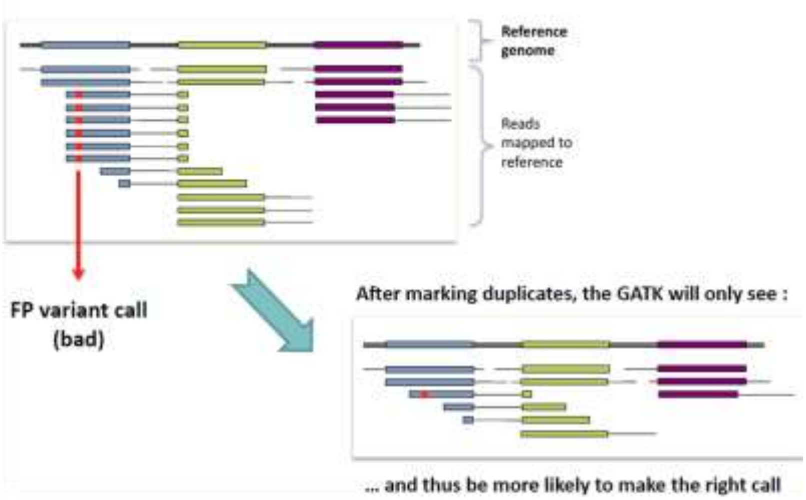


Figure 2. Remove or mark duplicate reads

Variant Calling

Recent advances in NGS and complementary bioinformatics analysis programs have provided a new and different approach to identify a greater number of variants. Therefore, it also allows the identification of rare SNPs so that the gene regions associated with complex phenotypic traits controlled by multiple genes can be identified (Handel et al., 2013). However, a variant must be detected in many short reads for reliable, since the detection of a variant in many reads is critical to distinguishing regions of real polymorphism. Many algorithms and software packages have been developed to identify SNPs in NGS data. Nowadays, programs such as GATK and Bcftools; their options, HaplotypeCaller and call argument, respectively; are generally used. The latest output format obtained in these programs is vcf files. Variant calling is briefly summarized, since the format to be used for genome annotation in many programs is the vcf format.

ENSEMBL

The Ensembl genome database project is a scientific project at the European Institute of Bioinformatics that was initiated in 1999 with the completion of the Human Genome Project (Flicek et al., 2010a). Ensembl is an organization for geneticists, molecular biologists, and the study of the genomes of humans, other vertebrates, and model organisms. Also, Ensembl aims to create a database for other researchers (Flicek et al., 2010b).

In the Ensembl project, the sequence data works with the gene annotation system (a collection of software "pipelines" written in Perl) that performs functional identification of genes and saves them in a MySQL database for subsequent analysis and viewing. Data recorded in Ensembl is freely presented for the world research community. All data and code generated by the Ensembl project can be downloaded (Ruffier et al., 2017), and there is also a public database server that allows remote access. In addition, the Ensembl web portal provides computer-generated visual representations of most of the data.

The other aims of the Ensembl are, continue to “expand” this biological integration to include other model organisms relevant to understanding human biology as they become available; “deepening” this integration to provide a more seamless connection than ever before between equivalent components of different types; and “enabling” further classification of functional elements that were previously difficult to find in the genome.

Ensembl Variant Effect Predictor (VEP) has been developed to manage and minimize the complexities of variant analysis. Vep annotation offers two different ways of annotation with web portal and script. However, annotation programs such as annovar, snpEff and vep have been initiated as part of the human genome project. In these projects, the software is adapted to the human genome. In other words, it is defined according to *Homo sapiens*, its default settings.

Web Portal of VEP

The web portal is best suited for first-time users or small-scale analysis. The maximum compressed (gzip) uploaded data file size is currently limited as 50 megabytes (MB), which is large enough for approximately two million rows of typical VCF data. The web portal results were given in the figure 3.

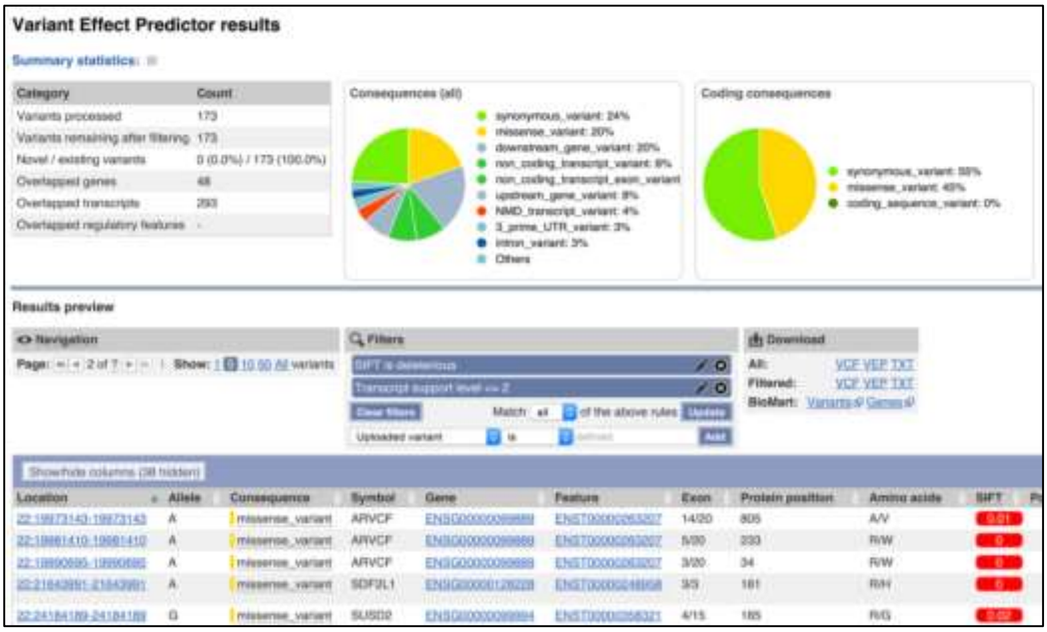


Figure 3. Results obtained from Web portal of VEP

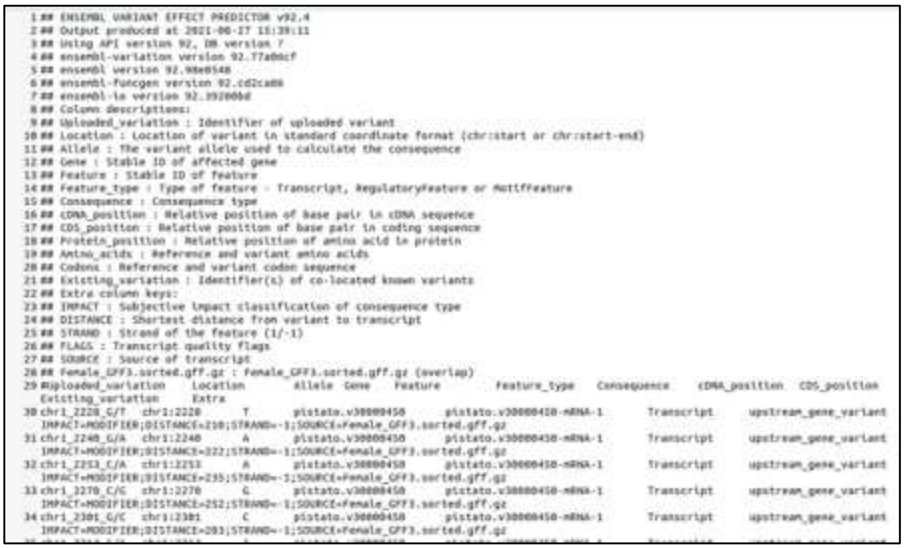
Script of VEP

The VEP script needs inputs in different formats with variable positions to run smoothly. In the simplest file formats, the variant should have chromosome number, position, and a pair of alleles (reference and alternative). Common formats such as the annotation VCF format with VEP should be used.

VEP script is first downloaded using https://github.com/Ensembl/ensembl-vep.git or "conda install -c bioconda ensembl-vep". Since the downloaded file extension is perl, the perl program must be installed. If it is not installed, it can be installed with the command "conda install -c bioconda perl" for easy and quickly. And to test that it is installed, the arguments are listed with the --help command. Then, INSTALL.pl with perl extension is run in the downloaded ensembl-vep folder in terminal. To test whether it is loaded, the arguments are listed with the ensembl-vep command. The basic codes are detailed in the below.

However, if you want to annotate a species that you are interested in, it is mandatory to have the genome (.fa) and genome annotation information (.GFF/.GFT) files of the species files. The output file, variant annotation file, were given in the below figure 4. If you edit and add arguments to vep script, visit running vep using this link (https://www.ensembl.org/info/docs/tools/vep/script/vep_options.html).

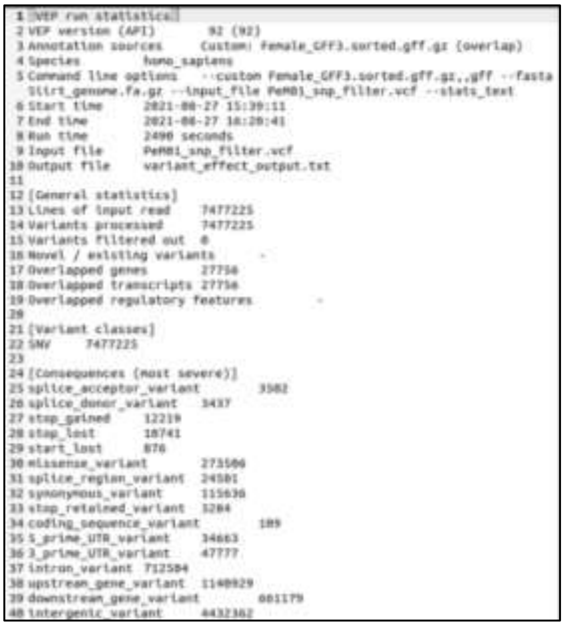
> vep --sf \
--i ${genotype}.vcf –custom genome_anno.gff.gz,,gff \
--fasta genome.fa.gz --o ${genotype}_A



```
## ENSEMBL VARIANT EFFECT PREDICTOR v92.4
## Output produced at 2021-06-27 15:39:11
## Using API version 92, DB version ?
## ensembl-variation version 92.77a0ccf
## ensembl version 92.98e0548
## ensembl-funcgen version 92.cd2ca08
## ensembl-io version 92.392006d
## Column descriptions:
## Uploaded_variation : Identifier of uploaded variant
## Location : Location of variant in standard coordinate format (chr:start or chr:start-end)
## Allele : The variant allele used to calculate the consequence
## Gene : Stable ID of affected gene
## Feature : Stable ID of feature
## Feature_type : Type of feature - Transcript, RegulatoryFeature or MotifFeature
## Consequence : Consequence type
## cDNA_position : Relative position of base pair in cDNA sequence
## CDS_position : Relative position of base pair in coding sequence
## Protein_position : Relative position of amino acid in protein
## Amino_acids : Reference and variant amino acids
## Codons : Reference and variant codon sequence
## Existing_variation : Identifier(s) of co-located known variants
## Extra column keys:
## IMPACT : Subjective impact classification of consequence type
## DISTANCE : Shortest distance from variant to transcript
## STRAND : Strand of the feature (1/-1)
## FLAGS : Transcript quality flags
## SOURCE : Source of transcript
## Female_GFF3.sorted.gff.gz : Female_GFF3.sorted.gff.gz (overlap)
#Uploaded_variation	Location	Allele	Gene	Feature	Feature_type	Consequence	cDNA_position	CDS_position
  Existing_variation	Extra
chr1_2228_G/T	chr1:2228	T	pistato.v30000450	pistato.v30000450-mRNA-1	Transcript	upstream_gene_variant
  IMPACT=MODIFIER;DISTANCE=210;STRAND=-1;SOURCE=Female_GFF3.sorted.gff.gz
chr1_2240_G/A	chr1:2240	A	pistato.v30000450	pistato.v30000450-mRNA-1	Transcript	upstream_gene_variant
  IMPACT=MODIFIER;DISTANCE=222;STRAND=-1;SOURCE=Female_GFF3.sorted.gff.gz
chr1_2253_C/A	chr1:2253	A	pistato.v30000450	pistato.v30000450-mRNA-1	Transcript	upstream_gene_variant
  IMPACT=MODIFIER;DISTANCE=235;STRAND=-1;SOURCE=Female_GFF3.sorted.gff.gz
chr1_2270_C/G	chr1:2270	G	pistato.v30000450	pistato.v30000450-mRNA-1	Transcript	upstream_gene_variant
  IMPACT=MODIFIER;DISTANCE=252;STRAND=-1;SOURCE=Female_GFF3.sorted.gff.gz
chr1_2301_G/C	chr1:2301	C	pistato.v30000450	pistato.v30000450-mRNA-1	Transcript	upstream_gene_variant
  IMPACT=MODIFIER;DISTANCE=283;STRAND=-1;SOURCE=Female_GFF3.sorted.gff.gz
```

Figure 4. The output of genome annotation file

After genome annotation, the classification of all variants is recorded in detail in the variant_effect_output.txt file. In addition, an output containing a summary of all variants is printed in the summary file (Figure 5). Also, file with extension html is created summary of the results with detailed graphs.



```
[VEP run statistics]
VEP version (API)        92 (92)
Annotation sources       Custom: Female_GFF3.sorted.gff.gz (overlap)
Species          homo_sapiens
Command line options     --custom Female_GFF3.sorted.gff.gz,,gff --fasta
 Slirt_genome.fa.gz --input_file PeM01_snp_filter.vcf --stats_text
Start time       2021-08-27 15:39:11
End time         2021-08-27 16:20:41
Run time         2490 seconds
Input file       PeM01_snp_filter.vcf
Output file      variant_effect_output.txt

[General statistics]
Lines of input read      7477225
Variants processed       7477225
Variants filtered out    0
Novel / existing variants        -
Overlapped genes         27756
Overlapped transcripts 27756
Overlapped regulatory features           -

[Variant classes]
SNV     7477225

[Consequences (most severe)]
splice_acceptor_variant          3582
splice_donor_variant     3437
stop_gained      12219
stop_lost        18741
start_lost       876
missense_variant         273506
splice_region_variant    24501
synonymous_variant       115636
stop_retained_variant    3284
coding_sequence_variant          189
5_prime_UTR_variant      34663
3_prime_UTR_variant      47777
intron_variant  712584
upstream_gene_variant    1140929
downstream_gene_variant          881179
intergenic_variant       4432362
```

Figure 5. VEP script result statistics belonging to VCF

The statistics parameters such as intronic, exonic, UTR5, UTR3, upstream, downstream, splicing, intergenic, stop gain, stop loss, frameshift, non-frameshift, synonymous and nonsynonymous in annotation file were demonstrated in figure 6.

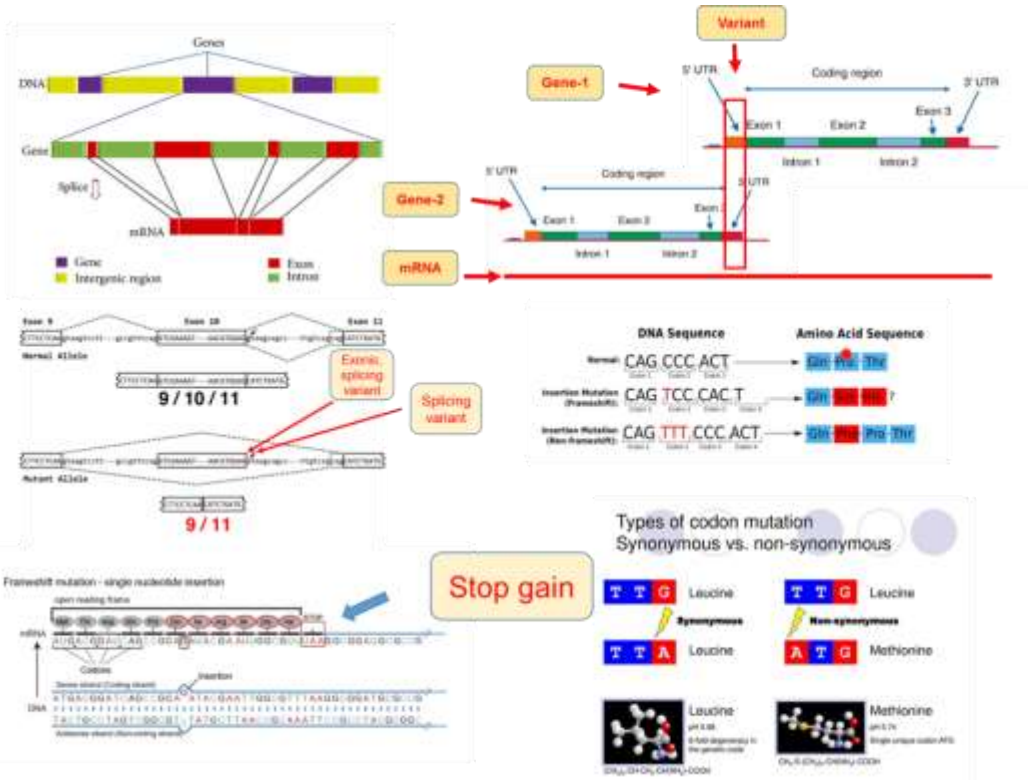


Figure 6. General illustration of the genome annotation file (the figures were cited from internet)

CONCLUSIONS

Genome annotation is analyzed in two different ways using vep such as web portal and perl script. Especially the web portal is very useful for small size genomes. Especially for the sequenced genomes in the Ensembl database, different from other annotation programs, it gives many different statistical results. These functions make VEP annotation highly preferable. The files obtained from all annotation programs are selected in accordance with the purpose of the study and the selected variants, gene ontology (GO) and KEGG pathways are performed. In this way, intra-species and inter-species information integration can be made.

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HYDROPRIMING AFFECTS SEED GERMINATION AND SEEDLING PERFORMANCE IN SWEET CORN CULTIVARS

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ABSTRACT

Seed priming methods have been used to increase seed germination and seedling emergence in several crops. Five different F1 hybrid sweet corn cultivars were hydroprimed (16 hours at 25 °C, dried 24 hours) and compared with the control seeds. Mean germination time was calculated by counting radicle emergence in every 2 hours between 24 and 192 h. Root length and shoot length measurements were made at the end of the 8-day period. Seedling emergence test was conducted at 20°C over 20 days in a climatic room. Daily counts were also made for the seedling emergence percentages. Root and shoot length measurements were made at the end of the 20th day. The results showed that hydro-priming increased total germination 1-17% and normal germination percentages about 6-85%, root length 17-42% and shoot length 21-63%, seedling emergence 2-36%, seedling root 29-40% and shoot length 16-22%, respectively. Mean germination time was reduced 1.94 from 1.53 days and mean seedling emergence time was reduced from 13.65 to 11.65 days in control compared to treated seeds. The effect of treatment was varied according to the cultivars. It can be concluded that hydropriming can be a simple method to improve seed quality in sweet corn.

Keywords: Priming, seedling quality, seedling establishment, seed treatments

1.INTRODUCTION

Corn or maize is one of the most important grains in the world due to its wide variety of uses and high nutritional content. Sweet corn, which is a subspecies of corn, whose consumption is increasing rapidly throughout the world, started to be cultivated in Turkey in the 1930s. It has a higher sugar content (6%) than other maize subspecies when harvested during the milk stage. Therewithal, sweet corn kernels have the highest content of fat and protein, as they have larger embryos than the other subspecies. (Öktem and Öktem, 2006). In addition to being consumed in different ways as fresh, frozen and canned sweet corn, it is also beginning to be used in beverages. In recent years, the use of corn syrup in packaged foods and beverages has also led to an increase in the cultivation of sweet corn.

Priming is a seed treatment involving the presowing, controlled hydration of seeds in a manner that allows for the pre-germinative metabolic activity to occur without protrusion of seed radicles. Priming of seeds enhances seedling establishment and crop production under stressed conditions. This could be due to faster emergence of roots and shoots, more vigorous plants, better drought tolerance, earlier flowering, earlier harvest, and higher grain yield under adverse conditions in the field (Bennet et al., 1992; Demir and Ellis 1994; Nouman et al., 2012). In sweet corn hybrids with super sweet genotypes (shrunken 2-sh2) have an inherent problem of low seed emergence in the field (El-Hamed et., al. 2011). Therefore, seeds may germinate slowly and exhibit poor seedling vigor (Wolf et al., 1997). Poor germination poses a particular problem for early spring planting in cold soils. Various priming techniques have been attempted to improve germination and emergence. One of the most studied techniques for achieving such benefits is hydropriming. Hydropriming involves allowing the seeds to absorb sufficient water to not allow germination to complete but to initiate the metabolic process. Several studies have shown this method to be effective in increasing the germination percentage and vigor of seedlings. For instance, Harris et al. (1999) demonstrated that on-farm seed priming by soaking seeds overnight in water followed by surface drying and then sowing in the normal way markedly improved the stand establishment and early vigor of maize. Nagar et al. (1998) observed a significant improvement in field emergence and seedling characteristics after hydro-priming maize for 16 hours. On the other hand, Lara-Viveros (2020) stated that hydroprimed seeds for 12 and 18 hours, germinated more rapidly in comparison with the control.

Moreover, a series of bioassays involving different priming agents such as $CaCl_2$ (Farooq et al., 2008b), GA3 or IAA (Afzal et al., 2008), salicylic acid (Farooq et al., 2008a), and moringa leaf extracts (Basra et al., 2011; Afzal et al. 2012) have been also tested to improve the maize performance under low-temperature stress. While, hydro-priming is a simple low-cost method of seed priming that requires no sophisticated equipment (Mabhaudhi and Moti 2011).

This study aimed to evaluate hydro-priming to improve seed germination and seeding performance of five commercial sweet corn hybrids.

2. MATERIAL AND METHOD

This study was conducted in the laboratory of the Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara during the period from March to April in 2021. Five different sweet corn cultivars (*Zea mays saccharata* Sturt. cvs Frigo, Filinta, Datome, Khan and Baron) were used and they were obtained from various commercial companies.

2.1 Laboratory Germination

The seeds washed under distilled water for 30 seconds before starting the experiment to remove the seed coating material and then they were divided into two sub-samples. One of the them was considered as control (unprimed) and the other sample was prepared for priming treatment. For hydro-priming, seeds were soaked in distilled water at 25°C for 16 h under dark conditions. Thereafter, the treated seeds were dried for 24 hours under paper towels. Three replicates of 50 seeds from each variety and priming treatment were germinated between double-layered, moistened paper towels. The paper towels were rolled, put into zip-lock bags, and incubated in a germination chamber at 25℃ (ISTA, 2020). Germination was considered to have occurred when the radicles were 2 mm long. Total germination percentage (%), normal germination percentage (%), seedling length (mm), and radicle length (mm) were recorded after 8 days of planting on filter paper. Mean germination time (MGT) was calculated according to Ellis and Roberts (1980) to assess the rate of seed germination. Radicle emergence was counted from 24 h to 192 h every two hours after setting up germination.

$$MGT = \frac{\sum n.t}{\sum n} \quad \text{where,}$$

n = number of newly germinated seeds (2 mm radicle emerged) at time t, t = days from planting, and $\sum$n = final radicle germination

2.2. Seedling Emergence

The seedling emergence test was carried out in three replicates of 50 seeds each, under field conditions in plastic germination trays (32 x 16 x 6 cm, length x width x depth) with garden soil at Ankara, Turkey, on 3 May 2021 and 23 May 2021. The soil was a sandy loam (pH: 7.57, EC: 0.98 dS/m, N: 0.09, P2O5: 4.5%, K: 0.16%, Na: 0.41%, Ca: 6.7%, organic matter: 3.1%). Daily minimum and maximum soil temperatures were recorded throughout both sowings and varied between 9 and 30 °C.

Nonprimed and hydroprimed treated seeds of five lots were sown at a depth of 5 cm in the soil. Twenty days after sowing, all seedlings in each replicate were taken, shoot length (mm/plant), and root length (mm/plant) were calculated. The number of seedling emergences assessed to have occurred when the cotyledons had unfolded above the surface was counted daily until 20 days after sowing.

Mean time to emergence was calculated using Bewley and Black (1994).

$MET = \frac{\Sigma(fx)}{\Sigma f}$ where, f = number of newly emerged seeds at a given time (day), and

x = number of days from date of sowing.

2.3. Statistical Analysis

Statistical analysis was carried out by using independent samples t-test significance of the difference between means. Means were considered significantly different for P<0.05.

3. RESULTS

Hydropriming had some positive effects on germination compared to control with no treatment. Priming treatment significantly affected all cultivars in terms of germination criteria like total germination percentages. For example Frigo, Filinta and Khan cultivars were significantly affected by priming treatment (Table 1). Hydropriming increased the germination percentages of the cultivars compared to the controls by 20.3%, 14.6%, 8.3%, 2.8% and 1.4 % for Khan, Frigo Filinta, Baron, and Datome, respectively (Table 1). Priming had an effect ($P < 0.05$) on mean germination time (MGT). For all varieties, MGT was reduced significantly by the treatment. Furthermore, there was a highly significant interaction ($P < 0.05$) between control and priming for root length and shoot length in all cultivars except in Baron (Table 1).

There are significant differences in normal germination percentages between hydroprimed sweet corn cultivars. As well as the normal germination percentage was lower in control seeds in all cultivars, the cultivar that was most affected by hydropriming was Khan (from 5.3% to 36%). While this was followed by Frigo with from 39.3% to 54.6%, there were 39, 27, and 7% increase in normal germination percentage in Filinta, Baron, and Datome, respectively after hydropriming for 16 h at 25°C (Figure 1).

Table 1. The effects of hydropriming treatment in 5 different hybrid sweet corn cultivars in terms of total germination (TG) mean germination time (MGT), root length and shoot length compared to control. * indicates the significant differences based on t-test (95% S.D.) in each cultivar and stress conditions, App: Application

Cultivars	Criteria							
	TG (%)		MGT (h)		Root length		Shoot length	
	C	HP	C	HP	C	HP	C	HP
Frigo	64.0	73.3*	53.65	42.31*	4.74	6.02*	6.17	7.19*
Filinta	72.0	78.0*	45.17	36.49*	4.22	7.23*	6.37	10.44*
Datome	97.3	98.6 ns	41.04	35.86*	7.79	9.34*	8.75	11.08*
Khan	82.0	98.6*	46.96	32.62*	2.08	3.47*	1.38	3.67*
Baron	94.6	97.3 ns	47.10	37.39*	3.10	4.07*	2.94	4.02 ns

Seedling emergence improved when seeds were primed for16 hours. But there were no differences (P > 0.05) in seedling emergence (Table 2) between hydropriming and control except Khan. The Hydropriming method significantly influenced the mean emergence time of sweet corn cultivars. Root length of seedlings of Datome and Khan were significantly different, but for the other 3 cultivars, it was not significant (p>0.05) compared to the controls. Hydropriming also increased shoot length for all varieties except Baron.

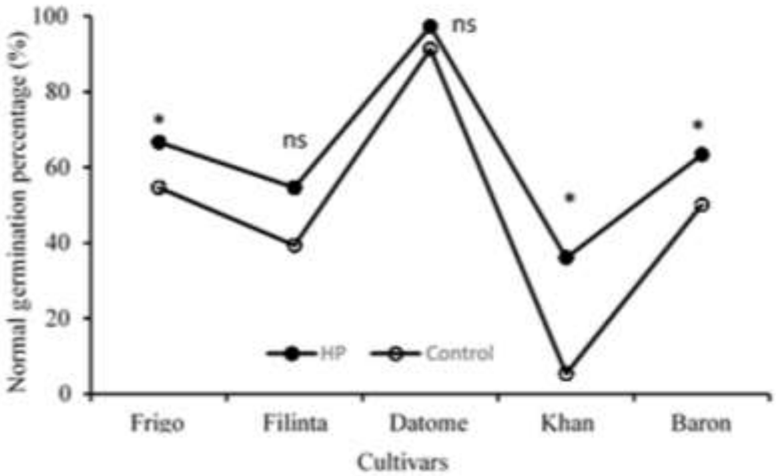

Figure 1. Normal germination percentage in hydroprimed and untreated seeds of five sweet corn cultivars.(* indicates the significant differences based on t-test (95% S.D.) in each cultivar.

Table 2. Seedling total germination (TG), mean germination time (MGT), root length, and shoot length of sweet corn seed cultivars. * indicates the significant differences based on t-test (95% S.D.) in each cultivar and hydropriming, App: Application

Cultivars	Criteria							
	TG (%)		MET		Root length		Shoot length	
	C	HP	C	HP	C	HP	C	HP
Frigo	29.3	42.0 ns	12.80	11.56*	6.83	11.34 ns	9.73	11.54*
Filinta	49.3	52.6 ns	16.11	13.18*	7.06	11.49 ns	10.83	12.91*
Datome	94.6	96.6 ns	10.71	10.33*	13.77	21.23*	16.66	20.70*
Khan	56.6	88.0 *	13.61	11.69*	9.83	13.79*	13.87	17.77*
Baron	82.6	88.7 ns	15.06	12.51*	12.22	17.27 ns	13.35	16.10 ns

Hydropriming is also like the effect on the normal seedling. Statistical analysis showed that treatment on normal seedling emergence made significant differences in Frigo, Khan and Baron. Hydropriming increased the normal emergence percentages of the cultivars compared to the controls by 38.6%, 28%, and 14% for Khan, Baron, and Frigo respectively. But there were no differences (P > 0.05) in normal seedling emergence on Filinta and Datome (Figure 2).

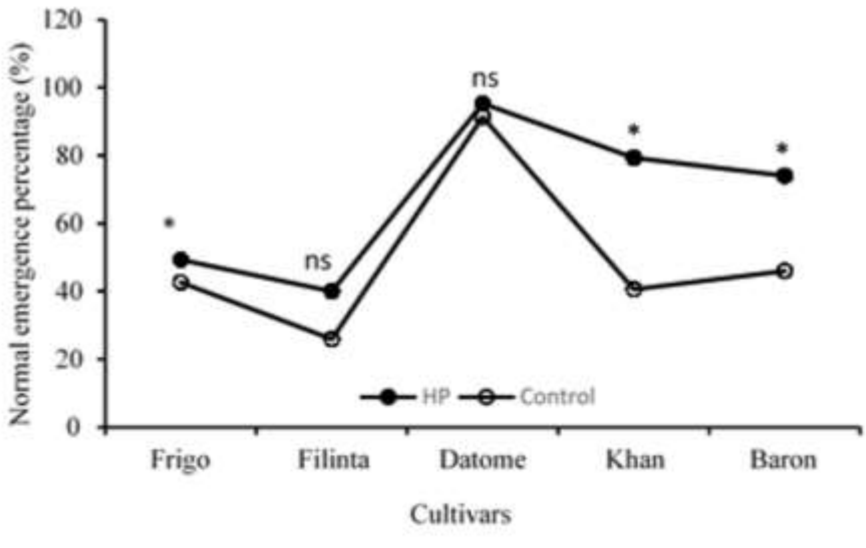

Figure 2. Normal emergence percentage in hydroprimed and untreated seeds of five sweet corn cultivars. * indicates the significant differences based on t-test (95% S.D.) in each cultivar

4. DISCUSSION

The objective of this study was to determine whether hydropriming can be used to improve vigor, with respect to germination attributes and seedling emergence in five different sweet corn cultivars. Priming of seed has been effectively used to enhance the vigor and emergence of seedlings under both optimal (Demir and Van De Venter 1999; Farooq et al., 2006) and sub-optimal conditions (Wahid and Shabbir, 2005). Results of the present study indicated that hydropriming resulted in a positive effect on the germination of sweet corn seeds. Germination percentages of the cultivars were higher than those of the controls, but the difference for Datome and Baron were not statistically significant. This probably was due to the high germination potential of Datome and Baron compared to the other cultivars. Even at the control level, Datome and Baron had a high germination percentage and the increase in germination with hydropriming remained insignificant. Hydro-primed seeds had shorter germination time and better root and shoot growth which agrees with earlier reports (Parera and Cantliffe, 1994; Taylor et al.,1998; Casenave and Toselli 2007; Bölek et al., 2013; Bhusal and Thakur 2020).

On the other hand, hydropriming did not affect the seedling emergence of all varieties except Khan. The rapid uptake of water during priming may have caused imbibition injury, failing seeds to germinate (Mabhaudhi and Modi, 2011). Secondarily, during re-drying starch hydrolysis, was slowed down due to reduced water availability. Upon availability of moisture on sowing, re-dried seeds took some time for the metabolism to restart, which caused a delay in germination and seedling growth (Farooq et al., 2010). Therefore, normal emergence seedling is significant especially poor seedling emergence. Priming improved emergence speed and reduced MET. Primed seeds germinated faster and more uniformly than unprimed seeds. Improved seedling growth in early or optimum planting for root, shoot lengths might be due to earlier emergence stimulated seedling vigor causing enhanced plant growth (Rehman et al 2015). A significant reduction in emergence time maybe since seed priming stimulates an array of biochemical changes such as hydrolysis, activation of enzymes, and dormancy breaking in the seed (Aziza et al., 2004; Farooq et al., 2010). Seed priming not only improved the normal seedling and MET but also grew root and shoot length. These results are similar to others reported in earlier reports (Mahboob et al., 2015; Bölek et al., 2013).

In conclusion, hydro priming at 16 hours duration followed by 24 hours surface drying could be used as a simple low-cost, environmentally, and useful technique for enhancing seedling emergence rate and percentage of sweet corn. These effects can also be effective in the seedling establishment.

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THE STRUCTURAL EVALUATION REGARDING TO AGRICULTURAL TOURISM OF VITICULTURE FARMS IN TEKİRDAĞ PROVINCE

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ABSTRACT
Agricultural tourism enables consumers to see a production pattern on the spot and have information by including them in agricultural activities in rural areas, either for a day or with accommodation. In addition, agricultural tourism is the purchase of the products harvested by the producers or sold locally by the visitors, thus providing an economic contribution to the producer, promoting the social and cultural characteristics of the region and raising awareness on the consumer. In other words, it is the farmers' marketing of their agricultural activities and rural cultures without breaking away from the existing agricultural production pattern. In this context, activities that can provide additional income to the producer; outdoor recreations, educational experiences, harvest festivals, accommodation services, direct sales from the farm. In this way, while the producers will ensure their sustainability in agricultural terms, it will also contribute to the development of individuals living outside the rural areas in terms of agricultural culture. In this study, its commentate the perspectives on agricultural tourism activities that can provide additional income to the farmer alongside their current agricultural production of viticulture farms in Tekirdağ province. In addition, were investigated in terms of their potential to apply of these activities and the structural adaptation to agricultural tourism of viticulture farms. For this purpose, were tried to be revealed agricultural tourism potentials of 104 viticulture farms in 27 neighborhoods of Süleymanpaşa and Şarköy districts in Tekirdağ province. Considering some of the parameters required for tourism, Tekirdag province has features such as having the sea-sand-sun, at the same time, natural attractiveness (forest ecosystem, etc.), hosting historical wineries and museums, revealing the strong side of the region. It seems that the willingness of the producers to give agricultural tourism activities is in a positive direction when the findings obtained as a result of the study conducted in Şarköy and Süleymanpaşa districts The biggest negativities for the viticulture businesses in the study area are; When the demographic characteristics of the rural neighborhoods, such as low income levels of the producers, high average age (about 55 years), the young population is almost non-existent, and the annual birth rate is below 1, the producers are shy about taking risks and being open to innovations.

Keywords: Agricultural Tourism, Tekirdağ, Viticulture

1. INTRODUCTION

Agricultural tourism enables consumers to see a production pattern on the spot and have information by including them in agricultural activities in rural areas, either for a day or with accommodation. In addition, agricultural tourism is the purchase of the products harvested by the producers or sold locally by the visitors, thus providing an economic contribution to the producer, promoting the social and cultural characteristics of the region and raising awareness on the consumer (Hurma ve ark., 2010). In other words, it is the farmers' marketing of their agricultural activities and rural cultures without breaking away from the existing agricultural production pattern. In this context, the activities that can provide additional income to the producer are listed as follows (Dorobantu ve Fieldsend 2011; Garcia 2006; Gümüş 2015; Tuna ve ark. 2020).

- ✓ Outdoor recreations, educational experiences, harvest festivals, accommodation services, direct sales from the farm.
- ✓ Outdoor recreations (Fishing, photography etc.)
- ✓ Educational experiences (School tours, technical trips, helping with farm work, product promotion programs, etc.)
- ✓ Harvest festivals (Harvest festivals, fields, fairs etc.)
- ✓ Accommodation services (Being a guest in the residences of the enterprise, etc.)
- ✓ Direct sales from the farm (Roadside stands, you-pick or pick-your-own vb.)
- ✓ Off-Farm direct selling (Farmers' markets, fair organizations, special day organizations etc.)

In this way, while the producers will ensure their sustainability in agricultural terms, it will also contribute to the development of individuals living outside the rural areas in terms of agricultural culture.

In this study, its was investigated in terms of their potential to apply and the structural adaptation to agricultural tourism activities that can provide additional income to the farmer alongside their current agricultural production of viticulture farms in Tekirdağ province.

2. MATERIAL AND METHOD

The target group of the present research is the viticulture enterprises in Tekirdağ province. The material of the study consists of the data obtained through the survey conducted with these enterprises in question. In this context, 27 neighborhoods were selected as the research area, and it was determined that there were 13750 decares of vineyard land in total. The formula used to determine the sample size is as follows (Newbold, 2000).

$$n = \frac{(n) \times t^2 \times (p) \times (q)}{d^2 \times (n-1) + t^2 \times (p) \times (q)}$$

n: Number of samples, p and q: They have been selected as 0.5 to keep the sample size high, t: Significance level (95%), d: Margin of error (10%)

According to the formula, the number of surveys to be conducted was determined as 104 equaling to 95% significance level and 10% margin of error. In order to determine how to distribute these 104 surveys to the neighborhoods to be studied, the distribution was carried out by applying the proportional sample volume formula. The locations of the 27 neighborhoods where the surveys were conducted are shown on the map (Figure 2.1)

Figure 2.1 Location map of the study area

3.RESEARCH FINDINGS

Research findings of the face-to-face survey conducted in the viticulture enterprises in Tekirdağ province on how they perceive agricultural tourism and how their vineyards and demographic background could be adapted to agricultural tourism have been pointed out in Table 3.1. Of the 104 viticulture enterprises participating in the survey, 95% (99 people) are male and 5% (5 people) are female entrepreneurs.

42% (44 people) of the producers participating in the survey are 59 years old and over, and 30% (31 people) are between 51 and 58 years old. Only 4% (4 people) of the producers are under the age of 34. It has been determined that the average age of the producers participating in the survey conducted in the districts of Şarköy and Süleymanpaşa, where 96% of viticulture of Tekirdağ take place, is approximately 55 (54.60).

When the distribution of the producers' education levels is examined, it is observed that the number of primary school graduate producers has the highest rate, constituting 84% (87 people). The rate of producers with high school and higher education levels is 6%, with only six people. Considering the enterprises' annual gross income distribution, we have observed that 28-37 thousand TL takes the first place with 42%, while 18-27 thousand TL takes the second place with 32%. Of the 104 viticulture enterprises that participated in the survey, the rate of enterprises with an annual income level of 48 thousand TL and above is only 11%. The highest income is 90 thousand TL, and the lowest income is 18 thousand TL. It has been determined that the annual average gross income of the producers is approximately 34 (33759.62) thousand TL (Table 3.1).

Fifty-one (49%) of the enterprises where the research was conducted have non-agricultural income as well. Retirement pension constitutes 90% of these 51 enterprises as a source of non-agricultural income. It is seen that the rate of those who have both a pension and different non-agricultural income sources is only 4%. It can be said that non-agricultural income sources are landlordism, rent through owning some stores, and income from commercial vehicles.

As to the share of the agricultural income within the household income, 47% of the participants claimed that agriculture constitutes their revenue in between 76% and 100%. This rate is followed by a 25% and 26%-50% share range, respectively, and the rate of producers with a 25% share and below agricultural income has been determined as 15%. In this context, when the share of agricultural income in non-agricultural income and household income is evaluated, it is seen that having a regular income such as a pension while residing in rural areas is an essential additional source of income.

Table 3.1. Descriptive Statistics on Demographics

	Groups	Frequency (n)	Ratio (%)
Sex distribution	Female	5	4,81
	Male	99	95,19
	Total		100,00
Age Groups	19-34	4	3,85
	35-42	8	7,68
	43-50	17	16,35
	51-58	31	29,81
	59-66	40	38,46
	67-74	4	3,85
	Total		100,00
Educational Status	Elementary School	87	83,65
	Secondary School	11	10,58
	High School	5	4,81
	University	1	0,96
	Total		100,00
Annual Income Distribution of Businesses	18-27 thousand TL	33	31,73
	28-37 thousand TL	44	42,31
	38-47 thousand TL	16	15,38
	48-57 thousand TL	7	6,73
	58 thousand TL and over	4	3,85
	Total		100,00
Distribution of Non-Farm Income Source (51 people)	Pension	46	90,20
	Others	3	5,88
	Both	2	3,92
	Total		100,00
Share of Agricultural Income in House Income	%0-%25	16	15,38
	%26-%50	26	25,00
	%51-%75	13	12,50
	%76-%100	49	47,12
	Total		100,00
Foreign Language Knowledge	Yes	9	8,65
	No	95	91,35
	Total		100,00

When foreign language knowledge in the households of viticulture enterprises is evaluated, it is observed that 9% of the viticulture enterprises participating in the survey have a family member who speaks at least one foreign language. Foreign language knowledge is an essential factor in tourism. Although this rate is generally low in the agricultural sector, the rate of 9% is observed to be positive in these viticulture businesses as 96% of them are small family businesses (Table 3.1).

Digital markets are a widely accepted phenomenon in the agricultural sector today, and internet websites constitute a vital role in providing market convenience to the producer. When asked whether the enterprises participating in the survey have a website, only 5% stated that they have a website, and it is seen that this rate is not at a sufficient level. When the farming experience of the producers participating in the research is examined, 58% (61 people) have an experience of 36 years or more, and the lowest rate is 8% (8 people) with 5-15 years of experience. The average farming experience is 36 years, the highest is 55, and the minimum is 5 years. The highest average age of the vineyards is between 15-30 constituting 69%. The average age of vineyards is 23 years; the oldest vineyard is 55, while the youngest is seven. When the producers were asked about their thremmatology pattern, 81% stated that they only carry out plant raising while 19% carry out plant and livestock raising. When the training system in viticulture enterprises is examined, 81% is based on goblets, 12% on wire cordons and 8% on both goblets and wire cordons.

When the locations and transportation means of the enterprises participating in the survey are evaluated, it is seen that the distance of the enterprises to the city center is 69 km on average; the farthest enterprise from the city center is 115 km, and the closest enterprise is 7 km from the city center. The distance of the viticulture enterprises to the district center is 18 km on average, the distance of the farthest enterprise to the district center is 40 km, and the nearest enterprise is 2 km. The average distance to the intercity highway is 23 km, the farthest from the intercity motorway is 60 km, and the closest enterprise is 3 km away. The distance of the enterprises to the inter-district road is 9 km on average, the distance of the farthest enterprise to the inter-district road is 35 km, and the distance of the nearest enterprise to the inter-district road is 2 km (Table 3.2).

Table 3.2. Accessibility of Viticulture Enterprises

	Average (km)	**Farthest (km)**	**Nearest (km)**
Distance to City Center	69,37	115	7
Distance to District Center	18,07	40	2
Distance to Intercity Highway	22,97	60	3
Distance to Road Between Districts	8,70	35	2

When the lands owned by the producers were evaluated in terms of accessibility, 48% (50 people) of the respondents stated that their vineyards had "Easy and Very Easy" accessibility, and 32% (33 people) stated that their lands had "Medium" accessibility. In addition, 20% of the producers (21 people) stated that the accessibility of their lands is not easy (Table 3.3).

It has been observed that there are no bus lines in 27 neighborhoods where the survey was conducted. 41% of these neighborhoods (11 neighborhoods) have a minibus line that leaves at least once a week, although it is not regular. 15% of the selected neighborhoods (4 neighborhoods) provide transportation with at least one tour company's vehicles. There are no tour companies in 85% (23 neighborhoods) of the neighborhoods.

When the producers were asked to evaluate the road quality of the neighborhoods and the region where the viticulture businesses are affiliated, 55% (57 people) of the survey participants positively evaluated the road quality of their neighborhood and region. 20% of the manufacturers (21 people) made a category selection in the direction of poor road quality.

Table 3.3. Transportation Characteristics of the Businesses (According to 27 Neighborhoods)

	Categories	Frequency (n)	Ratio (%)
Accessibility to Vineyard Lands	Very difficult	1	0,96
	Difficult	20	19,23
	Middle	33	31,73
	Easy	43	41,35
	Very easy	7	6,73
Total			100,00
Availability of Bus Line	Available	-	-
	Not available	27	100,00
Total			100,00
Availability of Minibus Line	Available	11	40,74
	Not available	16	59,26
Total			100,00
Presence of Tour Companies	Available	4	14,81
	Not available	23	85,19
Total			100,00

While 24 (23%) of 104 producers stated that their residences could be suitable for visitors, 80 (77%) stated that theirs were not (Table 3.4). Considering the ownership status of the houses where the surveyed producers live, 95% of them are homeowners. Manufacturers' houses are predominantly of the single-story or two-story residence types with a garden, with a rate of 79%. 37% of the manufacturers have cars, too (Table 3.5).

Table 3.4. Availability of Business-Owned Homes for Visitors

	Frequency (n)	Ratio (%)
Available	24	23,08
Not available	80	76,92
Total		100,00

Table 3.5. Infrastructure and Facility Features of Viticulture Enterprises

	Categories	Frequency (n)	Ratio (%)
Ownership Status of the Houses of the Farmers	Landlord	99	95,19
	Leaseholder	5	4,81
	Total		100
Type of House	One-Storey or Two-Storey House with Garden	82	78,85
	Garden Apartment (up to 5 floors)	7	6,73
	Multi-Storey Apartment with Very Spacious Garden	3	2,88
	Buildings	1	0,96
	Multi-Storey or Block Apartment Without Garden	11	10,58
	Total		100
Private Car Asset	Yes	38	36,54
	No	66	63,46
	Total		100
On-premises Accommodation Status	Yes	7	6,73
	No	97	93,27
	Total		100
Eligibility Status of Manufacturers' Out-of-Business Homes	Yes	17	17,53
	No	80	82,47
	Total		100

When the in-house accommodation opportunity is evaluated in the vineyard areas, the rate of lands with accommodation within the enterprise is 7% among the participants. When the producers who do not have accommodation in the enterprise were asked whether they could host those who want to visit in their own homes outside the enterprise, 18% stated that they could host guests (Table 3.5).

Twenty-four of the producers (23%) answered positively about the suitability of the residences for the guests, and these 24 producers were asked whether there is a separate kitchen for the guests in the houses belonging to the enterprise, and 87.5% of the producers answered "No." 96% (25) of the residences belonging to the enterprise have reinforced concrete buildings, and 4% have prefabricated residences. Considering the toilet conditions of these dwellings, we have observed that 58% are indoors and 37.5% are outdoor toilets, and the rate of those who own both is 4%. When the availability of the internet in the houses of the producers is evaluated, 58% of the producers stated that they do not have the internet at home, and 42% stated that they have the internet at home (Table 3.6).

Table 3.6. Structural Status of Houses Owned by Farms (24 Farms)

	Categories	**Frequency (n)**	**Ratio (%)**
Situation of Separate Kitchen for Guests in Houses Owned by the Business	Yes	3	12,50
	No	21	87,50
Total			100,00
Housing Information	Reinforced concrete	23	95,83
	Prefabricated	1	4,17
Total			100,00
Toilet Facilities	Inside	14	58,33
	Outside	9	37,50
	Both of them	1	4,17
Total			100,00
Wireless Internet	Yes	10	41,67
	No	14	58,33
Total			100,00
Electricity Supply	Electricity Grid	22	91,67
	Solar energy	2	8,33
Total			100,00
Heating information	Soba	20	83,33
	Doğalgaz Sobası	4	16,67
Total			100,00
Hot Water Condition	Available	20	83,33
	Not Available	4	16,67
Total			100,00

In terms of electricity source, the majority of the producers, 92%, stated that they benefited from mains power in their residences, and 8% said that they benefited from solar power. Regarding heating, 83% of the producers stated that they use stoves and 17% stated that they use natural gas stoves. When the hot water situation is evaluated, four producers who have houses in the vineyard stated that there is no hot water in the houses within the enterprise, and in general, 83% of them stated that there is hot water at their private houses (Table 3.6).

4. RESULTS AND DISCUSSION

Considering some of the parameters required for tourism in the research area, the features such as being suitable for swimming, being surrounded by natural attractions (forest ecosystem, etc.,), hosting historical wineries and museums reveal the strength of the region. In addition to these, being close to a big metropolitan city like Istanbul is another factor that reveals the strength of the enterprises in terms of tourism potential.

When a general evaluation of the research findings is made, 79% of the producers participating in the survey are between 51 and 66 years old, with an average age of 55. This may cause manufacturers to abstain from innovations and their eagerness to take risks may be low. The low level of education of the producers is effective on their communication skills, but it can also be effective on the perspectives and perception levels of the producers. However, in the survey, it was determined that the average annual gross income of the producers was 34 thousand TL. When all these situations are evaluated, it is not easy for producers to accept new approaches in agriculture in the short term.

Foreign language knowledge is an important advantage in tourism activities. However, only a few of the people in this sector can speak a foreign language. This fact is directly related to the low level of education in the agricultural sector. In the study, the number of individuals who know at least one foreign language in the households is relatively low.

Producers stated in the interviews that they are engaged in farming because of their high age and that they do not have enough financial power to start a new business. The producers have stated that they do not want their children to deal with farming. This situation may harm the sustainability of viticulture enterprises in the end. Young people are essential for agricultural tourism activities, so people in this field need to figure out solutions for young people to stay in the countryside.

It has been determined that there are problems in transportation in general in the 27 neighborhoods where the survey was conducted. When evaluated in this context, the lack of regular minibus/bus lines in the neighborhoods limits the accessibility of businesses.

It has been stated that the houses belonging to viticulture enterprises are not suitable for touristic purposes. The fact that the houses have insufficient features within the standard of living, the thought of embarrassing the tourists, the high average age, etc., cause the producers to abstain from visits requiring accommodation.

79% of the producers participating in the survey have a positive approach towards agricultural tourism activities. However, it is seen that there are problems and deficiencies in the structural suitability of businesses and the neighborhoods they are affiliated with for agricultural tourism activities. Considering the data obtained from the surveys and interviews conducted in the field, these deficiencies can be listed as follows:

- ✓ Problems related to transportation and communication infrastructures,
- ✓ Problems related to water resources and related infrastructure deficiencies,
- ✓ Inadequate accommodation facilities,
- ✓ The high average age of the producers, (It restricts the potential of physical strength to be compatible with agricultural tourism activities)
- ✓ The low level of knowledge of the producers on agricultural tourism,
- ✓ The scarcity of individuals with foreign language knowledge in the household,
- ✓ The lands are fragmented, and their accessibility is difficult,
- ✓ Problems like the lack of individual bathrooms in the neighborhoods can be listed.

With the agricultural tourism activities to be carried out in the study area, additional income opportunities will be created for viticulture enterprises in addition to their current production, and positive effects on the young population will be created by increasing the attractiveness of rural areas. This branch of tourism will also have positive effects on the consciousness levels of consumers.

Development breakthroughs in rural areas cannot be considered independent of ecology, social structure and economic factors. In this context, agricultural tourism activities emerge as an alternative tourism branch for the effective use of agricultural lands, which are considered natural resources, and for improving the living standards of rural producers.

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EVALUATION OF SOME ANNUAL RYEGRASS CULTIVARS IN GERMINATION AND EARLY SEEDLING STAGE UNDER DIFFERENT SALINITY LEVELS

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ABSTRACT

In recent years, salinity problems have started to emerge in the fields where agricultural production has been made for centuries. It is not possible to rehabilitate these areas in the short term. Therefore, cultivation of salinity-resistant plant resources in these fields is very significant for economic production. This study was carried out to determine the tolerances of some annual ryegrass (*Lolium moltiflorium* Lam.) cultivars (cv. Braulio, cv. Devis, cv. Hellen, cv. Trinova) in germination and early seedling stage under different salinity levels (Kontrol, 50, 100, 150 and 200 mM). In this research, germination ratio (GR), germination index (GI) and mean germination time (day) for germination traits and root length, shoot length and fresh weight (mg plant^{-1}) for early seedling stage traits were investigated. The experiment was laid out acoording to the factorial design (cultivar and salinity level) in randomized parcel with 4 replications and conducted in the climatic chamber conditions in the laboratory. According to the variance analysis, effect of cultivars (C), salinity levels (S) and C×S on germination ratio, germination index, mean germination time and root length was statistically significant while effect of cultivars on shoot length and fresh weight was not significant. Germination ratio of cultivars decreased as the salinity level increased. Germination ratios of Braulio and Hellen was higher than other cultivars. In addition, when the germination index and mean germination time was evaluated, cv Hellen gave the best result. Germination ratios were found to be higher at control, 50 and 100 mM salinity levels compared to 150 and 200 mM and they were statistically similar to each other. As the salinity level increased, germination index and mean germination time of cultivars increased. In addition, fresh weight and root and shoot length decreased as the salinity level increased. As a result, when the resistance of the cultivars against salt stress was fully evaluated, cultivars used in this study can tolerate 50 and 100 mM salinity levels. However, cultivars were sensitive to 150 and 200 mM salinity levels. Among the cultivars, Braulio and Hellen cultivars were found to be more tolerant to salt stress than other cultivars. It was determined that Braulio and Hellen cultivars would give good results in annual ryegrass cultivation in regions where irrigation water and/or soil have salinity troubles.

Key words: Germination, Early seedling stage, Salt stress, Annual ryegrass, *Lolium moltiflorium* Lam.

INTRODUCTION

It is reported that annual ryegrass (*Lolium multiflorium* Lam.) included in the forage cereal crops has an important potential for roughage production. (Açıkgöz, 2001). Although the annual ryegrass is one of the oldest cultivated plants, its cultivation has become widespread in recent years with the incentives given to forage plants in our country. This plant was planted on an area of approximately 25,331 ha in Turkey in 2020 and about 972000 tons of fresh forage was obtained. (Anonim, 2020).

Abiotic stress conditions are one of the factors that slow down or completely stop growth and development in most plants (Keles and Oncel, 2002; Ertekin et al., 2017; Ertekin et al., 2018; Ertekin et al., 2020; Ertekin and Bilgen, 2021). Environmental stress conditions such as salinity problems can negatively affect plant germination and seedling growth (McMaster and Wilhelm, 2003). Today, agricultural lands in the world are faced with salinity problem due to wrong irrigation methods, excessive use of agricultural inputs and the effect of global climate change (Akgül, 2003).

Responses of each plant species and different varieties within the same species to salt stress differ (Almansouri et al., 2001). The high level of salt in the soil or irrigation water causes an increase in the osmotic pressure in the root zone during the germination and first seedling development stage, so the water intake of the plant is prevented during the germination and seedling development stage (Essa, 2002; Sadeghian and Yavari, 2004). In some cases, the salt concentration in the germination medium can have a lethal effect (Ertekin et al., 2017).

This study was carried out to investigate the germination and early seedling development responses of some annual ryegrass cultivars widely cultivated as forage crop to different salt doses.

MATERIAL AND METHODS

In this study, 3 annual ryegrass cultivars (Hellen, Trinova, Devis) were used as plant material. These varieties were obtained from a commercial company.

This study was carried out in the laboratories of Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops. Before starting the germination stage, the seeds of the cultivars were surface sterilizing. For this purpose, the seeds belonging to each cultivar were kept separately in 2% sodium hypochlorite (NaClO) solution for 5 minutes in the shaker, and then the chemical residue on the surface was removed by rinsing with sterile distilled water 3 times (Bilgili et al., 2011). The sterilized seeds were placed to 50 Petri dishes (90 mm×15 mm) containing double-layer sterile coarse filter paper, which were also sterilized. Salt doses of 50, 100, 150 and 200 mM were prepared to create salt stress in the germination medium. In addition, sterile distilled water was used for kontrol application (without salt). Seed sowing and salt doses were applied to Petri dishes under a sterile cabinet to prevent contamination with air. 10 mL salt dose and control were applied to all Petri dishes (5 salt doses×4 cultivars×4 replications=80), and Petri dishes were closed with parafilm in order to preserve the moisture in the Petri dishes. Petri dishes were placed to the air-conditioning cabinet and left for germination and first seedling development for 10 days at 25 °C, 70% relative humidity, 8/16 day/night light rhythm. Germinated seeds were counted and recorded every day for 7 days. At the end of 10 days, Petri dishes were opened and root and shoot length were measured in 10 randomly selected seedlings. In addition, seedling fresh weights were weighed in 10 seedlings. Based on the number of germinated seeds recorded for each day, the germination ratio (GR), germination index (GI) and mean germination time (MGT) were calculated.

Germination Ratio %=(Number of germinated seeds / Total number of seeds)*100

Germination index= $\Sigma(Gt/Tt)$

Mean Germination Time= $\Sigma(fx)/\Sigma f$

Gt: number of seeds germinated on day t

Tt: number of days until day t

f: number of germinated seeds on count day

x: number of days counted

All the data obtained from the study were subjected to ANOVA test according to the factorial experiment design model in the JMP statistical program and TUKEY pairwise test was applied to the features found to be important.

RESULTS AND DISCUSSION

Significance levels and mean values with its results of comparison test of all investigated traits for germination and early seedling stages of annual ryegrass were given in Table 1. Cultivars affected statistically the GR, GI, MGT and SL but not the RL and SFW. GR values changed between 65.30% and 97.60% among the cultivars. The highest GR was determined in cv. Braulio and Hellen while the lowest was detected in cv. Devis. Many researchers have emphasized that germination rates in different plants affected by salt stress are reduced by the increase in salt dose (Day et al., 2008; Budakli-Carpici et al., 2009; Akhtar and Hussain, 2009; Dai et al., 2009; Çaçan and Kökten, 2014; Ertekin et al., 2017; Ertekin et al., 2018; Kızılşimşek and Süren, 2020). GI values ranged from 11.12 to 19.51 among the cultivars and cv. Hellen had the highest GI whereas cv. Devis revealed the lowest value. MGT values were between 2.72 and 3.30 day among the cultivars.

Table 1. Effects of cultivars and salt doses on some germination and early seedling stage traits of annual ryegrass

Cultivars	GR% ±SE	GI ±SE	MGT(day) ±SE	SL(mm) ±SE	RL(mm) ±SE	SFW (g/plant) ±SE
Braulio	$97.60±0.64^{a}$	$17.16±0.35^{b}$	$2.99±0.05^{b}$	$65.70±3.48^{b}$	73.44±4.05	0.04±0.00
Devis	$65.30±2.11^{c}$	$11.12±0.14^{d}$	$3.30±0.14^{a}$	$68.33±3.46^{b}$	70.14±4.50	0.03±0.00
Hellen	$97.60±0.43^{a}$	$19.51±0.14^{a}$	$2.72±0.14^{c}$	$75.95±4.26^{a}$	74.36±4.26	0.04±0.00
Trinova	$92.90±1.18^{b}$	$14.83±0.10^{c}$	$3.30±0.10^{a}$	$68.12±4.53^{b}$	72.59±4.81	0.03±0.00
p values	**	**	**	**	ns	ns
Doses (mM)						
Kontrol	$91.00±2.88^{a}$	$18.06±1.06^{a}$	$2.71±0.11^{d}$	$85.08±1.49^{a}$	$92.63±3.16^{a}$	$0.04±0.00^{a}$
50	$90.13±3.05^{a}$	$17.79±0.87^{a}$	$2.68±0.07^{d}$	$86.94±1.87^{a}$	$84.25±3.17^{b}$	$0.04±0.00^{a}$
100	$90.75±2.82^{a}$	$16.32±0.77^{b}$	$2.96±0.08^{c}$	$74.02±2.06^{b}$	$80.78±1.07^{b}$	$0.03±0.00^{b}$
150	$86.13±4.44^{b}$	$14.48±0.95^{c}$	$3.21±0.09^{b}$	$58.13±1.17^{c}$	$62.09±1.93^{c}$	$0.03±0.00^{b}$
200	$83.74±4.73^{b}$	$11.61±0.82^{d}$	$3.83±0.11^{a}$	$43.45±1.17^{d}$	$43.41±2.14^{d}$	$0.02±0.00^{c}$
p values	**	**	**	**	**	**
p $values^{int}$	**	***	**	**	**	ns

GR: Germination ratio; GI: Germination index; MGT: Mean germination time; SL: Shoot length; RL: Root length; SFW: Seedling fresh weight

$^{a-d}$Mean values given with different superscripts are statistically significant from each other. ns: not significant

$^{**}p<0.01$; $^{***}p<0.001$

The longest MGT was obtained from cv. Devis and cv. Trinova. The shortest MGT was recorded in cv. Hellen. SL values among the cultivars were ranged from 65.70 to 75.95 mm. The highest SL was obtained from cv. Hellen whereas the lowest value was detected in cv. Braulio. Some researchers reported that GI and SL of some plant species and/or cultivars decreased while MGT increased as the salt concentration increased in the germination field (Duan et al., 2004; Kusvuran, 2015).

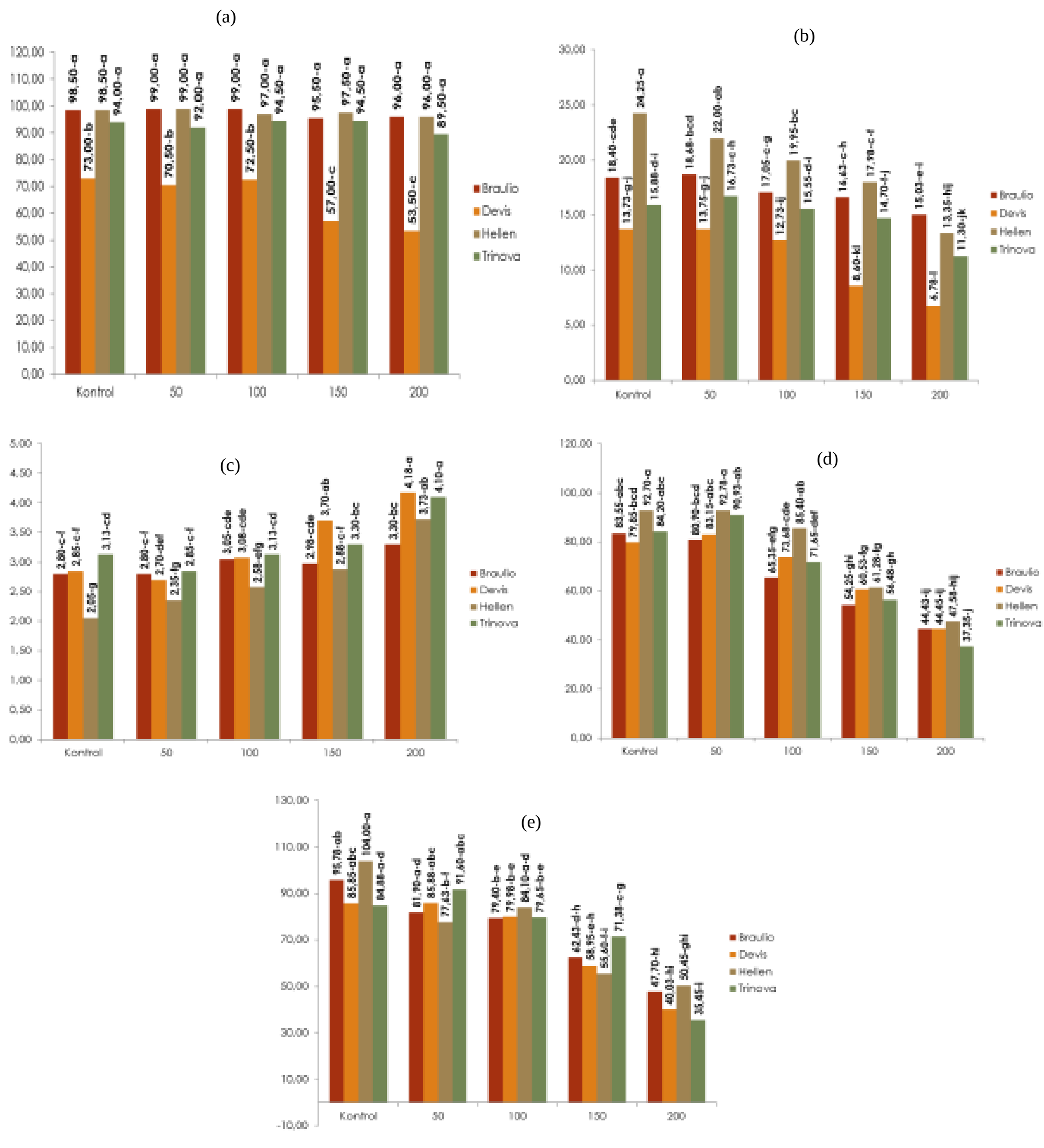

Figure 1. Values of GR, GI, MGT, SL and RL parameters affected from Cultivars×Dose interactions
(a) GR; (b) GI; (c) MGT; (d) SL; (e) RL

Salt doses affected significantly germination and early seedling parameters of annual ryegrass (Table 1). GR and GI values of doses ranged from 83.74 to 91.00% and from 14.48 to 18.06, respectively. As the doses increased the GR and GI values generally decreased. MGT among the doses changed between 2.68 and 3.83 day. After the 50 mM doses treated, as the salt dose increased MGT prolonged. SL and RL values was determined between 43.45 and 86.94 mm and 43.41 and 92.63 mm, respectively. As the salt doses increased, SL and RL was restricted. SFW values ranged from 0.02 to 0.04 g/plant. As the salt doses increased, RFW values decreased. In many studies on salt stress, it has been reported that as the doses of salt increases, germination and seedling growth of different plants are adversely affected (Day and Uzun, 2016; Ertekin et al., 2017; Ertekin et al., 2018; Kızılşimşek ve Süren, 2020).

Cultivars×Dose interactions affected significantly the all traits except for RFW (Table 1). GR values of interactions ranged from 53.30% to 99.00% (Figure 1). GR values of Devis affected from salt stress was lower than others. GI values changed between 6.78 and 24.25. The highest GI was determined in cv. Hellen×Kontrol interaction while the lowest value was detected cv. Devis×200 mM interaction. MGT values was found between 2.05 day and 4.18 day. MGT of annual ryegrass cultivars under salt stress prolonged as the salt concentration increased in the germination area. SL and RL values was recorded between 37.35 mm and 92.78 and 35.45 mm and 104.00, respectively. SL and RL values of annual ryegrass cultivars decreased as the salt concentration increased. Okçu et al. (2005), Carpıcı et al. (2009), Ertekin et al. (2017) and Ertekin et al. (2018) reported the similar results to this result.

CONCLUSIONS

As a result, when the resistance of the cultivars to salt stress is evaluated, the cultivars used can tolerate salinity levels of 50 and 100 mM. However, cultivars were found to be sensitive to salinity levels of 150 and 200 mM. It was determined that Braulio and Hellen cultivars were more resistant to salt stress than other cultivars. It has been determined that Braulio and Hellen varieties will give good results in annual ryegrass cultivation in regions with irrigation water and/or soil salinity problems.

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IMPROVING POSTHARVEST STORABILITY OF "CYPRUS LEMONS" BY THE APPLICATION OF OZONE, ROSEMARY OIL, STRAW COVERAGE AND/OR/WITH MODIFIED ATMOSPHERE PACKAGING

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ABSTRACT

This research was conducted to investigate the influence of modified atmosphere packaging, ozone treatment, rosemary oil application, wrapping in straw and some of their combinations on the postharvest storage of Cyprus local lemons (*Citrus lemon* L. Burman f.). Lemon fruits were collected at the beginning of the green-yellow stage on the 1st of November. This experiment consisted of fourteen different treatments and was planned to continue for 210 days (7 months). The measurement points were defined as 30 d, 60 d, 90 d, 120 d, 150 d, 180 d and 210 d (7 different measurement points). The results of the current study showed that all tested applications have an important effect on the fruit weight, visual quality, decay incidence, fruit firmness, vitamin C content and fruit colour. According to the results obtained, modified atmosphere packaging alone, or in combination with ozone, fungicide, rosemary oil (0.2%) or rosemary oil (1.0%) treatments were effective in protecting the postharvest quality and marketability of the lemons. All of those treatments were also found to provide around 13-16% weight loss, 2.6-3.2 visual quality score, 0.6-1.0 score of decay incidence, 0.46-0.48 kg cm-2 fruit firmness, 10.20-10.88% soluble solids concentration, 6.51-7.54 g 100 g-1 citric acid titratable acidity, and 44.88-52.99 g 100 g-1 vitamin C in 210 days of storage. Overall, results suggest that those treatments can be used to store lemon fruits for 210 days in the cold room with acceptable marketing quality.

Keywords: *Lemon colour, weight loss, titratable acidity, fruit decay, fruit quality*

INTRODUCTION

Lemon (Citrus limon L. Burman f.) fruits are rarely consumed as fresh fruit, however lemon juice is widely added to desserts, salads and other foods to enhance the flavour (Ladaniya, 2008). Lemon fruits are rich in phenolics, citric acid and ascorbic acid (vitamin C) and other nutrients, which accounts for it health benefits in preventing obesity, diabetes, cardiovascular disorders, some cancers and provides antibacterial and antiviral properties (Aruoma et al., 2012). Total lemon production (with limes) in the world was estimated to be around 19.4 million tons, on nearly 1.3 million ha area in 2018. According to FAO (2020), lemon production has been continuously increasing since 1961. Lemons are being produced on around 312.5 ha of land which represents 6.2% of citrus production in Turkish Republic of Northern Cyprus (TRNC) (TRNC MoA, 2020). Cyprus Lemon, Eureka, Enterdonat, Mayer and Verna are the important varieties being produced in TRNC and harvest starts mainly in January. In the current situation, lemons can be stored for 1 month, with little quality problem. However, after long storage, around half of the lemons are being lost; and rest, with poor quality are being sold at very high prices. Reducing postharvest losses is known to have positive effects on the number & amount of products presented to the market, and made it possible to reach fruits throughout the year, and to maintain the selling prices at an acceptable level.

Lemon fruits are very sensitive to postharvest storage due to respiration, transpiration and microbial decay (induced by *Penicillium italicum* Wehmer & *Penicillium digitatum* (Pers.: Fr.) Sacc.) (D'Aquino et al., 2017). Cold storage at 7-12 °C and 85-95% relative humidity are recommended to reduce respiration and transpiration (Arpaia and Kader, 2000) and fungicide (i.e.: imazalil, propiconazole and thiabendazole) application is suggested to control pathogenic decay at the lemon fruits (Kinay et al., 2007). However, there is an increasing public concern about the use of fungicides which increased the need for controlling postharvest decay in environmentally friendly methods (Feliziani and Romanazzi, 2013).

Numerous researches have been carried out about the efficacy of edible coatings (Chen et al., 2018; Chen et al., 2019) and plants essential oils (Kahramanoğlu et al., 2019; Kahramanoğlu and Usanmaz, 2019a; Chen et al., 2019) on the postharvest storability of different fruits and vegetables. Valuable results of those studies suggested that the edible coatings and essential oils reduce weight loss (by reducing respiration and transpiration) and provides slightly to high control of different postharvest pathogens. The combination of rosemary oil with thyme oil was also noted to provide a synergic effect on microbial growth (Nikkah and Hashemi, 2020). On the other hand, ozone (O3) fumigation treatment was also reported to have high sanitization potential against a wide range of pathogens (Khadre et al., 2001; Carletti et al., 2013). It is commercially used and was noted as GRAS (generally recognized as safe) for food handling. Ozone treatment was also noted to enhance the synthesis of some phenolic compounds in different fruits and vegetable (Artés-Hernández et al., 2007; Rodoni et al., 2010; García-Martín et al., 2018; Tabakoglu and Karaca, 2018). Another important technique for the improvement of the postharvest storability of fruits and vegetables is the modified atmosphere packaging (MAP) materials. These materials have the ability to reduce the oxygen concentration in the bags and thus reduce the respiration and improve storability. However, this technique is commodity dependent and despite the numerous successful applications of MAP for improving postharvest storability of several fruits and vegetables (Caleb et al., 2013; Kahramanoğlu, 2017; Kahramanoğlu et al., 2019), the number of studies with lemons are limited (Sandhya, 2010). Therefore, this research aimed to test the effects of MAP, ozone treatment, rosemary oil application, wrapping in straw and some of their combinations on the postharvest storage of lemon. It was believed that the results would be very beneficial for farmers, consumers and for the country by helping to reach sustainability in lemon production and marketing.

Material and Methods

Materials

Lemon fruits are generally being harvested at three different maturity stages: green, green-yellow and yellow stages. In this study, lemon fruits were collected at the beginning of green-yellow stage on 1st of November. Soluble solids concentration was recorded as 10.36% and the titratable acidity was measured as 7.06% at the harvest. Fruits were collected from a local citrus orchard planted in the Gemikonagi city in Turkish Republic of Northern Cyprus. Fruits were stored under shade during harvest and immediately after harvest (about 1.5 hours) were transferred to the Research Farm of European University of Lefke. MAP bags were received from Aypek & Biopac consortium with a brand of Lifepack® MA/MH. The bags were diversified from the regular MAP bags where they are capable of transferring and regulating the relative humidity inside the fruits. Rosemary (*Rosmarinus officinalis* L.) oil (100% pure) of present study was received from local shops with a brand of Arifoğlu. To generate ozone and use in the present study, a small ozone generator was purchased from the A2Zozone company (via Amazon). The capacity of the equipment is: 600mg h-1. It is mainly used for air, water and food sterilization. The fungicide of present study was selected according to the registered recommendations of the Turkey's Crop Protection Department and at the time of studies there was only one registered fungicide for lemons. This was Fosetyl-Al (450 g-1). Finally, the straw was collected from a local barley field.

Design of the Experiments

This experiment consisted of fourteen different treatments and was planned to continue for 210 days (7 months). The measurement points were defined as 30 d, 60 d, 90 d, 120 d, 150 d, 180 d and 210 d (7 different measurement points). Five replications (each with five fruits) were used for each experiment (#14) for each measurement point (#7). Thus, totally 2450 fruits were used in the storage studies and additional 25 fruits were used at the beginning of the experiments to determine the preliminary quality of the fruits. After treating the lemons with the below mentioned fourteen different applications, the fruits were air-dried for 30 min. Next, the weights of each fruit were measured and noted for further analysis. The fruits were then put in violas orderly, and stored at 9 ± 1 °C and 90-95% relative humidity. The explanations of each treatments are as follows:

- Treatment 1: Control. Lemons were immersed into tap water at ambient temperature conditions (20-22 °C) for 5 min.
- Treatment 2: Ozone treatment. Fruits were dipped into tap water and above-mentioned ozone generator was used to generate ozone into the water for 5 min.
- Treatment 3: Fungicide treatment. The Fosetyl-Al (450 g-1) was used at its recommended dose (500 ml 100 water L-1) and fruits were dipped for 5 min.
- Treatment 4: Modified atmosphere packaging. Fruits were treated with treatment 1 (tap water dipping) and after drying putted into MAP bags (25 fruits per bag).
- Treatment 5: Modified atmosphere packaging + ozone treatment. The treatment 2 and treatment 4 were combined.
- Treatment 6: Modified atmosphere packaging + fungicide treatment. The treatment 3 and treatment 4 were combined.
- Treatment 7: Rosemary oil application (0.2%). Rosemary oil was firstly dissolved in 70% ethanol (20/180 ml v/v) and then added up to 10 L with tap water (Kahramanoğlu et al., 2018). The dipping duration was 5 min.
- Treatment 8: Rosemary oil application (0.2%) + ozone treatment. The solution was prepared according to the method described in treatment 7 and ozone generator was also run into water during fruit dipping.
- Treatment 9: Rosemary oil application (1.0%). Same as treatment 7 but the rosemary oil ratio to 70% ethanol was 100/900 ml v/v.
- Treatment 10: Rosemary oil application (1.0%) + ozone treatment. The solution was prepared according to the method described in treatment 9 and ozone generator was also run into water during fruit dipping.
- Treatment 11: Rosemary oil application (0.2%) + MAP. After treating fruits with RO, as in treatment 7, fruits were put into MAP bags
- Treatment 12: Rosemary oil application (1.0%) + MAP. After treating fruits with RO, as in treatment 9, fruits were put into MAP bags.
- Treatment 13: Fruits were covered with barley straw and stored in cold rooms.
- Treatment 14: Fruits were covered with barley straw and stored under shade in ambient conditions.

Note: Dipping was performed with 10 L of water for 25 fruits for each treatment.

Fruit quality analysis

The experiments were continued for 210 days and 25 fruits from each treatment were taken out with 30-day interval to perform below listed quality analysis. A digital scale (± 0.01 g) was used to measure the initial and final weight of each fruit and then, standard ratio method was used to calculate the weight loss (%). Visual quality of each fruit was individually determined according to the 0-5 scale as recommended by Silvia et al. (2015) as follows. Decay incidence (DI) of each fruit, caused by *P. italicum* or *P. digitatum*, was determined according to the 0-4 scale recommended by Cao et al. (2011). The fruit firmness (kg cm-2) of each lemon fruit was measured with a hand penetrometer. For each fruit, four distinct locations around the fruit centre were used to measure the fruit firmness and the average was used in the calculations. Soluble solids concentration (SSC) of each fruit was measured with a hand refractometer. Titratable acidity of each fruit was determined according to the formula of AOAC (1990). Vitamin C (VC) content was assessed by titration with iodine solution (Skinner, 1997). And finally, the fruit colour of the lemons was assessed according to the method described by Kahramanoğlu and Usanmaz (2019b).

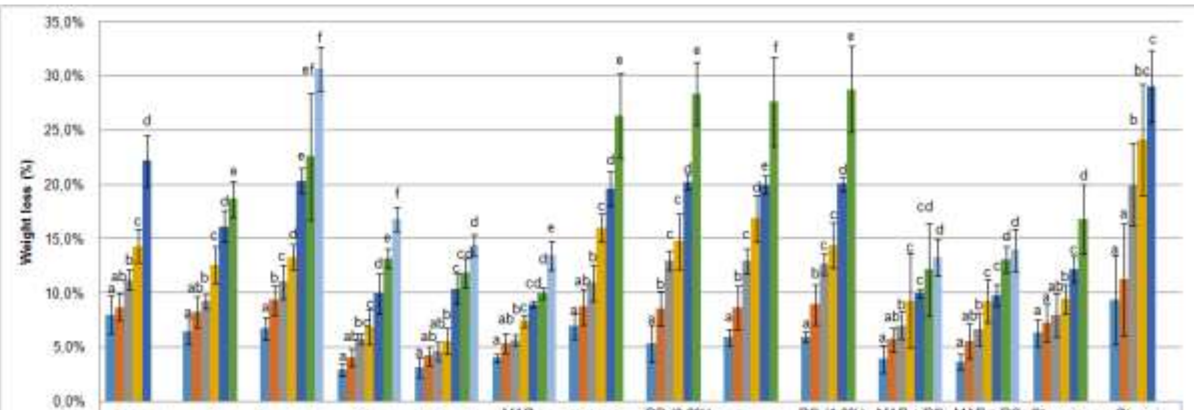
Data analysis

The means and standard deviation of each treatment was calculated with the Microsoft Office Excel 2007 from the raw data and the results were then used to create figures. The raw data of the experiments were then subjected to the analysis of variance (ANOVA) to determine any statistical differences and mean separations were assessed via Tukey's HSD test ($P < 0.05$) by using SPSS 22.0.

Results

Effects of treatments on the weight loss

During the 210 days of storage, weight loss increased at all groups of fruits, where some of the treatments were effective in reducing the weight loss (Figure 1.).



	Control	O3	Fungicide	MAP	MAP + O3	MAP + Fungicide	RO (0.2%)	RO (0.2%) + O3	RO (1.0%)	RO (1.0%) + O3	MAP + RO (0.2%)	MAP + RO (1.0%)	Straw in s room	Straw in open air
30 d	8,0% ef	6,4% cde	6,7% def	2,9% a	3,2% ab	4,0% abc	6,9% def	5,4% bcd	5,9% bcd	6,0% bcd	3,9% abc	3,7% abc	6,3% cde	9,4% f
60 d	8,7% cd	8,3% bcd	9,3% cd	4,1% a	4,2% ab	5,4% abc	8,7% cd	8,6% bcd	8,7% cd	8,9% cd	5,7% abc	5,6% abc	7,2% bcd	11,3% d
90 d	11,2% cd	9,2% bc	11,0% cd	5,8% a	4,6% a	5,7% a	10,9% cd	13,0% d	13,0% d	12,7% d	7,0% ab	6,7% ab	8,0% abc	20,0% e
120 d	14,3% cd	12,6% bc	13,3% cd	6,9% a	5,5% a	7,4% a	16,0% d	14,8% cd	16,8% d	14,4% cd	9,3% ab	9,3% ab	9,4% abc	24,1% e
150 d	22,2% c	16,1% b	20,4% c	9,9% a	10,4% a	9,0% a	19,6% c	20,2% c	20,0% c	20,1% c	10,0% a	9,7% a	12,2% a	29,0% d
180 d		18,7% bc	22,6% cd	13,2% ab	11,8% ab	10,0% a	26,3% d	28,4% d	27,6% d	28,8% d	12,2% ab	13,1% ab	16,8% abc	
210 d			30,6% c	16,8% b	14,4% ab	13,4% a					13,3% a	13,9% ab		

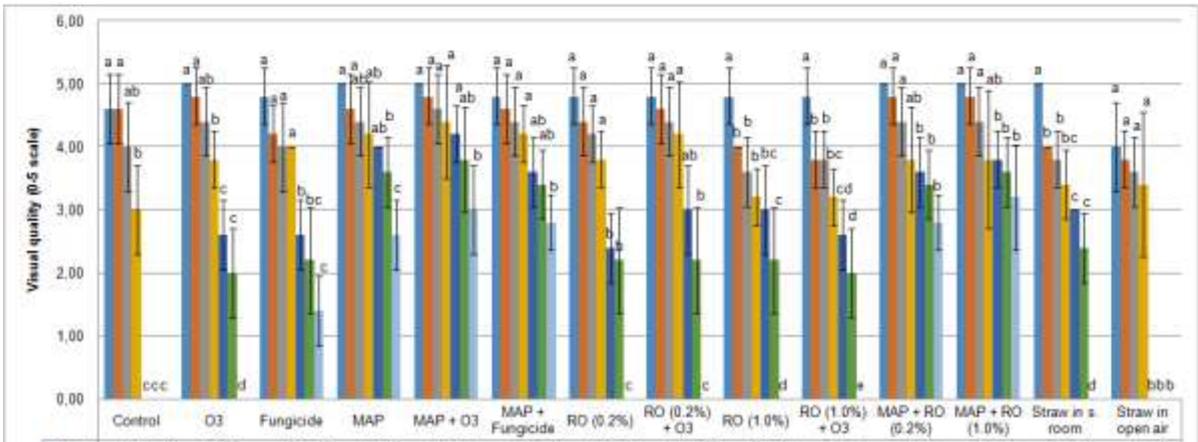
Figure 1. Weight loss of the lemon fruits as affected by the different treatments during 210 days of storage. (Letters on the columns are used to compare the means of measurement points for each treatment separately; and the letters in the table below the figure are used to compare the means of different treatments separately at each measurement point. At both conditions, different letters used to show scientific differences at $p \leq 0.05$ level according to Tukey's HSD).

In all groups, fruits were healthy until 150 days of storage. However, fruits of control treatment and straw in open air decayed all after 150 days of storage. Some of other treatments (O3, RO 0.2%, RO 0.2% + O3, RO 1.0%, RO 1.0% + O3 and straw in cold room) were also not effective after 180 days of storage. It was clear from the results that the most successful treatments for reducing the weight loss are all the treatments with modified atmosphere packaging. After 150 days of storage, the highest weight loss was noted from the control treatment as 22.2% and the least weight loss was 9.0% which was from the fruits treated with MAP and Fungicide. A similar trend was continued till the end and the least weight loss was noted from MAP +

RO (0.2%) and MAP + Fungicide treatments, as 13.3% and 13.4%, respectively. The combination of MAP with O3 was also effective in preventing weight loss and protecting fruit quality.

Effects of treatments on the fruit visual quality

The visual quality results of current research are in agreement with the weight loss results. It was clear from the results (Figure 2.) that the control fruits can only be stored for 120 days. The fruits covered with strow straw and stored in open air had similar results with the control fruits. On the other hand, the fruits in MAP were found to have higher visual quality scores, even in 210 days of storage. At the end of the storage period (210 days), the fruits of two groups were found to have visual quality scores of higher than 3.0. these treatments are MAP + O3 and MAP + RO (1.0%). Additionally, the MAP + Fungicide and MAP + RO (0.2%) treatment was found to have a score of 2.8. Overall, results suggest that the MAP, O3 and RO treatments, alone or in combination are effective in maintaining visual quality of lemon fruits.

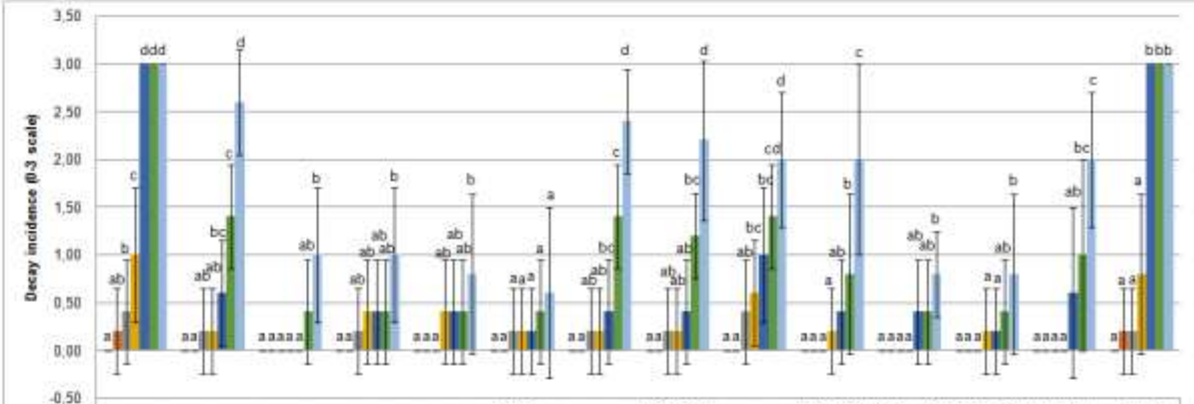


	Control	O3	Fungicide	MAP	MAP + O3	MAP + Fungicide	RO (0.2%)	RO (0.2%) + O3	RO (1.0%)	RO (1.0%) + O3	MAP + RO (0.2%)	MAP + RO (1.0%)	Straw in s. room	Straw in open air
30 d	4,60 ab	5,00 a	4,80 ab	5,00 a	5,00 a	4,80 ab	4,80 ab	4,80 ab	4,80 ab	4,80 ab	5,00 a	5,00 a	5,00 a	4,00 b
60 d	4,60 a	4,80 a	4,20 a	4,60 a	4,80 a	4,60 a	4,40 a	4,60 a	4,00 a	3,80 a	4,80 a	4,80 a	4,00 a	3,80 a
90 d	4,00 a	4,40 a	4,00 a	4,40 a	4,60 a	4,40 a	4,20 a	4,40 a	3,60 a	3,80 a	4,40 a	4,40 a	3,80 a	3,60 a
120 d	3,00 b	3,80 a	4,00 a	4,20 a	4,40 a	4,20 a	3,80 a	4,20 a	3,20 a	3,20 a	3,80 a	3,80 a	3,40 a	3,40 a
150 d	0,00 d	2,60 bc	2,60 bc	4,00 a	4,20 a	3,60 ab	2,40 c	3,00 abc	3,00 abc	2,60 bc	3,60 ab	3,80 ab	3,00 abc	0,00 d
180 d	0,00 d	2,00 c	2,20 bc	3,60 ab	3,80 a	3,40 abc	2,20 bc	2,20 bc	2,20 bc	2,00 c	3,40 abc	3,60 ab	2,40 abc	0,00 d
210 d	0,00 c	0,00 c	1,40 b	2,60 a	3,00 a	2,80 a	0,00 c	0,00 c	0,00 c	0,00 c	2,80 a	3,20 a	0,00 c	0,00 c

Figure 2. Visual quality scores of the lemon fruits as affected by the different treatments during 210 days of storage. (Letters on the columns are used to compare the means of measurement points for each treatment separately; and the letters in the table below the figure are used to compare the means of different treatments separately at each measurement point. At both conditions, different letters used to show scientific differences at $p \leq 0.05$ level according to Tukey's HSD).

Effects of treatments on the decay incidence

The decay incidence findings are in agreement with the weight loss and visual quality scores. The highest DI scores were noted from the control fruits and the straw in open air treatments (Figure 3.). As expected, the highest effect was noted from the Fungicide treatment and was followed by the MAP + Fungicide treatment. On the other hand, the O3 and RO treatments were also found to be effective in preventing DI.

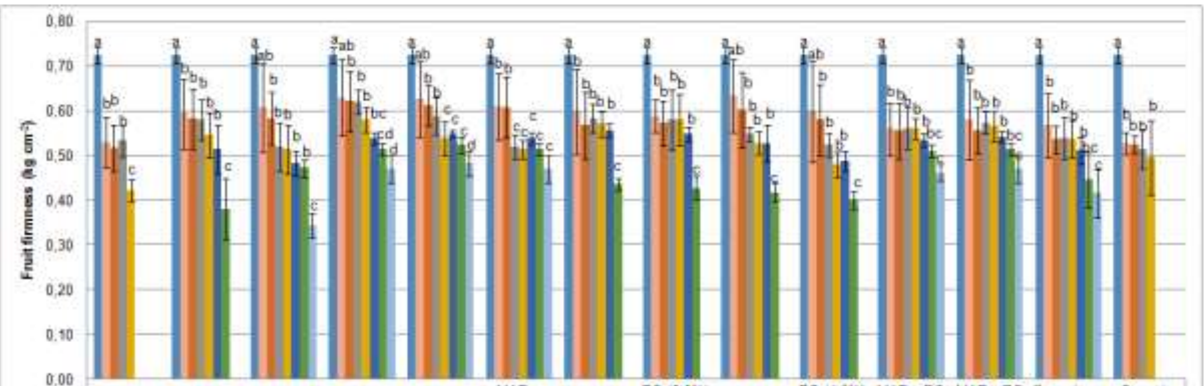


	Control	O3	Fungicide	MAP	MAP + O3	MAP + Fungicide	RO (0.2%)	RO (0.2%) + O3	RO (1.0%)	RO (1.0%) + O3	MAP + RO (0.2%)	MAP + RO (1.0%)	Straw in s. room	Straw in open air
30 d	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a
60 d	0,20 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,00 a	0,20 a
90 d	0,40 a	0,20 a	0,00 a	0,20 a	0,00 a	0,20 a	0,20 a	0,20 a	0,40 a	0,00 a	0,00 a	0,00 a	0,00 a	0,20 a
120 d	1,00 a	0,20 a	0,00 a	0,40 a	0,40 a	0,20 a	0,20 a	0,20 a	0,60 a	0,20 a	0,00 a	0,20 a	0,00 a	0,80 a
150 d	3,00 b	0,60 a	0,00 a	0,40 a	0,40 a	0,20 a	0,40 a	0,40 a	1,00 ab	0,40 a	0,40 a	0,20 a	0,60 a	3,00 b
180 d	3,00 b	1,40 ab	0,40 a	0,40 a	0,40 a	0,40 a	1,40 ab	1,20 ab	1,40 ab	0,80 a	0,40 a	0,40 a	1,00 a	3,00 b
210 d	3,00 d	2,60 d	1,00 ab	1,00 ab	0,80 a	0,60 ab	2,40 cd	2,20 bcd	2,00 bc	2,00 bc	0,80 a	0,80 a	2,00 bc	3,00 d

Figure 3. Decay incidence scores of the lemon fruits as affected by the different treatments during 210 days of storage. (Letters on the columns are used to compare the means of measurement points for each treatment separately; and the letters in the table below the figure are used to compare the means of different treatments separately at each measurement point. At both conditions, different letters used to show scientific differences at $p \leq 0.05$ level according to Tukey's HSD).

Effects of treatments on the fruit firmness

Fruit firmness of lemon fruits was observed to have a decreasing trend during storage (Figure 4.).



	Control	O3	Fungicide	MAP	MAP + O3	MAP + Fungicide	RO (0.2%)	RO (0.2%) + O3	RO (1.0%)	RO (1.0%) + O3	MAP + RO (0.2%)	MAP + RO (1.0%)	Straw in s. room	Straw in open air
0 d	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a	0,72 a
30 d	0,53 a	0,59 a	0,61 a	0,63 a	0,63 a	0,61 a	0,60 a	0,59 a	0,63 a	0,60 a	0,56 a	0,58 a	0,57 a	0,53 a
60 d	0,52 a	0,58 a	0,58 a	0,62 a	0,61 a	0,61 a	0,57 a	0,57 a	0,60 a	0,58 a	0,56 a	0,56 a	0,54 a	0,52 a
90 d	0,53 b	0,58 ab	0,52 b	0,62 a	0,59 ab	0,52 b	0,58 ab	0,58 ab	0,55 ab	0,52 ab	0,56 ab	0,57 ab	0,54 ab	0,51 b
120 d	0,42 c	0,55 ab	0,51 abc	0,58 a	0,54 ab	0,51 abc	0,57 ab	0,58 a	0,53 ab	0,48 bc	0,56 ab	0,57 ab	0,54 ab	0,50 bc
150 d		0,51 abc	0,48 c	0,54 abc	0,55 a	0,54 abc	0,56 a	0,55 a	0,53 abc	0,49 bc	0,53 abc	0,54 ab	0,51 abc	
180 d		0,38 c	0,47 ab	0,51 a	0,52 a	0,51 a	0,44 bc	0,43 bc	0,42 bc	0,40 c	0,51 ab	0,51 ab	0,45 abc	
210 d			0,34 b	0,47 a	0,48 a	0,47 a					0,46 a	0,47 a	0,42 a	

Figure 4. Fruit firmness of the lemon fruits as affected by the different treatments during 210 days of storage. (Letters on the columns are used to compare the means of measurement points for each treatment separately; and the letters in the table below the figure are used to compare the means of different treatments separately at each measurement point. At both conditions, different letters used to show scientific differences at $p \leq 0.05$ level according to Tukey's HSD).

The initial fruit firmness was 0.72 kg cm^{-2} and it decreased to 0.42 kg cm^{-2} at the control fruits in 150 days of storage. At the same time, the fruit firmness was noted as 0.58 kg cm^{-2} at the fruits stored in MAP. The combination of MAP with O3 or RO was also noted as successful in preventing the loss of fruit firmness. Results also suggested that Fungicide application and straw covering are also effective in preventing the loss of fruit firmness. The efficacy of Fungicide treatment can be due to the effects on decay prevention.

Effects of treatments on the soluble solid concentration

Results suggested that the treatments have no statistically important effect on the SSC of lemon fruits, but in some treatments, the means of SSC were found to have a significant difference during the storage period. Especially Specially for the MAP, MAP + Fungicide, RO (0.2%) and RO (1.0%) treatments, the SSC content was found to have an increasing trend during the first 90 days of storage and then it had a decreasing trend. This was fund to be slightly higher than the means of SSC at the control fruits (Figure 5.).

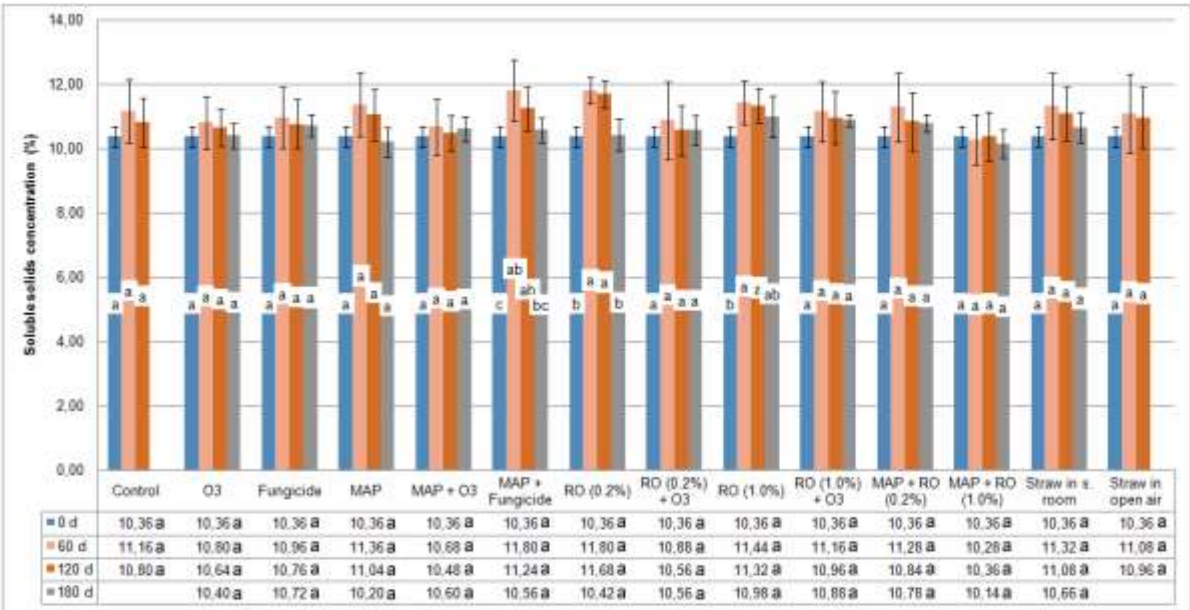
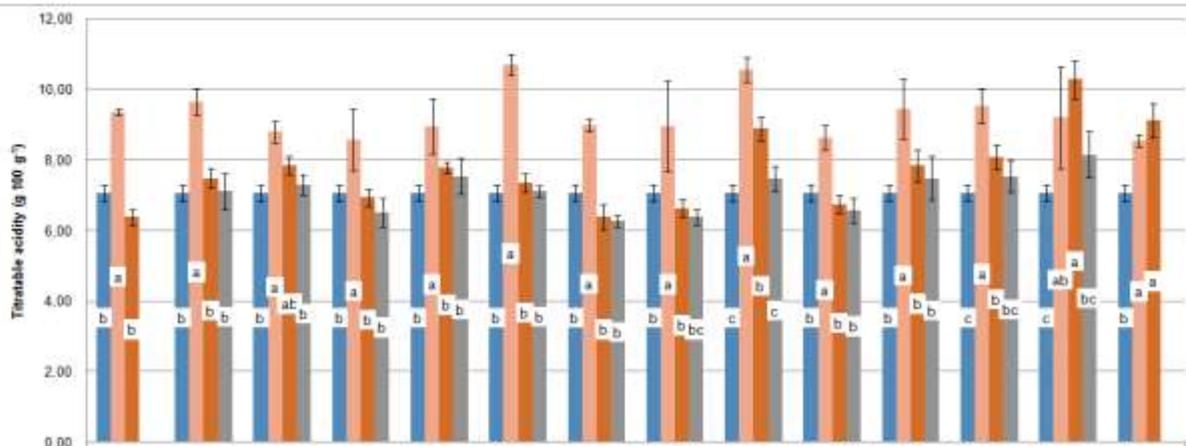


Figure 5. Soluble solids concentration of the lemon fruits as affected by the different treatments during 210 days of storage. (Letters at the centre of the columns are used to compare the means of measurement points for each treatment separately; and the letters in the table below the figure are used to compare the means of different treatments separately at each measurement point. At both conditions, different letters used to show scientific differences at p ≤ 0.05 level according to Tukey's HSD).

Effects of treatments on the titratable acidity

The results of TA were found to have a similar trend with SSC. Treatments were not effective in preventing changes in TA, but the storage duration is effective. More or less, a similar trend was observed for all treatments. The TA content had an increasing trend in the first 60 days of storage. It increased from 7.06 g 100 g-1 citric acid to 8.58-10.69 g 100 g-1 citric acid in 60 days of storage. Then, it showed a downward trend until 6.38-8.15 g 100 g-1 citric acid in 180 days of storage. Although the treatments are not effective in preventing changes in TA, straw coverage in the cold room and O3 treatments had slightly higher TA than the other treatments (Figure 6.).

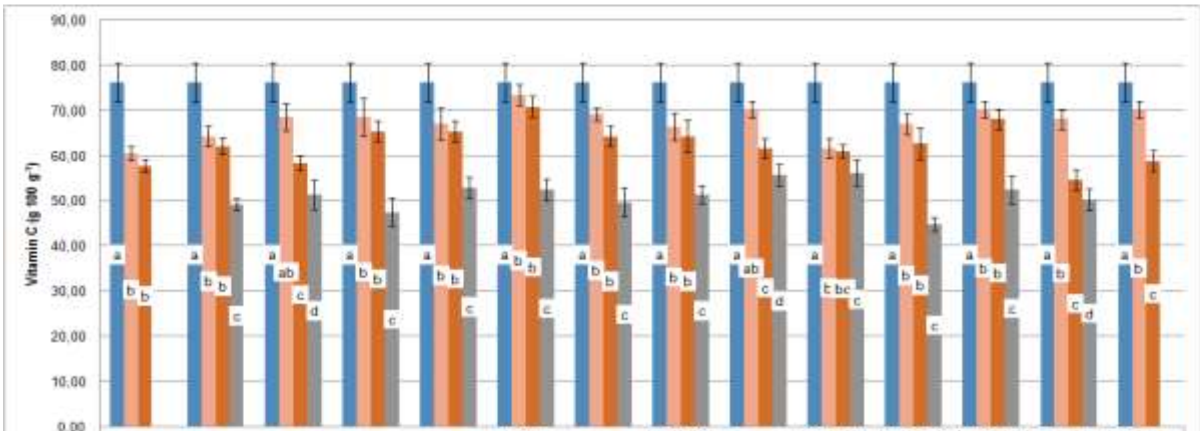


	Control	O3	Fungicide	MAP	MAP + O3	MAP + Fungicide	RO (0.2%)	RO (0.2%) + O3	RO (1.0%)	RO (1.0%) + O3	MAP + RO (0.2%)	MAP + RO (1.0%)	Straw in a room	Straw in open air
0 d	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a	7,06 a
60 d	9,37 a	9,65 a	8,79 a	8,58 a	8,95 a	10,69 a	8,98 a	8,95 a	10,56 a	8,62 a	9,45 a	9,54 a	9,20 a	8,55 a
120 d	6,38 a	7,48 a	7,84 a	6,93 a	7,78 a	7,36 a	6,38 a	6,63 a	8,88 a	6,75 a	7,84 a	8,09 a	10,28 a	9,12 a
180 d		7,11 a	7,30 a	6,51 a	7,54 a	7,11 a	6,26 a	6,38 a	7,48 a	6,57 a	7,48 a	7,54 a	8,15 a	

Figure 6. Titratable acidity of the lemon fruits as affected by the different treatments during 210 days of storage. (Letters at the center of the columns are used to compare the means of measurement points for each treatment separately; and the letters in the table below the figure are used to compare the means of different treatments separately at each measurement point. At both conditions, different letters used to show scientific differences at $p \leq 0.05$ level according to Tukey's HSD).

Effects of treatments on the Vitamin C content

VC was observed to have a different trend than the SSC and TA contents of the lemon fruits. During the storage time, the VC content had a decreasing trend. It was measured as 76.24 g 100 g-1 at harvest and it decreased to 44.88-56.23 g 100 g-1 in 180 days of cold storage. The highest VC content was noted from the RO (1.0%) + O3 treatment (Figure 7.). MAP alone was also effective in preventing the loss of VC.



	Control	O3	Fungicide	MAP	MAP + O3	MAP + Fungicide	RO (0.2%)	RO (0.2%) + O3	RO (1.0%)	RO (1.0%) + O3	MAP + RO (0.2%)	MAP + RO (1.0%)	Straw in a room	Straw in open air
0 d	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a	76,24 a
60 d	60,56 e	64,34 cde	68,67 abc	68,67 abc	67,05 abc	73,54 a	69,21 abc	66,51 bcd	70,29 ab	61,64 de	67,05 abc	70,29 ab	68,13 abc	70,29 ab
120 d	57,85 de	62,18 cd	58,40 de	65,42 bc	65,42 bc	70,83 a	64,34 bc	64,34 bc	61,64 cd	61,10 cd	62,72 cd	68,13 a	54,61 e	58,94 de
180 d		49,20 cd	51,37 abc	47,58 cd	52,99 abc	52,45 abc	49,74 cd	51,37 abc	55,69 ab	56,23 a	44,88 d	52,45 abc	50,28 cde	

Figure 7. Vitamin C of the lemon fruits as affected by the different treatments during 210 days of storage. (Letters at the center of the columns are used to compare the means of measurement points for each treatment separately; and the letters in the table below the figure are used to compare the means of different treatments separately at each measurement point. At both conditions, different letters used to show scientific differences at $p \leq 0.05$ level according to Tukey's HSD).

Effects of treatments on the fruit colour

The colour values (R, G, B) and lightness (L*) of the lemon fruits can be followed from the Figure 8. It is clear from the results that the percentage of L and R values increased during storage, while the G and B values decreased. The highest changes were observed from the R and B values, which increased and decreased, respectively. In other words, the colour of the lemon fruits changed from green to yellow during storage. The effects of treatments on the colour were negligible. However, it can be said that the MAP and RO treatments had a higher influence on the colour, in terms of inducing yellowing.

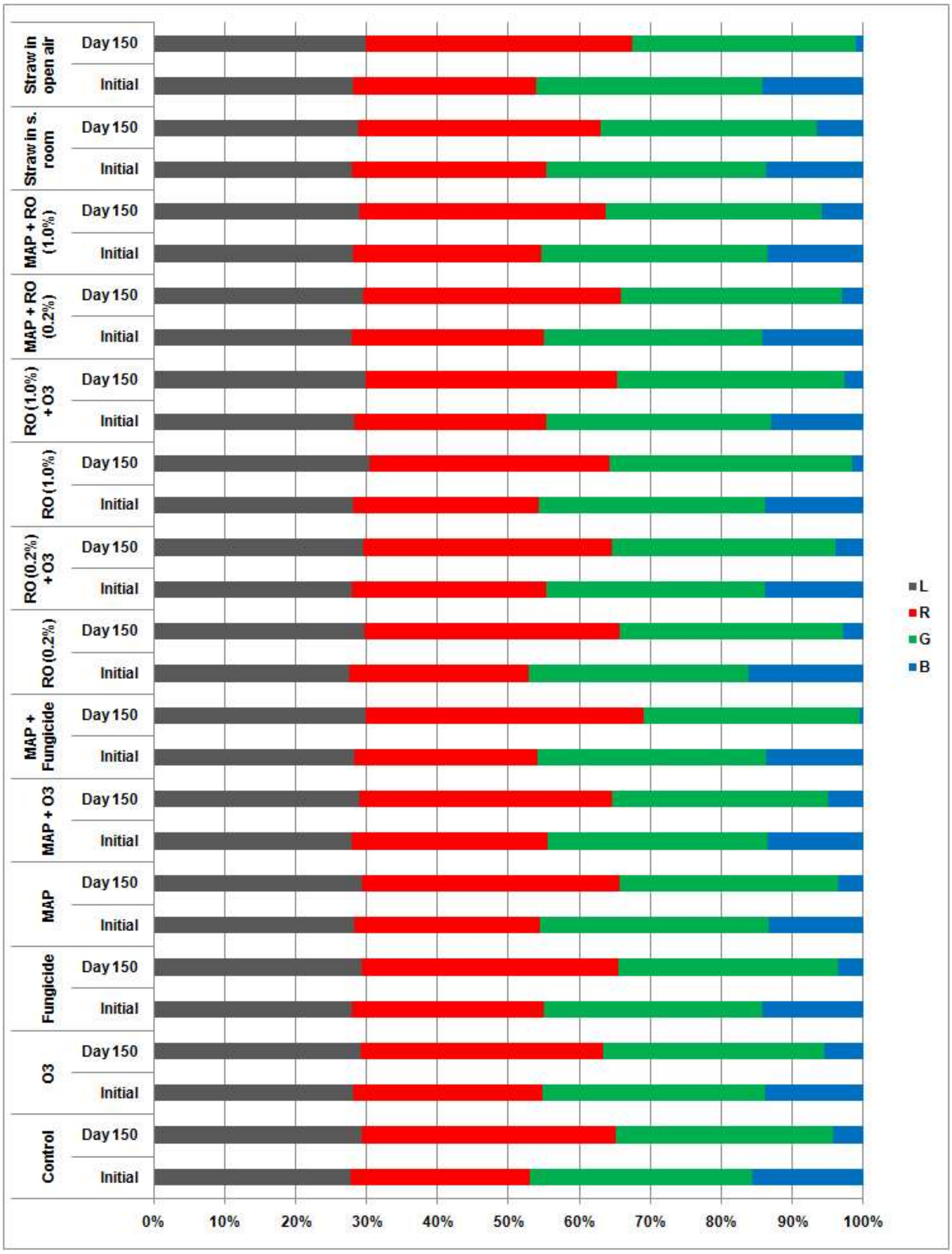


Figure 8. Comparison of the initial and 150th day colour (L: lightness, R: Red, G: Green, and B: Blue) of the lemon fruits as affected by the different treatments.

Discussion

Weight loss is an important characteristic for the postharvest quality of fruits and due to the nature of the fresh fruits, after harvest, weight loss of the fruits commonly has a decreasing trend during storage. These findings are in agreement with the findings of present study. Results of present study showed that some treatments, MAP + RO (0.2%) and MAP + Fungicide treatments were very effective in preventing weight loss. The combination of MAP with O3 was also effective in preventing weight loss and protecting fruit quality. O3 treatment was previously suggested to reduce weight loss and protect quality (de Souza et al., 2018). The higher efficacy of MAP was also previously reported for different fruits (Mangaraj and Goswami, 2009; Kahramanoğlu, 2017; Kahramanoğlu et al., 2019). It was also noted by Sandhya (2010) that the MAP is effective in preventing weight loss at in lemon fruits. However, there was no study about the efficacy of synergism of MAP and RO on lemon fruits. The RO was previously noted to reduce weight loss at the jujube fruits (Nikkhah and Hashemi, 2020).

According to the results obtained, the same treatments were found to be effective in reducing the DI in agreement with the weight loss and visual quality scores. The highest effect was noted from the Fungicide treatment and was followed by the MAP + Fungicide treatment. On the other hand, the O3 and RO treatments were also found to be effective in preventing DI. Similar results were previously noted by some researchers (Al-Qurashi et al., 2018; Chen et al., 2019) for different essential oils and for rosemary oil on different fruits (Servili et al., 2017; Nikkhah et al., 2017; Nikkah and Hashemi, 2020). On the other hand, the efficacy of O3 on pathogens is not a surprising result where some other previous studies recommended O3 as a potential sanitization agent for a wide range of pathogens (Khadre et al., 2001; Carletti et al., 2013).

Fruit firmness of lemon fruits was also observed to have a decreasing trend during storage. The combination of MAP with O3 or RO was but found to be effective in preventing the loss of fruit firmness. Similar results for O3 treatment were previously suggested by de Souza et al. (2018) on carrot fruits. Although the treatments are not effective in preventing changes in SSC and TA, straw coverage in the cold room and O3 treatments had slightly higher TA than the other treatments. These results are in accordance with the findings of Monaco et al. (2016) who reported that the O3 treatment is effective on TA at mangoes.

During this experiment, it was observed that the VC content had a decreasing trend. However, results suggested that the RO (1.0%) + O3 treatment and MAP alone were effective in preventing the loss of VC. Similar results for MAP were noted by Sandhya (2010), Mangaraj and Goswami (2009), Caleb et al. (2013) and Kahramanoğlu et al. (2019). The higher efficacy of essential oils on the prevention prevention of VC content is also a well-known and highly reported condition (Kahramanoğlu et al., 2019; Kahramanoğlu and Usanmaz, 2019; Chen et al., 2019). The results of O3 were also not surprising, where the results are in accordance with the reports of Tabakoglu and Karaca (2018) who noted that the O3 application is effective in preventing VC loss at mulberry fruits.

Conclusions

This study was designed to search the influence of MAP, ozone treatment, rosemary oil application, wrapping in straw and some of their combinations on the postharvest storage of Cyprus local lemon. The results of current study suggested that all applications have a significant influence on the fruit weight, visual quality, decay incidence, fruit firmness, vitamin C content and fruit colour. The effects on SSC and TA were negligible. According to the results obtained, MAP alone, or in combination with ozone, fungicide, rosemary oil (0.2%) or rosemary oil (1.0%) treatments were effective in protecting the postharvest quality and marketability of the lemons. Overall, results suggest that those treatments can be used to store lemon fruits for 210 days in the cold room with acceptable marketing quality.

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EFFECTS OF SODIUM BI-CARBONATE AND MODIFIED ATMOSPHERE PACKAGING ON THE POSTHARVEST STORAGE QUALITY OF SNAP BEAN PODS

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ABSTRACT

Present study was conducted to investigate the effects of postharvest applied sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) technique on the storage quality of snap beans (Phaseolus vulgaris L. cv. 'Blue Lake'). The main reason behind this aim was the high sensitivity of snap beans to storage conditions and low quality after storage. Snap bean pods of current work were hand-collected from a commercial farm found around Lefke city of Northern Cyprus. Experiments were conducted with a completely randomized block design with four treatments and twenty replications each with 50 bean pods. The treatments are: 1) control, dipping in pure water, 2) SB, dipping in 2% sodium bi-carbonate, 3) Control+MAP, dipping in pure water and storing in MAP and 4) SB+MAP, dipping in 2% SB and storing in MAP. After dipping, all fruits were air dried for 30 min, and transferred to the storage conditions at 5.5±0.5 °C and 95% relative humidity. Four replications from each treatment were taken out from storage rooms at 5th, 10th, 15th, 20th and 25th days of storage to measure quality parameters (weight loss, soluble solids concentration (SSC), chilling injury, firmness, browning, shrivelling and chlorophyll content). Results showed that snap bean pods have high weight loss even in cold storage conditions if they are not treated well. Weight loss reached to 24.08% in 25 days of storage at control beans, where the MAP application found to have only 4.34% weight loss. MAP was also found to have a slight influence on the prevention of loss in SSC. Results demonstrated that the storage of bean pods with the application of SB+MAP would be possible up to 25 days in terms of firmness, chilling injury and shrivelling. However, bean pods stored in MAP found to have moderate to extreme browning after 15 days of storage. Further studies come to the forefront of importance to prevent pods browning in MAP conditions for the better and longer storage of beans.

Keywords: *Weight loss, chilling injury, firmness, browning, chlorophyll content*

INTRODUCTION

Snap bean (*Phaseolus vulgaris* L.) have higher market value, shorter cultivation period until harvest and longer harvest duration as compared with dry beans (Ugen et al., 2013). Snap beans are beneficial for human health due to their high minerals (calcium and iron) and vitamins (A and C) contents and dietary fibre (Kelly and Scott, 1992; Carović-Stanko et al., 2018). However, snap bean pods are highly perishable, due to high respiration rate and sensitivity to low temperatures (Costa et al., 1994) and quickly deteriorate if not managed correctly. Due to the high perishability of the pods, snap beans mainly processed and stored as canned or frozen. Temperature is again an important factor not only for the fresh beans, but also for frozen beans (Vicent et al., 2018). The meaning of "quality" differs among consumers. For many of the consumers' quality primarily referred appearance for many years, but food safety issues come to the public awareness since the beginning of 21th century (Koch, 2017). However, colour is still an important feature for fresh produce including snap beans (Trail et al., 1992). Firmness and chlorophyll contents are the other important quality indices for snap beans (Martinez et al., 1995).

Storage temperature is the most important factor affecting postharvest life of snap beans (Proulx et al., 2010; Kinyuru et al., 2011). Recommended storage conditions for snap beans vary from 5 to 7.5 °C with 95 to 100% relative humidity and maximum shelf life at those recommended conditions for though to be 8 to 12 d (Proulx et al., 2010). Storage at lower temperatures than the recommended units, cause chilling injury (CI) and storage at above the suggested units cause discoloration and loss of firmness (Nunes, 2008). Besides to the storage temperature, surrounding atmosphere of fresh produce is an important factor highly affecting postharvest storage quality of fresh produce. Numerous previous studies reported the modified atmosphere packaging (MAP) technique makes it possible to alter surrounding atmosphere of the fresh produce, reduce respiration and thus protects postharvest quality (Gil et al., 1997; Day, 2001; Caleb et al., 2013; Kahramanoğlu et al., 2018; Kahramanoğlu, 2019). There are numerous previous studies about the postharvest storage of snap beans (Costa et al., 1994; Martinez et al., 1995; Proulx et al., 2010; Kinyuru et al., 2011) but very few about the use of MAP (Ubhi et al., 2014; Thenmozhi et al., 2016). On the other hand, it is well known that the responses of quality attributes to the storage conditions significantly vary depending on the commodity. Therefore, present study aimed to investigate the effects of postharvest applied sodium bi-carbonate and modified atmosphere packaging technique on the storage quality of snap beans cv. 'Blue Lake'.

Material and Methods

Materials

Snap bean pods (*P. vulgaris* L. cv. 'Blue Lake') of current work were hand-collected from a commercial farm found around Lefke city of Northern Cyprus. Bean pods were collected at commercial maturity based on skin colour and firmness on 1st of July 2019. 'Blue Lake' is a climbing bean cultivar dating from 1885 and resistant to adverse weather condition, even summer droughts and remain tender when cooked.

Test materials of present study are sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) bags. SB was purchased from local shops and 2% of it was prepared by dissolving it in pure water. MAP bags of present study were procured from Dekatrend (Trendlife®). The material used for development of bags is low-density polyethylene film and the target gas concentration of the bags was 2-5% O2 and 5-10% CO2.

Methods

Bean pods were firstly selected to eliminate damaged crops and to ensure similar colour and size for the study. Experiments were conducted with four treatments (1-control, 2-sodium bio-carbonate "2%", 3-MAP and 4-MAP+sodium bi-carbonate "2%"). Twenty replications were used for each treatment and each replication was formed from 50 bean pods. Application of sodium bi-carbonate (SB) was performed by dipping the bean pods into the 2% SB solution for 2 min at 24±1 °C. Control fruits were also immersed into pure water for 2 min. After dipping, all fruits were air dried for 30 min, and transferred to the storage conditions at 5.5±0.5 °C and 95% relative humidity. Before the experiments, total fruit weight of each replication was measured and noted to enable the calculation of weight loss. Eight bean pods were also selected at the beginning of the experiments to measure soluble solids concentration (SSC). Studies were planned to continue for 25 days and quality analysis of the bean pods were carried with 5-days interval (5, 10, 15, 20 and 25 d). At the mentioned days, four replications from each treatment were taken out from storage rooms to measure quality parameters. Fruit weight was measured with a digital scale sensitive to ±0.01 g and weight loss was calculated for each replication by using the initial weight data. SSC (% Brix) was measured with a hand refractometer. Furthermore, five bean pods were randomly selected from each replication to observe visual quality (chilling injury, firmness, browning and shrivelling) parameters by following the scales given in Table 1.

Table 1. Visual quality observation scale scores for snap beans

Quality	Scale scores					Reference for the Scale
	1	**2**	**3**	**4**	**5**	
	Very poor	**Poor**	**Acceptable**	**Good**	**Excellent**	
Chilling injury (CI)*	Severe pitting, large rusty spots, entire discoloration	Moderate pitting, medium rusty spots and moderate discoloration	Slight pitting and small rusty spots	Very small pitting and/or rusty spots	No abnormalities	Proulx et al. (2010)
Firmness	Extremely soft and does not snap	Soft, bents easily and does not snap	Tender but less firm, does not snap easily	Tender and firm, snap easily	Extremely tender and firm, snaps very easily	Quintana and Paull (1993)
Browning	Extreme browning	Severe browning	Moderate browning	Slight browning	No browning	Proulx et al. (2010)
Shriveling	Extremely shriveled and dry	Serious shriveling	Shriveling evident but not serious	Slight signs of shriveling	No shriveling	Quintana and Paull (1993)

*CI scores were observed after 1 additional day at 20 C.

Apart from the above-mentioned quality parameters, chlorophyll contents of the bean pods were also measured at the beginning of experiments, 10 days after storage (DAS) and 20 DAS. To calculate the chlorophyll contents, the method of Arnon (1949) as suggested by Sudhakar et al. (2016) was followed. A digital balance (sensitive to ±0.0001 g) was used to sample about 1 g of bean pods (only the valve “carpel”, not the seeds) and grind with 10 mL of 80% acetone in mortar using pestle. The sample was then filtered through what man filter paper no. 1. The optical density (OD) of the extract is measured at 663 and 645 nm wavelengths using spectrophotometer. Thus, the absorption coefficients were used to calculate Chl-a, Chl-b and total Chl contents of the samples by using the following equations (Arnon, 1949):

$$Chl - a\ (mg\ g^{-1}) = \ [12.7(A663) - 2.69(A645)] \times V/1000 \times W$$

$$Chl - b\ (mg\ g^{-1}) = \ [22.9(A645) - 4.68(A663)] \times V/1000 \times W$$

In the above given formula; A = is the absorbance at specific wavelengths, V = final volume of chlorophyll extract and W = fresh weigh of tissue extracted. Finally, beans pod color were also determined according to the method developed by Kahramanoğlu and Usanmaz (2019). A hand-made mini light box (width:22 cm, length:30 cm, height:22 cm) was used to measure capture images of the bean pods, and these images were transferred to a computer. Hereafter, luminosity and RGB values of the images were determined by using the histogram function of Photoshop 7.0 ME software.

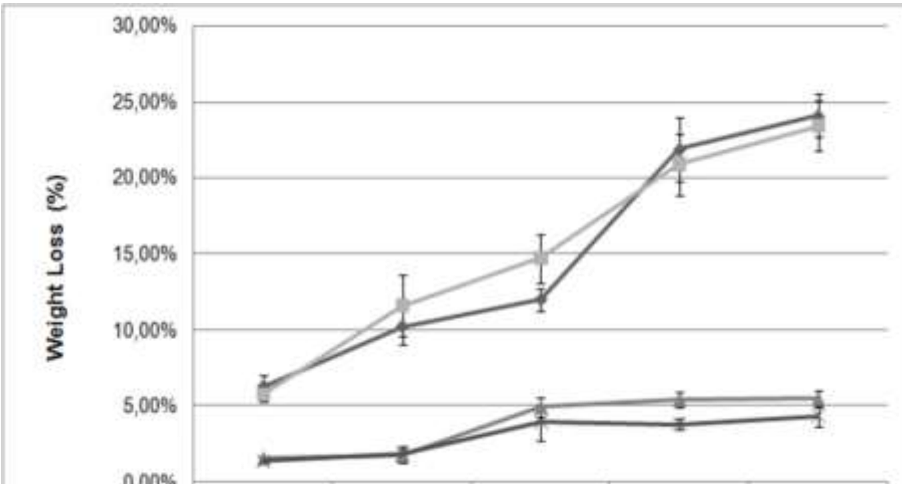
Data analysis

The effects of four different treatments at five different storage durations were analyzed separately by subjecting the raw data to the analysis of variance (ANOVA) at IBM SPSS 22.0 computer program. Separation of means were then performed according to the Tukey's Honestly Significant Difference (HSD) test at P = 0.05.

Results and Discussions

Effects on weight loss and soluble solids concentration

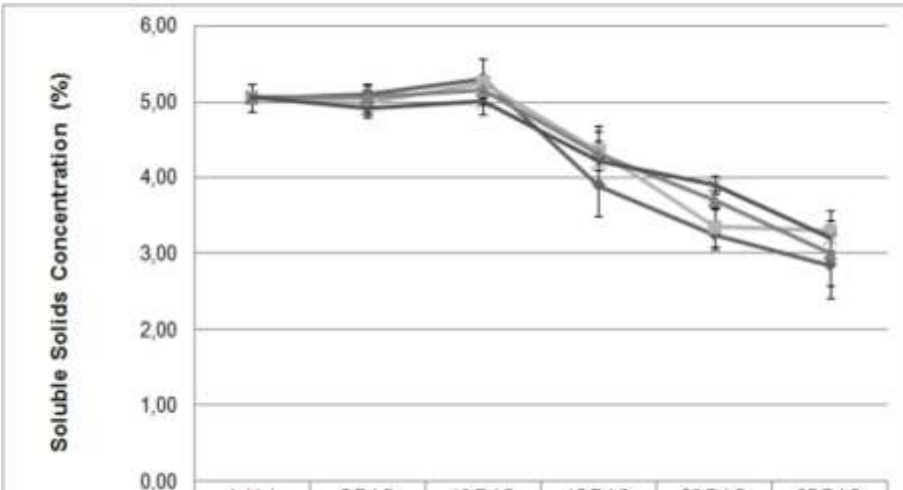
One of the most important results of present study is that modified atmosphere packaging (MAP) provides favourable conditions for storage of beans and prevents weight loss during storage. As expected, weight loss showed an increasing tendency during storage. Total weight loss in 25 days at the control bean pods was measured as 24.08% while the weight loss at bean pods stored in MAP and MAP+SB were 5.47% and 4.34%, respectively. The weight loss measured at the bean pods stored in MAP or MAP+SB in 25 days, were found to be less than the weight loss measured at the control bean pods in 5 days of storage. This result demonstrates the highest efficacy of MAP. Application of sodium bi-carbonate (SB) was on the other hand found to have slight influence on the prevention of weight loss. However, this effect was not statistically significant. Kinyuru et al. (2011) previously conducted a similar study and tested the effects of different storage temperatures on the beans pod quality. The results about the weight loss in present study are in conjunction with their findings. The positive effects of packaging on the prevention of weight loss at the bean pods are in conformity with the results of previous studies (Trail et al., 1992; Ubhi et al., 2014).



	5 DAS	10 DAS	15 DAS	20 DAS	25 DAS
Control	6,29% b	10,23% b	12,01% b	21,88% b	24,08% b
SB	5,76% b	11,58% b	14,71% c	20,87% b	23,38% b
Control+MAP	1,57% a	1,74% a	4,94% a	5,42% a	5,47% a
SB+MAP	1,38% a	1,87% a	3,95% a	3,82% a	4,34% a

Figure 1. Effects of sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) on the weight loss of snap bean pods during 25 days of storage [DAS: Days After Storage. Values followed by the same letter or letters at the same DAS are not significantly different at Tukey's HSD test at $p \leq 0.05$].

Soluble solids concentration (SSC) of the bean pods was 5.05%. During the first 10 days of storage, bean pods' SSC demonstrated a slight increase (except SB+MAP application) and then found to have a decreasing tendency (Figure 2.). The effects of treatments on the bean pods' SSC were also varying. At the end of the experiments, lowest SSC was measured from control treatments and the highest SSC from the applications of SB and SB+MAP. Similarly Kinyuru et al. (2011) reported that the SSC of bean pods decrease substantially during storage, even at the lower temperatures. Thenmozhi et al. (2016) also noted that SSC decrease during storage and modified atmosphere packaging have positive influence on the prevention of the SSC.



	Initial	5 DAS	10 DAS	15 DAS	20 DAS	25 DAS
Control	5,05 a	5,10 a	5,30 a	3,90 a	3,25 c	2,85 a
SB	5,05 a	5,00 a	5,25 a	4,35 a	3,35 bc	3,30 a
Control+MAP	5,05 a	5,05 a	5,15 a	4,30 a	3,70 ab	3,00 a
SB+MAP	5,05 a	4,90 a	5,00 a	4,20 a	3,90 a	3,20 a

Figure 2. Effects of sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) on the soluble solids concentration (SSC) of snap bean pods during 25 days of storage [DAS: Days After Storage. Values followed by the same letter or letters at the same DAS are not significantly different at Tukey's HSD test at $p \leq 0.05$].

Effects on visual quality characteristics

Firmness is one of the most important quality characteristics for bean pods. The pods should have an acceptable level of firmness. The 1-5 scale was used to determine the pod firmness during studies and acceptable limit was 3 which means tender but less firm and does not snap easily. The beans should be tender and could snap to be used for cooking (Figure 3.).

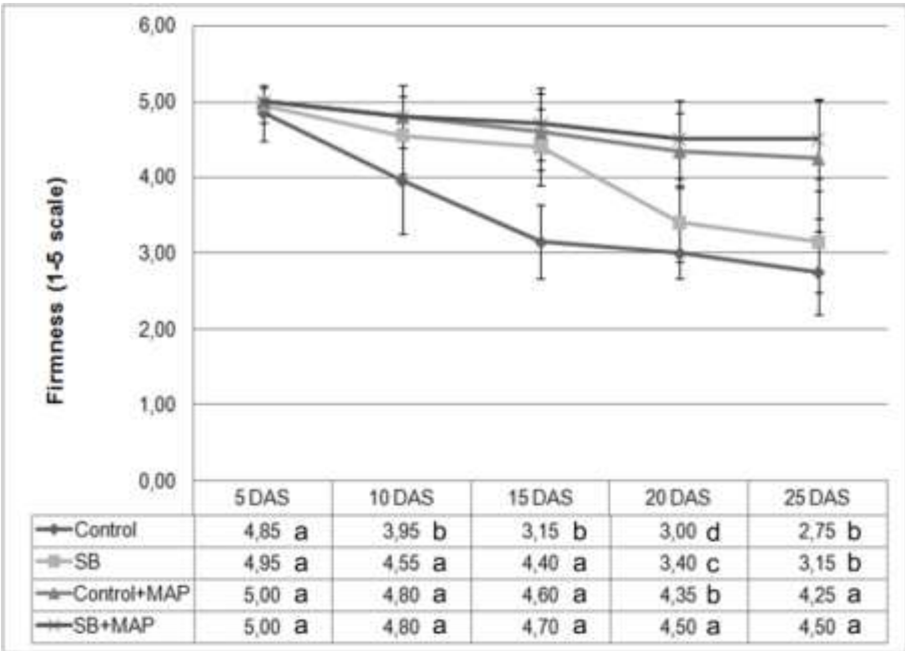


Figure 3. Effects of sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) on the firmness of snap bean pods during 25 days of storage [DAS: Days After Storage. Values followed by the same letter or letters at the same DAS are not significantly different at Tukey's HSD test at $p \leq 0.05$].

Results showed that treatments have important influence on the pod firmness. The firmness of control bean pods decreased to 3.15 in 15 days of storage where the firmness of treated fruits with any of the treatments was between 4.40 and 4.70. No significant difference was observed among the treatments in 15 days of storage. After 20 days of storage, the effect of SB on the protection of pod firmness decreased and firmness score get close to control treatment. The other treatments, Control+MAP and SB+MAP, were found to be effective still in 25 days of storage.

Results showed that modified atmosphere packaging is an effective method for the preservation of the firmness quality of the bean pods. Sistrunk et al., (1989) reported that the loss of turgidity and firmness is associated with the weight loss and increase in soluble pectin. Results of present study are in agreement with Sistrunk et al., (1989) in which the firmness was higher at the bean pods which have less weight loss. Another important quality parameter of the bean pods is browning. Similar with the firmness scores, the browning score is also should be above 3. Results showed that the SB application has an important positive influence on the prevention of browning, but the MAP has negative influence on it. The fruits with Control+MAP application had a browning score of 2.70 in 15 days of storage, which is close to moderate browning. All other treatments found to have a score of more than 4 equalling slight browning (Figure 4.). After 20 days of storage, beans stored in any of the MAP treatments, with or without SB, found to have a score of around 1, meaning extreme browning. Results on the other hand, suggested that the beans treated with SB could have an acceptable browning score even in 20 days of storage. All bean pods scored 3 or less after 20 days of storage. Browning is an important problem for snap beans stored at chilling temperatures (Nunes et al., 2001).

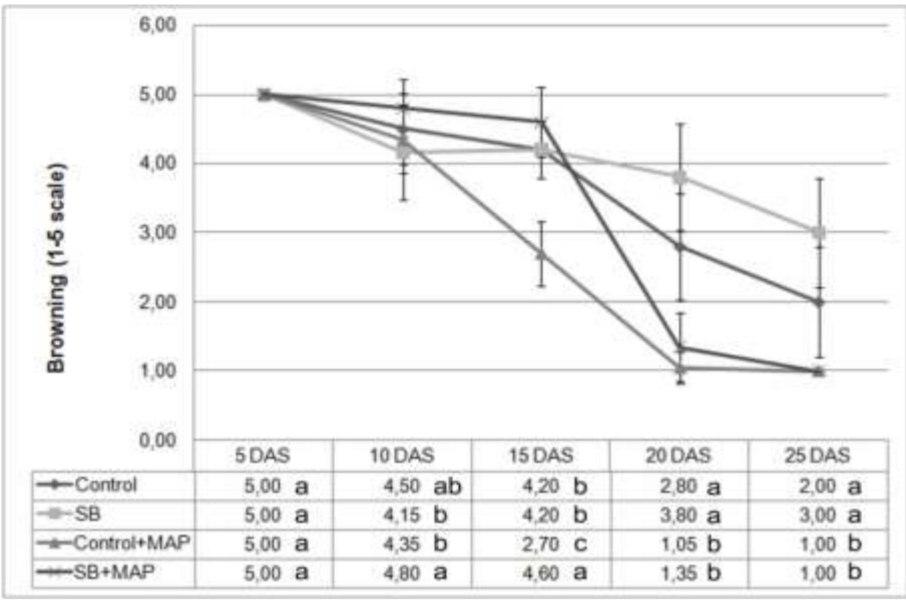


Figure 4. Effects of sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) on the browning of snap bean pods during 25 days of storage [DAS: Days After Storage. Values followed by the same letter or letters at the same DAS are not significantly different at Tukey's HSD test at p ≤ 0.05].

Pod discoloration, surface pitting and occurrence of rusty brown spots are the most chilling injury symptoms at snap beans (Proulx et al., 2010) and usually occur when beans are transferred to ambient temperatures (Abou Aziz et al., 1976). Results about the chilling injury scores of bean pods demonstrated that both SB and MAP have positive effects on the prevention of chilling injury. All treatments found to have positive influence on the prevention of the chilling injury. At 25 DAS, the lowest chilling score (so the highest chilling injury) was observed from control fruits, as expected, and other treatments found to have a score of more than 4 (Figure 5.).

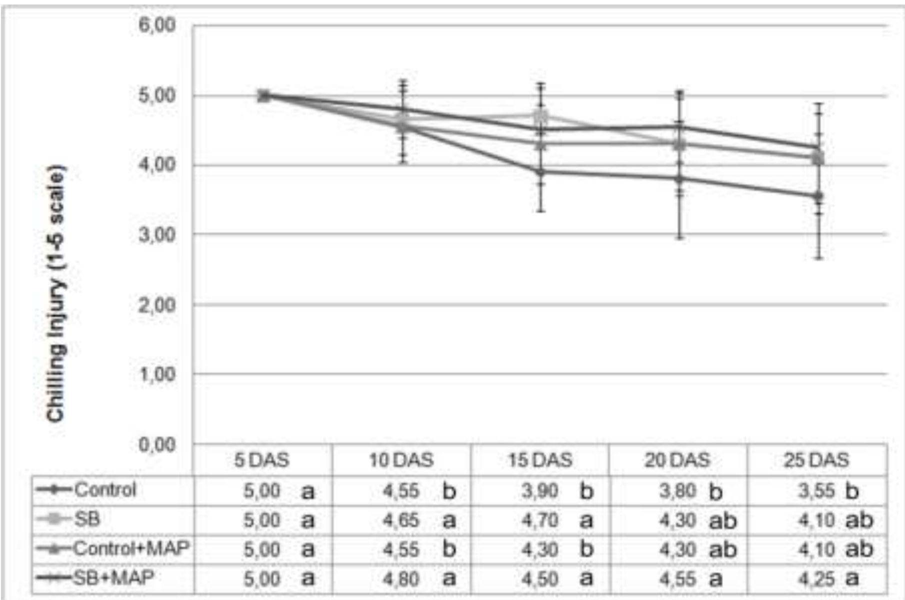


Figure 5. Effects of sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) on the chilling injury of snap bean pods during 25 days of storage [DAS: Days After Storage. Values followed by the same letter or letters at the same DAS are not significantly different at Tukey's HSD test at p ≤ 0.05].

The final quality characteristic of the experiments was shrivelling which was determined with the 1-5 scale. Similarly, with other characteristics, 3 was an acceptable level which was meaning shrivelling evident but not serious. Results showed that the fruits stored in MAP bags, either with or without SB, had acceptable shrivelling scores (Figure 6.). According to the results obtained, bean pods have acceptable quality in terms of shrivelling in 20 days of storage without any treatments, but the quality decreased to unacceptable (serious shrivelling) in 25 days.

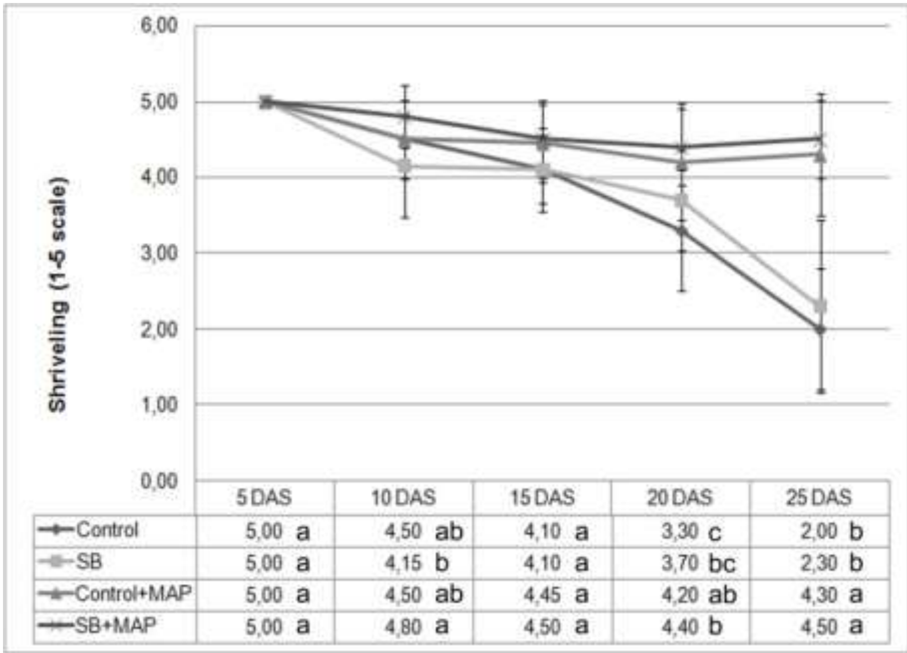


Figure 6. Effects of sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) on the shrivelling of snap bean pods during 25 days of storage [DAS: Days After Storage. Values followed by the same letter or letters at the same DAS are not significantly different at Tukey's HSD test at $p \leq 0.05$].

Effects on chlorophyll contents and color

Results demonstrated that the chlorophyll (Chl) content has a decreasing trend during storage and the tested treatments have potential to prevent the decrease in Chl content. In 20 days of storage, the Chl content decreased from 0.1968 mg g-1 to 0.0675 mg g-1 at the control treatment, while the Chl content was 0.1400 mg ml-1 at the SB+MAP treatment in 20 days of storage. Results also suggested that the SB application has a significant influence on the Chl content. Similarity Kinyuru et al. (2011) noted that the Chl content decrease during storage and closed packaging prevents the reduction in Chl.

Results of present study suggested that the increase in storage duration results in loss of luminosity but SB+MAP provides an increase after 15 days of storage. Similar results were also found for G "Green" value of the bean pods and those results are in conjunction with the findings of Trail et al. (1992) and Proulx et al. (2010). After 15 DAS, R and B values of snap beans treated with any of the treatments significantly increased. Results suggested that both SB and MAP provides better colour for the snap bean pods.

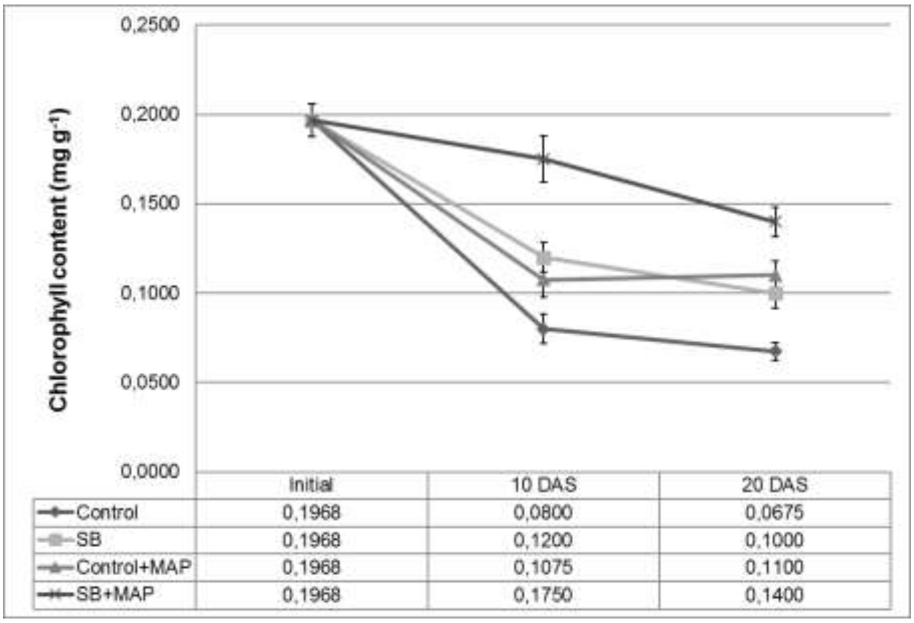


Figure 7. Effects of sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) on the chlorophyll contents of snap bean pods during 25 days of storage [DAS: Days After Storage. Values followed by the same letter or letters at the same DAS are not significantly different at Tukey's HSD test at $p \leq 0.05$].

Conclusions

Results showed that snap bean pods have high weight loss even in cold storage conditions (5.5±0.5 °C and 95% relative humidity) if they are not treated well. Weight loss reached to 24.08% in 25 days of storage at control beans, where the MAP application found to have only 4.34% weight loss. MAP was also found to have a slight influence on the prevention of loss in SSC. According to the results obtained, application of SB alone found to have slight or no effect on the quality parameters, but the combination with MAP provided best performance among the tested treatments. Results demonstrated that the storage of bean pods with the application of SB+MAP would be possible up to 25 days in terms of firmness, chilling injury and shrivelling. However, bean pods stored in MAP found to have moderate to extreme browning after 15 days of storage.

Therefore, further studies have to be performed to prevent browning in MAP bags to provide better storage of bean pods.

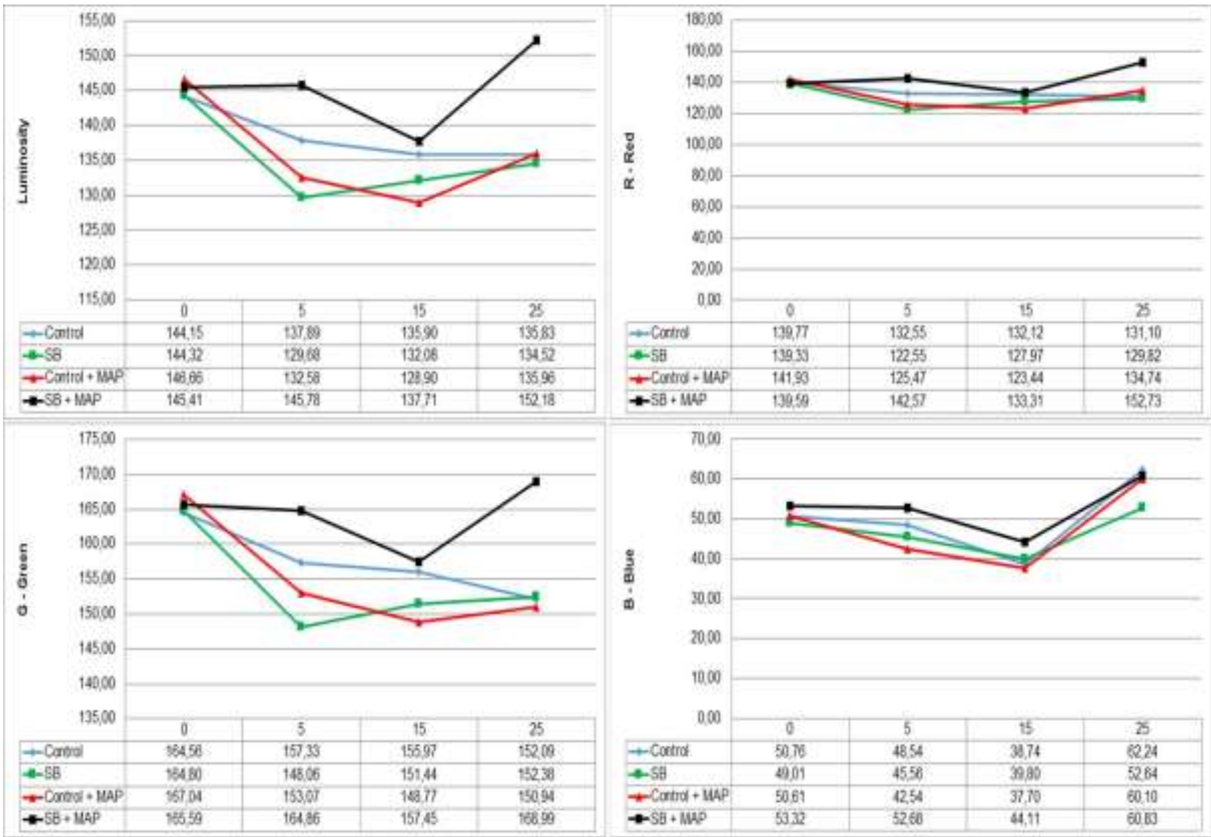


Figure 8. Effects of sodium bi-carbonate (SB) and modified atmosphere packaging (MAP) on the color indices (Luminosity and RGB values) of snap bean pods during 25 days of storage [DAS: Days After Storage].

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BIOACCUMULATION OF HEAVY METALS BY SELECTED SOME PLANTS CULTIVATED IN CENTRAL AND EASTERN BLACK SEA REGION

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ABSTRACT
The excessive accumulation of heavy metals in the soil, have significant effects not only on soil fertility and ecosystem functions, but also on animal and human health through the food chain. It is also known that heavy metal concentration in soil has certain effects on heavy metal concentration in plants. The aims of the current study are to i-) determine the amount of heavy metal in the leaves of vine, tea and hazelnut plants, ii-) to examine heavy metal transmission in soil systems where the selected plant grows and iii-) to determine the bioaccumulation factor (BAF) used in the evaluation of heavy metal accumulation in plants. For this purpose, leaves and soil samples of hazelnut, vine and tea plants were collected in from Samsun, Tokat and Rize provinces, respectively. Analysis of Cu, Cd, Cr, Pb, Co, Ni and Zn were performed in 7 soil and plant samples from hazelnut fields, 8 from vine fields and 8 from tea fields also BAF were determined. According to the study results, it was determined that Cd values vary between 0.001-0.038 mg kg^{-1}, Co values 0.023-0.146 mg kg^{-1}, Cr values 0.018-0.024 mg kg^{-1}, Cu values 9.663-73.756 mg kg^{-1}, Ni values 0.001-1.610 mg kg^{-1}, Pb values 0.347-0.684 mg kg^{-1} and Zn values 13.015-50.264 mg kg^{-1} in vine leaves. In addition, it was found that Cd values vary between 0.022-0.081 mg kg^{-1}, Co values 0.092-0.533 mg kg^{-1}, Cr values 0.025-0.037 mg kg^{-1}, Cu values 2.067-8.017 mg kg^{-1}, Ni values 0.663-2.803 mg kg^{-1}, and Zn values 13.103-26.953 mg kg^{-1} in tea leaves. Moreover, it was detected that Cd values vary between 0.001-0.056 mg kg^{-1}, Co values 0.082-0.652 mg kg^{-1}, Cr values 0.026-0.060 mg kg^{-1}, Cu values 1.603-8.419 mg kg^{-1}, Ni values 0.406-9.473 mg kg^{-1}, Pb values 0.001-0.191 mg kg^{-1} and Zn values 10.227-19.834 mg kg^{-1} in hazelnut leaves. Also, BAF values were changed between 0.00002-2.56 and were sorted as Zn>Cu>Pb>Cd>Co>Ni>Cr over average values in vine leaves. BAF values of tea leaves varied between 0.00003-0.64 and were sorted as Zn>Ni>Cu>Cd>Co>Cr>Pb on average values while BAF values of hazelnut leaves changed between 0.00006-0.48 and were sorted as Zn>Cd>Cu>Ni>Co>Pb>Cr on average values.

Key words: Heavy metal, Bio-accumulation factor, Tea plant, Hazelnut, Vine plant

INTRODUCTION

Today, with accelerated industrialization, urbanization and agricultural practices, environmental pollution has increased and many environmental problems have emerged. Heavy metals that lead to these problems cause pollution in air, soil and water sources. Soil pollution caused by heavy metal negatively affects plant physiology, causing a decrease in plant production (Yerli et al., 2020). Contaminated soil and heavy metals transmitted from the air threaten all living things through the food chain. Also, the accumulation of heavy metals taken from the soil in the tissues and organs of plants negatively affects the development of vegetative and generative organs of plants. In addition, many physiological events such as transpiration, stomatal movements, water intake, photosynthesis, enzyme activity, germination, protein synthesis, membrane stability, hormonal balance in plants are negatively affected (Kılıç et al., 2019).

Speciation and distribution of metals in the plant can provide important information helpful to understanding the mechanisms for their accumulation, translocation, and transformation. Besides, it is reported that the bio-accumulation factor is a good indicator for determining metal accumulation capacity in plants or environments (Özgür, 2019). By using this ratio, the absorption of heavy metal elements in the soil by the plant can be shown and the size of the transition of elements from soil to plant can be quantified.

Agricultural soils in many parts of the world are slightly to moderately contaminated by heavy metal toxicity, such as cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), chromium (Cr), lead (Pb), and arsenic (As). The bioavailability of heavy metals depends on soil conditions, the plant species, the kind of metal, and environmental factors. All of the above mentioned factors jointly determine what quantity of heavy metals will be absorbed by the plants, and what will be immobilized in the soil environment (Pachura et al., 2015). The mobility and bioavailability of heavy metals in contaminated soil is affected by a number of biological processes and physiochemical properties like soil pH, organic matter (OM), cation exchange capacity (CEC), soil texture, and soil microbiota. Bravo et al. (2017) indicated that some of the elements including heavy metals (Cu, Ca, Fe, Al, Ba, Pb, Sr, Zn, Mn) intake by plants were different in acidic and alkaline soils while, Zhang et al. (2020) reported that geological diversity that controls the distribution of heavy metals in soil to a large extent. The differences observed in the accumulation of heavy metals in different plants may be due to the fact that the rate at which plants absorb heavy metals is different (Pandey and Pandey 2009; Emurotu and Onianwa, 2017) and the mobility of heavy metals is different (Aladesanmi et al., 2019).

Tea, vine and hazelnut are three important products of the Central and Eastern Black Sea Region. 66.4% of tea production areas are located in Rize and 68.6% of production is made in this province (TEPGE, 2021). In addition, Tokat has 6.411 ha vineyard area and represents 39% of fruit production areas (Anonymous, 2020). The “brine leaf+grape production model” is at the forefront in the region and most of the enterprises produce brine leaves. Also, hazelnut production is carried out in 1167143 ha area in Samsun province and 17.17% of hazelnut planting areas in Turkey are located in Samsun (Anonymous, 2021).

Heavy metals have different effects on the health and quality of the atmosphere, soil, plants, water and living things. However, it is important to evaluate it because of the negativity it causes in all factors, polluting the atmosphere, disrupting the soil structure, reducing plant yield, affecting water quality and endangering living health. For this reason, the current study aimed to determine the amount of heavy metal in the leaves of vine, tea and hazelnut plants; i-) to examine the transition of heavy metal in the soil systems where these plants grow and ii-) to evaluate the accumulation of heavy metal in plants using BAF factor.

MATERIAL AND METHOD

Study Areas

The study area includes tea, vine and hazelnut areas in Rize, Tokat and Samsun provinces respectively (Figure 1).

Figure 1. Location map of the study area

Rize province, which has an area of about 381831 ha, ranges from 0 m to 3782 m above sea level. In most of the northern direction area of it is mountainous and has a hilly topographic feature, and with steep and very steep slopes (>50%) are common. In contrast, the land near the coast is the base land with almost flat slope. There are a total of seven different great soil groups, the most common of which are located within the borders of the province. It consists of Grey Brown Podzolic soils and Red Yellow Podzolic soils with about 60.83% as the highest distribution class in Rize and followed by Brown Forest Soils. According to the CORINE-2012 land use and land cover classification, about 24.2% of the province is grassland and pasture areas and sparse plant areas, while about 45.0% is forest areas. In addition, agricultural activity covers 20.3% of the province.

Tokat province, which has about 1027258 ha, varies between 75 m and 2415 m above sea level. Most of the land in part of the southeast direction and some land in the northwest part have a mountainous and hilly topographic feature and the slope of these areas consists of steep and very steep and steep slopes (>50% slope). The central areas where the plains are located are the areas close to the flat plain, where the slope is almost gentle. There are nine different large soil groups within the province, of which about 60% are Brown Forest Soils, followed by Red Chestnut soils (12.95%), Gray Brown Podzolic soils and Aluvial soils. According to the CORINE-2012 land use and land cover classification, about 16.5% of the province is grassland and pasture areas and sparse plant areas, while about 31.0% is forest areas. In addition, agricultural activity covers 38.2% of the province.

As for Samsun which has about 988294 ha located between 0 m and 1976 m above sea level. The north part of Samsun has a mountainous and hilly topographic feature and the slope of these areas consists of steep and very steep and steep slopes (>50% slope). Only, Vezirköprü, Havza and Ladik districts located on high plateau has low slope areas. On the other hand, areas located in the southern part and close to the coast consist of areas with flat and gently slope, especially the Bafra and Çarşamba Plains. Within the province, there are a total of eight different great soil groups, of which about 42% are Brown Forest soils as the highest distribution class, followed by Gray Brown Podzolic soils (24.94%), Chestnut soils and Aluvial soils. According to the CORINE-2012 land use and land cover classification, about 5.6% of the province is grassland and pasture areas and sparse plant areas, while about 34.6% is forest areas. In addition, agricultural activity covers 49.5% of the province.

Soil and Plant Samples and Analysis

Soil samples were taken after harvesting plants from the surface (0-20 cm) in the land where each plant sample was also collected. These samples in under laboratory, their gravel and plant particles were extracted conditions and left to dry in the air. Dried soils were passed through a 2 mm steel sieve and sifted soil samples were placed in polyethylene boxes. Some physico-chemical analyses were performed in the soil samples ready for analysis. Some physical and chemical analyses of soil samples and heavy metal content were made according to the methods given in Table 1.

Table 1. Analysis methods for physical and chemical features of soil parameters

Parameters	Methods
Texture	Hydrometer method (Demiralay 1993)
Organic matter, %	Walkey-Black method (Kacar 1994)
EC, dS m^{-1}	Conductivity metre in 1:1 (w/v) soil:water suspension (Bayraklı 1987)
pH	Potentiometric in 1:1 (w/v) soil:water suspension (Bayraklı 1987)
$CaCO_3$, %	Based on Scheibler method (Kacar 1994)
Total heavy metal (Cu,Cd,Cr,Pb,Co,Ni,Zn)	According to EPA 3051 for soils using ICP-OES

As part of the study, samples were made from the leaves of hazelnut, tea and vine plants. Hazelnut samples were taken from leaves that have completed their development in August while tea leaves were collected from young shoots in September. In addition, vine leaves were taken in May. Plant samples brought to the laboratory were washed with pure water. Later, these samples were dried in the study for 48 hours at 65 ^{o}C. Dried plant samples are milled in the plant mill and ready for analysis. Heavy metal contents of samples (Cu, Cd, Co, Cr, Ni, Pb, Zn) were determined by using the ICP-OES device of the filter obtained by dry incineration (reduced to ash at 500-550 oC and dissolved with 1N HCl at 1:10 ratio) method (Slavin, 1968; Yağmur ve Okur, 2018). Bio-accumulation factors (BAF) were calculated to assess the accumulation capacity of heavy metals in tea, vine and hazelnut leaves. BAF was calculated as the ratio of metal content in plant leaves to metal content in soil ($BAF=C_{leaves}/C_{soil}$); the fact that this value is greater than 1 indicates a significant accumulation of heavy metals from the soil in the plant (Pang et al., 2017, Rezvani and Zaefarian, 2011; Zhang et al., 2002).

RESULTS AND DISCUSSION

Some Physico-Chemical Properties and Heavy Metal Contents of the Soils

Descriptive statistics of some physico-chemical properties of soils were given in Table 2. According to the obtained results, the clay values of the soil samples taken from tea cultivation area varied between 6.49-27.56% and the soils were determined loamy soil namely medium-texture (CL, L, SiL, SL) soils. The pH values of the soils varied between 3.70-7.37 strong acid and neutral reaction. Organic matter values were determined between 2.32% and 7.28%. Clay values of the soil samples taken from vine cultivated area varied between 14.74-25.12 % and as tea soils it was determined that these soil samples had loamy soil namely medium-texture (SCL, L, SL) soils. The pH values of soils were between 6.92-7.83 called neutral reaction. In addition, organic matter values were determined between 0.86% and 2.24%. As for soil taken from hazelnut cultivation area, amount of clay varied between 13.95-52.24% and called as also medium and heavy texture (C, CL, SL, L). Their pH value was 5.45 and 7.48 that meant slightly acid and neutral soil reaction. Moreover, it was found that organic matter values showed between 1.58-3.42%. Looking at the CV values of the soil samples, in particular, it was determined that tea soils had higher values both in terms of physico-chemical properties and heavy metal content than soils from which other plants cultivation areas. In the selected parameter, the lowest CV values were determined in the soil samples in vine cultivated area.

Table 2. Descriptive statistics of physical and chemical properties of the soils

Statistical parameters	Sand %	Clay %	Silt %	pH	EC dS m^{-1}	$CaCO_3$ %	OM %
Soils taken from tea cultivated area							
Minimum	31.87	6.49	15.01	3.70	0.08	0.00	2.32
Maximum	76.22	27.56	53.90	7.37	0.55	1.59	7.28
Mean	59.52	12.43	28.06	4.66	0.26	0.20	3.83
Standard error	6.47	2.53	4.81	0.42	0.05	0.20	0.62
Standard deviation	18.31	7.14	13.61	1.19	0.15	0.56	1.74
Kurtosis	-1.42	2.47	0.38	4.61	0.84	8.00	1.11
Skewness	-0.72	1.60	1.10	2.08	0.93	2.83	1.31
CV	30.76	57.49	48.51	25.50	56.53	282.84	45.43
Soils taken from vine cultivated area							
Minimum	43.06	14.74	17.99	6.92	0.32	0.16	0.86
Maximum	60.73	25.12	35.10	7.83	1.26	12.96	2.24
Mean	51.19	21.31	27.50	7.50	0.58	6.67	1.61
Standard error	2.21	1.39	2.08	0.10	0.11	1.61	0.18
Standard deviation	6.26	3.94	5.89	0.28	0.31	4.54	0.50
Kurtosis	-0.94	-1.04	-0.84	2.21	3.82	-1.10	-1.10
Skewness	0.01	-0.73	-0.40	-1.36	1.85	-0.17	-0.15
CV	12.23	18.51	21.42	3.73	53.40	68.13	31.08
Soils taken from hazelnut cultivated area							
Minimum	22.26	13.95	22.36	5.45	0.14	0.00	1.58
Maximum	54.38	52.24	49.29	7.48	0.54	8.43	3.42
Mean	35.73	30.20	34.07	6.34	0.31	1.52	2.69
Standard error	4.32	4.13	3.52	0.27	0.05	1.09	0.23
Standard deviation	12.22	11.69	9.96	0.75	0.14	3.08	0.66
Kurtosis	-0.60	0.95	-0.82	-0.88	-0.70	4.00	-0.72
Skewness	0.76	0.74	0.68	0.59	0.69	2.08	-0.44
CV	34.19	38.73	29.24	11.87	44.90	202.95	24.45

EC: Electrical conductivity; OM: Organic matter

Descriptive statistics of the total heavy metal contents of soils were given in Table 3. According to the results of the analysis, it was determined that total Cu, Cd, Cr, Pb, Co, Ni and Zn contents of soils collected from tea cultivated area were 15.79-68.98 mg kg^{-1}, 0.20-0.89 mg kg^{-1} 6.14-27.17 mg kg^{-1}, 6.87-29.43 mg kg^{-1}, 0.35-19.13 mg kg^{-1}, 6.50-18.33 mg kg^{-1}, and 37.78-187.06 mg kg^{-1}, respectively. When based on Kloke (1980), according to the distribution of heavy metal elements in agricultural soils and the amount values of soils that can tolerate these elements all tea soils were below the tolerable amounts. However, at many points the elements Cu and at a few points Pb and Co gave a value above the determined threshold level.

Table 3. Descriptive statistics of heavy metal contents of the soils

Statistical parameters	tCu mg kg^{-1}	tCd mg kg^{-1}	tCr mg kg^{-1}	tPb mg kg^{-1}	tCo mg kg^{-1}	tNi mg kg^{-1}	tZn mg kg^{-1}
Soils taken from tea cultivated area							
Minimum	15.79	0.20	6.14	6.87	0.35	6.50	37.78
Maximum	68.98	0.89	27.17	29.43	19.13	18.33	187.06
Mean	42.98	0.48	17.16	15.38	8.82	10.09	77.40
Standard error	6.95	0.10	2.28	3.59	2.22	1.31	17.96
Standard deviation	19.67	0.28	6.46	10.16	6.29	3.71	50.80
Kurtosis	-1.58	-1.34	0.46	-1.82	-0.64	4.00	2.88
Skewness	0.24	0.60	-0.07	0.72	0.20	1.79	1.70
CV	45.76	57.08	37.63	66.10	71.26	36.75	65.64
Soils taken from vine cultivated area							
Minimum	31.10	0.22	28.56	2.59	4.14	40.11	19.67
Maximum	60.32	1.00	94.72	15.92	13.66	81.35	57.78
Mean	43.35	0.61	54.75	7.66	8.83	53.96	43.86
Standard error	3.37	0.10	7.40	1.45	1.01	5.13	4.43
Standard deviation	9.53	0.28	20.93	4.09	2.85	14.51	12.52
Kurtosis	-0.06	-1.04	0.94	1.75	0.71	0.50	0.69
Skewness	0.51	-0.20	1.10	1.15	0.17	1.16	-1.04
CV	21.99	46.08	38.23	53.46	32.31	26.88	28.55
Soils taken from hazelnut cultivated area							
Minimum	16.65	0.01	9.86	10.16	4.42	8.53	25.83
Maximum	46.88	1.08	83.95	17.20	19.12	131.75	56.83
Mean	28.60	0.44	31.14	12.90	11.96	39.51	41.40
Standard error	4.03	0.15	9.96	0.90	1.86	16.18	3.46
Standard deviation	11.40	0.43	28.16	2.54	5.25	45.76	9.79
Kurtosis	-0.43	-1.48	0.52	-0.54	-1.42	1.47	-0.29
Skewness	1.08	0.74	1.43	0.83	-0.10	1.62	-0.02
CV	39.88	97.73	90.45	19.66	43.91	115.82	23.65
Distribution range *	1-20	0.1-1	2-50	0.1-20	1-10	2-50	3-50
Tolerable value*	100	3	100	100	50	50	300

* Kloke, A., (1980). Orientierungsdaten für tolerierbare gesamtgehalte einiger elemente in kulturboden mitt. Vdlufa, H 1-3, 9-11.

Zhang et al. (2020) performed a study to determine the effect of geological diversity on cadmium (Cd), lead (Pb), thallium (Tl), mercury (Hg), arsenic (As), antimony (Sb), chromium (Cr), nickel (Ni) and manganese (Mn) contents of tea soils and 22 soil were taken from China's southern Guizhou region and their concentrations were determined as 0.33, 40.9, 0.39, 0.19, 26.3,2.0, 66,31.1, and 700 mg kg^{-1} respectively. Furthermore, 41%, 14% and 23% of soil samples for Cd, Pb and As, respectively, exceeded risk screening values (RSV) in agricultural soils in China (pH<5.5) (MEEPRC and SAMR, 2018). Bayraklı and Dengiz (2020), evaluated the risk of heavy metal contamination of some micro-basins growing tea in Rize province in the Eastern Black Sea region in Turkey by taking into account three different approaches (enrichment factor (EF), geo-accumulation index (Igeo), Contamination factor (CF)). They determined the average heavy metals in soils as Zn > Fr > Cr > Ni > Pb > Co > Cd. According to all three contamination risk models, they reported Cd as the riskiest heavy metal in terms of contamination.

It was determined that total Cu, Cd, Cr, Pb, Co, Ni and Zn contents of soils collected from vine cultivated area were 31.10-60.32 mg kg^{-1}, 0.22-1.00 mg kg^{-1}, 28.56-94.72 mg kg^{-1}, 2.59-15.92 mg kg^{-1}, 4.14-13.66 mg kg^{-1}, 40.11-81.35 mg kg^{-1}, and 19.67-57.78 mg kg^{-1}, respectively. When based on Kloke (1980), according to the distribution of heavy metal elements in agricultural soils and the amount values of soils that can tolerate, Ni element in half of the vine soil samples was over tolerable amounts. In addition to that, Cu, Cr and Zn values were also found over the threshold level. Mirzaei et al. (2020), sampled during the harvest period from 38 fertilized vine-grown fields in Chaharmahal and Bakhtiari provinces in Iran. According to their results, the heavy metal content in the soils were as Zn>Fr>Pb>Cr>Cd. They also compared heavy metal concentrations in the studied soil with permissible levels in Iran (Barzin et al. 2015) and the maximum concentration of Cu, Zn, Pb and Cr, excluding Cd, for all soil samples, was reported as below national permissible limits. Vázquez et al. (2016), in their study of Vineyards in the Ribeiro region (Ourense province) in north-western Spain, found also the total Cu and Zn in the soil in the range of 133-306 mg kg^{-1} and 98.9-156 mg kg^{-1}, respectively. In heavy metals, Pb was the most abundant element with 72.6-104 mg kg^{-1}, followed by Cr (3.13-5.09 mg kg^{-1}), Cd (43.9-101 µg kg^{-1}) and Hg (21.2–29.9 µg kg^{-1}).

It was found that total Cu, Cd, Cr, Pb, Co, Ni and Zn contents of the soils collected from hazelnut cultivated area were 16.65-46.88 mg kg^{-1}, 0.01-1.08 mg kg^{-1}, 9.86-83.95 mg kg^{-1}, 10.16-17.20 mg kg^{-1}, 4.42-19.12 mg kg^{-1}, 8.53-131.75 mg kg^{-1}, and 25.83-56.83 mg kg^{-1}, respectively. When based on Kloke (1980), according to the distribution of heavy metal elements in agricultural soils and the amount values of soils that can tolerate, two points for the Ni element were found above the tolerable value, and many points were found above the distribution range in terms of Cu and Co. In a study included heavy metal accumulation in the hazelnut soil, it was found as the total Cd concentration 3.1-5.8 mg kg^{-1}, Co concentration 13.2-15.5 mg kg^{-1}, Cr concentration 35.6-66.7 mg kg^{-1}, Cu concentration 221-280 mg kg^{-1}, Ni concentration 21-35.3 mg kg^{-1}, Pb concentration 94-680 mg kg^{-1}, the concentration of Zn 270-410 mg kg^{-1} in the Black Sea coastal region of Turkey (Özkutlu et al., 2009).

Heavy Metal Contents of Plants and Bioaccumulation Factors (BAF)

Descriptive statistics of the heavy metal contents of the leaves of each three plant were given in Table 4. According to the results obtained, Cu, Cd, Cr, Co, Ni and Zn values of the tea leaves were as 2.07 -8.02 mg kg^{-1}, 0.02-0.08 mg kg^{-1}, 0.03-0.04 mg kg^{-1}, 0.09-0.53 mg kg^{-1}, 0.66-2.80 mg $kg^{-1}$and 13.10-26.95 mg kg^{-1}, respectively. The heavy metal content of tea leaves was listed as Zn>Cu>Ni>Co>Cd>Cr>Pb based on their mean values. Zhang et al. (2006) in their study were determined different tea leaf samples from the field in Zhejiang province of China, on average, in Cu, Cd, Pb and Zn concentrations in mature leaves, respectively, 13.8, 0.022, 1.53, and 24.1 mg kg^{-1} and for old leaves as 11.1, 0.032, 4.09 and 16.2 mg kg^{-1}. The results obtained in both studies showed that the elements most commonly found in tea fronds were Zn and Cu.

As for vine leaves, Cu, Cd, Cr, Co, Ni and Zn values of the tea leaves were 9.66-73.76 mg kg^{-1}, 0.001-0.038 mg kg^{-1}, 0.018-0.024 mg kg^{-1}, 0.35-0.68 mg kg^{-1}, 0.02-0.15 mg $kg^{-1}$and 0.02-0.15 and 13.02-50.26 mg kg^{-1}, respectively and the heavy metal content of vine leaves was listed as Zn>Cu>Pb>Ni>Co>Cr>Cd. Beygi and Jalali (2019) determined the average heavy metal content in Vine leaves as 0.36 mg kg^{-1} for Cd, 5.91 mg kg^{-1} for Cu, 9.58 mg kg^{-1} for Ni and 27.04 mg kg^{-1} for Zn, respectively and metal contents were ordered as Zn>Ni>Cu>Cd. Cd and Cu contents in the above-ground part of plants showed a significant and positive correlation with the total and useful amounts of heavy metals in the soil while, Ni and Zn content in the above-ground part of plants showed significant and positive correlations with only useful amounts of heavy metals. Bora et al. (2015) in Turulung (Romania) were determined the average metal content of some vine leaves for their Cd 0.77 ± 0.03 mg kg^{-1} for Pb 4.25 ± 0.03 mg kg^{-1} for Ni 9.47 ± 0.75 mg kg^{-1} for Zn 25.20 ± 1.37 mg kg^{-1} for Cu 49.16 ± 3.40 mg kg^{-1} and Co 0.47 ± 0.07 mg kg^{-1}.

Table 4. Descriptive statistics of heavy metal content of leaf samples

Statistical parameters	tCu mg kg^{-1}	tCd mg kg^{-1}	tCr mg kg^{-1}	tPb mg kg^{-1}	tCo mg kg^{-1}	tNi mg kg^{-1}	tZn mg kg^{-1}
Tea leaf samples							
Mean	2.07	0.02	0.03	0.0010	0.09	0.66	13.10
Standard error	8.02	0.08	0.04	0.0010	0.53	2.80	26.95
Standard deviation	6.30	0.05	0.03	0.0010	0.25	1.80	18.06
Kurtosis	0.66	0.01	0.00	0.0000	0.05	0.27	1.81
Skewness	1.86	0.02	0.00	0.0000	0.13	0.77	5.13
CV	4.71	-0.26	-0.48	0.0000	2.40	-1.18	-0.05
Mean	-1.97	0.70	0.77	0.0000	1.33	-0.24	1.21
Standard error	29.53	41.62	14.89	0.0000	53.35	43.02	28.42
Vine leaf samples							
Mean	9.66	0.001	0.018	0.35	0.02	0.001	13.02
Standard error	73.76	0.04	0.024	0.68	0.15	1.61	50.26
Standard deviation	21.00	0.01	0.02	0.55	0.09	0.42	22.06
Kurtosis	7.60	0.01	0.00	0.04	0.01	0.20	4.27
Skewness	21.51	0.01	0.00	0.12	0.04	0.56	12.09
CV	7.58	1.06	-1.76	-1.00	0.25	2.60	5.48
Mean	2.73	1.59	0.11	-0.37	-0.82	1.73	2.23
Standard error	102.42	153.06	11.86	22.21	42.71	132.82	54.81
Hazelnut leaf samples							
Mean	1.60	0.001	0.026	0.001	0.08	0.41	10.23
Standard error	8.42	0.056	0.060	0.191	0.65	9.47	19.83
Standard deviation	3.99	0.022	0.036	0.040	0.26	2.11	13.92
Kurtosis	0.97	0.008	0.004	0.028	0.08	1.27	1.51
Skewness	2.57	0.022	0.011	0.073	0.22	3.37	3.99
CV	-0.28	-1.335	4.314	3.338	0.28	5.40	-1.14
Mean	0.91	0.592	1.955	1.930	1.33	2.31	0.86
Standard error	64.50	101.58	30.92	184.37	85.63	159.99	28.68

Cu, Cd, Cr, Co, Ni and Zn values of the hazelnut leaves were 1.60-8.42 mg kg^{-1}, 0.001-0.056 mg kg^{-1}, 0.026-0.060 mg kg^{-1}, 0.001-0.191 mg kg^{-1}, 0.08-0.65 mg kg^{-1} and 0.41-9.47 and 10.23-19.83 mg kg^{-1}, respectively and the heavy metal content of hazelnut leaves was listed as Zn>Cu>Ni>Co>Pb>Cr>Cd. Pehlivan and Aslantaş (2020), carried out an investigation about determination of heavy metals in hazelnut gardens located between Trabzon and Giresun. In hazelnut leaves, Cr content 0.43-4.64 mg kg^{-1}, Ni content 0.18 - 14.54 mg kg^{-1} and Pb 0.48-4.32 mg kg^{-1} were found.

The bio-accumulation factor values calculated for three plants were given in Table 5. According to results, BAF values of tea leaves were detected between 0.00003-0.64 and were sorted as Zn>Ni>Cu>Cd>Co>Cr>Pb on average values while BAF values of tea leaves were detected between 0.00002-2.56 and were listed as Zn>Cu>Pb>Cd>Co>Ni>Cr on average values. In addition, BAF values of hazelnut leaves were detected between 0.0001-0.48 and were listed as Zn>Cd>Cu>Ni>Co>Pb>Cr on average values.

Table 5. Bio-accumulation (BAF) values of the samples

Sample number	tCu	tCd	tCr	tPb	tCo	tNi	tZn
Tea leaf samples							
T-1	0.09	0.22	0.005	0.00013	0.02	0.31	0.34
T-2	0.12	0.06	0.002	0.00011	0.02	0.05	0.35
T-3	0.50	0.08	0.001	0.00010	0.08	0.23	0.43
T-4	0.07	0.06	0.001	0.00014	0.01	0.39	0.32
T-5	0.21	0.07	0.001	0.00015	0.01	0.06	0.43
T-6	0.09	0.36	0.002	0.00004	0.02	0.14	0.16
T-7	0.22	0.04	0.002	0.00003	0.64	0.16	0.14
T-8	0.20	0.23	0.003	0.00003	0.10	0.34	0.14
Min	0.07	0.04	0.001	0.00003	0.01	0.05	0.14
Max	0.50	0.36	0.005	0.0001	0.64	0.39	0.43
Mean	0.186	0.139	0.002	0.0001	0.113	0.211	0.290
Vine leaf samples							
V-1	0.30	0.038	0.0005	0.07	0.02	0.00002	0.45
V-2	2.37	0.005	0.0007	0.26	0.01	0.008	2.56
V-3	0.42	0.111	0.0005	0.09	0.02	0.006	0.39
V-4	0.28	0.001	0.0004	0.02	0.01	0.002	0.25
V-5	0.16	0.005	0.0002	0.13	0.01	0.011	0.27
V-6	0.33	0.007	0.0003	0.05	0.01	0.002	0.41
V-7	0.44	0.002	0.0003	0.06	0.01	0.032	0.35
V-8	0.26	0.001	0.0004	0.11	0.01	0.00002	0.63
Min	0.160	0.001	0.0002	0.02	0.01	0.00002	0.25
Max	2.371	0.111	0.001	0.26	0.02	0.03	2.56
Mean	0.568	0.021	0.0004	0.099	0.011	0.008	0.664
Hazelnut leaf samples							
H-1	0.13	0.07	0.0004	0.0001	0.005	0.032	0.41
H-2	0.18	0.44	0.0004	0.0070	0.039	0.072	0.42
H-3	0.20	0.04	0.0018	0.0171	0.015	0.027	0.38
H-4	0.11	0.00	0.0040	0.0001	0.027	0.055	0.18
H-5	0.07	0.00	0.0013	0.0001	0.021	0.021	0.48
H-6	0.07	0.38	0.0029	0.0001	0.025	0.024	0.22
H-7	0.15	0.08	0.0020	0.0001	0.009	0.033	0.31
Min	0.07	0.001	0.0004	0.0001	0.01	0.02	0.18
Max	0.20	0.44	0.004	0.02	0.04	0.07	0.48
Mean	0.131	0.146	0.002	0.003	0.020	0.038	0.343

Hu et al. (2020) in their study, which examined the accumulation of heavy metals of plants in different crop-soil systems, they calculated BAF values for tea fronds as 0.013 for Pb, 0.022 for Hg, 0.501 for Cu, 0.396 for Zn, 0.246 for Ni and 0.413 for Cd. Zhang et al (2020) reported that 63.6% of young tea leaves were BAF>1 for Mn and BAF<1 for Cd, Pb, Tl, Hg, As, Sb, Cr and Ni. Moreover, Beygi and Jalali (2019) found the transfer factor in vine fronds in the form of Zn>Ni> Cu>Cd.

The most accumulating element of all three plants was Zn. In tea leaves, the least accumulating element was Pb, while in Vine and hazelnut leaves, this element was determined as Cr. It is known that metals accumulate in plants at different rates depending on factors such as plant differences, physicochemical properties of soils, usefulness of heavy metals in soils (Sharma et al., 2018; Hu et.al.2017; Bravo et.al. 2017). Only BAF values for Cu and Zn elements of a sample on vine leaves were found above 1 and in all samples other than this, BAF values were below 1. Looking at the soil analysis results of the land where this sampling was performed, it was determined that Cu (31.10 mg kg^{-1}) and Zn (19.67 mg kg^{-1}) were significantly lower than tolerable values. But, because the clay content and organic matter value of the soil in which they were located was low, the retention of these elements in the soil was low and plant protection applications to vine may have caused BAF to be high.

CONCLUSIONS

The present study was carried out to determine the concentration of heavy metals and bio-accumulation case in soil and leaf samples belonging to the three most important agricultural products (hazelnut, tea, vine) of the Karadeniz Region. For this purpose, soil and plant leaves from Samsun, Rize and Tokat provinces were collected. Physical and chemical properties also heavy metal contents of the soils were determined. According to the study results, it was determined that Cd values vary between 0.001-0.038 mg kg^{-1}, Co values 0.023-0.146 mg kg^{-1}, Cr values 0.018-0.024 mg kg^{-1}, Cu values 9.663-73.756 mg kg^{-1}, Ni values 0.001-1.610 mg kg^{-1}, Pb values 0.347-0.684 mg kg^{-1} and Zn values 13.015-50.264 mg kg^{-1} in vine leaves. In addition, it was found that Cd values varied between 0.022-0.081 mg kg^{-1}, Co values 0.092-0.533 mg kg^{-1}, Cr values 0.025-0.037 mg kg^{-1}, Cu values 2.067-8.017 mg kg^{-1}, Ni values 0.663-2.803 mg kg^{-1}, and Zn values 13.103-26.953 mg kg^{-1} in tea leaves. Moreover, it was detected that Cd values vary between 0.001-0.056 mg kg^{-1}, Co values 0.082-0.652 mg kg^{-1}, Cr values 0.026-0.060 mg kg^{-1}, Cu values 1.603-8.419 mg kg^{-1}, Ni values 0.406-9.473 mg kg^{-1}, Pb values 0.001-0.191 mg kg^{-1} and Zn values 10.227-19.834 mg kg^{-1} in hazelnut leaves. Also, the most accumulating element of all three plants was Zn. BAF values were also changed between 0.00002-2.56 and were sorted as Zn>Cu>Pb>Cd>Co>Ni>Cr over average values in vine leaves. BAF values of tea leaves varied between 0.00003-0.64 and were sorted as Zn>Ni>Cu>Cd>Co>Cr>Pb on average values while BAF values of hazelnut leaves changed between 0.00006-0.48 and were sorted as Zn>Cd>Cu>Ni>Co>Pb>Cr on average values.

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EVALUATING COMMERCIAL BERRY JAMS FOR THE PRESENCE OF POLYPHENOLS IN SAMSUN AREA

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ABSTRACT

Biologically active components such as polyphenols are becoming an area of interest in the research of many scientists. Furthermore, polyphenols have a very important role as functional foods in our nutrition having many effects on prevention and protection from several specific diseases i.e. certain polyphenols can stop several diseases' development via certain mechanisms. Berries are rich in the content of polyphenols. Due to the stated facts, berries are recommended for regular consumption. At the same time, fresh berries are subject to rapid deterioration and, consequently, they are generally processed into different kinds of products to extend their shelf-life and made easily accessible all year round. Customers are more likely to buy commercial fruit products than to make them at home, so the purpose of this paper was to estimate the amount of polyphenolic content in four different kinds of jams of fruit from locations 'Samsun', Black sea region Turkey. Individual polyphenols were detected and quantified by Ultra-Fast Liquid Chromatography (UFLC) technique. Most of the analyzed polyphenol compounds were detected in the blueberry jam sample.

INTRODUCTION

Traditional berry is an important foodstuff in the local population's nutrition, and it has been used in folk medicine since ancient times. And in last years berry fruits, are more available on the Turkish market, both for fresh consumption during the growing season and are also manufactured into juices, jams, fruit under the syrup, etc. Awareness of the health benefits correlated to berries' consummation has also favored the local commercialization of blueberries, strawberries, raspberries, and blackberries and also wild species of berries spontaneously grow in mountainous areas. Berry fruits are rich in biologically active components. Biologically active components such as polyphenols are becoming an area of interest in the research of many scientists. Phenolics are defined as compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known (Dai and Mumper, 2010). Their chemical diversity is matched by their various role in the plant world, for example, phenolics can function as antibiotics, to prevent water loss, as natural pesticides, to signal substances for the establishment of symbiosis with rhizobia, as attractants for pollinators, to protect the plant from harmful ultraviolet solar radiation and as structural materials to give plants stability (de la Rosa et al., 2010; Shahidi and Naczk, 2006). Furthermore, polyphenols have a very important role as functional foods in our nutrition having many effects on prevention and protection from several specific diseases i.e. Certain polyphenols can stop several diseases' development via certain mechanisms thus increasingly gaining importance in the food industry today. Focusing on fruit jams, consumers are highly interested in health claims of polyphenols. The main commercial jams are based on the use of red fruits like bilberry, blackberry, cranberry, strawberry, raspberry, cherry. Labeling commercial fruit jams detailing polyphenol composition would be of great interest to consumers and would increase market demand. Our goal was to analyze some of the most common commercial jams of purple-red berries blueberry, strawberry, blackberry, and raspberry for their contents of total phenolics, total flavonoids, DPPH concentration and to determine their metal chelating activity.

MATERIAL AND METHOD

Commercially available berry jams made of blueberry, blackberry, raspberry, and strawberry were bought from a supermarket in Migros, Samsun – Turkey. All four jams were produced in 2019, purchased, and analyzed in the summer of 2020. Extraction of selected fruit sample analysis of individual polyphenols was performed
according to the method of WU.M.M. Et al. (2015), with some modification.

Table 1. Time-scheduled gradient elution program

	Time	Module	Action	Value
1	0.2	Pumps	C	10
2	28.00	Pumps	C	40
3	39.00	Pumps	C	60
4	50.00	Pumps	C	90
5	55.00	Pumps	C	10
6	65.00	Pumps	C	10
7	65.00	Controller	Stop	

Total Phenolic Determination

Analytical standards were: gallic acid, p-coumaric acid, caffeic acid, neochlorogenic acid, procyanidin B_1, procyanidin B_2, epicatechin, quercetin 3-glucoside, quercitrin 3-ramnozid, keracyanin chloride, and rutin. The chromatographic separation was performed using the Shimadzu Ultra Fast Liquid Chromatograph system (UFLC, Shimadzu Corporation, Kyoto Japan) equipped with a pump LC-20AD, diode array detector SPD-M20A, autosampler SIL-20AHT, room temperature. Chromatographic separation was performed on a C18 Intersil ODS-3 column (GL Science Inc., Japan) analytical column (4.6x250mm, 5 µm). A gradient consisting of (A) 1% acetic acid and (C) acetonitrile was in chromatographic separation. Wavelength 280nm and 520nm. The injected volume was 10 µL. The studied phenolic compounds were successfully separated in 65 min and the flow rate was 0.7 mL/min.

Total phenolic compounds were determined according to the method proposed by Slinkard and Singleton (1977), using Folin-Ciocalteu reagent linked to phenolic standard gallic acid. After 1 mL of plant extract was taken into a test tube, 4.5 mL of water and 0.1 mL of Folin–Ciocalteu reagent was added, and the solution was kept in the dark for 5 minutes, and 0.3 mL of sodium carbonate (2%) was added. Tubes were covered with parafilm and kept in the dark for 1 hour. Measurements were made at 765 nm in a spectrophotometer and a comparison was made with the gallic acid calibration curve. Results are expressed as mg gallic acid/g (mg GAE/g) in the dried sample.

Total Flavonoid

Total flavonoid content was determined with quercetin standard solution using the methods suggested by Park et al., (2008). 1 mL of extract was placed in test tubes, followed by 2 mL of distilled water, 0.15 mL of 0.5M $NaNO_2$ and 0.15 mL of 0.3 M $AlCl_3$ reagent. 5 minutes after this application, 1 mL of NaOH was added and the absorption was measured at 510 nm with a spectrophotometer, and compared with the quercetin calibration curve. Total flavonoids were defined as mg equivalents of quercetin (mg QE/g) per g of the dried fraction.

Antioxidant Activity Determination (2,2-difenil-1-pikrilhidrazil- DPPH)

In our study, DPPH Free Radical Removal method was used to determine antioxidant activity. This method is based on the reaction of hydrogen donor antioxidants with a stable free radical, DPPH (1,1-diphenyl-2-picryl-hydrazil) and converting this radical to αα-diphenyl-β-picryl hydrazine with scavenging activity. The color change (lightening of the characteristic purple color) refers to the scavenging potential of the antioxidant compounds, and the absorbance changes of the samples are measured at 517 nm in the spectro (Brand-Williams et al. 1995). Antioxidants reduce absorption by giving protons to radicals, low absorption of the reaction mixture indicates high free radical scavenging activity (Gülçin et al., 2004). Plant material was prepared at different concentrations (25-400 µg/mL) and 0.5 mL of these extracts were taken and 3 mL of ethanol and 300 mL of 20 mg/L DPPH solution were added. After adding butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trolox (100–500 µg/mL) to this solution, the mixture was shaken vigorously and the decrease in absorbance at 517 nm was measured. As a control, 0.75 mL of water was used instead of the sample. The percent inhibition activity was calculated with the formula:

$$\text{DPPH}^{\bullet}\ \text{radical scavenging effect}(\%)$$

$$\% = [(A0 - A1) / A0] \times 100$$

Metal Chelating Activity (MCA)

The chelating activity (Fe^{2+}) of the extracts in iron ions, which is an approach used to express antioxidant activity, was determined according to the method proposed by Decker and Welch (1990). For this purpose, 1mL of extract sample at different concentrations (25-400 µg/mL) was mixed with 3.7 mL of deionized water and the mixture was incubated with $FeCl_2$ (2 mM, 0.1 mL) for 30 minutes. After incubation, the reaction was started by adding ferrozine (5 mM and 0.2 mL) to the mixture and after it was kept at room temperature for

25 minutes, absorbance was measured at 562 nm. The chelating activity of the extracts on Fe^{2+} was compared with the same concentrations of EDTA and calculated with the same formula as DPPH activity.

RESULTS

The total phenolic and total flavonoid content, and total antioxidant activity (DPPH) in fruits of blackberry and blueberry are shown in Table 1.

Jam sample	Total Phenolic (mg GAE/g)	Total Flavonoid mg (QE)/g	Antioxidant Activity (%)
Blackberry	63,85	13,31	14,80
Strawberry	57,80	15,04	20,99
Raspberry	58,85	14,22	20,68
Blueberry	133,38	20,22	22,50

Randomly selected four berry jams (blueberry, blackberry, strawberry, and raspberry) were analyzed for their total phenolic content. Based on the results shown in Table 1, statistically significant differences among the total phenolic contents of the jams were recorded. The content of total phenolics ranged between 57,80 and 133,38 mg of GAE/g. Disagreeing with the literature range of 174,20–484,15 mg GAE / 100 g as reported by Mendes Rodrigues et al. (2017) on blueberry, blackberry, and strawberry jams. The blueberry jam contained the highest total phenolic content (133.38 mg GAE/g), whereas raspberry jam was demonstrated to have the lowest phenolic content (57,80 mg GAE/g). Although the total phenolic obtained in our study were generally lower than the values obtained in other studies, the results are still considered to say that the commercial jams bought in the Samsun, Black Sea Region of Turkey are good sources of phenols.

The total flavonoid contents of the samples were evaluated by using Park´s method, and the results are shown in Table 1. The levels of the total flavonoids ranged between 13,31 mg QE/g in the blackberry jam and 20,22 mg QE/g in the blueberry jam. Similar to the total phenolic content, blueberry jam contained the highest total flavonoid concentration. Additionally, in the case of blackberry jam, the total flavonoid content was significantly lowered 13,31 mg QE/g compared to that of blueberry jam 20,22 mg QE/ g. Raspberry and strawberry jams appear to have similar total flavonoid contents. Raspberry jam contained 14,22 mg QE/ g and strawberry 15,04 mg QE/ g. Their quantitative differences to our results could be due to different cultivars used for jam preparation, environmental growing conditions, different harvesting time, the manufacturing procedure, or even the storage conditions used.

The antioxidant activity of the samples was measured by DPPH assay and found to be ranging between 14.80 and 22,50 (%) with the highest antioxidant activity being observed for blueberry sample 22,5 %, and the lowest blackberry jam 14,80% . Noticeably, strawberry and raspberry jams presented a similar antioxidant potential (20,99 and 20,68%, respectively). We believe that the high antioxidant capacity in blueberry jam is due to its higher content in phenolic compounds and total flavonoid.

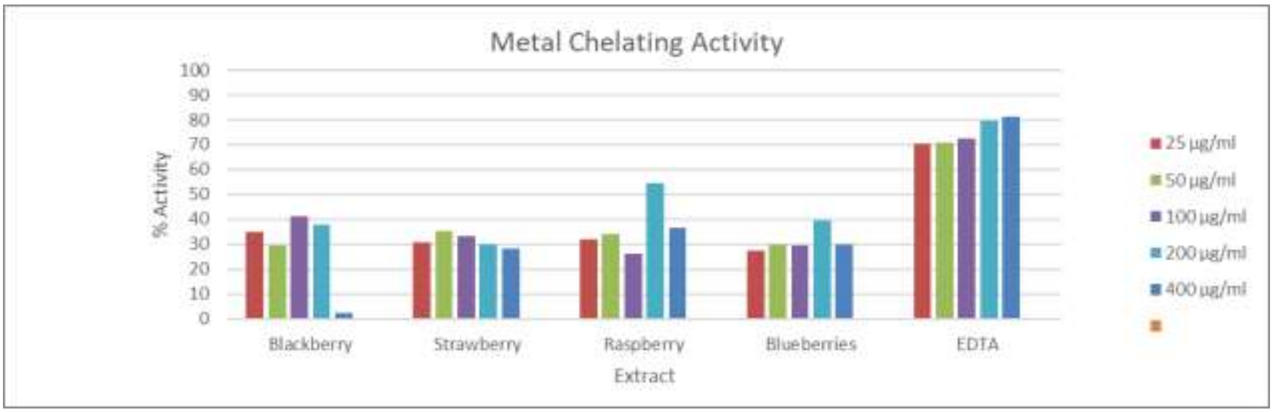

Figure 1. Metal chelating activities of jams extracts at different concentrations. Data are reported as mean values (n=3).

As excess free irons have been implicated in the induction and formation of free radicals in biological systems, we tested our jams samples in a metal chelating assay. Tested in the different concentration ranges, all 4 extracts demonstrated strong chelating activities in concentration-dependent manners (Figure 1).

The contents of individual phenolic compounds among the strawberry, raspberry, and blackberry, and blueberry jams examined varied considerably. The dominant individual phenolic compound in the blueberry jam was epicatechin 19,71 mg/100g, in blackberry jam (8,26 mg/100g) and raspberry jam (7,80 mg/100g) was gallic acid, in strawberries jam procyanidin B_2 4,90 mg/100g was the dominant individual phenolic compound.

Gallic acid was detected in all four samples.The levels of gallic acid ranged between 3,94 mg/100g in the blueberry jam and 8,26 mg/100g in blackberry jam. In the raspberry jam sample, gallic acid was determined 7,80 mg/100g, and in strawberry jam 4,58 mg/100g. Content of p-coumaric acid 0,80 mg/100g was detected only in blueberry jam. Caffeic acid was detected in the following jams: blueberry sample 6,98 mg/100g, blackberry sample 3,49 mg/100g, raspberry sample 7,69 mg/100g. Neochlorogenic acid has similar content in the next three samples: blueberry jam 4,68 mg/100g, blackberry jam 4,18 mg/100g, and strawberry jam 4,30 mg/100g.

Procyanidin B_1 was detected only in strawberry jam and its quantity was 4,90 mg/100g. Procyanidin B_2 was detected in three of four samples: blueberry jam 3,60 mg/100g, raspberry jam 3,23mg/100g, and strawberry jam 3,2 mg/100g.

Quercetin 3-glucoside (3,20 mg/100g), quercitrin 3-ramnozid (1,05 mg/100g) and epicatechin (19, 71 mg/100g) were detected only in blueberry jam. Most individual polyphenols were found in blueberry jam.

Rutin was detected in two jam samples, blueberry 0,81 mg/100g and strawberry 0,60 mg/100g.

The only anthocyanin that was analyzed in this study was keracyanin chloride and identified and quantified in three samples of jam, blueberry jam 2,31 mg/100g, raspberry 1,28 mg/100g, and the greatest value detected in blackberry jam 2,6 mg/100g.

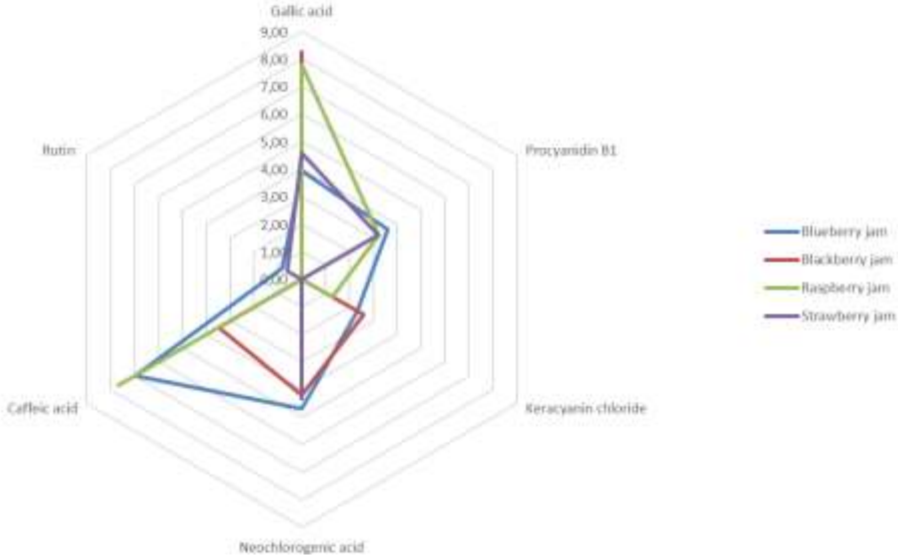

Figure 2. Review of some individual polyphenols in tested jams

CONCLUSION

Berry fruit jams are good sources of natural antioxidants and possess potent antioxidant activity. In the present study, we showed that total phenolic, total flavonoid, and DPPH activity values of blueberry jam were higher than the rest of the three jams. Most individual polyphenols were found in blueberry jam. The dominant individual phenolic compound in the blueberry jam was epicatechin 19,71 mg/100g, in blackberry jam (8,26 mg/100g) and raspberry jam (7,80 mg/100g) was gallic acid, in strawberries jam procyanidin B2 4,90 mg/100g was the dominant individual phenolic compound. Gallic acid was detected in all four samples. These berry fruit jams species are rich in phenolic compounds and thus recommended for everyday consumption.

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EFFECTS OF LIVING AND NON-LIVING MULCH APPLICATIONS ON FRUIT YIELD AND QUALITY IN ORGANIC KIWIFRUIT PRODUCTION

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ABSTRACT
Kiwifruit cultivation, whose production has increased rapidly in recent years, started in the Black Sea region in the 1990s and according to 2020 TUIK statistics, 63,798 tons of kiwifruit is produced in an area of 21,325 da. Organic production constitutes 841 tons of this production. With the technological developments at the global level, increasing population and industrialization, environmental pollution is increasing proportionally and the balance of nature is deteriorating. This situation led to the need to protect the natural balance and new concepts such as good agriculture, organic agriculture and sustainable agriculture revealed. Organic agriculture forbids the use of synthetic chemicals as pesticides, which are foreign to nature, intrusive and put the life of living things at risk by creating residues. This study was carried out in the farmer's garden in Aydınlar Village of Giresun province between 2016-2019 to determine the effects of living and non-living mulch materials on fruit yield and quality in organic kiwifruit production. As living mulch materials, soil surface covering plants, hazelnut husk + straw and weeds were cut and left on the soil. Geotextile (weed mat) cover was used as inanimate mulch material. The study was established according to the randomized complete blocks experimental design, in 20 plots with 5 applications x 4 replications and 4 kiwi vines in each plot. In the study; yield, fruit weight, fruit width, fruit length, fruit flesh color, vitamin C content, total phenol, TSS, pH, TETA and fruit hardness parameters were examined. In our study, in which a sustainable agricultural technique and nature-friendly methods were discussed, no statistical differences were found apart from yield and TETA data. However, it is hopeful to transfer the results obtained one-to-one into practice. In terms of fruit weight (100.1 grams), fruit width (51.91 mm) and vitamin C content (100 mg/100 gr), hazelnut husk + straw application gave the highest results. On the other hand, geotextile in terms of fruit size (66 mm) and pH, and surface covering plant applications (9.32) in TSS values came to the fore. When the yield per decares was evaluated according to the years, it was determined that there were statistical differences. According to the results obtained, the control application was in the same group as the surface covering plant application (1157 kg/da) with a fruit yield of 1281 kg per decares, while the hazelnut husk + straw application was in the last group with 827 kg/da.

Keywords: kiwifruit, weed mat, hazelnut husk, mulch, yield

INTRODUCTION

Kiwi was first brought to our country from Italy in 1988 through the Atatürk Horticultural Central Research Institute. With the saplings brought this year, adaptation-demonstration gardens in 15 different ecologies have been established in our country. As a result of several studies, it has been determined that some areas close to the coast in the Marmara and Black Sea Regions and the Aegean are suitable for kiwi cultivation (Yalçın, 1999).

Black Sea and Marmara regions are the kiwi production centers of Turkey due to their climate. According to 2020 TUIK statistics, 63,798 tons of kiwi are produced. The highest production was obtained from the provinces of Yalova, Bursa, Ordu, Rize and Samsun, respectively. Organic production constitutes 841 tons of this production (TUIK, 2021).

The increase in food demand brought about by rapid population growth puts pressure on agricultural areas. This situation imposes more production tasks on researchers with less input. The fact that producers in most countries lack the resources to use high inputs increases the threat to environmental quality.

The inputs used in agricultural systems pose a great threat to the environment and human health, and the pollution in natural resources is increasing day by day. In addition, the necessity of sustainable friendly production techniques in agricultural systems emerges. Therefore, the importance of organic agriculture has increased considerably in recent years. Organic agriculture does not allow use of synthetic chemicals like pesticides, which are foreign to nature, intrusive and put the life of living things at risk by creating residues (Demiryürek, 1999). The use of living and non-living mulches in organic agriculture is important in order to prevent environmental pollution, achieve agricultural sustainability, ensure the continuity of resources, and reduce agricultural costs. In many studies conducted in this area, black and white geotextile, black woven geotextile, black and transparent polyethylene, and green heavy and durable plastic mulch products were used as inanimate mulch material (Ashworth and Harrison,1983; Brown and Osborn, 1989; Wien et al.,1993; Wan and El-Swaify ,1999; Verdu and Mas, 2007.). As a living mulch material; many plant materials such as shredded bark, straw, vetch, rye, clover and legumes were used (Wagger, 1989; Asiegbu, 1991; Clark et al.,1994; Iles and Dosmann, 1999; Olsen and Gounder, 2001). As a result of the researches, it has been observed that the use of living and non-living mulch materials according to mulch-free applications has a significant positive effect on many factors such as soil structure, soil organic matter content, increase in soil nitrogen amount, increase in fruit yield and quality, increase in shoot length, earliness, and weed control. (Ashworth and Harrison, 1983; Smith et al., 1987; Brown and Osborn, 1989; Asiegbu, 1991;, Wien et al.,1993; Creamer et al., 1996; Walsh et al., 1996; Iles and Dosmann, 1999; Buck et al., 2000, Olsen and Gounder, 2001; Barrales-Dominguez, J.S. and Alejo-Santiago G., 2002; Brault et al., 2002; Sanchez et al., 2007; Kitiş, 2009, Nieto, Castro & Fernandez-Ondono, 2012).

In this study, it was aimed to develop a sustainable farming technique with the effects of living and non-living mulch on fruit yield and quality in organic kiwi cultivation.

MATERIAL AND METHOD

This study was carried out in the farmer's garden in Aydınlar Village of Giresun province between 2016 and 2019 to determine the effects of living and non-living mulch materials on fruit yield and quality in organic kiwifruit production. The producer's garden consists of the Hayward kiwi variety, which was established in 2002 with the T cultivation system on its own root. As living mulch materials, soil surface covering plants, hazelnut husk + straw and weeds were cut and left on the soil. Geotextile (weed mat) cover was used as inanimate mulch material. The study was established according to the randomized complete blocks experimental design, in 20 plots with 5 applications x 4 replications and 4 kiwi vines in each plot.

Vicia villosa and *Scale cereale* mixture (beginning of flowering form) was used as cover plant on the plot ground, hazelnut husk + straw mixture as mulch 1 material (end of March-early April), geotextile (end of March-early April) was used as mulch 2. In addition, a total of 5 applications were made as cutting the existing weeds and leaving them in the soil (once every 2-3 weeks) and control (leaving them to natural vegetation without any application). In the experiment, 500 grams of hairy vetch and 800 grams of rye seeds per plot were sown in October-November considering the weather conditions. In the group where husk + straw was applied, 75 kg of straw per parcel and 55 kg of hazelnut husk obtained in the previous production period were used. In the study; yield, fruit weight, fruit width, fruit length, fruit flesh color, vitamin C content, total phenol, TSS, pH, TETA and fruit hardness parameters were examined.Variance analyzes were evaluated with the JMP13 statistical package program, taking the average of 3 years. Differences between data were determined by the LSD multiple comparison test.

Figure 1. Application plots (Control, Geotextile, Hazelnut husk + Straw mix, *V. villosa* + *S. cereal* mix and mowing existing grass, respectively.)

RESULTS AND DISCUSSION

The harvest date was determined according to the weekly brix values in the trial area, starting from October every year, and harvesting was carried out when it reached 12%. As a result of the statistical analysis; there was no statistical difference in terms of fruit weight, fruit width, fruit length, firmness, TSS and pH values. On the other hand, while the fruit weight values ranged between 96.77 g and 100.1 g, the highest value was obtained from the Hazelnut husk + Straw applied plots. When the fruit width and length values were examined, the highest values were obtained from the fruits in the parcel where geotextile was applied, with 51.91 mm and 66 mm, respectively. When the hardness values were examined, the lowest value was obtained from the parcels with geotextile cover with 6.12 kg, while the highest value was obtained from the Control application with 7.23 kg.

As the reason for the lack of difference in these values, it is thought that studies covering longer years should be carried out in the changes made in the production technique in perennial species.

Table 1. Harvest values

	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Fruit hardness (kg)	TSS	TA	pH	Kg/da
Geotextile	98.84	51.19	66.00	6.12	8.78	1,65 b	3.46	1026 ab
Mowing existing grass	98.99	50.88	64.59	6.25	9.12	1,67 b	3.41	1000 ab
Hazelnut husk + Straw	100.1	51.91	63.50	6.57	8.57	1,59 b	3.43	805 b
V. villosa + *S. cereal* mix	98.18	50.25	64.17	6.86	9.32	1,74 ab	3.35	1157 a
Control	96.77	50.76	63.39	7.23	8.74	1,85 a	3.35	1250 a
	-	-	-	-	-	P<0,05	-	P<0,05

When the yields per decare were evaluated according to years, statistical $p<0.05$ significance level differences emerged. Accordingly, Control application was in the same group with the plots where *V. villosa* + *S. cereal* mixture was applied with 1281 kg/da, while Hazelnut husk + Straw application was in the last group with 827 kg/da (Table 1).

TSS values were obtained by measuring the samples taken from fruit juice prepared for sugar analysis with a pH meter. As a result of the analysis, the difference between the applications was not statistically significant, but the highest pH values of 3.46 were obtained from the parcels with Geotextile cover. When Table 2 is examined, there is no statistical difference between the TSS values, but the values varied between 9.74 and 10.50.

In the study, there was no difference between the statistically evaluated data, but TPvalues varied between 480 and 620 mg/GA. While the highest total phenolic content was obtained from the cover crop application, the lowest value occurred in the geotextile application.Vitamin C analysis was made by spectrophotometer method, and as a result of the analysis, the amounts of vitamin C among the applications were not statistically significant, but the values varied between 80 and 100 mg/100 g.

Table 2. Eating values

	TSS(%)	TA (%)	pH	Fruit hardness (kg)	Total phenol (TP) content (mg/GA L)	Vitamin C content (mg/100gr)
Geotextile	10.07	1.66	3.54	1.24	480	80
Mowing existing grass	9.74	1.66	3.52	1.14	570	90
Hazelnut husk + Straw	9.78	1.67	3.52	1.68	565	100
V. villosa + S. cereal mix	10.28	1.65	3.49	1.46	622	95
Control	10.50	1.65	3.36	1.20	548	80
	-	-		-	-	-

Figure 1. Harvest (2019)

Similar to the results obtained from our study; In the study of Shirgure et al. (2003) to investigate the effects of different mulch applications on plant growth and yield in mandarin cultivation; It has been reported that the best results in terms of fruit yield, fruit weight, total soluble dry matter amount and acidity were obtained from black polyethylene mulch application. Kumar and Srivastaka (1998), in their study, found that covering the soil surface increased the total fruit yield percentages, significantly affected the specific weight and volume of the fruit, and had no effect on total soluble dry matter and vitamin C. Another study, Işık et al. (2006), investigated the possibilities of using cover crops in the fight against weeds in hazelnut orchards, and as a result of the study, they obtained the highest yield from the plots on which the cover crop was applied. In this research study, it is thought that the reason why the plots on which the cover crop was applied were lower than the control, may be related to the distribution of the plots in the random blocks in the field.

Table 3. Fruit flesh color

	L		a		B		Chroma		Hue	
	interior color	exterior color	IC	EC	IC	EC	IC	EC	IC	EC
Geotextile	47.43	40.60	5.03	7.29	5.35	4.79	3.44	5.23	06.10	22.93
Mowing existing grass	45.36	35.25	4.66	6.10	4.17	2.25	2.14	2.03	05.62	12.73
Hazelnut husk + Straw	51.61	42.97	4.86	7.57	6.18	5.39	5.16	6.89	05.40	12.33
V. villosa + S. cereal mix	50.71	41.57	5.51	7.33	5.58	4.85	3.36	5.54	06.52	13.06
Control	42.30	35.28	4.52	6.46	4.04	2.64	2.64	2.17	06.29	13.02

CONCLUSIONS

In our study, in which a sustainable agricultural technique and nature-friendly methods were discussed, no statistical differences were found apart from yield and TETA data. However, it is hopeful to transfer the results obtained one-to-one into practice. In terms of fruit quality, there is no statistical difference in harvesting and eating. However, in order to transfer the positive effects to practice, especially perennial plants, extending the trial period and evaluating it together with the data obtained in the following years will yield more effective results. However, the highest value in terms of fruit weight was obtained from the plots with Hazelnut husk+ Straw applied, and the values closest to this application were also obtained in the plots where *V. villosa + S. cereal* mix and Geotextile were applied. The lowest fruit weight was shown in the Control. Considering the importance of fruit weight in marketing, Hazelnut husk + Straw or Geotextile applications are recommended according to the possibilities of the producers. Our work will contribute to the country's economy in terms of recycling and reintroducing the husk, which is not evaluated especially in regions with hazelnut production areas. It is beneficial to continue similar studies in order to fully reveal the effects of sustainable environmentally friendly practices on fruit yield and quality.

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DETERMINATION OF PESTS (DIPTERA: TEPHRITIDAE) IN FRUIT GROWING ON TURKISH REPUBLIC OF NORTHERN CYPRUS

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ABSTRACT

Güzelyurt region of Turkish Republic of Northern Cyprus has a very important place in terms of fruit growing. Most of the subtropical fruits, especially citrus, olive, pomegranate and fig, are grown in the region. In order to determine the species belonging to the Tephritidae family, McPhail traps were prepared to be used in the orchards with 9 different fruit species selected in Kalkanlı, Yedidalga and Yeşilırmak villages of Güzelyurt between 10th September and 29th November 2016 and were hung in the specified orchards and renewed every week. As a result of this study, 5 species belonging to the Tephritidae (Diptera) family and 1 species of the Drosophilidae (Diptera) family, which are important for fruit growing, were determined. These species; Olive fruit fly (*Bactrocera oleae* (Rossi.)), Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)), Peach fruit fly (*Bactrocera zonata* (Saunders)), Celery fly (*Euleia heraclei* L.), *Trupanea amoena* (Frauenfeld) (Tephera) and Spotted wing drosophila (*Drosophila suzukii* (Matsumura)). Among these species, *B. zonata*, *E. heraclei*, *T. amoena* and *D. suzukii* were detected for the first time in the Turkish Republic of Northern Cyprus, and it is the first record for the insect fauna of the Turkish Republic of Northern Cyprus. *B. zonata* and *D. suzukii* are species that cause economic damage for fruit growing, while *T. amoena* is a phytophagous species that has also been reported in Turkey and is found in the natural flora. *B. zonata* is a polyphagous species and has been reported to cause damage during the growing season, especially in peach, mango and guava fruits. On the other hand, *D. suzukii* causes damage to different fruit species throughout the year and also causes fungal and bacterial diseases, which are secondary microorganisms, and is also a vector for secondary microorganisms such as fungal and bacterial diseases. In this study, fruit flies (Diptera: Tephritidae), which are also a problem in fruit growing, were detected in Kalkanlı, Yeşilırmak and Yedidalga villages in the Güzelyurt region. The results of this research consist of the first comprehensive data from Turkish Republic of Northern Cyprus and provide the basis for future studies.

Keywords: Fruit, Diptera, pest, TRNC

1. Introduction

The area of 187.069 hectares, which corresponds to 56.7% of the total land assets of the Turkish Republic of Northern Cyprus (329,891 hectares), is agricultural land. 157,483 hectares (84.2%) of this is arable land. More than 88% of agricultural land use is reserved for cereals and legumes produced for animal husbandry. Considering the irrigated lands, the total irrigated land is constituted approximately 39% of citrus fruits, 40% other fruits, 17% vegetables and greenhouses, 20% other fruits, 2% vineyards and 2% legumes (Kahramanoğlu et al., 2018). Fruit flies are the main pest in fruit growing on TRNC and therefore cause significant damage. Tephritidae family is a large family of nearly 4,500 described species arranged in about 500 genera. Fruit flies are characterized by the long extendible ovipositor of the female that is used to lay eggs under the skin of fruits and vegetables, which then provide food for the developing larvae. A number of fruit fly species attack citrus fruit in the most important growing areas in the world. These pests are tropical and subtropical multivoltine species with a broad host range of pulpy fruits, characterized by a great dispersal ability and high reproductive potential (Delrio and Cocco, 2012). The Güzelyurt region of the TRNC has a very important place in terms of fruit growing. Many subtropical fruits, especially citrus, olive, pomegranate and fig, are grown in the region. During this study, traps were prepared and hung on different fruit species to detect pests belonging to the Tephritidae family in Güzelyurt and Kalkanlı and Yeşilırmak villages of Güzelyurt.

2. Material and Method

In order to determine the species belonging to the Tephritidae family, which contains important pests such as Olive fruit fly and Mediterranean fruit fly in orchards, McPhail traps were hung in the orchards containing 9 different fruit species determined in Kalkanlı and Yeşilırmak villages of Güzelyurt and Güzelyurt between 10 September and 29 November 2016 on TRNC (Table 1, Figure 1 and 2). The traps were checked weekly and the flies were taken into Eppendorf tubes with the help of a pliers. After that, these tubes were sent to Çukurova University Faculty of Agriculture, Department of Plant Protection, Biological Control Laboratory to be identified by writing label information on them. The work continued throughout 2016 and the traps were replaced with new ones as needed in the orchards.

The insect species obtained in the study were identified and confirmed commonly known pests by Prof. Dr. M. Rifat ULUSOY (Çukurova University Faculty of Agriculture, Department of Plant Protection Biological Control Laboratory), unknown Tephritidae species by Assoc. Prof. Dr. Murat Kutuk (Gaziantep University, Department of Biology) and Drosophilidae species by Assoc. Prof. Dr. Gulay Kacar (Bolu Abanat İzzet Baysal University, Faculty of Agriculture).

Figure 1. Appearance of McPhail traps in peach and plum trees

Table 1. General information about the orchards where the McPhail traps were hung

No	Location	Area of orchards (da.)	Number of trees	Age of trees	Species
1	Yeşilırmak	1,3	25	20	Persimmon
2	Güzelyurt	15,9	800	10	Apple
3	Güzelyurt	3,9	170	15	Plum
4	Güzelyurt	3,3	175	15	Apricot
5	Güzelyurt	5,3	243	12	Peach
6	Kalkanlı	2,6	110	60	Orange
7	Kalkanlı	1,3	37	8	Fig
8	Kalkanlı	15,9	130	32	Carob
9	Kalkanlı	26,6	1200	7	Pomegranate

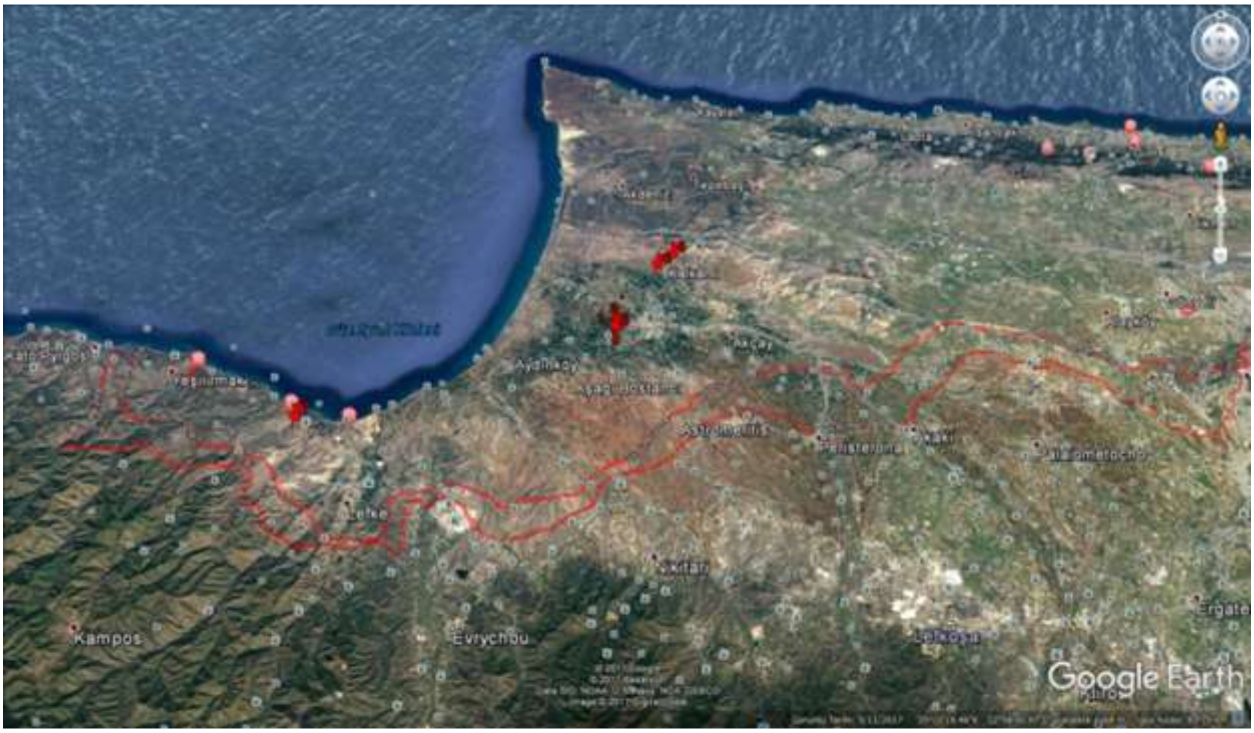

Figure 2. Orchards selected and trap-hung for the detection of fruit flies

*1. Zafer Niyazi-Apple, 2. Zafer Niyazi-Apricot, 3. Zafer Niyazi-Peach, 4. Zafer Niyazi-Plum, 5. Erten Kurnaz-Fig, 6. Hilat Sıva-Valencia, 7. Salih Çakır-Persimmon

Traps were hung in the persimmon (Ebenaceae) orchard in Yeşilırmak village of Güzelyurt region ; in the Güzelyurt region, apple, apricot, peach, plum (Rosaceae); in Kalkanlı village of Güzelyurt, orange (Rutaceae), fig (Malpighiaceae), pomegranate (Lythraceae) and carob (Fabaceae) orchards on the specified dates. Apple vinegar and a teaspoon of dissolved sugar were placed in the traps as an attractant to fill 1/4 of the traps, and these traps were hung on every two trees selected from these orchards. Insects, which were taken from these traps, were collected in tubes containing %70 ethyl alcohol and kept in the refrigerator at +4 ^{0}C.

3. Results and Discussion

The Güzelyurt region of the TRNC has a very important place in terms of fruit growing. Many subtropical fruits, especially citrus, olive, pomegranate and fig, are grown in the region. During this study, it is aimed to detect harmful fruit flies (Tephritidae) on fruits in in Güzelyurt and Kalkanlı and Yeşilırmak villages of Güzelyurt where fruit growing is intense. For this purpose, McPhail traps were prepared to be used in the orchards with 9 different fruit species selected in Kalkanlı and Yedidalga villages of Güzelyurt and Güzelyurt between 10 September and 29 November 2016 in order to determine the species belonging to the Tephritidae family, and they were hung in the determined gardens and renewed with 1 week intervals.

As a result of the study, 5 species belonging to the Tephritidae (Diptera) family and 1 species of the Drosophilidae (Diptera) family, which are important for fruit growing, were determined. These; Olive fruit fly (*Bactrocera oleae* (Rossi.)), Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann)), Peach fruit fly (*Bactrocera zonata* (Saunders)), Celery fly (*Euleia heraclei* L.), *Trupanea amoena* (Frauenfeld) (Tephera) and the spotted wing drosophila (*Drosophila suzukii* (Matsumura)) (Figure 3). Among these species, *B. zonata*, *E. heraclei*, *T. amoena* and *D. suzukii* were detected for the first time in the TRNC and it is the first record for the TRNC insect fauna. On the other hand, it is recorded that *E. heraclei* pre-existed only in Southern Cyprus (Krivosheina ve Ozerova, 2016).

According to the literature, *B. zonata* (EPPO, 2010) and *D. suzukii* (EPPO, 2010) are species that cause economic damage to fruit growing, while *T. amoena* has been reported in Turkey (Giray, 1969) and is found in natural flora as a phytophagous species. *B. zonata* is a polyphagous species and has been reported to cause damage during the growing season, especially in peach, mango and guava fruits (EPPO, 2010). *D. suzukii* causes damage to different fruit species all year round and also causes fungal and bacterial diseases, which are secondary microorganisms, and is also a vector for secondary microorganisms, fungal and bacterial diseases (EPPO, 2010).

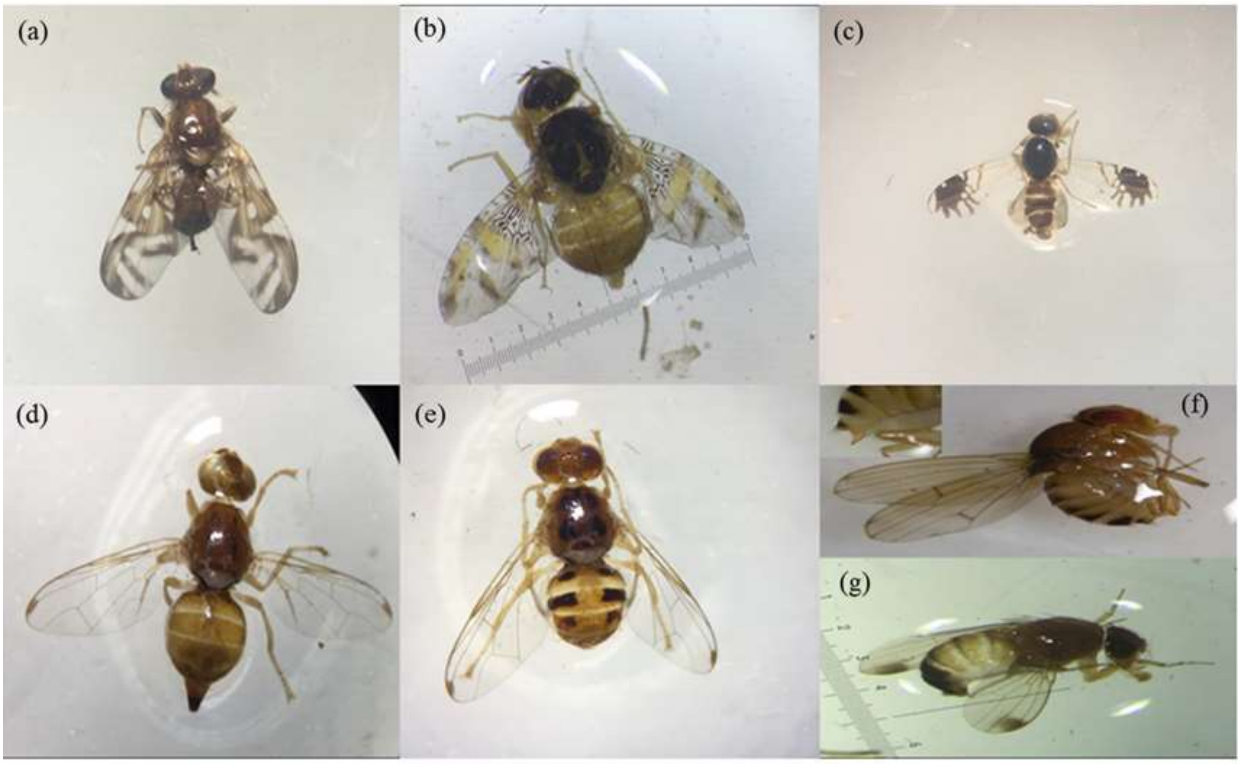

Figure 3. General view of fruit fly adults. *Euleia heraclei* (a), *Ceratitis capitata* (b), *Trupanea amoena* (c), *Bactrocera zonata* (d), *Bactrocera oleae* (e), *Drosophila suzukii* female and serrated egg-laying organ (f) and male (g)

4. Conclusions

In order to determine the species belonging to the Tephritidae family, McPhail traps were hung in the orchards with 9 different fruit species selected in Kalkanlı and Yedidalga villages of Güzelyurt and Güzelyurt region between 10 September and 29 November 2016 and were renewed with 1 week intervals. As a result of the study, 5 species belonging to the Tephritidae (Diptera) family and 1 species of the Drosophilidae (Diptera) family, which are important for fruit growing, were determined. These species are; Olive fruit fly (*Bactrocera oleae* Gmelin.), Mediterranean fruit fly (*Ceratitis capitata* Wiedemann), Peach fruit fly (*Bactrocera zonata* Saunders), Celery fly (*Euleia heraclei* L.), *Trupanea amoena* (Frauenfeld) (Tephritidae: Diptera) the spotted wing drosophila (*Drosophila suzukii* Matsumura) (Figure 3). The results of this research consist of the first comprehensive data made in Northern Cyprus and provide the basis for future studies. Besides, the results of our preliminary study indicate that vinegar traps can be used for detection and monitoring of fruit flies. These pests could be a potential agricultural pests for fruits cultivated in the area, and pest management studies should be considered hereafter.

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EFFECT OF SEED PRIMING ON GERMINATION AND SEEDLING GROWTH OF CICER ARIETINUM UNDER DROUGHT STRESS

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ABSTRACT

Cicer arietinum chickpea cultivation is practiced around the world for its agronomic economic ecological and food importance. Chickpeas seeds production is affected by several abiotic stresses especially drought. To evaluate chemical stimuli effect on three chickpea genotypes, Flip 84 92 C, Flip 93 93 and Flip 1-33 seeds germination and seedling growth under drought stress, a factorial experiment in a completely randomized design with four replicates per treatment were performed. First factor of priming includes different levels of salicylic, ascorbic acids, zinc sulfate and distilled water as a control and the second factor was drought stress levels.

Keywords: Sunflower, Sustainable production, Drought tolerance, Hybrid, Yield traits, Yield performance,

INTRODUCTION

Chickpea cultivation is influenced by biotic and a biotic constrain. Germination and seedlings establishment are the crucial phases for plant development and species expansion strongly affected by drought. Priming with chemical or vitamin solutions can improve seeds growth parameters for possible water stress tolerance. Therefore, *Cicer arietinum* seeds pretreatment with ascorbic acid, salicylic acid, water and zinc sulfate was conducted and there growth parameters assessed under different levels of osmotic pressure

MATERIAL AND METHOD

Three chickpea genotypes attested, Flip 84 92C, Flip 93 93 and Flip 1-33 seed germination parameters are stated after pretreatment with ascorbic acid salicylic acid and zinc sulphate under different levels of simulated water stress with PEG6000 at 0,-0.3,-0.6 and -0.8 bar. Different PEG6000 concentration are prepared according to Michel and Kofmann (1973) formula.

$$\Psi H=-(1{,}18\times10^{-2})\,C-(1{,}18\times 10^{-4})C^2+(2{,}67\times 10^{-4})CT+(8{,}39\times 10^{-7})\,C^2T$$

T: incubation temperature

C: PEG6000 concentration (g/l H_2O)

Germination rate

GR%=(Gn/Tn)x100

Gr: germination rate at 8th day, Tn: total seeds number

Seedling vigor index

The seedling vigor index is determined after 8 days of growth according to the formula suggested by Abdul-Baki and Anderson (1973). It is and indicator that assesses seed behavior under poor environmental conditions.

VI=(RL+SL)xGR

RL: root length in cm

SL: aerial part length in cm

GR: germination rate

RESULTS AND DISCUSSION

The percentage of germination, the length of the seedlings their fresh and dry weight as well as vigor index are enhanced by priming including ascorbic acid for Flip 84 92 C and Flip 93 93 and Flip 1-33 under water stress. For seeds of the third genotype, zinc sulfate seems more favorable

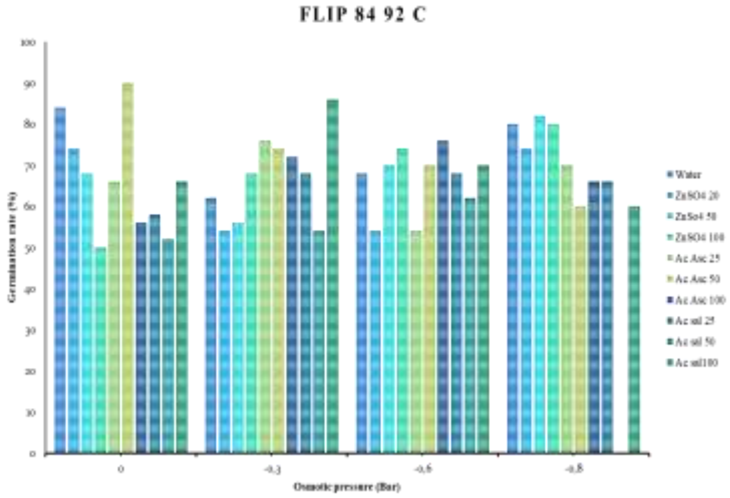

Figure 1 Germination rate primed seeds under drought stress

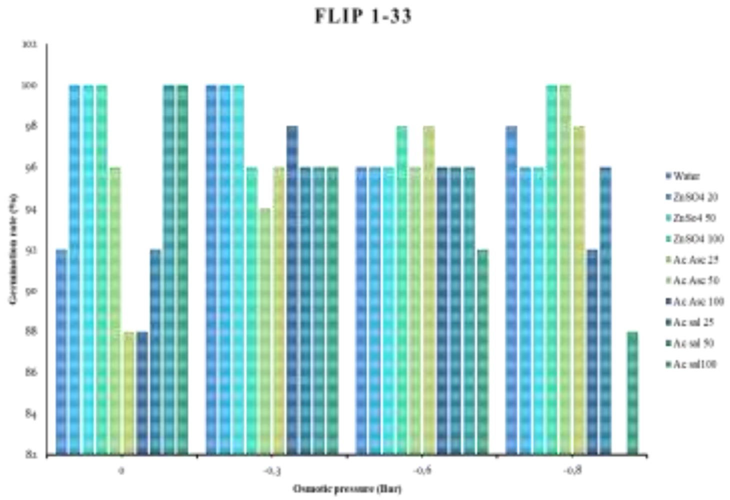

Figure 2 Germination rate primed seeds under drought stress

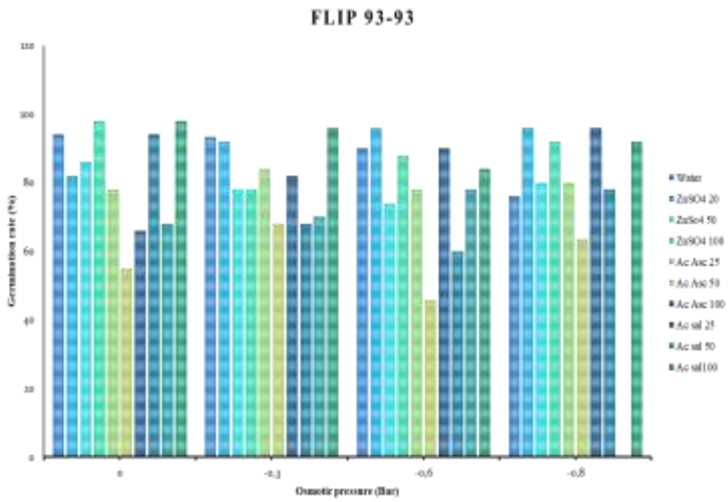

Figure 3 Germination rate primed seeds under drought stress

Table 1 Correlation between priming, drought stress and germination and seedling parameters

PEG	FwR	DwR	FwS	DwS	Rhg	Shg	LT	Germrate	Index V
PEG									
FwR	0,142**								
DwR	0,049	0,153**							
FwS	0,128**	0,718**	0,094*						
DwS	0,047	0,063	0,121**	0,197**					
Rhg	-0,033	0,539**	0,046	0,350**	-0.,009				
Shg	0,172**	0,646**	0,103*	0,575**	0,083	0,408**			
LT	0,014	0,632**	0,066	0,450**	0,014	0,972**	0,608**		
Germrate	-0,003	0,273**	0,078	0,085	0,015	0,256**	0,148**	0,260**	
Index V	0,013	0,621**	0,075	0,410**	0,006	0,945**	0,552**	0,962**	0,468**

Overall priming improves the germination of seeds under stress or not (Murungu et al., 2003). The results show that ascorbic and salicylic acid significantly improves the studied growth parameters. Under stress, ascorbic acid can suppress free radicals. Moreover, for the Flip 1 -33 genotype, zinc sulphate priming promoted the germination of seeds under water stress. Indeed, it is reported that seed priming with zinc sulphate on the characteristics of germination and growth of chickpea plants (*Cicer arietinum* L) (Khan et al, 2011).

Salicylic acid showed a positive effect on all genotype's seeds germination parameters. According to Ceritoğlu and Erman (2020) it's due to its characteristics and it's considered as a signal affecting some physiological processes of germination.

CONCLUSIONS

For the Flip 84 92 C genotype, seeds soaked in distilled water and not subject to water stress have a higher vigor index than the seeds of other pretreatments and the use of ascorbic acid at 50 to 100 and 25 mg/l allows for high index under stress at -0.8 Bar. For Flip 93 93 C, it is also observed that pretreatment with ascorbic acid at different concentrations tested resulted in the optimization of seedling vigor especially in stressed seeds) when used at 100 mg/l. For the Ghab-4 genotype, zinc sulphate pretreatment appears to be more conducive to improving seedling vigor in stressed seeds

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ADULT FLIGHT PERIOD AND HOSTS OF Tropinota hirta (PODA, 1761) (COLEOPTERA; CETONIIDAE) IN ÇANAKKALE PROVINCE

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ABSTRACT

The study was conducted with the purpose of determining the adult flight period and hosts of *Tropinota hirta* (Poda, 1760) (Coleoptera: Cetoniidae) in Central (Saraycık and Dardanos villages) and Ezine (Akköy village) districts of Çanakkale province, in 2021. Adult flight period was determined by capturing adults with basin traps containing 1/3 water and an attractant (1:1 Trans-anethol and Cinnamil Alcohol) on plum, peach, cherry, and quince orchards, on a broad bean field and on a meadow between 12th of February and 17th of May. It is determined that *T. hirta* adult flight has started in early February and has lasted until mid-June with highest adult numbers in March and April, in the study. In this timeframe, 44 adults in the meadow, 75 adults in the plum, 135 adults in peach and 79 adults in cherry were counted in Ezine, Akköy. Also, 22 adults in quince and 16 adults in broad bean were captured in the traps between 12.04 and 17.05. In Dardanos, 129 adults were counted in a field with abundant wild mustard. In Saraycık, number of adults was limited as 9 adults in cherry and 16 adults in peach with no adults in plum and quince. Population density of *T. hirta* was found to be significantly different in relation to vegetation regions and sampling locations. A total of 16 weed species from 7 families were determined as hosts during the adult flight period of *T. hirta* on different dates until 16th of June. First adult flight of *T. hirta* in Dardanos was recorded on the flowers of *Taraxacum officinale* on 22nd of February and on *Sinapis arvensis* and *Capsella bursa pastoris* on 4th of March. These hosts were observed as important in adult feeding for *T. hirta* at early spring. Also, adults of *T. hirta* were recorded on *Leontice leontopetalum*, *Raphanus raphanistrum*, *Bunias erucago, Lamium amplexicaule, Anthemis* sp., *Lamium albüm, Crepus rubra, Papaver* spp.*, Carthamus tinctorius, Onopordum illyricum, Rubus ulmifolius, Picris hieracioides* and *Dacus carota* on different dates and locations. As the result, in adult flight period of *T. hirta*, in addition to different fruit species, host weed species are also important as adult nutrition sources.
Keywords: Çanakkale, *Tropinota hirta, Sinapsis arvensis, Taraxacum officinale*

INTRODUCTION

The adults of *Tropinota hirta* (Poda, 1760) (Coleoptera; Cetoniidae) adult are matte black color and their body is covered with dense hairs. White spots on its elytra make it easy to identify. Although the adults feed on the flowers of the plants and the larvae feed on the roots of the weeds as saprophytes. Therefore, the larvae are not harmful to cultivated plants. *T. hirta*, has one generation per year, spends the winter in the adult stage in areas such as pasture where soil cultivation is not done (Kara,1995). Although the first adult flight varies according to the regions, it starts in mid-February when the daily temperature rises above 10^{o}C. Adult flight continues until July, reaching the highest population density in March and April (Özbek, 2008; Aydın, 2011; Arslan and Aslan, 2016; Avcı and Özpınar, unpublished). It has been determined that while the adult activity is high on sunny and hot days, but it decreases in rainy weather. (Erbay and Özpınar, 2019).

As Subchev et al. (2011) reported, Popova (1962) stated that 37 plant species are hosts of adults of *T. hirta* in the geography including of Turkey, and this number continues to increase with new studies. An important part of these hosts is hard (almond, plum, cherry, cherry, peach, apricot) and pome fruit (apple, quince, pear) fruit species that cause economic damage by eating the flowers of adults in early spring. In addition to these, oily plants such as safflower, broad beans and cereals are among the important hosts of *T. hirta* adults. Especially the damage of adults to the flowers of some fruit trees causes significant economic losses in the product. It has been reported that in cases where no control is made against *T. hirta* adults in Bulgaria, it causes damage up to 70% in cherry fields (Kutinkova and Andreev, 2004). In Turkey, it has been recorded that adult damage reaches 90% in pear orchards (Kara,1995). Chemical control against *T. hirta* adults during the flowering period of fruits is not recommended due to the presence of pollinator species. For this reason, research has been carried out on the development of blue color traps with attractant to control of *T. hirta* adults (Vuts et al., 2010; Gezer and Özpınar, 2015; Arslan and Aslan, 2016; Yaşar and Dahham, 2019; Özpınar and Erbay, 2020). Determination of other hosts during the flight period is important for the success of control of *T. hirta* adults in fruit horticulture.

In this study, were determined the population density of *T. hirta* adults on different fruit species and their host plants during the flight period.

MATERIAL AND METHOD

The study was carried out in agricultural (plum, peach, cherry, quince and broad bean), non-agricultural and no tillage areas in the province of Çanakkale in 2021 (Table 1). According to Özpınar and Erbay (2020) found to be effective in attracting *T. hirta* adults, a 50x35x20 cm basin, painted with #4D9AC9 hexadecimal coded blue color (Table 2), was placed at the sampling sites as of 12.02.2021. The basins, which were fixed with iron rods driven on three sides, were filled with 1/3 water and the ropes passed over them were tied to the iron rods. Attractants (1:1 Trans-anethol and Cinnamyl Alcohol) are attached to these strings (Figure 1).

Table 1. Sampling locations, characteristics and dates.

Sampling locations	Vegetation field (*****)	Characteristics of locations			Sampling interval
		Age	Field (da)	Coordinates	
Center-Saraycık	*Prunus domestica* (plum)	13	3.0	N40°08'08" E26°21'19"	19.02-16.04
	Prunus persica (peach)	8	2.0	N40°08'06" E26°28'08"	19.02-26.04
	Prunus avium (cherry)	8	5.5	N40°08'04" E26°28'06"	16.04-17.05
	Cydonia vulgaris (quince)	8	4.0	N40°08'09" E26°28'09"	28.04-17.05
Center-Dardanos	*Sinapsis arvensis* (wild mustard)+	-	25.0	N40°04'27" E26°21'05"	12.02-26.04
Ezine - Akköy	Mera (**x**)	-	30.0	N39°43'10" E26°21'19"	13.02-17.05
	Prunus domestica (plum)	9	6.0	N39°49'11" E26°20'42"	13.02-17.05
	Prunus persica (peach)	6	9.5	N39°49'11" E26°21'19"	13.02-17.05
	Prunus avium (cherry)	6	22.5	N39°49'13" E26°21'04"	13.02-17.05
	Cydonia vulgaris (quince)	8	6.0	N39°49'10" E26°21'16"	12.04-17.05
	Vicia faba (bean)	-	4.0	N39°49'14" E26°21'15"	19.04-17.05

(*****) Sampling areas with traps; + There is a fruit collection garden near the sampling area in Dardanos
x Meadow field; 200 m. to the peach, quince and broad bean plot, 400 m. to the cherry plot. and 1500 m to the plum plot. located at a distance.

Table 2. Color measurement values of the basin trap.

Rank / Value	x	y	z	light transmittance	redness	jaundice	HUE	Croma
Measurements	19.86	20.50	62.57	47.65	-1.00	-44.12	275.02	10.17

Note: Color measurement values were made with the COLORMETRE 200 device.

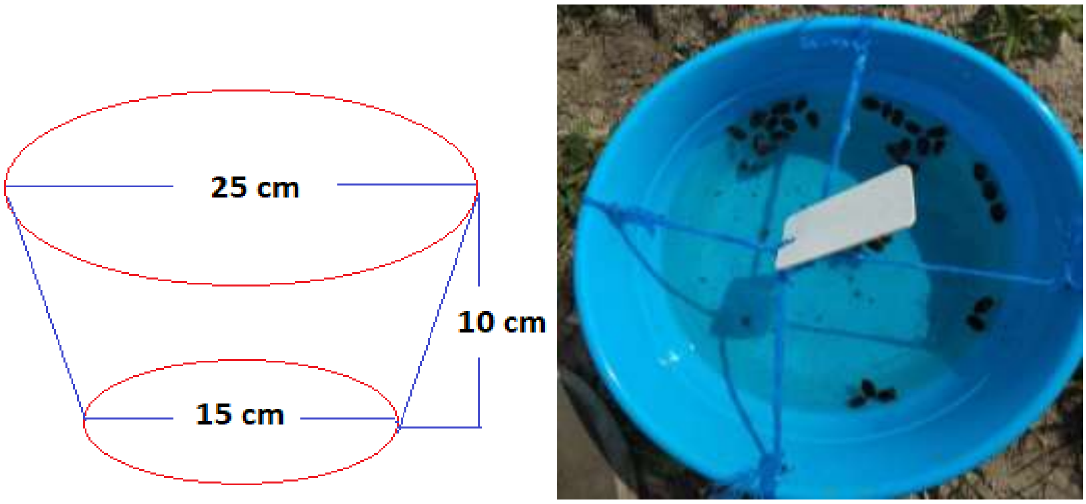

Figure 1. Dimensions and basin trap with attractant attached

In the sampling interval in Table 1, the adults caught in the traps were counted every day, by taking them in the water with the help of a strainer. The traps were cleaned for the next count and the missing water was completed. In addition, pictures of weeds on which *T. hirta* adults were taken in the fruit areas and outside, and those that could not be identified in the field were identified by the relevant experts. The number of adults caught in the traps in the sampling areas was compared with graph. Identified hosts of *T. hirta* were recorded.

RESULTS AND DISCUSSION

Host Weed Species of *Tropinota hirta*

In this study, weed species that determined as hosts of *T. hirta* in Çanakkale province are given in Table 3 and Figure 2. *T. hirta* adults were recorded on the flowers of 16 wild/weedy plants belonging to 7 families at different dates and sampling locations. During the adult flight period, T. *hirta* adults were recorded on dandelion (*Traxacum officinale*) plant in the early period, on 23.02.2021. Then, on 04.03.2021, *T. hirta* adults were detected on wild mustard (*Sinapsis arvensis* L.) and shepherd's purse (*Caspella bursa pastoris*) together with dandelion at all sampling sites. *T. hirta* adults were mostly detected on wild mustard and dandelion plants. T. *hirta* adults continued to be recorded in different places and different hosts until 16.06.2021 (Table 2). Stankevch et al. (2020) reported a different number of host species in Ukraine, including as *T. officinale* and *S. arvensis*. Uzun (2019) determined that *Taraxacum* spp, and *Lamium amplexicaule* species in Adıyaman province. Çetin et al., (2006) reported the *Rubus fruticocus* species in Bursa and Yalova. The safflower plant identified in this study, which was recorded by Esfahani et al., (2012) in Iran. Giray (1985) recorded poppy (*Papaver somnuferum* L.) as plant hosts *T. hirta* adults in Turkey. In Iran, adults of *T. hirta* have been recorded on plants such as rose, wheat, pomegranate, citrus, apple, pear, blossom apple, white thorn, elm, almond, cherry, plum, peach, black poplar, alder, corn acacia, and locust (Awall, et al. 2006). According to these studies, the presence of T. *hirta* adults has been reported in many plants that bloom in early spring.

Table 3. Hosts of *Tropinota hirta* adults determined at different dates and places in Çanakkale province.

Date	Species	Familia	Sampling locations
23.2.2021	*Traxacum officinale* Weber ex Wiggers	Asteraceae	Dardanos
4.3.2021	*T. officinale*	Asteraceae	Ezine Akköy
4.3.2021	*Caspella bursa pastoris* (L.) Medik	Brassicaceae	Dardanos
4.3.2021	*Sinapsis arvensis* L	Brassicaceae	Dardanos
4.3.2021	*T. officinale*	Asteraceae	Saraycık köyü
15.3.2021	*Leontice leontopetalum* L.	Berberidaceae	Ezine Akköy
22.3.2021	*L. leontopetalum*	Berberidaceae	Ezine Akköy
1.4.2021	*S. arvensis*	Brassicaceae	Dardanos
1.4.2021	*T. officinale*.	Asteraceae	Güzelyalı köyü
2.4.2021	*Raphanus raphanistrum* L.	Brassicaceae	Ezine Akköy
2.4.2021	*S. arvensis*	Brassicaceae	Ezine Akköy
2.4.2021	*Bunias erucago* L.	Brassicaceae	Ezine Akköy
6.4.2021	*S. arvensis*	Brassicaceae	Güzelyalı köyü
10.4.2021	*Lamium amplexicaule* L.	Lamiaceae	Güzelyalı köyü
14.4.2021	*Raphanus raphanistrum* L.	Brassicaceae	Ezine Akköy
20.4.2021	*Anthemis* sp.	Asteraceae	Ezine Akköy
22.4.2021	*Lamium albüm* L.	Lamiaceae	Ezine Akköy
22.4.2021	*Crepus rubra* L.	Asteraceae	Ezine Akköy
5.5.2021	*Papaver* spp.	Papaveraceae	Ezine Akköy
25.5.2021	*Carthamus tinctorius* L.	Asteraceae	Eceabat (Ilgardere)
16.6.2021	*Onopordum illyricum* L.	Asteraceae	Dardanos
16.6.2021	*Rubus ulmifolius* Schoott	Rosaceae	Dardanos
16.6.2021	*Picris hieracioides* L.	Asteraceae	Dardanos
16.6.2021	*Dacus carota* L.	Apiaceae	Dardanos

Figure 2. Images of host plants of *Tropinota hirta* adults detected in Çanakkale in 2021.

Adult Number and Population Development of *Tropinota hirta* in Different Vegetations

The first adults flight of *Tropinota hirta*, with an average daily temperature of 8.5°C (Figure 3) caught in the trap in Dardanos where wild mustard (*Sinapis arvensis*) plant is found on 12.02.2021 (Figure 4). Adult flight ended due to decrease in temperatures and increased rainfall and when the average daily temperature was 8 °C (Figure 3) in Akköy, the first adults were caught in traps on plum, cherry and peach on February 24,. If in the meadow, the first adult was recorded on February 27 (Figure 5).

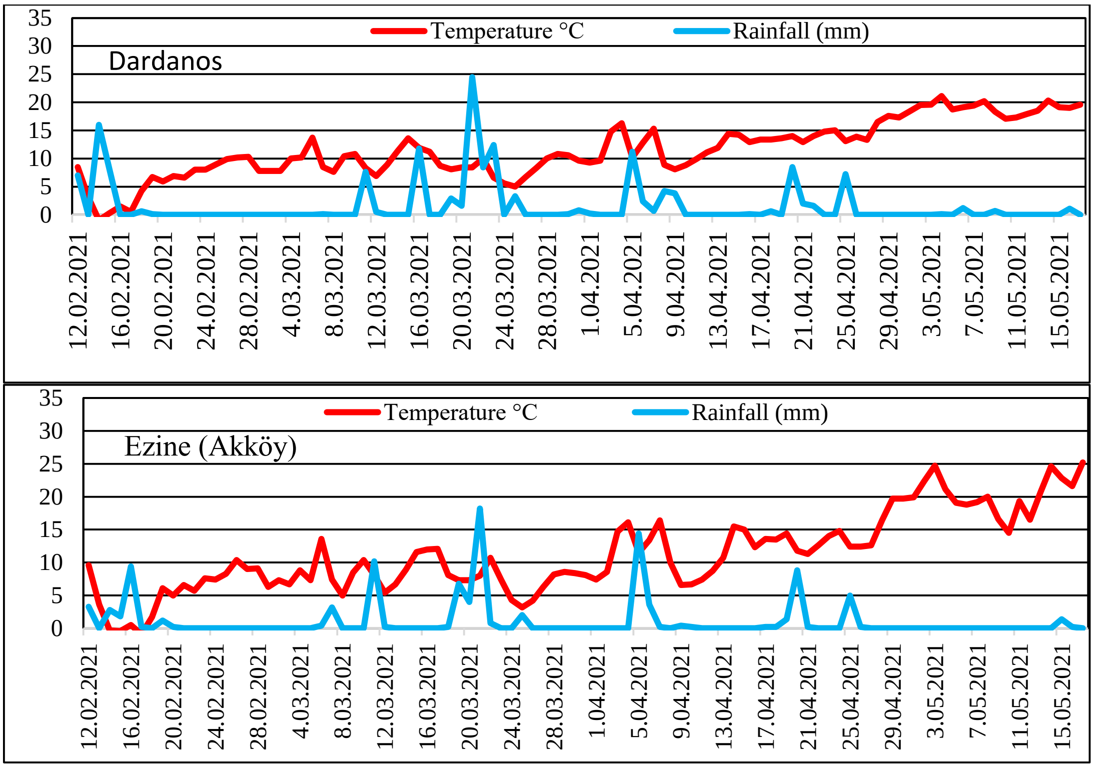

Figure 3 Temperature and rainfall values of Dardanos and Ezine during the flight period of *Tropinota hirta*.

The first adults were found in Hungary on March 27 (Schemera et al., 2004), in the peach orchards in Çanakkale on March 04 (Erbay and Özpınar, 2019), in the cherry orchards in Afyonkarahisar on March 31 (Sağdaş and Yaşar, 2013), in the almond orchards in Adıyaman on February 18. (Uzun, 2019) stated that they were caught in traps on April 9 in apple orchards in Isparta, (Dahham and Yaşar, 2019) and on April 3 in pear orchards (Erdoğan and Sağdaş, 2016).

As can be seen, although the adult flight of *T. hirta* took place in different vegetations at different times, it was observed that it mainly took place from the beginning of March. Adult flight density varied depending on temperature and rainfall. Between 16.03-23.03, which was rainy, the number of adults caught in the traps decreased in both Dardanos and Akköy.

It has been observed that wild mustard and dandelion plants are an important as adult food for *T. hirta* adult in February. The fact that a total of 129 adults were caught in traps during the adult flight period in Dardanos also confirmed this situation (Table 3, Figure 4). Adult flight, which started at the end of February in Dardanos and Akköy, reached its highest level in mid-March, and then continued with interruption of adult flight due to rainfall (Figure 4 and 5).

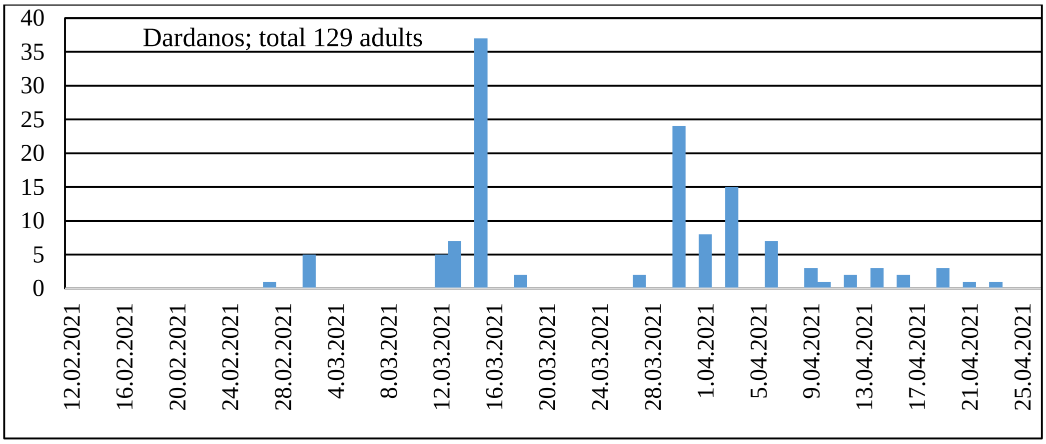

Figure 4. Population development of *Tropinota hirta* adults in Dardanos (Çanakkale) in 2021

Table 3. Number of *Tropinota hirta* adults in different vegetations during sampling

Sampling locations	Vegetation	Sampling interval	Ergin sayısı
Center- Saraycık	*Prunus domestica*	19.02.21-16.04.21	0
	Prunus persica	19.02.21-26.04.21	9
	Prunus avium	16.04.21-17.05.21	16
	Cydonia vulgaris	28.04.21-17.05.21	0
Center- Dardanos	Meadow (*Sinapsis arvensis*)	12.02.21-26.04.21	129
Ezine -Akköy	Meadow	13.02.21-17.05.21	44
	Prunus domestica	13.02.21-17.05.21	75
	Prunus persica	13.02.21-17.05.21	135
	Prunus avium	13.02.21-17.05.21	79
	Cydonia vulgaris	12.04.21-17.05.21	22
	Vicia faba	19.04.21-17.05.21	16

Adult population development of *T. hirta* occurred on different fruit trees and meadow in Akköy as seen in Figure 5. The highest number of adults were caught in the trap in the peach orchard, followed by the trap in the cherry and plum orchard (Table 3). In the first adult flight period, the number of adults in the meadow was higher than the fruit areas, the total number of adults caught in this trap remained behind the fruit areas. Flowering in fruit trees was effective in attracting adults. Based on the flowering period, in traps placed in plots of bean and quince on April 12 and 19, the adults of 16 and 22 *T. hirta* were recorded respectively, confirming this situation (Table 3). However, adult flight continued until 17 May without interruption in the meadow, as in other areas.

As a result, flowering periods are effective in attracting adults. The wild/weeds, which are the hosts of the adults of *T. hirta* in the post-flowering and pre-flowering periods of fruit areas and in the meadow was observed to play a role as the host to continue the adult flight. The fact that adults were detected in the flowers of weeds at the end of flowering in the culture areas supports this opinion. The efficacy of wild host plants as adult food of *T. hirta* allows it to spread over a longer flight period.

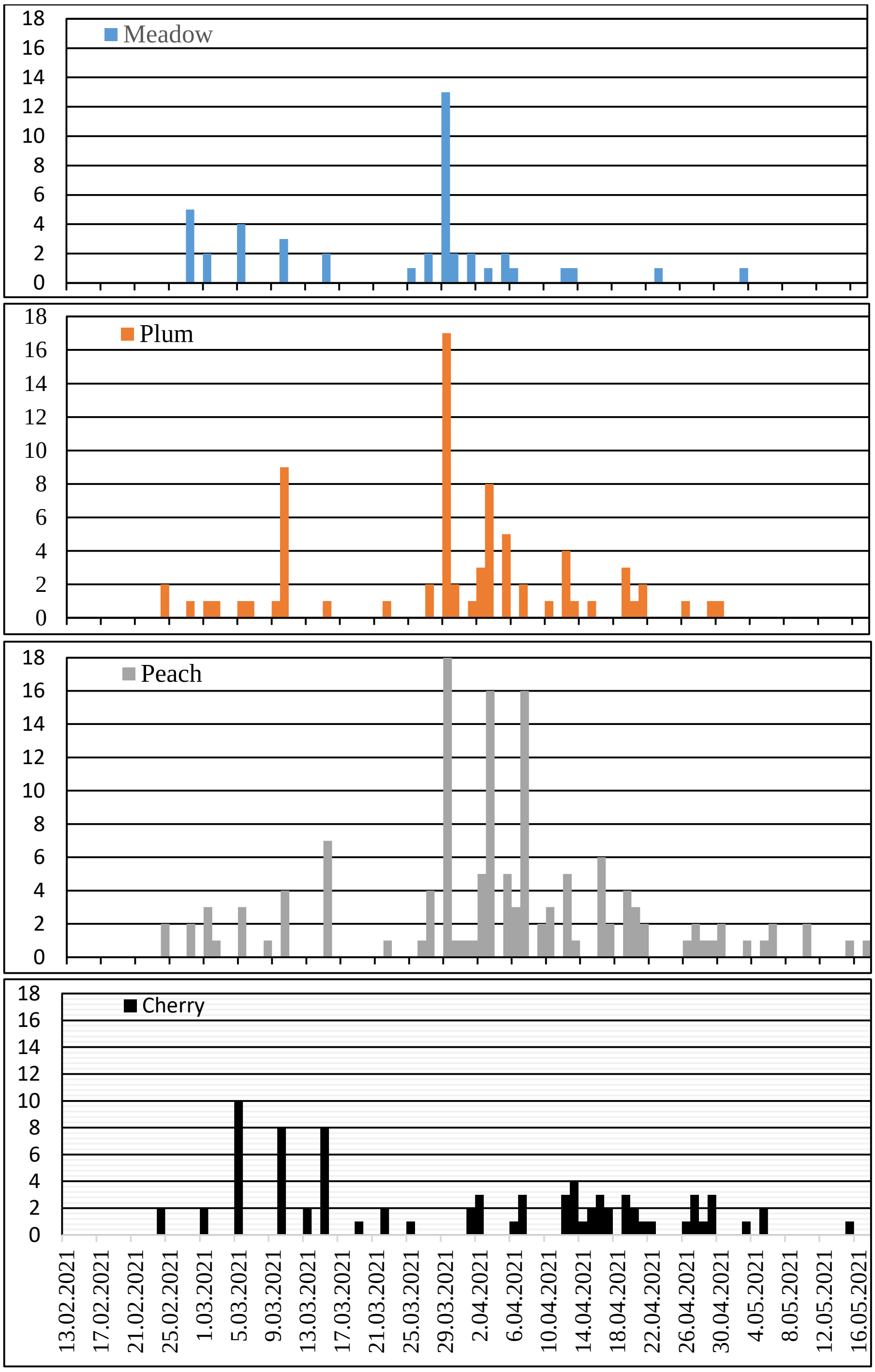

Figure 5. Population density of *Tropinota hirta* adults of in different area in Akköy (Ezine) in 2021.

CONCLUSIONS

In early spring, together with fruit trees, host plants such as *S. arvensis* and *T. officinale* in orchards or pastures are important in the adult flight period of *T. hirta*. Adult flight continued from mid-February until mid-June, and adult population density varied according to hosts and regions. The highest population density was in March and April, and adult flight was interrupted by rainfall. In this study, 16 wild host plant species belonging to 7 families were determined in Çanakkale. Despite the end of flowering in fruit trees, it has been observed that these host plants have an important place for adult nutrition. It was noted that the adults were concentrated in fruit areas according to the flowering periods, but the adult flight continued over the other hosts.

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DEVELOPMENT OF QUALITY OIL SUNFLOWER (HELIANTHUS ANNUUS L.) HYBRIDS

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ABSTRACT

The development of a hybrid with high oleic acid is an important breeding goal for sunflower. Hence, inbred lines differing for oleic acid were selected and hybrids having one or both parents with high oleic acid were developed and evaluated at various sites at Pakistan in spring and autumn seasons. DNA profiling of hybrids differing for oleic acid contents was characterized using N1-3F/N2-1R, which successfully confirmed the presence of high oleic acid gene in hybrids. Oleic acid and seed yield components were higher in spring than in autumn due to the high degree days availability during the former. Among these hybrids, H5 had stable high oleic acid content during the spring season with high seed yield and kernel to seed percentage than the cultivar check. Combining ability analysis revealed a high relationship with mean oleic acid contents at two sites, thus showing the effectiveness of selection for developing high oleic acid inbred lines. Newly developed inbred C.112.P was a positive combiner for oleic acid across sites except Sargodha, while restorer populations such as RH.344, RH.345 and RH.347 were positive male combiners.

Keywords: Combining ability, degree days, kernel to seed%, seed yield

Introduction

Sunflower seed is rich source of edible oil (30–50%), protein (20–30%), tocopehrols, and fatty acids. Its oil contained up to 90% polyunsaturated fatty acid (60 % linoleic acid) (Rauf *et al.*, 2017). Improvement and modification of sunflower oil and fatty acid is an important sunflower breeding goal. Regular sunflower oil is more suitable as salad dresser. This is partly due to presence of poly-unsaturated fatty acid in sunflower oil. Traditional sunflower oil is rich in linoleic acid, which is ideal for the edible uses but such an oil show poor oxidative stability as cooking oil or deep-frying (Rauf *et al.*, 2017). Oil rich in polyunsaturated fatty acids, rancidify quickly due to its susceptibility to the oxidation that reduces its shelf life (Rauf *et al.*, 2017). On the other hand, saturated fatty acids or mono-unsaturated fatty acid (oleic acid) show more stability during cooking and have greater shelf life than polyunsaturated fatty acids. However, saturated fatty acid increases blood cholesterol level and have health hazards (Rauf *et al.*, 2017, 2020). Research showed that rising the oleic acid could increase the stability of oil with increased shelf life. Fatty acid profile showed that mid-high oleic hybrids had oleic acid ranging from 44 to 85%, 9 to 46% linoleic acid, 4 to 6% palmitic acid, and 2 to 6% stearic acid (Carvalho *et al.*, 2019). Sunflower accessions has been characterized into three types with respect to oleic acid contents such as standard sunflower types (< 50%), mid oleic acid (50–70%) and high oleic acid content (> 80%) (Rauf 2019). High oleic acid content was controlled by a single dominant gene mutation induced through ethyl methane sulfonate (EMS) (Dimitrijević *et al.*, 2017, Rauf *et al.*, 2017). The ‘Pervenent’ population was selected and further used as source of high oleic acid by sunflower in breeding programs.

Oleic acid contents in ‘Pervenent’ population were highly unstable and ranged between 15.0–51% and 87–91% across range of environments depending upon the reproductive phase temperature (Alberio *et al.*, 2017). ‘NuSun’ has been released for general cultivation in the USA as mid-oleic acid commercial cultivar of sunflower (Gupta, 2014). Development of a new source that may yield stable high oleic acid sunflower has been a main goal of sunflower breeding (Alberio *et al.*, 2017). Mutants with high oleic acid content may, however, affect agronomic traits and could only be used as a source for pre-breeding.

Temperature and intercepted solar radiation had additive effects on oleic acid content in sunflower. Intercepted solar radiation increases the oleic content that also depends on minimum night temperature (Echarte *et al.*, 2010). Likewise, sowing dates had differential effects on oleic acid of sunflower. Sowing date affects growth because temperature, humidity and rainfall changed oleic acid content (Akkaya *et al.*, 2019). Early sowing date decreased oleic acid and palmitic acids, while increased linoleic and stearic acids simultaneously under irrigation (Flagella *et al.*, 2002). Moreover, irrigation decreased oleic acid contents in sunflower (Flagella *et al.*, 2002). Regular sunflower hybrids were grown in spring and autumn season. The spring season had a significant effect increasing oleic acid when compared with the autumn season. Plant growth from early sowing date was under low temperature while delayed sowing time gave high temperature that increased oleic acid in sunflower (Qadir *et al.*, 2006).

Stability analyses has been carried out to select the appropriate hybrids with high oleic acid content since sustainability of its production across environments depends on suitable sunflower genotypes. In this regard, Van Der Merwe *et al.* (2013) evaluated 16 sunflower hybrids and found that one high oleic (80%) hybrid was stable across environments. Unstable hybrids may be sensitive to temperature, rainfall and humidity.

Combining ability analyses of 15 hybrids generated from breeding lines differing for oleic acid contents revealed dominance or partial dominance in the F_1 upon specific parental lines (Joksimović *et al.*, 2006). Inbred line differed for their ability to produce high oleic acid hybrids. Inbred lines that produced high oleic acid hybrids were positive general combiners (Joksimović *et al.*, 2006). Oleic acid traits showed preponderance of additive gene action, while dominance and epistatic effects were non-significant (Joksimović *et al.*, 2006).

On the basis of these background, the aim of our research was to select inbred lines with high oleic acid for further crossing to develop hybrids, and to evaluated these in various locations during spring and autumn seasons in Pakistan.

Material and Methods

Development of breeding lines

‘Pervenent’ high oleic acid sunflower (89% oleic acid) was introduced from USDA and superior plants with high fertility were selected and backcrossed to cytoplasmic male sterile lines to develop two inbred lines ‘C.112.P’ and ‘C.116.P’. High oleic acid restorer populations (RH.345, RH.347 and RH.345) were introduced from the United States Department of Agriculture (USDA) and subjected to selection and maintained for purity for several generations. These restorers originally had more than 80% oleic acid content. Low oleic acid inbred lines C.250, C.249, RSIN.82 and R.365 were also included in the crossing. Seeds of the inbred lines were multiplied and produced hybrids as indicated in Table 1. Those hybrids having sufficient seeds were evaluated in multisite trials and over seasons. Hybrid seed was produced by growing cytoplasmic male sterile line (CMS) and restorer (R) lines (2:1). Synchronized plants of CMS and R combinations were bagged together before opening of ray florets to avoid from insect pollinators and CMS lines were manually pollinated from R lines to improve the seed setting. CMS lines were also maintained from their "B" lines while restorer and maintainer lines were maintained through sib mating by bagging two plants of same inbred lines. Mature hybrid seed was manually threshed, dried and put in kraft paper bags and stored at room temperature.

Evaluation of hybrids

The F_1 hybrids were evaluated at two locations during the autumn season (planting: 15th August, 2020), and at four locations during the spring season (planting: 16th February, 2020). Details of all locations and meteorological features are given in Table 2. Soil of Faisalabad site had sandy clay loam type with a pH of 7.4 ± 0.27, EC equal to 2.19 ± 0.1, 20 g ± kg^{-1}, and water holding capacity 18.5% by weight determined through gravimetric method. Sargodha had sandy loam type of soil with pH of 7.6 ± 0.11, EC equal to 1.67 ± 0.08, and 17.5% water holding capacity by weight. Location at Multan had loam type of soil with pH of 8.86 ± 0.19, EC equal to 1.85 ± 1.08, organic matter being 0.42 ± 0.05, k^{+} equal 177.5 ± 5.00 mg kg^{-1}, 7.47 ± 0.72 mg kg^{-1}, 20 g ± kg^{-1}, and 18.3% water holding capacity by weight. Bahawalpur location had sandy soil having chemical properties such as pH of 7.63 ± 0.09, EC equal to 2.15 ± 0.22, organic matter 0.64 ± 0.04, k^{+} of 124.5 ± 7.13 mg kg^{-1}, 11.37 ± 1.01 mg kg^{-1}, 30 g ± kg^{-1} and 15.2% water holding capacity. Each hybrid was sown in three rows of 6 m following a randomized complete block design with three replications. Plants to plant distance was maintained at 22 cm while distance between ridges was 75 cm. Soils were fertilized with in organic fertilizer, diammonium phosphate (60 kg ha^{-1}) and 40 kg ha^{-1} of urea during field preparation. Pre-emergence herbicide S-metolachlor was sprayed at all sites to control the growth of weeds. Pest scouting was done during entire seasons and recommended pesticide was sprayed when army worm population reached above threshold level during spring season. No specific infestation was seen at both sites during autumn season.

Measurement of seed yield and components

Five heads from consecutive plants within each row of 1 m were harvested and threshed manually. Seed from all hybrids from each replication within each location were dried to constant moisture (8%). Seed yield (g m^{-1}) was determined on digital weighing balance. Seed lots of 100 g were derived after dehulling using a rotary machine. Mass of dehulled seed was determined to calculate kernel to achene ratio as follows:

$$Kernel\ to\ achene\ ratio\ =\ mass\ of\ kernel\ after\ dehulling\ (g)\ \times\ 100$$

mass of 100 g of achene

Kernel oil content after dehulling was determined on soxhlet apparatus. About 10 g of kernel were crushed and put in thimble to extract oil through petroleum ether. Kernel oil contents were determined by following equation:

$$Kernel\ oil\ contents\ (\%)\ =\ [KM\ before\ extraction\ (g) - KM\ after\ extraction\ (g)]\ \times\ 100$$

KM before extraction

where $KM\ =\ kernel\ mass\ (g)$

Fatty acid profile

A manual oil extractor was used to obtain a small quantity of oil, which was put in Eppendorf tubes for fatty acid profile. Small amounts of seed (10g) were put in the extractor and pressed to obtain about 1.5 g of oil. A sample of this oil (50 µL) was methylated using 4ml KOH for one hour at room temperature. Methylated fatty acids were extracted with hexane. Fatty acid profiles of all edible oils were analyzed using gas chromatography (M-3900, Varian, USA). Analysis was done by using the fused capillary column, flame ionizing detector and nitrogen gas carrier @ 3.5 ml min^{-1}. Injector and detector temperature were set at 260°C, while column oven temperature set at 222 °C. Methylated esterified fatty acid were injected manually while fatty acids were identified through peak retention time when compared with standard.

DNA profiling

DNA profiling was carried out Agricultural Biotechnology Research Institute, Pakistan. Sunflower seed samples of various hybrids were first frozen in liquid nitrogen and grounded into fine powder. 200 mg of samples was used to isolate the total genomic DNA using CTAB methodologies as described by Rogers and Bendich (1985) with some modifications. About 100 ng of isolated DNA was used in PCR for onward verification of High oleic (HO) acid using specific primers i.e. N1-3F/N2-1R given by Bilgen *et al.* 2018. The PCR profile was comprised on 35 cycles of 95 ^{o}C for 30 sec, 56 ^{o}C for 45 sec and 72 ^{o}C for 1 min with a final step of 72 ^{o}C for 07 min. The amplified PCR product was separated on 1.5% agarose gel and visualized under UV gel documentation system.

Statistical and biometrical analyses

All data of hybrids were analyzed using computer based "R" software (Ferreira *et al.* 2014) considering a randomized complete block design with 3 replications at 4 locations during the spring season, while 3 replications in each of the 2 locations were used for autumn season. R functions GGEbiplotGUI were used for GGE biplot analysis, which was originally outlined by Yan and Kang (2002). Stability parameters such as ecovalence (W_i^2; Wricke 1962), regression coefficient (b_i; Eberhart and Russel, deviation from regression (s^2d_i; Finlay and Wilkinson 1963), stability variance ($\sigma^2_{i,}$; Shukla 1972), coefficient of variation (CV, Francis and Kannenberg 1978); mean and genotype × environment variance component, ($\theta_{(i)}$ and θ_i: Plaisted and Peterson's 1959), and ranking (KR; Kang 1988) were estimated using STABILITYSOFT (Pour-Aboughadareh *et al.* 2019). Combining ability effects were estimated on an Excel worksheet following Kempthorne (1957). Mean values of traits were compared using least significant differences at probability below or equal to 0.05.

RESULTS

DNA of sunflower hybrid seed obtained after crossing among various parental lines was subjected to the amplification with primer N1-3F/N2-1R. The primer has been extensively utilized for identification of high oleic acid sunflower germplasm (Bervillé *et al.* 2009, Dimitrijević *et al.* 2017, Bilgen *et al.* 2018). DNA profiling of the hybrids provided a low cost and reliable method of identification of high oleic acid. Field screening of the high oleic acid sunflower is very complicated due to instability of the gene and high temperature driven expression of this genes as identified in our studies.

DNA of seven hybrids were extracted which included high, medium and low oleic acid hybrids. PCR successfully identified high oleic band of 870 bp in medium and high oleic acid sunflower hybrids (2, 3, 4 and 6; lane 2= C.112.P × R.SIN.82 (H2); 3= C.112.P × RH.344; 4=C.249 × RH.344; 6= C.250 × RH. 344); while high oleic acid band was absent in hybrids (lane 1 = Hysun.33, 5= C.224 × R.SIN.82; 7=FH.33), thus confirming the successful hybridization between sunflower inbred lines and integration of high oleic acid band in various cross combinations (Figure 1).

There were significant ($P \leq 0.05$) differences among the hybrids, locations and their interaction hybrids × locations according to the analysis of variance (Table 3). The significant ($P \leq 0.05$) hybrids × location interaction indicated that hybrids changed their relative performance across locations. Hence, means were compared with reference to their specific location. Oleic acid had a medium heritability estimate, while kernel oil contents percentage (KOC%) and SY had low heritability estimates across four locations during the spring season (Table 3).

Among inbred lines B.250, B.249 and R.SIN.82 had the highest dehulling percentage (KTS%). while RH.344 had the highest KOC%. Oleic acid contents of parental lines during the spring season are given in Table 4. Parental lines B.116.P and B.112.P, which were selected from 'Pervenent' population, had the highest oleic acid content as female lines. Introduced restorer populations produced 70 to 75% oleic acid averaged across the four locations (Table 4).

The hybrids had the highest seed yield (g m^{-1}) (SY) at Multan. Among the hybrids, H5 had the highest seed yield at Multan and Faisalabad. Lowest SY were noted at Sargodha during the spring season (Figure 2A), where hybrid H1 had the highest SY. Hybrid H3 had the highest SY at Bahawalpur (Figure 2A).

With respect to locations responses for KOC% and kernel to seed percentage (KTS%), mean values of these traits were highest at Sargodha, where on average the lowest SY was observed during the spring season. Hybrids FH.331, H3, H5 and H10 had the highest KTS% at Sargodha, while H4 had the highest KTS% at Multan. H1 had the highest KTS% at Bahawalpur, and FH.331 at Faisalabad during spring season (Figure 2B).

Hybrid FH.331 (check) and hybrid H1, H2, H3 and H7 had the highest KOC% at Sargodha (Figure 2A). H1 had the highest KOC% at Faisalabad, Bahawalpur and Multan (Figure 3A). There were significant differences among the highest for oleic acid (%). Both commercial hybrids had lower oleic acid percentage at all locations (Figure 3B). The highest oleic acid was obtained at Multan and Bahawalpur while hybrids showed lowest values of oleic acid% at Sargodha and Faisalabad during spring season. H1 and H5 had the highest oleic acid percentage (≥ 80%) at Bahawalpur, while hybrid H9, H2 and H5 (≥ 80%) had the highest oleic acid (≥ 70%) at Multan. H1 had the highest oleic acid% at (≥ 70%) at Faisalabad while H7 and H8 showed the highest oleic acid% (≥ 60%) at Sargodha (Figure 3B). Multan and Bahawalpur had favorable environmental conditions for SY and oleic% during the spring season.

Sargodha had the highest oleic acid percentage vis-à-vis Faisalabad during the autumn season. Hybrid H10 had the highest oleic acid percentage (≥ 60%) at Sargodha and Faisalabad followed by H3 at both locations (Figure 4A). FH.331 and H10 showed similar SY across both locations during autumn season. Hybrid H7 had the highest SY during autumn season followed by H2 and H3. H10 had the highest SY at Faisalabad during the autumn season (Figure 4B).

Biplot analysis of oleic acid% showed that H4, H5 and H10 had relatively stable oil content across locations during the spring season (Figure 5). FH.331 had stable but low oleic acid %. H1 had the highest oleic acid at Multan and Bahawalpur during the spring season. Although Hysun.33 was characterized as a low oleic acid hybrid but it produced relatively higher oleic acid at Multan than in other locations (Figure 5).

Stability parameters estimated for oleic acid% and seed yield are given in Tables 5 and 6, respectively. Hybrid H5 had the highest oleic acid contents across locations (Table 5), followed by H7 and H8 during spring season. The estimated eco-valence (wi^2) is the contribution of each genotype to its interaction with the environment. A low value tells trait stability. As per wi^2, H10 (mid-oleic acid%) had the lowest wi^2 value followed by H1 (high oleic acid hybrid). H7 and H10 had the smallest Shukla's $\sigma^2{}_i$ stability values, thus being stable. The regression coefficient (b_i) also allows identifying stable genotypes. Genotypes with b_i about 1 and non-significant $S^2{}_{di}$ were regarded as stable. Hybrids H10, H4 and H5 had regression coefficient about 1 with lowest non-significant $S^2{}_{di}$, and high SY. The hybrids H5, H4, H7 and H10 were the most stable and with the highest oleic acid content.

Hybrid H5, H1 and H6 had the highest SY (g m^{-1}) across locations (Table 6). H8 had the lowest eco-valence and Shukla's $\sigma^2{}_i$, thus it should be regarded as a stable hybrid. H7, H1 and H10 had b_i about 1. H8 had a non-significant $S^2{}_{di}$ that confirms its stability for SY. Hybrid H8 also had the lowest Kang's ranking (KR), which indicates that this hybrid as having the highest stability along with high SY when grown in the spring season.

Combining ability effects showed high relationship with mean oleic acid contents of inbred lines at Faisalabad and Sargodha, thereby showing that selection for high oleic acid inbred lines were effective for developing high oleic acid hybrids at these locations. The variability among inbred lines was masked by the environment at both Sargodha and Multan, where relationship between combining ability effects and mean oleic acid contents were not high (Figure 6). Among inbred lines, RH>347, RH.344 and B.112.P were positive and good combiners for oleic acid content across locations except Sargodha, where RH.347 and RH.345 were positive combiners. B.112.P had high mean oleic acid content but with negative combining ability effects (Figure 6).

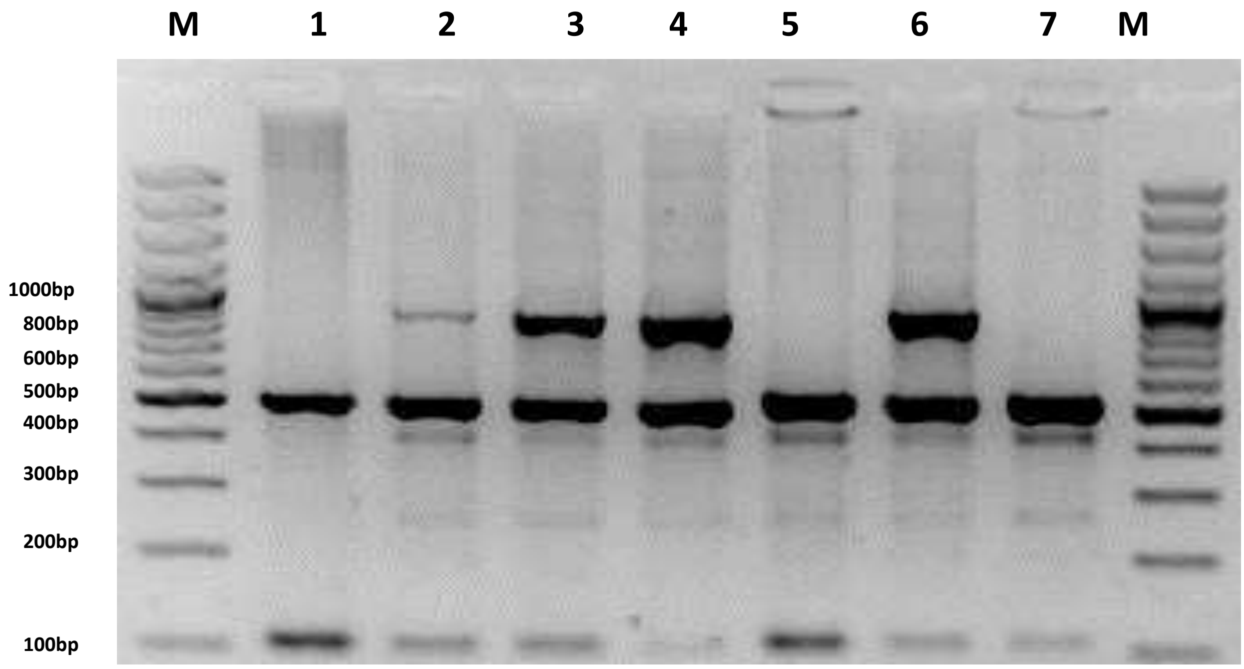


Figure 1. Amplification 870 bp fragment in lanes 2, 3, 4 and 6 that are specific for HO, thus confirming the successful cross combinations for HO in sunflower. Lane 1 = Hysun.33, 2= C.112.P × R.SIN.82 (H2); 3= C.112.P × RH.344; 4=C.249 × RH.344; 5= C.224 × R.SIN.82; 6= C.250 × RH. 344; 7=FH.331

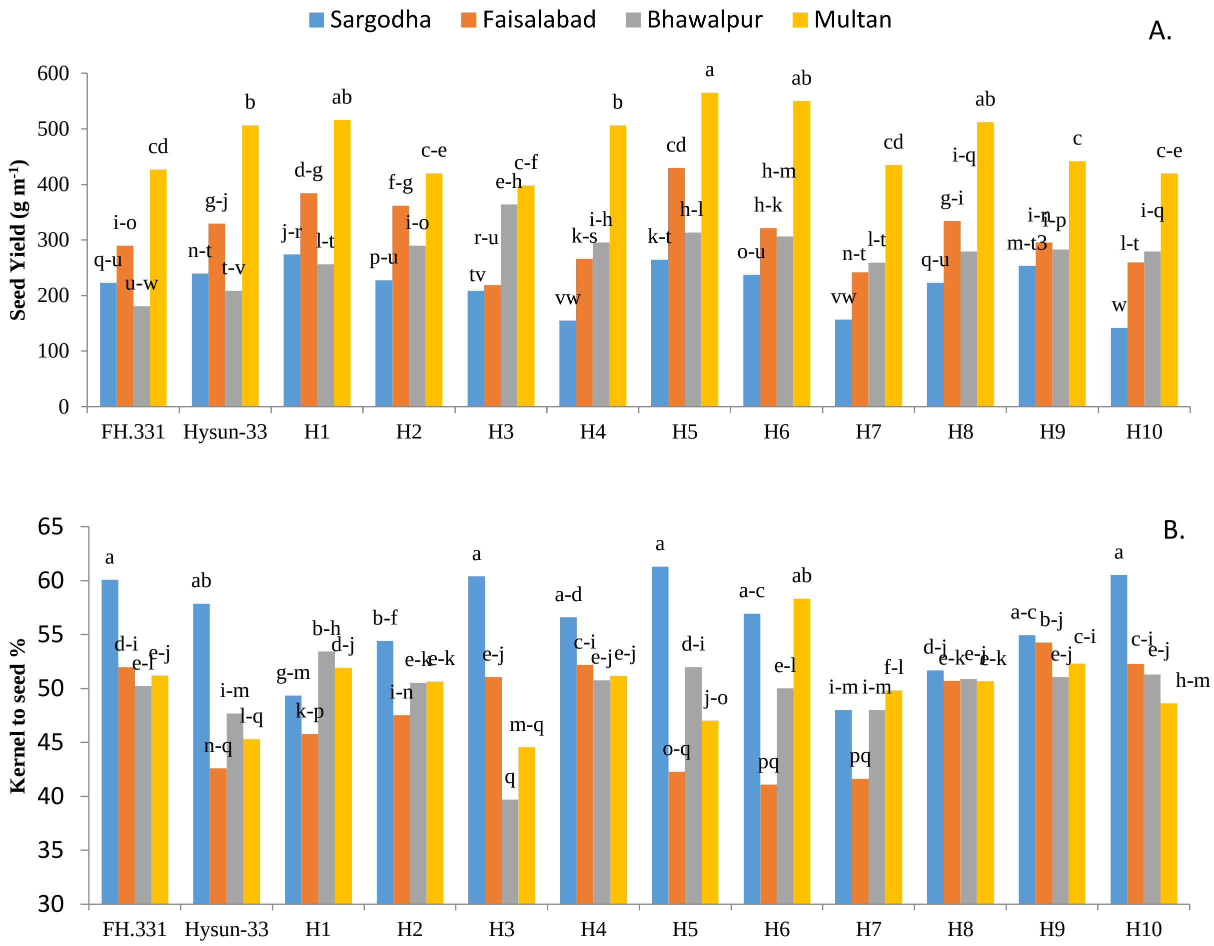


Figure 2. Characteristics of newly developed hybrids along with cultivar checks in the spring season:

A. Seed yield (g m^{-1});

B. Dehulling percentage (kernel to seed percentage).

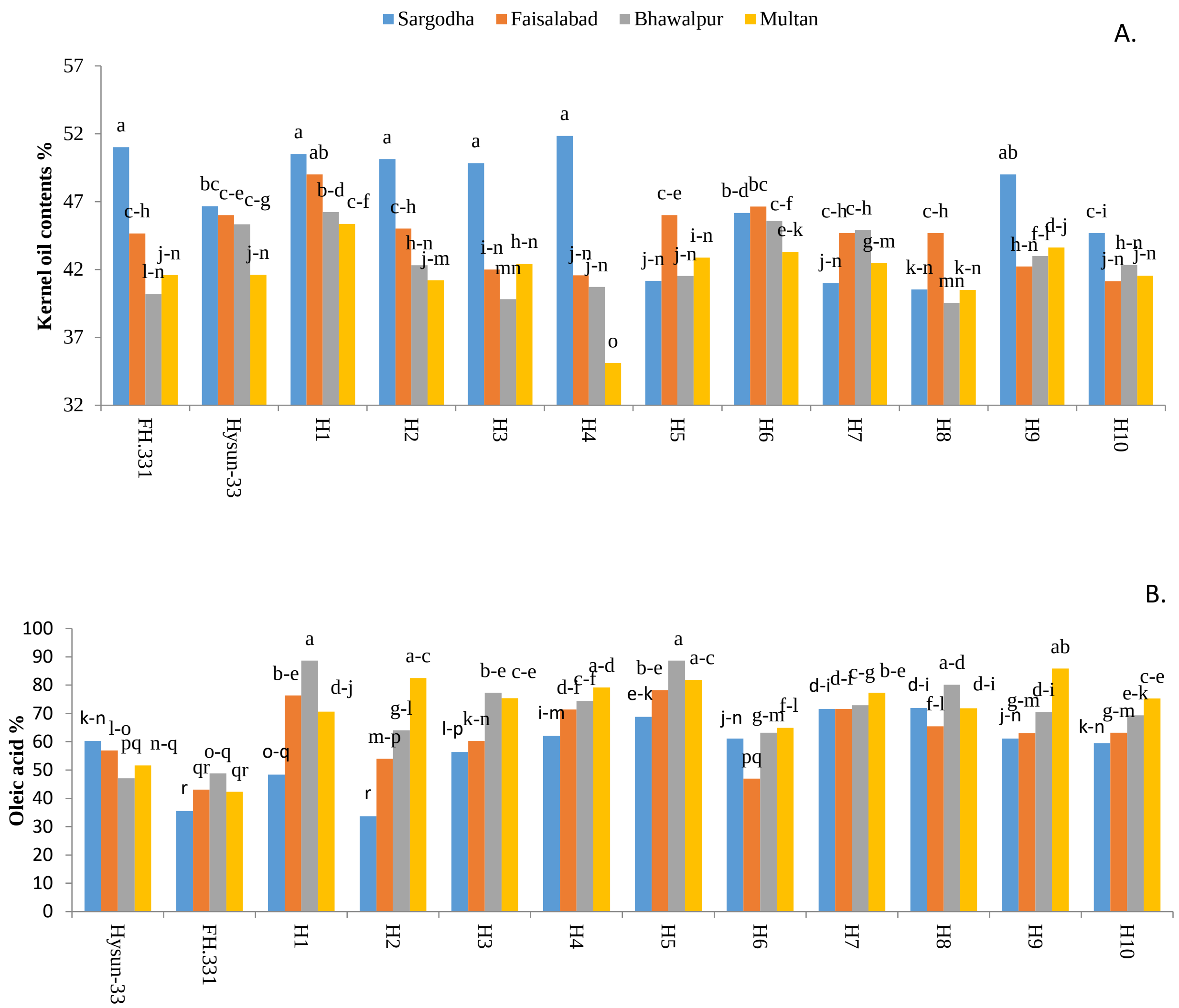
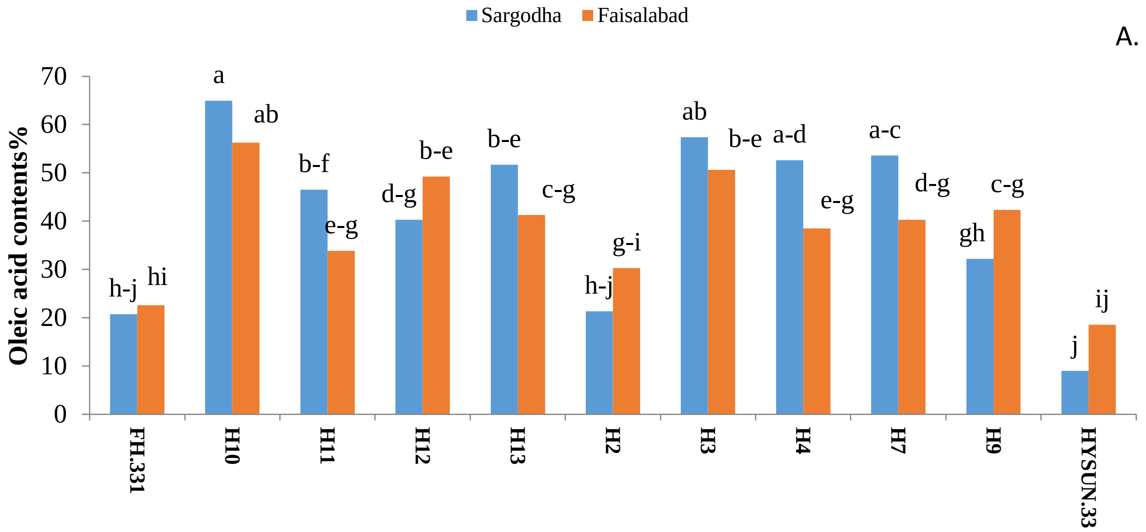


Figure 3. Characteristics of newly developed hybrids along with cultivar checks in the spring season:

A. Kernel oil content (%);

B. Oleic acid percentage.



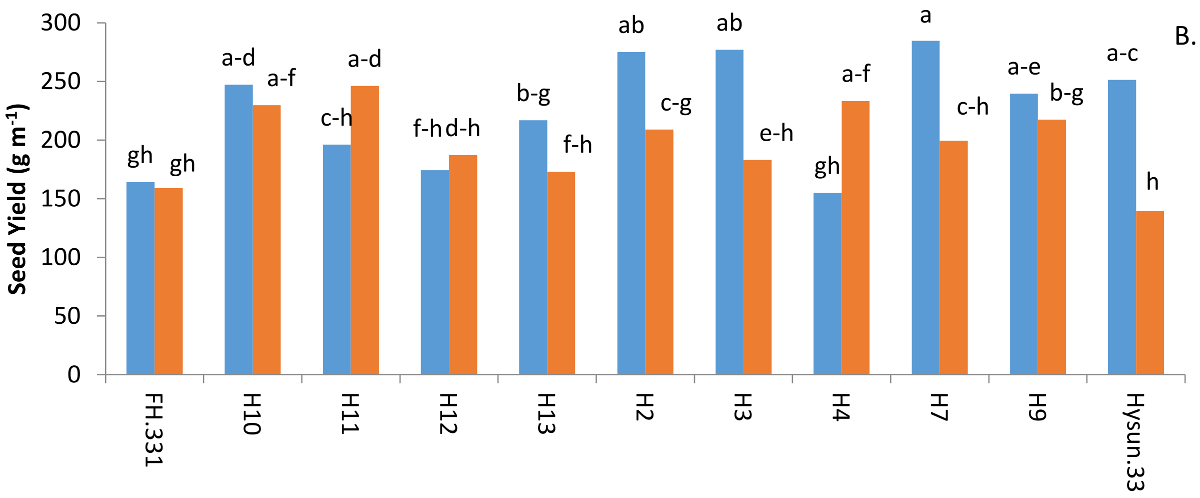


Figure 4. Characteristics of newly developed hybrids along with cultivar checks in the spring season:

A. Seed yield (g m^{-2});

B. Oleic acid percentage.

Same letters above each bar indicate that they are not significantly different (*P* $\geq$ 0.05)

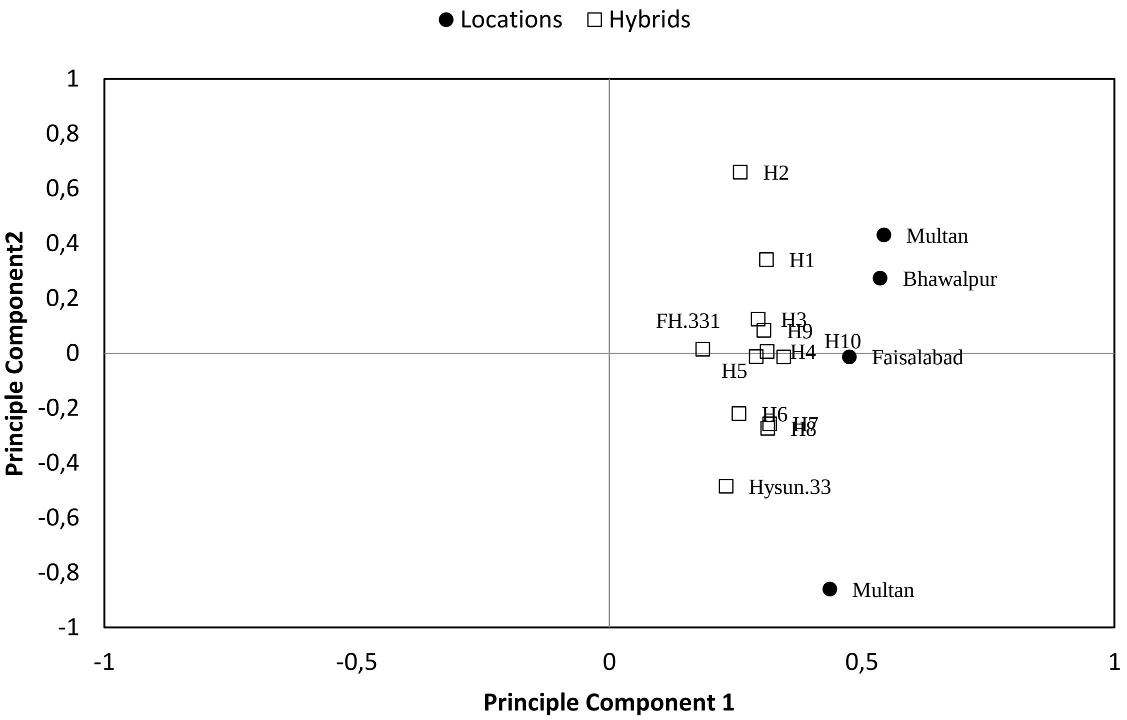


Figure 5. Biplot analysis of hybrids + (hybrid × location) for oleic acid content in newly developed hybrids

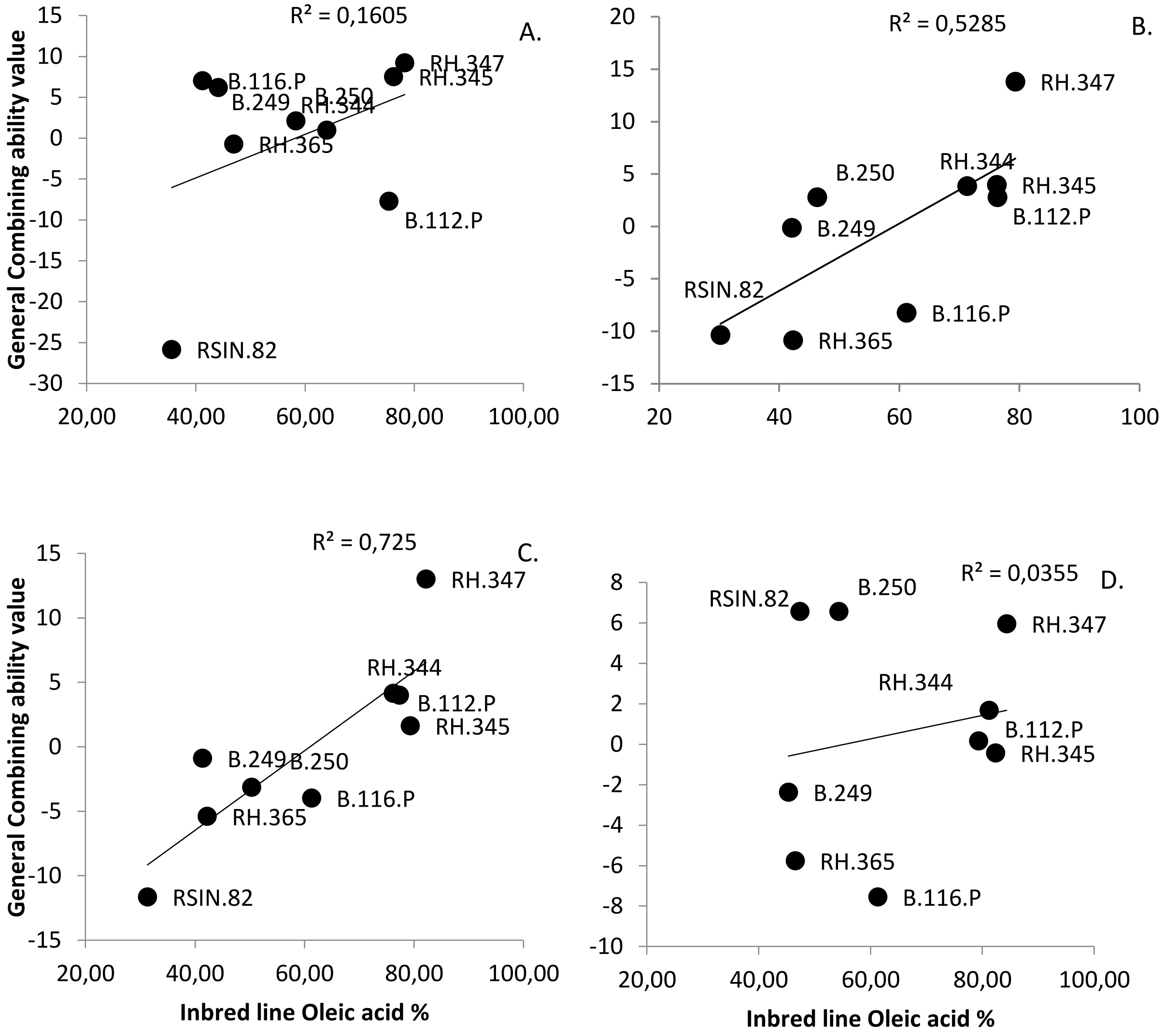


Figure 6. Relationship between combining ability effects and mean oleic acid contents of inbred lines at four locations, namely, Sargodha (**A**), Faisalabad (**B**), Bahawalpur (**C**) and Multan (**D**) in Pakistan.

Table 1. Code and salient feature of hybrids developed and evaluated in the study

Code	Parentage	Salient feature
FH.331	Cultivar check	Local hybrid, dwarf and early maturing hybrid, low oleic acid hybrid
Hysun-33	Commercial check	High yielding, tall and late maturing hybrid, low oleic acid hybrid
H1	C.112.P × RH.344	Both high oleic acid parents
H2	C.112.P × RSIN.82	High × low oleic acid parent
H3	C.112.P × RH.365	High × low oleic acid parent
H4	C.250 × RH.345	Low × high oleic acid parent
H5	C.112.P × RH.347	Both high oleic acid parents
H6	C116.P × RH.365	High × low oleic acid parent
H7	C.249 × RH.345	Low × high oleic acid parent
H8	C.116.P × RH.344	Both high oleic acid parents
H9	C.250 × RH344	Low × high oleic acid parent
H10	C.249 × RH.447	Low × high oleic acid parent

T**able 2**. Characteristics of the experimental sites used for field trials across seasons in Pakistan

	Spring				**Autumn**	
Trait	Sargodha	Faisalabad	Bahawalpur	Multan	Sargodha	Faisalabad
Location	32.07° N, 72.69° E	31.45° N, 73.14° E	29.35° N, 71.69° E	30.16° N, 71.52° E	32.07° N, 72.69° E	31.45° N, 73.13° E
Elevation (m)	190	186	214	122	190	186
Vegetative days	721	880.5	1406.5	1240	861.5	931.75
Reproductive days	835	969.5	1336.5	1570	830	678
Rainfall (mm)	181.5	170.0	71.6	120.6	43.1	56
Supplemental irrigation	1.0	1.0	4.0	4.0	1.0	1.0
Relative humidity	52	62	29	39	68	73

Table 3. Analyses of variance for seed yield and quality traits during spring season in sunflower and estimated variances (σ^2)

Sources of variation	**Degrees of freedom**	**Mean squares**			
		Oil contents (g)	Oleic acid (%)	Kernel to seed (%)	Seed yield (g m^{-2})
Blocks	2	1.30^{NS}	70.05^{NS}	3.60^{NS}	377.55^{NS}
Hybrids (H)	11	35.38**	1256.37**	51.64**	16088.46**
Locations (L)	3	183.26**	1607.93**	456.04**	438595.16**
H × L	33	22.97**	203.18**	52.01**	5863.14**
Residual	94	3.16	35.20	9.29	1254.55
Total	143	13.96	201.38	31.70	12621.85
$\boldsymbol{\sigma^2_{genotype}}$		1.03	87.77	□	852.11
$\boldsymbol{\sigma^2_{phenotype}}$		10.80	176.03	□	3642.86
Heritability (h^2)		0.10	0.49	□	0.23

** and ^{NS} indicate significant at $P \leq 0.01$ and non-significant at $P > 0.05$, respectively.

Table 4. Average seed quality traits of various maintainer and restorer lines used for the development of hybrids to be grown in the spring season

Parents	Kernel to seed (%)	Kernel oil content (%)	Oleic acid (%)
B.208	56.00 ± 3.18	43.10 ± 1.74	35.55 ± 1.47
B.116.P	36.40 ± 2.27	40.12 ± 2.21	71.24 ± 1.52
R.SIN.82	62.00 ± 2.38	45.34 ± 1.92	35.55 ± 1.67
RH.344	56.40 ± 4.15	52.22 ± 1.34	70.00 ± 2.12
B.250	68.40 ± 1.67	44.11 ± 2.12	52.31 ± 2.33
RH.345	51.00 ± 2.93	42.15 ± 1.69	69.27 ± 1.22
RH.365	55.60 ± 3.71	44.10 ± 3.54	40.93 ± 1.39
B.112.P	48.00 ± 3.57	43.19 ± 3.19	78.38 ± 4.12
B.249	63.20 ± 4.19	45.33 ± 2.54	44.09 ± 3.19
RH.347	61.20 ± 3.38	50.13 ± 1.69	75.23 ± 2.16
RH.447	53.24 ± 4.19	47.21 ± 1.86	74.29 ± 3.19

Table 5. Oleic acid contents % and stability parameters [Z] estimated from four locations in Pakistan during spring season

Hybrid	Y	W_i^2	σ^2_i	s^2d_i	b_i	CVi	$\theta_{(i)}$	θ_i	*K*R
Hysun-33	53.96	446.94	172.00	2.18	-0.79	10.70	58.25	118.51	21
FH.331	42.45	55.25	15.33	5.20	0.63	12.81	72.49	47.30	17
H1	71.01	484.03	186.84	54.77	1.87	23.71	56.90	125.25	16
H2	58.55	607.65	236.29	19.78	2.87	34.77	52.40	147.73	22
H3	67.32	54.86	15.17	2.25	1.54	15.67	72.50	47.22	11
H4	71.76	16.15	-0.31	2.30	1.02	10.03	73.91	40.19	6
H5	79.38	41.59	9.86	5.68	1.12	10.46	72.99	44.81	4
H6	59.06	186.90	67.99	23.02	0.56	13.94	67.70	71.23	18
H7	73.33	74.16	22.89	1.34	0.30	3.68	71.80	50.73	8
H8	72.32	125.15	43.29	11.86	0.44	8.33	69.95	60.00	10
H9	70.14	130.99	45.62	15.37	1.42	15.98	69.74	61.07	14
H10	67.10	11.29	-2.26	1.59	1.03	10.67	74.09	39.30	9

[Z] Y: mean seed yield (g m^{-1}) across all locations; Wi2: Wricke's (1962) ecovalence; σ^2i: Shukla's (1972) stability variance; s^2d_i: Finlay and Wilkinson 's (1963) deviation from regression; b_i: Eberhart and Russell's (1966) regression coefficient, $\theta_{(i)}$ and $\theta_{(i)}$: CV: Francis and Kannenberg's (1978) coefficient of variation; $\theta_{(i)}$ and θ_i: Plaisted and Peterson's (1959) mean and genotype × environment variance component; KR: Kang's (1988) ranking.

Table 6. Seed yield (Y, g m^{-1}) and stability parameters [Z] estimated from four locations in Pakistan during spring season

Hybrids	Y	W_i^2	σ^2_i	s^2d_i	b_i	CV_i	$\theta_{(i)}$	θ_i	*K*R
FH.331	280.17	5587.83	2039.70	744.97	0.90	38.42	1946.58	2090.85	18.00
Hysun-33	321.04	6426.11	2375.01	806.63	1.15	41.66	1916.09	2243.27	16.00
H1	357.63	4941.27	1781.07	703.68	1.02	33.48	1970.09	1973.30	9.00
H2	324.92	5792.15	2121.43	386.23	0.71	25.81	1939.15	2128.00	14.00
H3	297.33	20103.92	7846.14	2104.95	0.62	32.86	1418.72	4730.14	21.00
H4	305.71	6440.79	2380.88	461.98	1.30	48.02	1915.56	2245.94	19.00
H5	393.13	4323.06	1533.79	454.16	1.18	34.07	1992.57	1860.90	7.00
H6	353.92	2489.33	800.30	91.97	1.22	38.42	2059.25	1527.49	6.00
H7	273.17	1608.61	448.01	222.89	1.04	42.70	2091.28	1367.36	14.00
H8	337.29	774.11	114.21	18.15	1.13	37.13	2121.62	1215.63	5.00
H9	318.42	2662.75	869.67	67.03	0.76	26.47	2052.94	1559.02	11.00
H10	275.17	3343.18	1141.84	476.58	0.99	41.37	2028.20	1682.74	16.00

[Z] Y: mean seed yield (g m^{-1}) across all locations; Wi2: Wricke's (1962) ecovalence; σ^2i: Shukla's (1972) stability variance; s^2d_i: Finlay and Wilkinson 's (1963) deviation from regression; b_i: Eberhart and Russell's (1966) regression coefficient, $\theta_{(i)}$ and $\theta_{(i)}$: CV: Francis and Kannenberg's (1978) coefficient of variation; $\theta_{(i)}$ and θ_i: Plaisted and Peterson's (1959) mean and genotype × environment variance component; KR: Kang's (1988) ranking.

DISCUSSION

Modification of sunflower oil is an important breeding goal for sunflower breeding. Oleic acid is omega-9 mono-unsaturated fatty acid that is considered suitable for cooking due to its high stability at high temperatures (Smith *et al.*, 2007, Rauf *et al.*, 2017, de Carvalho *et al.*, 2019). Several breeding lines with high oleic acids have been developed. These breeding lines had, however, poor agronomic traits, which also yield poor hybrids when used as parents (Rauf *et al.*, 2017). Hence, our research was initiated to develop high oleic acid hybrid with better SY. Breeding lines with high oleic acid and high fertility were derived from 'Pervenent'. These newly developed breeding lines were further used as female lines in the breeding program. They were mated with low oleic or high oleic acid restorer populations. Ensuing hybrids were evaluated at four locations, which differed for the total degree days received during their reproductive phase.

Generally, hybrids whose parents show high oleic acid produce high oleic acid hybrids at warmer and low humid sites, especially Bahawalpur. This finding agrees with previous research showing that high temperatures trigger the production of high oleic acid (Echarte *et al.*, 2010, Akkaya *et al.*, 2019). However, there was no clear-cut advantage of using both parents in breeding programs to develop hybrids with stable high oleic acid (Van Der Merwe *et al.*, 2013, Alberio *et al.*, 2017). Among evaluated hybrids, H1 (both parents with high oleic acid), H4 (only one parent with high oleic acid), H5 (both parents with high oleic acid) and H10 (one parent with high oleic acid) were the most promising due to their high oleic acid content across locations during the spring season (Figure 2B). Among these hybrids, H10 was the most promising due to its stable and medium oleic acid content (67%) across locations. This hybrid also showed medium oleic acid content during the autumn season (Figure 3A). Another promising hybrid H5 (oleic acid 79%) was also stable according to the biplot analysis and KR. Previous research demonstrated that high oleic acid content was due to a dominant mutation, which seems contradictory to the response of hybrids in our study; i.e., hybrids with one oleic acid parent had medium oleic acid showing partial dominance (Joksimović *et al.*, 2006). Some high oleic acid hybrids such as H5 also had the highest SY across locations and had higher yield than a low oleic acid standard hybrid. Overall, there was a positive relationship between high oleic acid and seed yield ($r2$ = 0.27) across locations where high oleic acid hybrids also had high SY. However, H8 was the most stable hybrid with lowest environmental variances for SY.

Combining ability effects showed high relationship with mean oleic acid contents at Faisalabad and Bahawalpur (Figure 5). This finding confirms that selection for high oleic acid may result in high oleic acid. Furthermore, heritability of oleic acid content was medium across locations. This may be due to the response of the high oleic acid mutant genes to environmental factors such as temperature, availability of water, humidity (Echarte *et al.*, 2010, Akkaya *et al.*, 2019). Our research also confirmed the response of high oleic acid gene under high temperature. In addition, it was also shown that Several genomic regions are known to modify the oleic acid content in sunflower (Premnath *et al.*, 2016). Inbred lines RH.344, RH.347. RH.345 and C.112. were positive combiners for high oleic acid content and are regarded as promising lines for the development of high oleic acid hybrids.

Acknowledgement

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Authors Contributions

Mr. Masood Hussain worked as PhD. Scholar under the project and did the agronomic evaluations of this project. Dr. Shahid Nazir conducted DNA profiling of hybrids. Dr. Abdul Naveed was co-principal investigator of project. Dr. Saeed Rauf was the principal investigator of the project who conceived the research ideas behind it. Professor Rodomiro Ortiz made major improvement in the manuscript and technical aspects of research.

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ENHANCEMENT OF SOYBEAN (GLYCINE MAX L.) ADAPTABILITY AND PRODUCTIVITY THROUGH MOLECULAR METHODS

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Abstract

Soybean is an important protein and oilseed crop of the masses, which is highly sensitive to the environmental factors such as photoperiod, and altitude making it to adaptable to specific regions of the world. Poor adaptability of soybean cultivars caused significant decreased in seed yield due to disrupting of flowering time or maturity time. Moreover, global climate changes triggering factors such as high temperature and water stress condition are further challenging its sustainable production in various parts of the world. Therefore, there is need to understand the genetic response induced by the factors. Review of the current literature showed that flowering is affected by several QTLs and show Photo-thermal responses. Review also highlighted cyto-genetical stock such as chromosome segment substitution lines and their role in introgression of novel genes from wild soybean and was also discussed. All genes showing responses to various environmental factors and adaptability were reviewed in the study and their putative function was discussed. There was also need to enhance the tolerance against global climate change induced factors such as high temperature and water stress. Genes related with enhancement of temperature and heat stress were also discussed which may help to develop stress tolerant soybean cultivars.

Key words: altitude, tolerance, global climate change, CSSLs

Introduction

Soybean is the most important and widely grown crop as it is a good source of oil, protein and provide many industrial products (da Silva et al. 2017). It is known for its nutritive qualities and called as the protein king for its high quality protein content. It is also characterized as an oilseed crop by the Food and Agriculture Organization (FAO 2019). Oil is rich source of polyunsaturated fatty acid, tocophrols and sterol which may help to cure diseases due its antioxidant properties (Kusuma 2015). The seed also contained many minerals and vitamins which are good for human health.

Soybean is prized in the global seed trade for its high quality protein, and premium quality oil. Soybean has wider consumption in human diet and animal feed. Soybean bean milk and meal is exploited in large number of formula feed sausages, ice creams. It has many byproducts which included soybean milk, tofu (soybean curd), miso (fermented soy paste), soy sauce, doufuru (fermented soy cheese) and soybean sprout. Soybean is number one source of edible oil and 60.02 million metric tons of soybean oil was produced worldwide (Statista, 2020). According to recent studies, the seed production of soybean is 333.67 million tones globally during 2019 (FAO, 2019). Its seed contained about 19-24% oil and 39-35% protein (FAO 2020).

Soybean is cultivated in more than 97 countries of the world and its cultivated areas ranged from low to high latitude (Liu et al. 2017). Its cultivation is generally concentrated in the north and south American continent where Brazil (114.26 million tons), USA (96.79 million tons) and Argentina (55.26 million tons) were major producer of soybean during year 2019 (USDA, 2020), These countries together account for about 80% of the total soybean production. Asia produced (31.72 million tons) during 2019 which was only 10% of the world soybean production. This show that soybean was highly concentrated and successful in American continent despite its origin and center of diversity in Asian countries. These statistics show an alarming situation for Food security of the world and require concrete efforts to expand the adaptability of soybean in other continents of the world including Africa and Asia where popularity of this crop for consumption is ever increasing.

Soybean demand will continue to rise due to its enormous health benefits, high quality proteins, and premium quality edible oil (Arif et al. 2010). Moreover, soybean may also increase the food security of the nation and may be a popular pulse crop due to cheaper and rich source protein and high quality oil which may also be used as cooking oil. However, its production need to be continuously improve by developing breeding material with high yield potential, adaptability to broad range of climate, resistant to diseases and environmental stresses which may help to expand its production in developing countries where adaptability of locally developed cultivars having poor pod formation and seed setting was major challenge for breeders and biotechnologist (Gao 2018; Li et al. 2019b). Moreover, there is constant need to improve the product quality such as improvement of specific amino acids in proteins and modification of fatty acid and tocopehrols or sterol contents within oil.

Adaptation of Soybean

Soybean is often recognized as "Golden Beans," or as "plant flesh" since its seeds contained 30-40% protein in general (Ali et al. 2017). In order to improve the availability and sustainability of soybean seed supplies, continuous efforts are required for the expanding the crop in new areas for the global soybean production. Production of soybean is generally affected by several soil ((i.e. pH, soil nutrition, moisture contents) and climatic factors i.e. temperature, humidity and photoperiod (Rorris and Carballo 2014; Nandagawali, 2015). These factors generally affect traits such as germination, leaf area, days to flowering, pod formations, anthesis, grain filling or harvest index.

Photoperiod sensitivity play a key role in plant adaptation and production to particular environment. Extended photoperiod during pod formation and grain filling stage (R3-R6) increased the photosynthate allocation and show positive relation with pod formation and seed yield (Kantolic and Slafer 2001). A number of photo sensitive loci have been identified in soybean which will be discussed in another section. These loci caused the extension of the soybean cultivation from center of origin (China ∼35 ∘N latitude) to both north (up to ∼50 ∘N) and south (up to 40 ∘S) latitude (Gupta et al. 2017). Temperate adapted cultivar generally induced early flowering under short day length with yield losses when grown at low latitude (20 º) (Liu et al. 2017a).

Photoperiod and thermal degree days dictate the maturity time in soybean and define the adaptability of cultivar to particular climate. Soybean cultivars were characterized into 13 maturity groups, due to photoperiod sensitivity, significantly differing in days for maturity (Liu et al. 2017). Cultivar maturity time varied between 75-210 days when world soybean collection was evaluated at Nanjing (Liu et al. 2017). In another study maturity ranged between 97 – 127 days in soybean accessions and seed yield increased with days to maturity up to 112 days (Cober et al. 2010). It was noted that new maturity group continue to arise with expansion of soybean cultivation in new areas of the world (Liu et al. 2017). MG0000- cultivars are adapted to high latitude areas where as X cultivars are known to excel in the low latitude.

Photoperiod sensitivity and growth habit of cultivar explain the time of maturity in determinate and indeterminate cultivars. Determinate were shorter than indeterminate accessions with similar time of maturity (Cober et al. 2010). Yield in determinate cultivars was dependent over plant height, maturity, and lodging resistance (Cober et al. 2010). Photoperiod insensitive isolines were 8-13 days earlier, low yield and lodging resistant (Cober et al. 2010).

Global climate change

Soybean production will be challenged by the global climate change and may affect its sustainable production in its key production areas. Global climate change has activated various climatic factors, such as weather patterns, heat, disease outbreaks, and insect outbreaks, which may affect the global food security and agricultural productivity (Ali et al. 2017). Climate change also caused an observable and predictable increase in the likelihood of flood events (Muis et al. 2015), changes in severe weather events (Donat et al. 2016), the intervention of sea water into aquifers (Dasgupta et al. 2015), changes in soil composition (Brevik 2013), and atmospheric circulation structure, such as ozone levels. These factors were responsible for yield losses or crop failures of the soybean crop in various regions of the world. Yield losses may occur due to suboptimal conditions for growth and productivity of soybean crop.

Some positive impact of global climate change were also noted such as continues increase in CO_2 concentration may accelerate photosynthetic rate, higher CO2 fixation, and high temperature may induce early crop maturity with accelerated degree days accumulation **(**Fox et al. 2014**).** Cultivar response of 5 -90% increase in soybean yield was obtained under elevated CO_2 level due to increase in the pod number and number of seed per pod but generally positive impacts of global warming and climate change are assume to outweigh by negative impacts (Li et al. 2019). Heat and water stress and increased infestation of pest were known as principal agent which may cause serious decline in soybean yield and production which were triggered by climate change (St-Marseille et al. 2019). Global yield losses due to climate change may be 5-6 % in the 2030-31 and intensifies to 30-32% in the 2080-81 (Havlík et al. 2015).

Soybean is very sensitive to soil moisture contents and base water stress tolerance was (-0.67 MPa) and generally cause the inhibition of germination, seedling growth reduction of leaf area, flower abscission, pod formation and grain yield (Khalil and Mex 2010). Despite soybeans can tolerate a broad range of temperatures (11 to 41°C) depending upon specific cultivar, high temperature or greater fluctuation in temperature cause abscission of flowers, failure of pollination or grain filling causing significant yield losses (Luo 2011). An increase of 1-2°C may result in average 2.5% yield losses in various states of USA states (Mourtzinis et al. 2015).

Development of new cultivar was key to sustain soybean production under climate change scenario and adaptability to specific climatic and growth conditions (Fodor et al. 2017). Conventional approaches to release a cultivar generally involve two steps for development of highly adaptable cultivars.

The first step is to characterize soybeans accessions. Characterization determines the expression of heritable traits related to stress tolerance. Accessions may be characterized on multiple traits such as smaller leaf area, hairiness, cuticular waxes, gametophytic fertility, pod formation under adverse conditions or physiological traits such as high canopy temperature discrimination, water use efficiency, heat shock proteins, and other molecules related to stress tolerance (Vandana et al. 2017). Genotypes must be evaluated in order to make their better use in breeding programs or their cultivation in targeted zones (Prince 2016).

Development of environmental ideotype which may better fit to specific environmental condition could also help architect climate adaptable varieties. In 60s, Donald coined the word "Ideotype" to describe various types of wheat for irrigated or rainfed conditions. The association among morphological and physiological, metabolic, molecular, and physiological features with production is evaluated for the construction of an ideotype, and then an appropriate plant type is offered.

Climatological ideotypes, which concern with climatic adaption features, are appropriate for the production of soybean ideotypes in various agro-climatic zones (Table 1). It demonstrates the significance of studying climatic variation and genotypic characterization.

Table 1. List of various ideotypes of soybean specific for various environment and industrial uses

Traits	**Use**	**Results**	**Reference**
High oleic acid ω-9 fatty acid or stearic acid	Better cooking quality and deep frying and biodiesel production	144 RIL lines were selected though BLUP analysis.	Woyam et al. (2019)
Erect, early and high yield	High yield under targeted environment	FAI-BLUP index was used to select best progenies. Model was effective to improve the genetic gains.	Volpato et al. (2020)
High root depth, root density and narrow root angle	Drought resistance	Positive relationship between the root length, shoot length and above ground biomass	Dayoub et al. (2021)
Cultivar with maturity group 7.8 and plant density 50 m^{-2}	Climatic proof ideotype	Supplementary irrigation was beneficial to improve seed in various climatic scenario	Battisti et al. (2018)

Mixed Model Methodology: to increase soybean adaptability and productivity

Adaptability of genotypes is assessed under range of environments helping to estimate genotype × environment interactions under mixed model arrangement (LI et al. 2017). Genotype × environment is an integral component of phenotypic variance when breeding material was tested over range of environment (Baraskar et al. 2014). Differential performance of specific genotypes to different set of environment indicated adaptability of genotypes to specific environment and may not be recommended for general cultivation over range of agro-ecological conditions (Bernardo 2020).

In order to estimate GE interactions, elite breeding material was evaluated in various environmental or climatic scenarios through mixed model approach which indicated low estimate of genotypic variance while high GE across various climatic scenarios (Table 1).

Conventional approaches have contributed in the development of cultivars with increased adaptation to targeted environment. However, conventional techniques are slow and unable to provide insight for the genes and their role in determining the phenological responses to various environmental factors and stresses. However, cyto-genetical studies may help to identify the genes related to the environmental responses of the soybean cultivar and may able to develop cultivar with greater productivity and more adaptable to targeted environment.

Soybean genome

Soybean has 2n= 4× = 40 chromosomes, which are obtained from 2 rounds of chromosome duplication. However, soybean behaves like a diploid by inducing disomic inheritance at most of the loci in contrast to tetrasomic inheritance generally prevailing in tetraploid species (Walling et al. 2006; Rauf et al. 2021).

Karyotyping of chromosome was done on the basis of centromeric position which showed that it comprise of 24m+14sm+2st (Jilin et al. 1990). In another study chromosome were characterized as 28m+12m+2st (Zhaosheng et al. 1999). Pachytene chromosome had heterochoromatin on both side of centromere (Singh and Hymowitz, 1988). Cytogenetics of soybean has been lag behind than other agronomical important species due to difficulties in identification of individual chromosome, except 2 st chromosome (Chung & Singh, 2008). Contrastingly, florescent insitu hybridization (FISH) technique was optimized to identify all 20 chromosomes through differentially labeled probes (Findley et al. 2010). The same technique was also successfully applied to identify chromosome of other wild species i.e. *Glycine soja*. FISH technique also led to the identification of translocations between chromosome 11 and 13 (Findley et al. 2010).

Soybean was domesticated about 4000 year ago in China (East Asia), while *Glycine soja* a wild annual species was considered its possible parental species (Chung and Singh 2008). The genus glycine was divided into two subgenus *soja* which included cultivated soybean species and its wild progenitor *Glycine soja* Sieb. & Zucc. The annual wild species *Glycine soja* had center of diversification in China, Korea and Japan and Far East region of Russia (Liu 2004). Asian countries such as Laos, Afghanistan, Cambodia, Mayanmar contained rich diversity of this crop. Subgenus glycine carried 26 wild perennial species distributed in Australia (Singh 2017). Some species belonging to subgenus soja were distributed in various Islands of Japan, Subgenus Glycine comprise of secondary gene pool while three species *G. max, G. soja* and *G. gracilis* comprise of primary gene pool. *Glycine gracilis* is an interspecific hybrid of *G. max* and *G. soja* (Liu 2004) which is weedy form of soybean. Accessions belonging to secondary gene pool has been extensively collected and characterized and contained stress resistant genes (Doyle et al. 2003).

Glycine soja is considered to be closest relative of glycine max with 2n = 40 chromosome. The chromosome of the species are smaller in size which were found difficult for karyotyping. It comprise of 12 metacentric and 8 sub-metacentric chromosome (QN et al. 1984). Chromosome of *Glycine soja* species were 6-7% smaller than the Glycine max (QN et al. 1984), while 16 chromosome of the species were similar to Glycine max (QN et al. 1984). Both *Glycine max* × *Glycine soja* hybridized rapidly and set normal pods. The pairing behavior of hybrids showed 18II and I IV with pollen fertility ranged of about 50% (Singh and Hymowitz 1988).

Cytogenetic tools for Karyotyping of soybean genome

Conventional root and bud squash methods and various staining techniques were used for soybean chromosome counting and painting to differentiate hetero and euchromatin among its basic set of chromosome. Soybean has 40 small sized chromosome which were difficult to karyotype on the basis of various chromosome feature but has been subjected to studies on the basis of various banding techniques such as Florescent Banding (Imran et al. 2015). Scatter plot were constructed on the basis of quantitative traits such as arm ratio, total length of chromosome to identify various homologous chromosome of soybean (QN et al. 1983). Some other studies also identified variation in chromosome on the basis of arm ratio, centrometic index and relative total length of chromosome (Imran et al. 2015). Current tools include different inset hybridization methods which were used for the understanding the structure, function and mapping of the genes related to various economically important and adaptability traits **(**Chaturvedi et al. 2011**).** Techniques included the florescent insitu hybridization (FISH) or genomic insitu hybridization (GISH). Association mapping was done for marker assisted selection while potential techniques such as CRISPR/cas9 was used to induce modification in soybean genome. Current soybean genomic resources include chromosomal segmental substitution lines, high density linkage map, genome sequences, and high density mutant libraries (Harada and Kaga 2019).

Chromosome segment substitution lines

Chromosome segment substitution lines (CSSLs) represent a complete genomic segments of distant genotype into adaptive genetic background. Molecular analysis provides a valuable tool to tag various chromosomal segments or QTLs with agronomically important traits. Introduced chromosomal segments represent complete genomic library of distant accession in adaptive background. Each CSSL potentially had single chromosome segment with 99.9% genome of recurrent parent as adaptive genetic background. They are generally developed by the population backcrosses with recurrent parent along with high density genome wide marker assisted selection which is followed by self-pollination to constitute lines (Fig 1). Each line contains a chromosomal segment from distant genotype such as wild relative, the segment is tagged by markers from donor parent.

CSSLs are evaluated for targeted traits and lines showing significant differences from parental types are selected (Balakrishnan et al. 2019). The selected CSSL lines may be used to identify quantitative trait loci (QTL), genes of interest. CSSLs are valuable tools for basic and applied genetics and considered as genetic stocks or pre-breeding material which may also help to widen the genetic base of the population. Moreover, they are important tool to introgress genes from wild relatives with minimum linkage drag. A full review on CSSL lines are available in various crops i.e. rice (Balakrishnan et al. 2019).

CSSL lines have also been developed in soybean (Wang et al. 2013). In a study 151 soja (CSSLP1) were developed which covered about 96% donor genome from Glycine soja in adaptive genetic background (Wang et al. 2013). CSSL were used to identify QTLs related to valuable traits such as flowering (Liu et al. 2018), nematodes resistance (Huang et al. 2021), adaptability (Qingyuan et al. 2021), seed coat color (Liu et al. 2021). Other examples are presented in Table 2. A major QTL qFT12.1 was tagged with flowering time which explained about 40% of the phenotypic variability and its recessive allele was related with delayed flowering (Liu et al. 2019). Parental lines from cultivated and wild accessions were used to develop CSSL lines through back cross breeding program. Late flowering in soybean was introduced from wild accession through CSSL lines (Liu et al. 2017).

Table 2. Utilization of chromosome segment substitution lines (CSSLs) as breeding material in improving adaptability of soybean

Parents	CSSLs	Results	Reference
Jackson × JWS156-1	120	Recessive allele qFT12.1 delayed flowering	Liu et al. (2018)
NN1138-2(*max*)×*N24852*(*soja*)	177	Six genes for days to flowering and 2 for seed coat color	Liu et a (2021)
‘Khao Dawk Mali 105’ (‘KDML105’)	Lines CSSL11, CSSL12, and CSSL16	DHL212 chromosome 1 was related with salt tolerance and photosynthesis rate	Chutimanukul et al. 2018
Enrei × Peking	103	50 reproducible QTLs for flowering, maturity and yield	Watanabe et al. (2018)

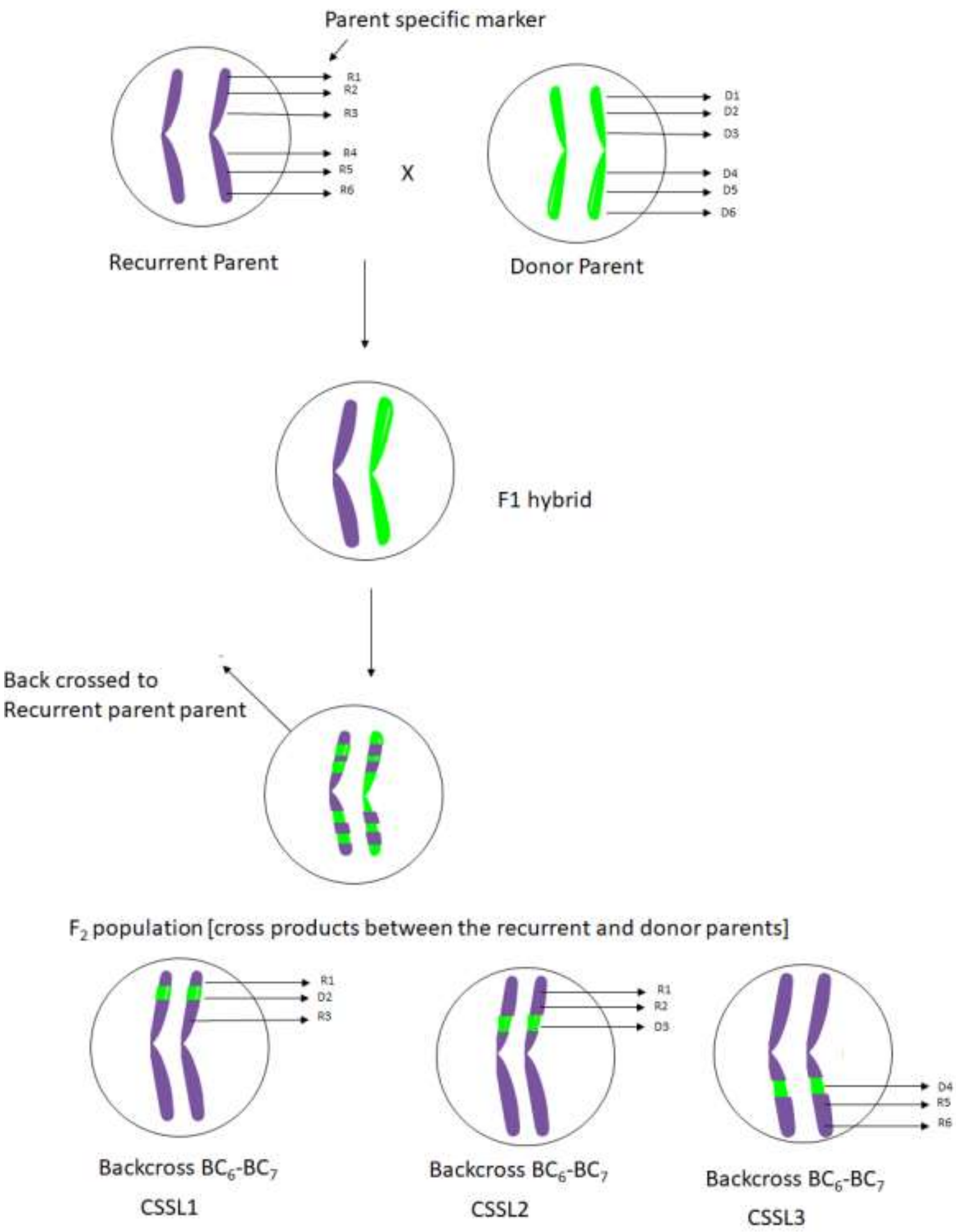

Fig 1. Scheme for development of chromosome segment substitution lines (CSSLs)

Genomic analysis soybean

Whole genome sequence assembly of several cultivars i.e. US cultivar ‘William 82’, Japanese ‘cultivar Enrei’, and Chinese ‘cultivar Peking’ are available. In addition several projects have been initiated for online availability of whole genome sequence consortia on various web tools such as https://www.ncbi.nlm.nih.gov/assembly/GCF_000004515.6; https://www.naro.go.jp/english/laboratory/nics/genomics/soybeans_genomics/index.html#id and Benko-Iseppon et al. (2012).

Recent genomics system apps have made large amount of genomic data available on number of webpages maintained by various organizations with extensive computational power. These computing resources aid in the analysis and storage of data, which is then made accessible through user-friendly systems as "databases." Numerous databases for soybeans have been created in this aspect, such as SoyBase, SoyNet, SoyFN, SoyDB and SoyKB The Soybean Knowledge and experience is a vital database that provides a complete web page for genomics data provided by diverse platforms (Joshi et al. 2013).

Genome size of Japanese cultivar ‘Enrei’ was about 928 Mb total bases and 221,674 contigs after sequencing and assembling through shot gun method (Shimomura et al. 2015). The sequenced genome of Enrei may be compared with reference genome of ‘William 82’ cultivar for single nucleotide polymorphism (SNP) induced through insertions/deletions. Sequence analysis of ‘Enrei” also led to the identification of genes related to important pathways such as initiation of flowering which was known to be control by genes whose expression was modified by the environmental factors such as temperature and photoperiod. Identification of temperature and photoperiod sensitive flowering initiation genes may lead to the better understanding of photoperiod sensitivity in soybean and may also be helpful for the development of phtoperiod insensitive cultivars which may increase their adaptability to various agro-ecological conditions. Several genes related to photoperiod sensitivity has been identified in soybean which were designated as “E”. QTL analysis led to the identification of FT2 linked with locus E2. The locus E2 was show to be affected by latitude in various regions of Japan. Mutant plant (e2/e2) for locus E2 initiated early flowering as compared to the wild plants. The genotype e2 had elevated expression of GmFt2 (Table 3).

“Omics “ tools may help to analyses genomes at various levels starting from the structural genomics to the metablomics. Whole genome analysis of soybean may help to uncover genes related to various traits or pathways and may be helpful to discover single nucleotide polymorphism (SNP) within genes of adaptability. SNPs may helpful in devising marker assisted selections in gene pools and could help selection in segregating populations or back cross schemes

Adaptability of soybean to climate change using genomic tools

Soybean evolved as short day plants and its cultivation was confined to 22°N till 1970s due to precocious flowering (Gupta et al. 2021). Flowering time is dictated by photo-thermal response of the genes, environment and their interactions. Alleles such as E1, E2, E3 and E4 were known to affect the maturity in soybean and allelic variation among soybean cultivars cultivated in various geographical regions was determined (Miladinović et al. 2018). Allelic combinations show complex interaction or additive effects but generally dominant allelic had later flowering. Contrastingly, loss of function and hypoactive alleles of “E” series induce early maturity, photoinsensitivity under long days (Gupta et al. 2017). For instance genotype e1-nf were earlier in flowering than E1, while average difference between the e1-asE4 vs E1E4 flowering time was about 6 to 14 days (Zhai et al. 2014). e1-asE2(E3)E4 vs E1E2E3E4 had difference of 8-28 days in various geographical region of china (Zhai et al. 2014). Recessive alleles e3 and e4 had inhibitory effect over E1. Cultivars with various combination of dominant alleles such as E1E2E3E4 (62 days), E1E3E4 (53 days), E1E4 (44 days) and E1 (36 days) had variable flowering (R1) in various geographical region of china (Zhai et al. 2014). Accession with similar allelic combination of E may have different flowering time under similar or multiple environment showing that flowering time was complex in soybean which was triggered by several factors including thermal degree days (Jiang et al. 2014).

Varieties belonging to specific geographical region carried a specific haplotype of E ranging from E1 to E4. Specific haplotype was representative of more than 50% of the cultivars within geographical area, with exception from Europe where more than one haplotype was present in soybean ancestral accessions (Miladinović et al. 2018) showing higher diversity within land races for maturity time. Cultivars belonging to Novisad Serbia carrying e1-as/e2/E3/E4 were highly productive and this combination of alleles was recommended for cultivars of central Europe (Miladinović et al. 2018). Similarly, majority of cultivars of Kazakhstan were genotyped as e1-as/e2/E3/E4 and cultivars carrying either of dominant E3 or E4 were found productive (Abugalieva et al. 2016). Molecular markers (dCAP or CAP) were used for tagging alleles *e1-as* and *e3-fs* in indian soybean cultivar which confer early maurity (Gupta et al. 2017).

FT2a and FT5a were involved in the induction of flowering and their expressions was controlled by the various allelic combination of “E” alleles mentioned above in response to photoperiod (Takeshima et al. 2016). Delayed flowering was noted due to down regulation of two genes *FT2a* and *FT5a* in soybean Thai cultivar K3 (Sun et al. 2019). 829 SNP markers detected a major QTL containing FT2a affecting flowering time in soybean under all tested environment (Sun et al. 2019). Down regulation of the FT2a was due to nonsynonymous substitution in exon 4 of the gene (Sun et al. 2019). Genome wide association studies identified 37 SNP linked with flowering time and other related traits to maturity (Li et al. 2019). Five candidate genes i.e. (Glyma.05G101800, Glyma.11G140100, Glyma.11G142900, Glyma.19G099700, Glyma.19G100900) were similar to the flowering genes identified in Arabidopsis thaliana (Li et al. 2019). Above mentioned genes encoded proteins such as FRI (FRIGIDA), PUB13 (plant U-box 13), MYB59, CONSTANS, and FUS3 and may have role in controlling growth period of soybean (Li et al. 2019). Large effects loci Gm 11, Gm 16, Gm 20 were known to control the flowering (Mao et al. 2017). The number of detected loci affecting the flowering increased with day length. Variation for flowering among cultivars arises due to epistatic × environment and additive × environment of various loci affecting the traits. Loci such as Gm04_4497001 (near GmCOL3a), Gm16_30766209 (near GmFT2a and GmFT2b) and Gm19_47514601 (E3 or GmPhyA3), the Gm04_4497001 interact with other loci or environment to induce flowering response of soybean (Mao et al. 2017).

The discovery of long juvenile (LJ) trait had broken down the latitude boundaries for soybean cultivation. LJ traits helped to increase the biomass under short day (Tripathi et al. 2021). Genes E6, J and E9 were the major genes involved in the expression of long juvenile traits such as late flowering and maturity in varieties under short day length (Gupta et al. 2021). E6 gene was mapped on GM04 with marker HRM101 (Li et al. 2017). The gene E6 had suppressive effect over E1, and it function via E1 gene. Tagging of genes with molecular may help to breed wide adaptation and stability in soybean (Li et al. 2017). Another study linked LJ traits with a SNP variation in FT2a loci (Tripathi et al. 2021). Traits helped the soybean cultivation expansion in Brazil. Short duration LJ varieties combined with photo insensitivity may help to adapt under stress condition with low rainfall (Gupta et al. 2021). Early flowering was known to be affected by locus e2 which carried 3 nonsynchronous SNP, identified when exon of e2 in 40 varieties were sequenced **(**Kim et al. 2018**).** SNP variation in coding sequence of E1Lb induced a recessive alleles which caused premature termination of translation and induced early flowering under long days (Zhu et al. 2019). List of various locus associated with phenology of soybean has been presented in Table 3 while impact of allelic combinations or modified alleles related to maturity and flowering on productivity in various ecological zones has been shown in Table 4.

Table 3. Locus conferring adaptability to climatic condition

Locus	Adaptability	Gene	Reference
Long-Juvenile trait (LJ)	Low latitude or tropical condition	E1 repressor and J protein suppress E1 inducing early flowering	Lu et al. (2017)
QTL LG4 Donnong 50 × Williams 82	Trait related to flowering time, maturity, and reproductive duration	Single QTL for all traits related to maturity and adaptability to various latitude	Kong et al. (2018)
FT2a and FT2b Down regulation	Long juvenile traits	FT2a carried a SNP mutation	Tripathi et al. (2021)
E1, *E2*, *E3*, and *E4*	Geographic adaptation	Genes related with flowering time	Kim et al. (2018)
PIF4	Early maturity	Reduced plant height, low leaf area, decreased branches, early flowering, robust flowering to pod maturity	Arya et al. (2021)
GmFT7	Transition to flowering	GmFT7 show strong expression during transition phase	Zhang et al. (2021)
e1 and e2 alleles in Indian genotypes (e1-as/E2/E3/E4, E1/e2/e3/E4, E1/e2/E3/E4, E1/E2/e3/E4)	Photo insensitive alleles	Reduced days to flowering and photperiod sensitivity	Tripathi et al. (2021b)
eI-Lb	Recessive alleles induce photo period insensitivity	Dominant allele retard flowering by suppressing the expression of FT2a and FT5a while recessive allele induce earlier flowering along with e3/E4 or E3/E4 under long days	Zhu et al. (2019)
Gmprr37-ZGDD mutant allele	Induce early flowering under natural long days	CRISPR/Cas9 induced modification of *GmPRR37*	Wang et al. (2020)
GmPRR37	Flowering inhibiting gene	Inhibit the function of flowering genes FT2a and FT5a and upregulated FT1a.	Wang et al. (2020)
e3 /e4 , e1 (e1-nl or e1-fs) with e3 or e4	Photoperiod insensitive	Induce photoinsensitivity within accessions, identified through screening of soybean germplasm	Gupta et al. (2017)

Genome editing

Genome editing is used to manipulate the genome in different ways through different techniques like CRISPER (explain) technique or use the ZEN (zinc finger nuclease) for improving the adaptabilities of the crop (Curtin et al. 2011; Chen and Gao, 2014; Du et al. 2016). A new protocol name as DNA free based on cleavage site has devised for induction of genome modification in soybean genome (Woo et al. 2015).

Various genome editing systems generate cleavage sites on targeted genomic regions The endogenous DNA repairing mechanism causes an addition or a deletion via non-homologous end-joining of the cleavage sites, resulting in a variation in the targeted sequence. Additionally, homology-directed DNA repairs can be used to exchange the target region with a homologous sequence.

Genome editing is considered to be safe than other traditional method due to non-induction of the foreign DNA (GAj et al. 2013). CrRNAs are more reliable because it targeted the desired genome more accurately than the DNA binding protein. CRISPR/Cas9 was used to induce mutation in the E1 locus which suppress early flowering under long day length. Mutation was induced at two sites with in genome i.e. 11 and 40 bp deletion causing early termination of translation ending up the production of truncated protein (Han et al. 2019). Induced modification of E1 locus caused early flowering within soybean under long day length to increase the harvest index (Han et al. 2019). Decrease in the translation of E1 protein caused subsequent increase in the GmFT2a/GmFT5a. E1 allele encode a bipartite protein comprising of nuclear localizing signal and region B3 domain, while mutation in E1 caused nonsense mutation causing loss of localizing specificity of protein inducing early flowering (Xia et al. 2012).

Table 4. Impact of various allelic combination on maturity and productivity of soybean cultivar

Allele	Environment	Flowering	Productivity	Reference
Gmprr37	High altitude	Early	Enhanced	Wang et al. (2020)
ft2aft5a	Short day length	Delayed	Increased pod and seed yield	Cai et al. (2020)
Induced modification in E1	Long day length	Early	High harvest index	Han et al. (2019)
Iso-lines developed from e2e2e3e3 ‘Enrei’, E2E2e3e3 ‘Sachiyutaka’ E2E2E3E3 ‘Fukuyutaka’	36°0′26N, 140°1′19E 34°13′47N, 133°46′36E	Early (E1), medium (E3) vs Late flowering (E2)	E2 and E3 isoline increased adaptability and more productivity in southern Japan	Yamada et al. (2012)
e1-n/E2/ E3/E4	Eastern Kazakhstan	670 thermal time length for flowering, Maturity group “0”	Highest yield in Eastern Kazakhstan	Abugalieva et al. (2016)
J locus	Short day length	Long Juvenile	Yield enhancement	Lu et al. (2017)

A double mutant ft2aft5a induced through CRISPR/Cas9 caused flowering to be delayed by 31.3 days under short days which increased pods and seed yield (Cai et al. 2020). It was concluded that delayed flowering may be beneficial in tropical environment (Cai et al. 2020). A modification of flowering inhibiting gene *GmPRR37* through CRISPR/Cas9 induced early flowering (Wang et al. 2017). A natural mutant allele *Gmprr37* enabled cultivation of soybean at higher altitudes and provide adaptability to specific region (Wang et al. 2020).

One of the challenge arouse after soybean genome sequencing project was to identify the functions of 47–57 thousand identified genes (Bai et al. 2019; Schmutz et al. 2010). CRISPR/Cas9 may able to identify the functions of genes through comparison of modified with wild phenotype. For instance, truncated proteins encoded by *GmHsp90A2* caused loss of heat tolerance in soybean identifying the possible function of gene (Huang et al. 2020).

Induced abiotic stress tolerance

Soybean was found sensitive to various types of stresses, however, global climate change has increased the chances of crops failures at early establishment phase or during reproductive phase causing significant decline in seed yield of crops including soybean. Soybean cultivar belonging to various maturity groups were exposed to sub-optimal or super-optimal temperature which caused significant decline in seedling parameters showing its negative impact during early establishment phase (Alsajri et al. 2019). Cultivar belonging to maturity group III showed more decline for seedling parameters when compared with MG IV and V (Alsajri et al. 2019). Variability among soybean cultivars was observed in response to induce abiotic stress. Differential responses to abiotic stress was due to various stress signals, and activations of molecular pathways which enhanced tolerance to abiotic stress. Genes such as ethylene responsive factor GmERF75 was triggered by stress signal of abscisic acid induced multi stress tolerance (Zhao et al. 2019). Genotype over expressing the transcript of GmERF75 had higher chlorophyll contents than wild types (Zhao et al. 2019). GmWRKY12 is an important regulator of plant responses to abiotic stress. Transcript was activated by the abscisic acid, drought or salt tolerance and involved in increased proline content and decreased malondialdehyde (MDA) in transgenic soybean seedling (Shi et al. 2018). Transcriptomic analysis in selected soybean cultivars revealed stress tolerant transcriptomes which was confirmed through transformation in model or soybean cultivars (Zhao et al. 2019). A two folds increase in the expression of differentially expressed transcriptome when soybean was exposed to high temperature affecting the expression of 1100 – 10000 genes (Gillman et al. 2019). Transgenic soybean having over expression of *GmHsp90A2* had high tolerance to heat stress as transgenic soybean had higher expression of chlorophyll and lower malondialdehyde contents (Huang et al. 2019). Function of various transcripts involved in various types of stress tolerance of soybean is shown in Table 5.

Table 5. Role of transcripts involved in ameliorating the effects of abiotic stresses in soybean

Transcript	Suggested role	Stress tolerance	Reference
*GmEF4 elongation factor (*EF1α*)*	Over expression reduced the leaf wilting, longer root, high proline contents, lower hydrogen peroide and malodialdehde	Drought and salt	Gao et al. (2019)
GmHsp90A2	Higher chlorophyll and lower malondialdehyde	Heat	Huang et al. (2019)
AtSZF2	Higher cell membrane stability and chlorophyll contents than wild types	Salt	Kim et al. (2017)
GmFDL19	High biomass and seed yield	abscisic acid, polyethylene glycol (PEG 6000) and high salt stresses	Li et al. (2017)
GmPIP2;9 Plasma membrane intrinsic protein	High root growth and leaf gas exchange properties	Drought	Lu et al. (2018)
GsWRKY20	High yield, cell membrane stability lesser malondialdehyde, high antioxidants and proline contents	Drought and salt stress	Ning et al. (2017)
AtABF3	High cell membrane stability, less damage to chlorophyll, high seed mass	Drought and salt	Kim et al. (2018)
*GmSAP1*6 Encode stress associate protein	Higher proline contents, lower water loss, lower malondialdehyde	Drought, salt, and abscisic	Zhang et al. (2019)
GmGLP7 Germin like Protein	Seed germination and root elongation	Salt, drought and oxidative stress	Li et al. (2017)
GmDREB1	Activate expression of numerous stress responsive gene i.e. GmPYL21, ABRE mediate gene expression	Cold, drought, heat, and salt	Kidokoro et al. (2015)

Non-coding RNA in soybean

Long non-coding (LncRNAs) is a class of ncRNA which are 200 nt long with a promoter substructure and poly A tail. LncRNA are specific to a tissue and time. Initially it was thought LncRNA were transcriptional noise due to their extremely low expression level. However, it has been identified that LncRNA were regulator of key cell processes and affect the transcription level as cis or trans regulator of gene expression. They were known to involve in adaptation to the stress tolerance (Chen et al. 2019). Stress responsive LncRNA had less number of exons and their concentration increased in response to the salt stress where they bind with proteins to enhance their catalytic activity (Chen et al. 2019). In addition, up regulation of differentially expressed 525 LncRNA were identified through root transcriptomic analysis when soybean accessions were exposed to phosphate starvation (Zhang et al. 2021). These differentially expressed LncRNA in nitrogen fixation, catalytic activity and lipids metabolic processes (Zhang et al. 2021). Recently, role of LncRNA in fatty acid synthesis were identified (Ma et al. 2021). Identified LncRNA were involved in fatty acid transport, synthesis of oil (Ma et al. 2021).

12 differentially expressed miRNA were identified in response to water deficit and half of which were upregulated by 1 fold (Zheng et al. 2016). Differentially expressed miRNA were targeting genes such as auxin related genes, superoxide dismutase etc (Zheng et al. 2016). Stress responsive genes were post transcriptionally regulated by miRNA (Cadavid et al. 2020). Regulated gene were involved in ethylene biosynthesis, DNA repair and other cellular processes under salt stress (Cadavid et al. 2020).

Conclusions

Molecular tools helped to understand the genes function related with adaptability to specific environmental condition. Genotyping of soybean cultivars were carried out to identify the allelic combinations related with maturity time and flowering initiation. Specific molecular markers such as SNPs, CAPs and deCAP were developed to characterize germplasm carrying various allelic combinations in various parts of the world. Genotyping of soybean cultivars help to identify most frequent and adaptable allelic combination to specific environmental conditions. It was elucidated that maturity and flowering time was response of several loci which interacted with photo-thermal condition of the specific area. Global climate change may impart several kind of stresses on soybean production which may threaten soybean sustainable production. Transcriptomic analysis was done to identify the transcript related with heat, cold, drought and salt stress. These transcript improved cell membrane stability, root length, increase activity of anti-oxidants. Role of LncRNA and MiRNA in improving stress tolerance was identified which showed that these transcript were related the diverse cellular function such as regulation of stress genes, binding with stress related proteins and had catalytic properties, ethylene biosynthesis, repairing of DNA, and other cellular functions.

Conflict of interest

Author(s) declare no conflict of interest

Reference

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SESAME AN UNDERUTILIZED OIL SEED CROPS: BREEDING ACHIEVEMENTS AND FUTURE CHALLENGES

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ABSTRACT
High oil concentration (50%) and balanced fatty acids, adaptability to water stress conditions are the ingredient which may help sesame for global expansion in its area and production. However, low yield, poor response to farm inputs and agro-climatic conditions, and high post-harvest losses due to dehiscence capsule hinder its production worldwide. CRISPR/Cas9 has great potential to downregulate the alleles having undesirable effects on plant phenotypes including modifications in fatty acid profile. Crop has entered the genomics era which may help to formulate the effective molecular breeding programs for its improvement. and may help to find genes related with important traits.

Key words: CRISPR/Cas9, Capsules, Genomics, SNPs, Yield, abiotic stress

Introduction
Sesame (*Sesamum indicum* L.), is among the oldest oilseed crop known to humanity and called as “queen of oilseed crops”. Its seed is rich in edible oil which contained more than 50% oil contents. Moreover, sesame seed is rich source of tocopherol, sterol and other compounds known as sesamol having medicinal properties. Seed meal contained 35-50% protein, having rich source of amino acids such as methionine and tryptophan. Despite its usefulness as an oilseed crop, or wide use in confectionary products, little efforts were done for its varietal improvement to enhance its yield potential or low research priorities were given by scientific academia and industry. It is considered as orphan crop and was grown on an area of 12.81 million ha and production of 6.55 million tons was obtained worldwide during the year 2019 (FAO, 2019). It was thought to be originated in Africa having center of diversity and several species were identified in wild. It is still grown on large scale in Africa with Sudan as its largest producer in the world followed by Mayanmar and India (FAO, 2019).
Sesame is self-pollinated, semi erect plant which grow to the size of 0.6 – 1.2 m. Leaves are ovate or lanceolate while basal leaves are tri-lobed and upper leaves are irregular and serated. It is drought tolerant crop prefer to grow on well drained soils having pH 5.5 – 6.8. Temperate to tropical climate was suited for its cultivation. Flowers are bell shaped with variable petal colors ranging from pale yellow to purple color. It has low yield potential and generally give poor response to the farm inputs, which make it an unattractive choice for growers around the world due to poor yield and low profitability when compared with other oilseed crops. Continuous cultivation of sesame in particular field show decline in yield, protein and mineral contents over the year (Wacal et al., 2019). Moreover, shattering problem at the maturity make it difficult for the crop harvest or mechanization. Other problems include, insect and disease infestation which caused yield losses around the globe, and require concrete efforts through breeding and genetics to develop elite high yielding sesame cultivars with resistance to disease and insect.
On the basis of this background, current development in breeding, genomics and marker assisted selection was accounted to high light the importance of this crop, along with breeding achievements through innovative molecular techniques and future challenges and opportunities for this oilseed crop.

Germplasm resources

Sesamum indicum is a diploid species (2n=26), however, chromosome number variability was identified in species of genus *sesamum* (Onkware et al., 2015). Species such as *calycinum*, *latifolium*, *agngolense* had 2n=32 chromosome (Onkware et al., 2015). Evidences suggested that sesame originated from wild population native to Indian continent or parts of Pakistan or Afghanistan. Studies of 4.4 Mbps related to various loci controlling the fatty acid metabolism provided evidence of bottlenecks and loses of genetic diversity (37-47% nucleotide diversity) during the process of domestication (Mondal et al., 2016). Wild population of *Sesamum orientale* show close morphological and cytogenetically resemblance with cultivated types (*S. indicum*). Both species are diploid with chromosome no. 26 and show fertile hybrids under spontaneous or artificial reciprocal hybridization. cpDNA based finger printing also suggest close relationship between the *orientale* and *indicum* (reviewed by Bedigian, 2010). Few authors suggested its origin from African population *Sesamum latifolium*. The crosses between the *indicum* × *latifolium* were unsuccessful and only few seeds were produced due to chromosomal number and structural difference of *latifolium* (2n = 32). Both species also showed qualitative differences such as sesamolin were absent in the latifolim showing both cyto- and morphological differentiation among both species. Several wild species of the genera *sesamum* are native to Africa but no study specifically classified any species which may considered as possible progenitor of cultivated sesame (reviewed by Bedigian, 2010).

Sesame is also considered as model species for sequencing, transcriptomic analysis, understanding metabolic pathways such as oil and fatty acid metabolism. Sesame have strong characteristics such as drought tolerance, short duration (90 days), large amount of seed setting (3000-10000 seeds plant^{-1}) small diploid genome of (350MB) which may considered as an ideal crop for research and development. Genome of sesame landraces ("Mishuozhima" and "Baizhima") and cultivar ("Zhongzhi13") was subjected to the sequencing through project such as Sinbase (http://ocri-genomics.org/Sinbase/)20, the Sesame Genome Project (http://www.sesamegenome.org/), and SesameHapMap (http://ncgr.ac.cn/SesameHapMap/)**.** Sequencing data was related with genes and phenotypic analysis to construction of functional genomics data FGsesame (http://ncgr.ac.cn/SesameFG/). These projects lead to the development of molecular markers based on simple sequence repeats (SSR) and identification of single nucleotide polymorphism (SNP) (Dossa et al., 2017). Genome wide association studies lead to the understanding of genes related to metabolic pathways such as oil and fatty acid synthesis. Construction of linkage groups lead to the identification of the quantitative traits loci associated with economical traits.

FGsesame data (http://ncgr.ac.cn/SesameFG/)was based on the core collection of 705 sesame accessions belonging to 29 countries. Each accession has been identified in the database on the basis of its origin, ecotype, sequencing coverage and group information. Result of sequencing were used in genome wide association studies of 56 traits obtained in four Chinese environment. These included yield components, disease resistance, seed or oil quality phenological and morphological traits (Wei et al., 2017). Accessions photographs of various phenological traits were also included. Expression profile of various traits such as capsule, seed leaf and root development of variety "Zhongzhi13", while transcriptomic profile of gene related to water logging were identified in tolerant "ZZM2541" and oil contents in high oil yielding variety "Zhongfengzhi1 (Wei et al., 2017). Candidate gene related with seed yield, lipid metabolism, phenological traits or diseases resistance were available in the data base (Wei et al., 2017). Information on the presence of the microsatellite (SSR) was identified through computer based software (MISA) while single nucleotide polymorphism (SNP) was designed through Primer 3 software for each locus (Wei et al., 2017). SNPs variants were identified through genome comparison of sesame. Sesame genome was surveyed for SSR markers which may be exploited for determining the genetic diversity and QTL mapping of the economical traits. There were 23,438 SSRs (five repeats), while dinucleotide was the most prevalent repeat pattern which was about 84.24% of the total, followed by 13.53% trinucleotide, 1.65 percent tetranucleotide, 0.3 percent pentanucleotide, and

0.28 percent hexanucleotide (Wei et al., 2014). Identification of microsatellite in sesame genome led to the development of 218 polymorphic SSRs markers which may be used to screen sesame germplasm for genetic diversity analysis (Wei et al., 2014). Whole genome sequences of sesame cultivar “Sweeta” was done through illuminia paired end sequencing and 454 shotgun sequence technologies. “GINMicrosatDb’ data base of microsatellite was developed having 5 sets of primer pairs for each microsatellite loci (Puru et al., 2018). Data base also contained GC contents, melting point flanking sequence (Purru et al., 2018).

Transcriptomic profile across various development phase identified genes such as MADS-box which code for transcription factors that were essential for sesame development and growth. There were 57 genes related to the MADS-box genes across 14 linkage groups. Similar types of MADS-box genes were also identified in model species *Arabidopsis thaliana* and *Solanum lycopersicum* and sesame genes were compared with the species. Structural analysis showed that MAD-box had 1 to 22 exons and there were 4 groups of sesame MADS-box genes i.e. 28 MIKCc-type, 5 MIKC-type, 14 M-type, and 10 M-type. The MIKCC-type MADS-box genes were related with sesame flower initiation and seed development which was revealed through transcriptomic profile of the MADS-box gene. There was structural and functional difference among the MADS-box genes of sesame when compared with other species.

Diversity within sesame germplasm and utility

Studies have shown that wild relative had high genetic diversity, and cultivated germplasm selected in various regions of the world also showed divergence when compared with each other. Wild species of sesame is treasurer of unique alleles which were absent in cultivated germplasm and require in situ conservation. Wild resources of sesame germplasm may get extinct due to over exploitation of sesame leaves and seed in culinary, medicines and cosmetics by local population from their native ecosystem (Bedigian, 2004). Germplasm resources of sesame played an important role in the sustainability of ecosystem and provide source of nutrition to human population during period of scarcity. Molecular markers were used to determine diversity among cultivated and wild accessions of sesame. An SSR based study showed that genetic diversity based on Shannon index was 0.28 and 0.34 (Nyongesa et al., 2013), while Indian accessions showed genetic divergence with rest of the evaluated germplasm (Nyongesa et al., 2013). Isozyme analysis also differentiated cultivated and wild species. Esterase bands were used to identify various sesame species. Polymorphic co-dominant cathodic bands differentiated wild species which were variable among the species ranging 2-6 alleles per species (Onkware et al., 2015). A comparison of various land races originated in various regions of the world was done through morphological traits and EST-SSR. The most variable traits were growth habit, branches angle, leaf blade, stem pubescence (Stavridou et al., 2021). Landraces were divided into 4 major clusters, and EST-SSR markers were highly polymorphic showing diversity within land races but landraces from same geographical areas may not grouped together showing different heterotic group (Stavridou et al., 2021). Wild relative may be utilized to fulfill various breeding objectives such as cytoplasmic male sterility, heat tolerance, disease resistance, modification of fatty acid. *S. radiatum* contained higher proportion of linolenic acid (9.43%) as compared to other species and cultivated germplasm (Azeez & Morakinyo, 2011). Kulkarni et al., (2011) noted cytoplasmic male sterility in crosses S. *malabaricum* × *S. indicum* and *S. mulayanum* × *S. indicum* and their backcross generation when interspecific hybrids were backcrossed to cultivated species as male parent. Wild species (*Sesamum mulayanum*, *Sesamum capense*, *Sesamum laciniatum*, *Sesamum latifolium*, *Sesamum occidentale* and *Sesamum schinzianum*) showed wide variability for fatty acids % in oil especially for palmitic acid and stearic acid which may be exploited in sesame breeding programs to modify fatty acid% in sesame oil (Hiremath et al., 2007). A high expression *sesamin synthtase* (CYP81Q1) was coupled with high sesamin level in wild species *S. malabaricum* (Pathak et al., 2015). *S. laciniatum* contained the highest total polyphenol contents and anti-oxidant property of its seed meal followed by species *S. indicum* subspecies *malabaricum* and *S. radiatum* (Pathak et al., 2020). Wild species such as *Sesamum alaturn* Thonn., *Sesamum malabaricum* Bum. and *Sesamum yanairmalaiensis* were resistant to mycloplasma like organism

(Saravanan & Nadarajan, 2005). *S. indicatum* was used to introgress charcoal rot resistance in cultivated species. Interspecific crosses were attempted and immature embryo was rescued to successfully develop hybrids which were backcrossed to establish breeding populations (Yang et al., 2017). F_1 hybrids were confirmed using cytological and SSR markers. Screening test showed that established population had higher resistance to the diseases than cultivated parent (Yang et al., 2017). Another study claimed the presence of tolerance to charcoal rot in *Sesamum mulayanum. The developed F_6 population and parents were inoculated with pathogen spore at seedling stage and transcriptomic analysis of the plant material was done which may paved the path to identify genes related with charcoal rot resistance in sesame (Dutta et al., 2020).*

Seed composition

Oil contents among sesame accessions varied and depending upon genotypes and its interaction with environment. It ranged between 45 – 53% among accessions collected from Turkey and 11 different countries (Kurt, 2018). Oil contents of white and black cultivars were 37% and 25% respectively (Kang et al., 2000). Fiber contents ranged between 2-5% and carbohydrate were between 7-12% (Gadade et al., 2017). Predominant fatty acids such as oleic acid ranged between 36-43%, linoleic acid 39-46%, and palmitic acid (8-10%) (Kurt, 2018). It also contained trace amount of linolenic acid which ranged between 0.28-0.4%. Oil predominantly contained unsaturated fatty acid and balanced amount of mono and poly unsaturated fatty make it perfect as salad dresser or cooking oil and wide used in industrial application. Sesaminn (3-11 g L^{-1}) and Sesamolin (3-14 g L^{-1}) are the major lignans present in sesame seed (Kancharla & Arumugam, 2020). Variation for ligans was higher in black seeded varieties while the concentration was higher in white seeded varieties (Kancharla & Arumugam, 2020). There was positive relationship between oil and lignans and accession with high oil contents and lignans may be selected for cultivation and parents in development of pedigree (Kancharla & Arumugam, 2020). In another study, white seeded varieties had higher lignans than black seed cultivars (Kang et al., 2000). Calcium was predominant cation among minerals which ranged between 3.02 to 9.66 mg/kg among Yemini cultivars (Mohammed et al., 2018). other mineral include K^+ (0.8 – 4.25 mg kg^{-1}) and Mg (0.81 – 4.27 mg kg^{-1}), while toxic metal were low. Sesame was rich source of various phenolic compounds which impart antioxidant properties when ingest and may helpful to cure diseases. Phenolic compounds such as rosmarinic acid hexoside (12.6 μg/g), 3-coumaroylquinic acid (11.3 μg/g), hydroxybenzoic acid (7.74 μg/g), quercetin-3,4-diglucoside-(6-feruloyl glucoside) (5.25 μg/g) and ellagic acid pentoside (4.98 μg/g) (Zeb et al., 2017). 36.7–52.4, 30.4–51.6, 9.1–14.8 and 0.0–8.0 oleic, linoleic, palmitic and erucic acids.

Table 1. **Natural variation for oil contents (%) fatty acid composition (%) in sesame germplasm**

Accession	Oil contents	Protein	16:0	18:0	18:1	18:2	18.3	22:1	Reference
22 cultivars, 4 landraces of *S. mulayanum* and 7 accessions of 4 wild species	-	-	9-15	-	37-52	30-52	-	0-8	Modal et al., (2010)
4 Pakistani sesame varieties	50-54	19-23	3-19	5-22	10	5-13	-	16	Asghar & Majeed (2013)

Market sample	48		5	13	43	36	-	-	Carvalho et al., (2012)
Brown non roasted vs roasted sesame	49 vs 51	-	5.6 vs 7.1	5.3 vs 6.8	40.3 vs 58.7	46.1 vs 25	0.4 vs 0.2	-	Hama (2017)
103 sesame land races	41-63	-	8-10	-	29-41	41-49	-	-	Uzun et al., (2008)
Collection of 12 countries	45-53	-	5-6	8-10	36-44	39-46	0.3-04		Kurt (2018)
6 cultivated (C) 3 wild species (W)	53-55 (C) 54-59 (W)	-	4-9 (C) 4-8 (W)	4.5-10 (C) 4-5 (W)	33-38 (C) 35-37 (W)	42-52 (C) 43-46 (W)	4-9.5 (C) 5-10 (W)	-	Azeez & Morakinyo, (2011).
Indian sesame cultivar	42-54	16-27	8-12	4-8	41.5-50	32-43	0.2-0.35	-	Awasthi et al., 2006

Fatty acid and oil contents biosynthesis in seed

Fatty acids are synthesized denova within chloroplast, and they may either enter the pathway as substrate for the synthesis of complex fatty acids or transported to the endoplasmic reticulum where they esterified with glycerol to synthesize triglyceride which is further stored as oil bodies within parenchymatic tissues of plants. Fatty acid composition within oil dictate the end use of oil. Oil rich in polyunsaturated fatty acid (18:2; 18:3) may not be used in deep frying or cooking due to oxidation and rancidity at high temperature and could be used as salad dresser. Hydrogenated fat is generally preferred for the cooking and deep frying which also impart negative effects over the serum cholesterol (Santoos et al., 2020). Modification of fatty acids in various crop such as Brassica, sunflower and soybean has been achieved through mutation, antisense or CRISPR/Ca9 to improve the oxidative stability of the oil (Rauf et al., 2017; Huang et al., 2020; Lee et al., 2021). Target fatty acids were ω-9 oleic acid or steric acid. However, breeding material developed through mutation breeding had yield drags and unstable expression of oleic acid and was dependent over warm environment for high expression (Rauf et al., 2017). Steric acid was neutral fatty acid as it neither increase or decrease blood serum cholesterol (van Rooijen & Mensink, 2020) while monounsaturated oleic acid lowers the cholesterol level and oil rich in oleic acid had significant higher oxidative stability as compared to oil having high concentration of polyunsaturated fatty acid (Bowen et al., 2020).

Increasing the concentration of steric acid could help to produce highly heat stable fat and may be done through either increasing the activity of Acyl-ACP thioesterase which elongate 16:0 as substrate. Second strategy was to down regulate the gene related with enzyme steoryl. Thioesterase enzymes had two classes i.e. FATA and FATB (Salas et al., 2021). Gene encoded by FATB hydrolyses the thioesterase bond releasing it to the cytoplasm as Acetyl-COA where it became the part of the TAG. Increased activity of FATB gene may help to increase the concentration of steric acid in sesame. Down regulation of SAD1 gene through antisense in brassica and other crop species which encode steroyl-Acyl carrier protein desaturase enzyme in seed led to the increased concentration of steric acid (Dar et al., 2017). The same gene may be downregulated in sesame to increase steric acid concentration.

The microsomal enzyme ω-6 fatty acid (FA) desaturase is involved in the conversion of linoleic acid (18:2) from 18:1, oleic acid as substrate (Nayeri & Yarizade 2014). Blocking the activity of ω-6 FA desaturase could enhance the 18:1 FA in sesame oil which originally had 40-50% oleic acid (Table 1). Increased concentration of oleic acid in sesame oil may not only help to improve the nutritional value of sesame oil but also impart oxidative stability and increased shelf life of oil (Jin et al., 2001). FAD2 gene was identified in sesame which encode desaturase enzyme involved in the conversion of 18;1 to 18:2 (Jin et al., 2001; Kim et al., 2006). FAD2 gene was sequenced in 12 accessions differing for oleic acid which led to the identification of the polymorphism among cultivated and wild accessions (Chen et al., 2014). These sequences polymorphism led to the change in the active site of enzyme causing altered activity of the enzyme (Chen et al., 2014). Seed specific expression of SeFAD2 gene was induced through abscisic acid signals and had intron mediated regulation (Kim et al., 2006). The gene was down regulated through anti-sense to raise the concentration of oleic acid. Sequence homology among the FAD2 complete mRNA sequences of soybean, sunflower and sesame was compared and shown in Fig. 1 which differentiated the species on the basis of sequence homology and FAD2 gene, and sequence orthologue independently evolved in each species. However, sesame transcript sequences were related to the sunflower (Fig 1).

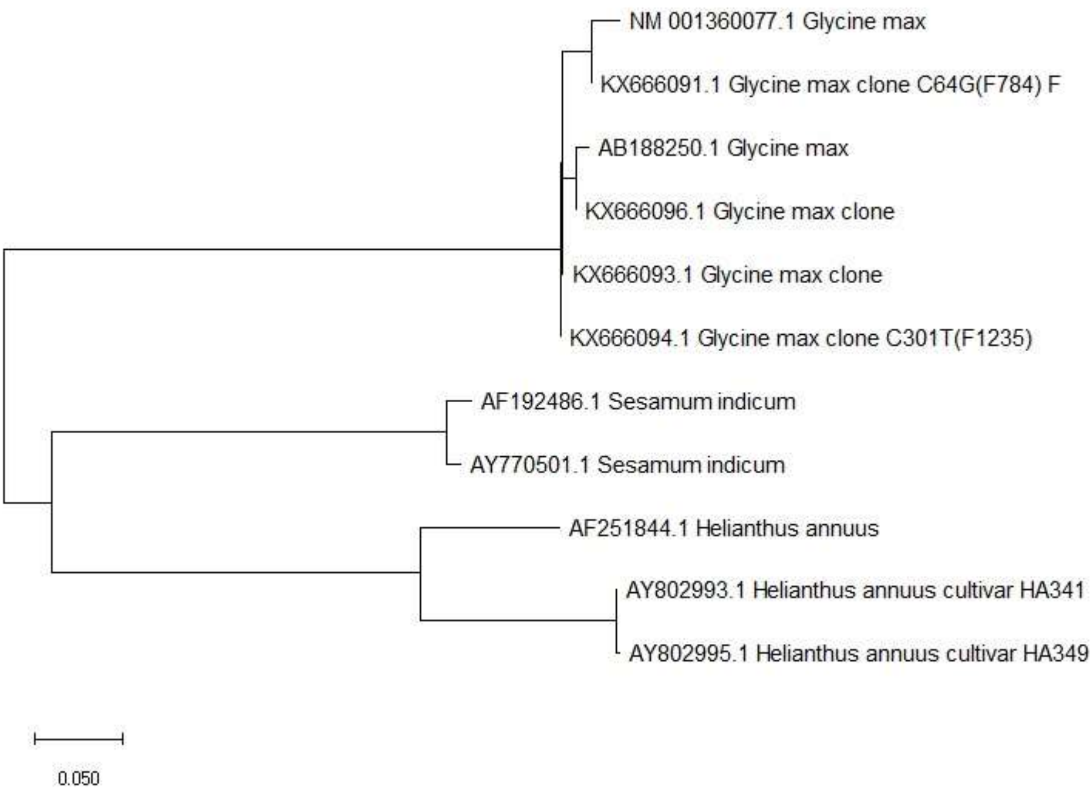

Fig 1. Sequence variability within FAD2 gene belonging to three species mRNA complete cDNA developed by authors obtained through MEGA X

Sesame seed has great taste for roasting and confectionary purpose which may require to enhance ω-3 fatty acid in sesame to avoid deficiencies, as sesame contained only traces of linolenic acid (Table 1). Required consumption of ω-6 to ω-1 fatty acid is 1, however increased intake of vegetable oil has drastically changed the relative ratio of ω-6 to ω-1 to several fold causing various chronic diseases of humans. α-linolenic acid is precursor for long chain fatty acids such as docosa hexenoic acid and pentaenoic acid which impart several key functions within human body (Rauf et al., 2017). FAD3 gene encode an enzyme which catalyze the conversion of 18:2 to 18:3 (Celik Altunoglu et al., 2018). Edible oil rich in ω-3 fatty acid may not be exploited for cooking purpose due to rapid oxidation at high temperature and poor storage ability. However, oil may be recommended as salad dresser to make the deficiency of this vital fatty acid in large human populations. Gene causing the accumulation of linolenic acid in sesame has not yet identified which may be identified for enhancement of linolenic acid. FAD7 isolated from sesame encode plastidal ω-3 enzyme which show close homology with microsomal with respect to amino acid sequence ω-3 enzyme except N-terminal organelle transit peptide. FAD7 gene was modified to induce expression of entoplasmic peptidal signal and C-terminal endoplasmic retention signal of ω-3 enzyme. Modified ω-3 enzyme showed over expression in transgenic tobacco which produced α-linolenic acid having 5-7% (Bhunia et al., 2017).

Sesame adaptability

Sesame is drought tolerant crop, with nitrogen recovery ability which make it a good choice under arid climate with low rainfall and soil fertility (Baath et al., 2021), Climate change with continuous increase in CO_2 and atmospheric temperature causing significant change in rainfall pattern, inducing drought and temperature fluctuation which may threaten sesame production in arid zones. Sesame vegetative growth show good performance at high temperature while temperature beyond 25ºC show decline in reproductive period and reproductive failures were observed after 33 ºC (Baath et al., 2021). Development of new cultivars with enhanced drought and heat tolerance by inducing traits such as longer root length, small leaf area, cuticular waxes, determinate growth habit, response to farm input, combined with high yield potential along with stable performance is pre-requisite to increase production of sesame cultivars under current scenario of global climate change (Kim et al., 2009). Increased CO_2 concentration in the atmosphere may be sequestered through high and prolonged photosynthesis rate in sesame which may be partitioned into reproductive biomass for increased harvest index (Najafabadi & Ehsanzadeh, 2017).

Selection for high yield potential and stability has been effectively done through QTL mapping of economical traits linked with yield, resistance against stresses and stability (section Genetics of economical traits) (Table 2). Availability of sesame genome sequences allowed to carried out genome wide association studies and to tap genes or QTL with traits of interest and development of SNP markers may help to effectively select favorable alleles in breeding population (Zhou et al., 2018). Molecular markers based studies may also help to identify interaction among QTLs related to various yield components. Thus may help to plan effective breeding programs for the enhancement of yield potential of cultivars. There were 547 genome wide QTLs associated with 39 traits which identified 48 candidate genes related with traits such as seed and biomass formation (Zhou et al., 2018).

Transcriptomic root profile of drought tolerant sesame accessions showed that gene related with catalytic activity, ion binding and transferase activity were differentially expressed in tolerant types when compared with susceptible sesame accessions (Song et al., 2020). Study helped to identify the genes related with osmotic stress.

Flowering in sesame is initiated in response to day length and temperature, and day length has profound effects over the flowering time in sesame (Zhang et al., 2019). Sesame is short day plant and flowering is initiated in response to the day length. A gene “CONSTANS” (CO) was identified which dictate vegetative growth period of sesame. Biometrical analysis of flowering time in 6 generation analysis showed that two genes controlled the flowering time in sesame which may have additive, dominance and epistatic effects (Zhang et al., 2019). Days to flowering initiation was negatively related with traits such as main stem length, capsule number plant^{-1}, 1000-seed mass (Shim et al., 2020). Accessions with later flowering had small reproductive period.

Table 2. Transcriptomic profiling of sesame accessions for various stresses

Transcriptome	**Results**	**Stress tolerance**	**Reference**
HD-Zip I-IV)	75% of SiHDz gene were differentially expressed in response to drought and salinity stress	Drought and salinity	Wei et al., (2019)
SiWRKY	65 genes were mapped on 15 linkage group	Growth and development Water logging and drought stress	Li et al., 2017
ERF and WRKY	47 genes related water logging resistance	Water logging	Wang et al., (2021)
SibZIPs	Differential expression in response to abiotic stress	drought, water logging, osmotic, salt and cold stress	Wamg et al., (2018)
SindOLP	Drought, salinity, oxidative stress, and charcoal resistance	High expression of ROS-scavengers, chlorophyll contents, proline and low lipid peroxidation	Chowdhury et al., (2017)
fIRAK1/4	Drought tolerant gene differentially expressed in sesame accessions	Peroxidase, heat shock protein, interleukin protein, APETALA2 / ethylene-responsive element-binding protein and mitogen activated protein kinase	Dossa et al., (2017)

Genome sequencing and annotation may be helpful for understanding the adaptability gene for particular environment. Gene regulation of flowering initiation in sesame could help to understand the mechanism lying for flowering and maturity under specific set of environment. Two homologues “SiCOL1 and SiCOL2” of gene CO were identified. SICOL2 lacked B-box motif and its expression delayed the flowering when compared with genotypes having SiCOL1 sequence. SICOL1 was possible functional homologue of CO gene which showed high expression in leaves before flowering and showed diurnal rhythmic expression in response to long or short day length (Zhou et al., 2018). SICOL1 had 16 haplotypes within Asian genotypes and mutant type did not show any response to day length and was considered as non functional alleles or photo insensitive (Zhou et al., 2018). Genotype having mutant allele of SICOL1 initiated early flowering under long days (Zhou et al., 2018).

Inducing new variability within sesame germplasm

Mutation breeding

Physical or chemical mutagen were used to expand genetic variability within breeding population (Table 3). 25 mutants from 8 countries were released for general cultivation, out of 5 were released in China with improved agronomic and disease resistance (Mu et al., 2017). Exposure of sesame accessions with 0.5% or 1% ethyl methane sulfonate (EMS) induced novel genetic variability such as tetra or tri capsule per leaf axil, determinate growth habit, seed color (Kouighat et al., 2020). Optimum dose for inducing LD_{50} for EMS in sesame was 0.34% and 0.44% (Sandhiya et al., 2020). The optimum lethal dose for gamma radiation was 389-417 Gy (Parthasarathi et al., 2020). Advanced mutant lines (M5) were evaluated in replicated trials and selected as candidate lines (SM-06, SM-07 and SM-04) which yielded higher than control and novel traits such as earliness, and the highest capsule plant^{-1} were observed within mutant population (Bhuiyan et al., 2019). Genetic divergence among the mutant lines showed that phenological traits such as height of first capsule and days to 50% flowering had the highest divergence (Patil et al., 2018).

CRISPR/ Cas9 has great potential to develop breeding with least detrimental effect over yield and components. With availability of sequence information about key genes related to fatty acid and photoinsensitivity, CRISPR/Cas may be efficiently used to down regulate the gene related to linoleic acid to develop breeding lines with high oleic acid without yield drags. Moreover, gene related to shattering problem may also be down regulated which help to induce shattering resistance in soybean. Shattering resistance may also facilitate sesame for mechanized harvesting apart from reduction of yield losses. Sesame specific adaptability may be improved by downregulating genes related to photoperiod, Photoperiod insensitivity help to initiate flowering under long day length (Ashri, 2019).

Table 3. Exposure of sesame for establishment of mutant population

Mutagen	**Established population**	**Novel variation**	**Reference**
Soium azide	M2	Glabrous stem	Weldemichael et al., (2021)
Sodium azide (15mM)	M4 and M5	Early maturity, yield advantage of 21% during Kharif season	Jayaramachandran et al., (2020)
600 Gy Co	M4	Salinity tolerance, number of capsule, 1000 seed mass	Aristaya et al., (2019)
300 Gy gamma rays	416 M_3 mutant	P 97-1 *Phytophthora nicotianae* resistance	Kumari et al., (2019)
40KR gamma rays 1.5 mM	M2, M3	Early maturity, no. of capsule, seed yield plant^{-1}	Ravichandran & Jayakumar (2018).
-	M2	Tetra carpillate per leaf, higher number of seeds capsule^{-1}	Gadri et al., (2020)

Semi dwarf plants and various mutant types to understand the traits significance and identification of genes

Development of semi dwarf sesame (*dw607*) genotypes had reduced intermodal length, stem lodging resistance. Semi dwarf genotype also had higher 1000-seed mass. The gene *Sidwf1* was known to affect the plant height as determined by the molecular analysis of 824 germplasm accessions carrying 58 genomic variants. *Sidwf1* inducing semi dwarf genotypes carried SNP mutation of nucleotide substitution of C to T causing amino acid change from proline to serine (Miao et al., 2020). An EMS-induced sesame mutant (sc1) with few number of seed capsule^{-1} and short capsule was due to mutation of *SICS1* alleles (Wei et al., 2019). The *SICS1* contained point mutation at intron 5 and exon 6 junction which caused frame shift of the reading frame causing defective protein (Wei et al., 2019). Indeterminate growth habit in sesame was dominant (*Dt*) over the determinate type (dt1) (Zhang et al., 2016). Crosses among the indeterminate and determinate types identified multiple recessive alleles of determinate growth such as *dt1*, *dt2* (Zhang et al., 2016). In cross between long and dense with short and sparse capsule, an F_1 showed dominance for long and dense capsule. A segregating ratio of 12:3:1 was obtained which showed that two genes had epistasis interactions (Engin, 2017). A yellow leaf dwarf mutant (YL1) had lower chlorophyll and carotenoid contents than wild types. YL1 also had longer growth duration with slower photosynthesis rate, later flower initiation, shorter plant height than wild type (Liu et al., 2017).

Indehiscence capsule

Capsule shattering is major impediment for the cultivation and mechanical harvesting of sesame crop. Generally farmer harvest the crop having partial pod maturity and than thresh to obtain the seeds. Conventional approaches understand the genetics of indehiscence capsule by crossing them with dehiscent parent, resultantly an F1 was produced which dominance of dehiscence capsule (Engin and Bulent, 2019). A segregating ratio of 3;1 was obtained which show a single recessive allele was controlling the indehiscent capsule (Engin and Bulent, 2019). Gene controlling shattering or non shattering type was mapped on onto SNP marker S8_5062843 (78.9 cM) near the distal end of LG8 (chromosome 8) (Zhang et al., 2018). Sequence of capsule nonshattering allele carried a frame shift mutation which caused a shift in the splicing and introduced SNP variation when compared with the shattering alleles (Zhang et al., 2018). In order to facilitate selection of non shattering types CAPS markers were developed which differentiated both types in sesame (Zhang et al., 2019).

Male sterility and hybrid vigor

The possibility to breed hybrid varieties of sesame has been documented in the past. Number of articles described heterosis in sesame for yield and components indicating possibility for commercial exploitation of hybrid vigor (Duhoon 2004; Jeeva et al., 2020). Heterosis ranged between 9.5 – 327% estimated from 1636 crosses (Duhoon 2004). However, commercial development of hybrid seed was only possible through exploitation of genetic or cytoplasmic male sterility. List of various nuclear alleles identified or induced in sesame has been shown in Table 4. Cytoplasmic male sterility sources was identified through wide hybriziation and species such as *S. malabaricum* was identified as possible source of cytoplasmic male sterility (Prabakaran et al., 1995). Genetics male sterility was controlled by the single recessive nuclear male sterility alleles and generally maintained in heterozygous condition. Spontaneous recessive male sterility was identified in Chinese cultivar "Zhuzhi" which was transferred and maintained through sib mating and crossing with male fertile plants (Liu et al., 2013). Developed male sterile line was named as "D248A" (Liu et al., 2013). "D248 A" had small green anther which did not contain pollens. Absence of pollen occurred due to abnormality in the microspore mother cells and microsporocytes had less cytoplasm and anucleate (Liu et al., 2018). Moreover, microspore mother cells were not able to undergo normal meiotic division (Liu et al., 2018). Study showed that male had segregating ratio of 1:3 in F_2 generation. Dominant genetic male sterility allele was also identified in sesame (Liu et a. 2015), which may be easy to maintain than recessive genetic male sterility within inbred line (Liu et al., 2018). Genetic male sterility required visible markers to effectively select plant with genetic male sterility at seedling stage (Liu et al., 2020). In sesame genetic male sterility was linked with wrinkled leaf to differentiate male sterile plants from normal plants (Liu et al., 2020). Chemical restoration agents have been recommended for maintenance of genetic male sterility in sesame (Zheng et al., 2000). CRA-02 showed the highest restoration of pollen fertility when sprayed at bud initiation stages and restored pollen fertility by 33% in same (Zheng et al., 2000). Bulk segregant analyses followed by next generation tools identified the putative regions of the 4 chromosomes related with pollen sterility in male sterile plants along with leaf shape (Liu et al., 2020), SSR and indel markers further narrowed down the region associated with traits in backcross population (BC1). qPCR identified suppression of 4 genes in sterile pollen and 1 gene during leaf development (Liu et al., 2020). CWINV1 (cell wall invertase) were involved in the supply for the carbohydrate to the developing pollen and their suppression in anther caused male sterility (Zhou et al., 2019). Gene Sicwinv1 induced male sterility in sesame was cloned to study the function of allele which indicated its accumulation during tetrad stage in tapetum cells of anther (Zhou et al., 2019).

Table 4. Sources of male sterility in sesame

Male sterility	Source	Effects	Reference
Genetic male sterility 95ms-5AB	Mutation in Yuzhi 4 Gamma rays Co^{60}	Defective pollen, shriveled anthers, recessive gene *Sms1*	Zhao et al., (2013)
GMS line (W1098A)	Wild accession Yezhi2 (*Sesamum mulayanum* Nair	Dominant genetic male sterility pollen abortion due to abnormal tapetum	Liu et al., (2015)
RGMS (D248A)	Spontaneous male sterility in cultivar Zuzhi	Recessive male sterility. Ms gene may be selected by SB2993 and LG1-170.	Liu et al., (2013)
RGMS	95ms-5A induced mutant vs 95ms-5A.	27 differentially expressed transcript identified in sterile vs fertile buds. 11 transcript involved in energy metabolism, signal transduction and cell development	Wu et al., (2014)
CMS	*S. malabaricum*	Interspecific hybridization showed male sterility. Reciprocal effects were identified indicating cytoplasmic inheritance of organelles	Prabakaran et al., (1995)

Genetics of economical traits

Generally, yield and its components are affected by numerous QTLs across the genome and their inheritance was further complicated by the interactions between loci, and environment. Yield components also show pleiotropic loci. Genome wide association mapping resulted in the identification of major QTLs related to the yield and its components such as 1000-seed mass, seed size and seed coat color (Du et al., 2019). Major QTLs for seed size and seed coat were mapped on LG04 and LG11 (Du et al., 2019). These linkage groups contained 155 candidate genes for seed coat and size (Du et al., 2019). Whole genome sequencing payed a way for high density markers such as single nucleotide polymorphism (SNP) or simple sequence repeats (SSR) which helped to map quantitative traits of sesame (Dossa et al., 2016; Du et al., 2019). For instance a study in sesame identified 2159 SNP markers on 13 linkage map, the distance between the marker was about 0.99 cM (Du et al., 2019). 83,135 non-redundant SSR marker positions and motif sequences has been published obtained from151 published genomic sequences (Dossa et al., 2016). Genetic regions containing cluster of genes in sesame was considered as 'hot spot' for economical traits. In another study a high density linkage map of 19309 markers was constructed which revealed 84 QTLs associated with yield components and seed mineral components (Teboul et al., 2020). 13 QTLs on 7 LG and 17 QTLs on 10 LG were identified for yield components identified through 12,30 markers in recombinant inbred lines (RIL) population (Wu et al., 2014). A high density map of specific locus amplified fragment (SLAF) was constructed which comprised of 3528 markers with an average distance 0.37 cM (Mei et al., 2021). SLAF makers revealed 46 significant QTLs for 7 yield components across 4 environments (Mei et al., 2021). QTLs were spread over 11 linkage groups and there were 23 stable QTLs detected in all environments (Mei et al., 2021). Favorable QTLs were concentrated in chinese line Yuzhi4 (Mei et al., 2021). QTLs for traits related to water stress condition i.e. root length and relative root length were identified on chromosome 12 of sesame (Liang et al., 2021). A density linkage map spanned over 12 chromosome comprising of 1354 markers developed through whole genome re-sequencing strategy (Liang et al., 2016). There was significant genetic variation for yield components such as capsule size, capsule number and seed size related traits (Zhou et al., 2018). Two novel candidate genes i.e. *SiLPT3* and *SiACS8*c affecting capsule length and capsule number traits were identified (Zhou et al., 2018). A major QTL *qLS15-1 which was stable across the environment located on LG15 was shown to affect leaf size and development (Sheng et al., 2021). Sequencing of QTLs and transcriptome analyses revealed three candidate genes SIN_1004875, SIN_1004882,* and *SIN_1004883 associated with leaf size (Sheng et al., 2021). Major QTLs for flowering date and yield components located on LG2 identified through high density linkage map of 20,294 SNP markers (Sabag et al., 2021). Significant QTL identified through SNP markers located on chromosome 10 related with phytophthora blight resistance which explained up to 13.34% of phenotypic variation (Asekova et al., 2021). Identified region on chromosome 10 revealed 5 genes related with PB resistance (Asekova et al., 2021). A candidate gene SIN_1019016 showed higher expression in resistant parents and may be introgressed in sesame germplasm to improve PB resistance (Asekova et al., 2021)***.**

Future challenges

Sesame is an oilseed crop with great potential to be ranked among leading oilseed crop of the world. In order to expand the area of this crop, new germplasm with adaptability to diverse environmental conditions may be developed. Sesame has low yield potential due to poor response to farm inputs. Sesame accessions having characteristics such as determinate growth, dwarfness, lodging resistance may be required to improve its response to the farm input and same ideotype for mechanized harvesting. Moreover, accessions with indehiscence capsule may be bred to reduce yield losses during harvesting. Some of the work has already been done and germplasm accessions with novel agronomic traits were developed through mutation breeding, selection or hybridization. Molecular techniques such as CRISPR/Cas9 has potential to remove undesirable traits such as long vegetative growth, indeterminate growth habit, dehiscent capsule and may also be effectively used to modify fatty acid profiles. CRISPR/Cas9 may not effectively downregulate the gene but may also reduce the linkage drags and thus useful elite germplasm breeding material may be created. However, sesame cultivation is potentially grown in the developing or resource poor country which may have seldom research priorities for advanced molecular techniques. Therefore, international collaboration among the institutes may be required for germplasm exchanges, so that potentially useful breeding or genetic stocks may be available for further research.

Expanding yield potential of elite cultivars is an important breeding objective of sesame breeding to increase sesame yield per unit area. Rapid increase in yield potential may be brought through hybrid breeding. However, inbred lines with high fertility, synchronization, and good combining ability may be developed to high heterotic sesame hybrids. Commercial success of hybrid seed production may only be achieved when reliable male sterile lines are available which may be exploited as females in hybrid seed production.

Breeding lines with unique characteristics may increase phenotypic diversity within germplasm whereas rapid improvement of agronomic traits may help to develop breeding lines with high yield potential. QTL mapping of the economical traits such as capsule size, number of capsules plant^{-1} and number of seeds capsule^{-1} may help to increase yield potential of sesame crop. Moreover, inducing resistance against factors inducing climate change may help to increase resilience against abiotic stress factors. Genomics such as transcriptomic or proteomics may help to identify potential regions of the genome related with resistance against stress conditions.

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EFFECT OF ORGALIFE FERTILIZER ON SOME QUALITY PROPERTIES OF THE SEED AND GROWTH POTENTIAL OF TWO RICE VARIETIES (ORYZA SATIVA L.) CULTIVATED IN NORTH MACEDONIA

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ABSTRACT

The aim of this study was to examine the effect of Orgalife (liquid organic microbial fertilizer based on manure from californian earthworms) on the seeds of two rice varieties, *San Andrea* and *Opale*, obtained from three production years: 2018, 2019 and 2020. They were treated with three different concentrations (3.3%, 6.7% and 9.9%) compared to control (distilled water). Germination energy, total seed germination, germination index, seedlings growth (root length, shoot length, dry seedling weight) and vigor index (I and II) were examined. One-way analysis of variance (ANOVA) was carried out and separation of means was performed using the LSD test at P=0.05 and P=0.01 significance level. The values of all treatments for all parameters were compared with the control. Germination energy and total germination for *Opale* in 2020 showed statistical significance for the 3.3% concentration at P=0.05, while for *San Andrea* the same significance was noticed only for the germination energy. Germination index for *Opale* gave statistically significant results only for the 2020 production year at P=0.05. For *San Andrea* the situation is slightly different where the treatments affected the germination index only for 2018 and 2020. Orgalife treatments increased the vigor index I and II in 2020 for both varieties and for both levels of significance. The length of the seedlings for *Opale* in 2020 was positively affected by the 3.3% concentration in both levels of significance, while *San Andrea* in the same year had statistical significance for the 9.9% concentration only for P=0.05. The results for the dry matter showed statistical significance for S*an Andrea* for the 6.7% concentration in 2018. As an overall conclusion, we can say that the use of the Orgalife fertilizer showed to be reasonable for the seedling growth and development, better than the effect on the germination parameters.

Key words: organic fertilizer Orgalife, rice seed, growth promotion, germination energy, total germination and seedling growth

Introduction

One of the most extensively cultivated cereals of the world is the rice (*Oryza sativa*) which belongs to the family of *Gramineae* and the genus *Oryza*. The rice is possibly the oldest domesticated grain (10 000 years), and is the staple food for 2.5 billion people (IRRI, 2002). The production of rice depends on a lot of factors. One of the important factors for production of rice is the seed quality which is characterized by the good germination capacity. Quality seed ensures quality seedling (Hasan et al., 2016).

In field conditions, for the purpose of good germination and growth of young seedlings, the seeding material can be treated with materials that affect germination, such as nutrients and growth regulators (Dimitrovski et al., 2020). Direct use of microorganisms to promote plant growth and plant pests/disease control is becoming a rapidly expanding research area. The capacity of specific root-colonizing bacteria, or rhizobacteria, to increase growth and yield of crop plants is attracting great attention (Suslow et al., 1979).

One of the most important nutrients for plant growth is nitrogen and hence heavily applied in agricultural systems via fertilization. Approximately 50% of the N fertilizer applied to plant production is not absorbed by plants, but lost to the environment as ammonia (NH_3), nitrate (NO^{3-}), and nitrous oxide (N_2O, a greenhouse gas with 300 times the heat-trapping capacity of carbon dioxide), raising agricultural production costs and contributing to pollution and climate change. These losses are driven by volatilization of NH_3 and by a matrix of nitrification and denitrification reactions catalysed by soil microorganism. Hence, the crucial player in nitrification is ammonia-oxidizing archaea (Beckman et al. 2018).

Vermicompost is an organic waste rich in nutrients and plant growth-promoting rhizospheric bacteria such as *Pseudomonas, Rhizobium, Bacillus, Azosprillium, Azotobacter,* which is produced from earthworms that is beneficial in enhancing the soil condition (Pathma and Sakthivel, 2012). It has been reported to help in improving the crop yield and quality. Vermicompost can be used as a fertilizer to increase the fruit chemical quality and maintain the soil quality for agricultural sustainability (Mahmud et al.2020). Vermicomposts contain higher humic acid substances (Albanell et al. 1988), compared to the parent material that has been used for composting. Earthworm gut connected microbes supplement vermicomposts with highly water-soluble and light-sensitive plant growth hormones, which gets absorbed on humic acid substances in vermicompost making them extremely stable and helps them persist longer in soils whereby influencing plant growth (Atiyeh et al. 2002; Arancon et al. 2003).

Extracts of humic and humic-like substances positively affected seed germination and growth of seedlings from lettuce and tomato plants (Piccolo et al., 1993), safflower plants (Başalma, 2015), maize fodder (Daur & Bakhashwain, 2013) significantly promote cucumber growth (Xu et al., 2012).

In rice production, effects of liquid extract of microbiological fertilizers were also reported on stimulating growth, germination, productivity, and quality of wheat and rice seedlings. The extract especially influenced the growth of shoot and root system (Wang et al., 2020).

In this study was examined the effect of Orgalife fertilizer on germination and young seedling growth in rice. The fertilizer contains growth promoting microorganisms such as nitrogen-fixation bacteria, nitrification bacteria, aerobic cellulolytic microorganisms and ammonification bacteria. The seeds treated with Orgalife fertilizer tend to emerge faster, and have better rooting capacity, plant growth and development (based on product label information).

Materials and Methods

Materials

Orgalife is commercial organic microbial fertilizer, biostimulator and soil improver prepared from Californian red worms casting base (not to be confused with worm tea). Orgalife - a liquid organic microbial fertilizer, prepared from a solid fertilizer base (casting) of California worms was used during the experiment. This fertilizer is dark-brown colored with a pH of 6.83 containing 48.35% organic matter, of which 2.44% is total N and microorganisms such as nitrogen fixing bacteria - 86% and nitrification bacteria - 79% (based on data from the manufacturer's label). The effect of Orgalife was tested in three different concentrations (3.3%, 6.7% and 9.9%) compared to the control treatment where no fertilizer was added but only distilled water (Table 1). For the purposes of treatment, rice seeds of two varieties, *San Andrea* and *Opale*, produced from three consecutive harvests (2018, 2019 and 2020) were used. The seed from all three production years was kept under controlled conditions.

Table 1. Content of organic matter and nitrogen in the fertilizer and in the prepared solutions

Fertilizer concentration	Orgalife concentration	3.3%	6.7%	9.9%	Control
Organic matter (%)	48.35	1.595	3.239	4.786	0%
Total N content (%)	2.44	0.08	0.163	0.241	0%
Volume of solution per replication of 100 seeds	/	20 ml	20 ml	20 ml	20 ml H_2O

Content of the organic matter and nitrogen is based on product label information

Methods

The examination was performed in the Department of Rice in Kochani at the University "Ss. Cyril and Methodius ”- Skopje. Total seed germination (TG), germination energy (GE), germination index (GI) and seedlings growth (fresh and dry seedlings weight, root length and shoot length), vigor index (I and II) were examined. A total of 12 variants were set up; four replications of 100 grains for all three production years (2018, 2019, 2020) and for all four treatments (33.33 ml / 1L (3.3%), 66.66 ml / 1L (6.7%), 9.99 ml / 1L (9.9%) and control-distilled water). The seeds of the control treatments were soaked in distilled water for 24 hours before transferring them to Petri dishes, while the seeds of the three treatments were submerged with a solution of fertilizer in the three concentrations listed above. Each of the replicates was placed in Petri dishes in an interfilter (IF) germination medium and placed in a germination chamber at a temperature of 25 ° C.

The first counting of germinated grains was performed on the fifth day of immersion of the seeds according to the treatments. The second count was performed on the 14th day after the immersion. The seed was considered germinated when the coleoptile and the root appeared together and the root length was at least 2 mm. Seed germination energy (ER) was determined on the 5th day, and total seed germination (ER) on the

14th day after immersion.These parameters were calculated as percentage from the total number of inoculated seeds in the Petri dish (Official gazette of the Republic of Macedonia No 61/2007).

Germination energy is the ratio between the number of germinated seeds on the fifth day (first counting) and the total number of grains, multiplied by 100.

Percentage of total germination is calculated as the ratio between the number of germinated grains and the total number of seeds, multiplied by 100.

The germination index is calculated by the formula of AOSA (1983), which reads: **Germination index**

$$= \left[\frac{\text{Number of germinated seeds}}{\text{Day of the first count}}\right] + \ldots + \left[\frac{\text{Number of germinated seeds}}{\text{Day of the last count}}\right]$$

In order to estimate the vigor index I and II, seeds from each treatment were germinated in an open Petri dish in the germination cabinet along with the replications. The RL and SL of 40 randomly picked up seedlings in each of the variants developed in dark conditions were measured at the end of the experiment (day 14).

Vigor index I: VI–I = Seedling length (cm) × Germination (%)

Vigor index II: VI–II = Seedling dry weight (mg) × Germination (%)

The 40 seedlings in each variant (treatment-year combination) were dried in air-oven at 100°C to constant weight. The **total dry seedling weight** was measured for each variant.

The results were statistically analyzed by two-way ANOVA with replication (Table 2) and LSD test at 0.05 and 0.01 significance level. Correlation analysis was determined by calculating the Pearson's correlation coefficient. The two way ANOVA was calculated based on the statistical methods for agricultural research provided by Hadživuković (1973).

Results and discussion

The germination energy was slightly affected by the treatments compared to the control. Mean values for *Opale* variety were in a range of 93.92% to 95.00% compared to the control which is 92.67%. The treatment with 3.3% concentration in 2020 gave the best result (99.25%) compared to the control (96.50%) regarding the germination of the seed. In the other variety - *San Andrea*, the mean values for the germination energy were varying from 86.83% to 91.58% compared to the control (91.00%). Also for *San Andrea* positive effect showed only 3.3% treatment in 2020 with 97.50% compared to the control (94.25%). This kind of situation might be dependent on the seed age and the production conditions. According to Andov et al. (2012), the germination energy in three years old rice seed decreases rapidly.

Table 2. Germination energy for *Opale* and *San Andrea* variety

Germination energy (%)							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD (α 0.05)**	**LSD (α 0.01)**
2018	92.25±1.50	91.00±1.63	93.25±2.87	95.25±1.50	**92.94b**	4.66	7.06
2019	91.75±2.50	92.00±1.41	93.25±1.26	86.25±4.35	**90.81a**	5.85	8.86
2020	99.25±1.50	98.75±0.96	98.50±1.00	96.50±1.00	**98.25c**	2.29	3.47
AVG (%)	**94.42ab**	**93.92ab**	**95.00b**	**92.67a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD (α 0.05)**	**LSD (α 0.01)**
2018	82.50±3.11	79.75±3.30	75.00±70.39	85.50±4.04	**80.69a**	11.13	16.85
2019	94.75±1.71	88.75±5.91	89.00±1.41	93.25±3.40	**91.44b**	7.38	11.18
2020	97.50±2.08	97.00±1.41	96.50±1.29	94.25±0.50	**96.31c**	2.74	4.15
AVG (%)	**91.58b**	**88.50ab**	**86.83a**	**91.00b**			

Comparable to the results from the germination energy, also the total germination was driven in the same way for the *Opale*, while for *San Andrea* the control treatment had the highest mean value of 96.83% and the lowest one was the 9.9% concentration with mean value of 93.67%, which can be considered as a negative effect of the maximum concentration treatment on the seed germination.

The germination index for *Opale* showed statistically significant results only for the 2020 production year at P=0.05 and the highest mean value was 26.97 for 3.3% concentration, while for the control it was 26.28. *San Andrea* had a little different situation where the treatments affected the germination index only for 2018 and 2020. In 2018 the 9.9% concentration had the lowest mean value (22.97) compared to the control (23.81) which means it might have some negative effect on the germination. In 2020 the 3.3% concentration had the highest mean value (26.54) compared to the lowest value of the control (25.83).

Table 3. Total germination for *Opale* and *San Andrea* variety

Total germination (%)							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD (α 0.05)**	**LSD (α 0.01)**
2018	93.00±1.41	92.75±0.50	95.25±1.89	96.50±0.58	**94.38b**	2.47	3.75
2019	92.25±2.50	92.75±1.71	94.00±1.41	87.75±3.77	**91.69a**	5.50	8.33
2020	99.75±0.50	99.25±0.96	99.00±0.00	97.75±0.96	**98.94c**	1.42	2.16
AVG (%)	**95.00ab**	**94.92ab**	**96.08b**	**94.00a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD (α 0.05)**	**LSD (α 0.01)**
2018	90.50±1.73	88.25±0.96	87.50±4.51	94.00±1.41	**90.06a**	3.78	5.72
2019	97.50±1.29	97.25±1.26	96.50±3.11	98.75±0.50	**97.50b**	3.96	6.01
2020	98.50±1.00	98.25±1.50	97.00±1.41	97.75±0.96	**97.13b**	2.26	3.43
AVG (%)	**95.50bc**	**94.58ab**	**93.67a**	**96.83c**			

Table 4. Germination index for *Opale* and *San Andrea* variety

Germination index							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG**	**LSD (α 0.05)**	**LSD (α 0.01)**
2018	25.09±0.38	24.82±0.36	25.45±0.70	25.95±0.28	**25.33b**	1.08	1.63
2019	24.94±0.68	25.03±0.39	25.37±0.34	23.52±1.14	**24.71a**	1.55	2.35
2020	26.97±0.34	26.84±0.25	26.77±0.20	26.28±0.24	**26.72c**	0.53	0.80
AVG	**25.67ab**	**25.56ab**	**25.86b**	**25.13a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG**	**LSD (α 0.05)**	**LSD (α 0.01)**
2018	22.97±0.74	22.26±0.64	21.25±2.37	23.81±0.89	**22.57a**	2.42	3.67
2019	25.92±0.41	24.70±1.21	24.69±0.40	25.70±0.66	**25.25b**	1.55	2.35
2020	26.54±0.47	26.42±0.37	26.23±0.34	25.83±0.13	**26.25c**	0.66	1.00
AVG	**25.14b**	**24.46ab**	**24.06a**	**25.11b**			

The seedling vigor index I and II were affected by all Orgalife treatments for both varieties in 2020 for $P=0.05$ and $P=0.01$ levels of significance. VI-I for *Opale* showed the highest results for 3.3% concentration (2310) compared to the control (1629.74) which was the lowest value. VI-I for *San Andrea* showed the highest mean value for 9.9% concentration (1848.09) compared to the control (1556.98).

Table 5. Seedling vigor index I for *Oppale* and *San Andrea* variety

Seedling Vigor Index I							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	2035.77±171.85	2121.80±89.79	1902.14±132.99	1966.68±192.85	**2006.60a**	324.29	491.28
2019	1862.77±158.27	1893.71±161.25	2150.72±80.30	1990.12±90.04	**1974.33a**	241.83	366.35
2020	2310.00±148.24	2048.31±67.64	2224.53±124.96	1629.74±65.20	**2053.15a**	184.84	280.02
AVG (%)	**2069.51b**	**2021.27ab**	**2092.46b**	**1862.18a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	1502.07±115.48	1289.26±87.57	1576.97±47.94	1580.14±103.52	**1487.11a**	180.76	273.84
2019	1701.13±105.00	1649.72±57.24	1879.82±116.81	1923.14±67.40	**1788.45c**	208.21	315.42
2020	1697.89±61.29	1541.34±65.12	1848.09±44.25	1556.98±92.80	**1661.08b**	83.93	127.15
AVG (%)	**1633.70b**	**1493.44a**	**1768.29c**	**1686.75bc**			

The treatments with 6.7% and 9.9% concentrations gave the highest values for VI-II for *Opale* (19.80) compared to the control (19.60). For *San Andrea* the treatments with 3.3% concentration showed the highest results (24.63) compared to the control (24.45).

Table 6. Seedling vigor index II for *Opale* and *San Andrea* variety

Seedling Vigor Index II							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	16.28±1.78	16.28±1.20	16.63±1.65	16.98±1.84	**16.54b**	3.72	5.63
2019	16.10±0.53	16.28±2.21	14.10±0.71	15.40±1.14	**15.47a**	3.10	4.70
2020	17.50±1.29	19.80±1.40	19.80±0.81	19.60±1.13	**19.18c**	2.60	3.94
AVG (%)	**16.63a**	**17.45a**	**16.84a**	**17.33a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01**
2018	20.36±1.17	22.05±0.72	19.69±0.51	21.15±1.21	**20.81a**	1.80	2.72
2019	21.94±1.26	24.33±0.79	21.71±0.56	24.70±1.80	**23.17b**	2.25	3.41
2020	24.63±1.39	24.58±1.79	21.83±1.25	24.45±0.80	**23.87b**	2.80	4.23
AVG (%)	**22.31b**	**23.65c**	**20.90a**	**23.43c**			

The length of the 14 days old seedlings for *Opale* in 2020 was positively affected by the 9.9 % concentration (24.47 cm) in both levels of significance, as well as, *San Andrea* in the same year had statistical significance for the same concentration (18.85 cm) only for P=0.05. Similarly, when bean and pea seedlings were treated with vermicompost aqueous extracts showed positive effect on the growth and development of the seedlings (Ievinsh, G., 2011). In parallel, in a study evaluating the influence of humic acids derived from earthworm-processed organic wastes on plant growth (Atiyeh et al., 2002), the results indicated significantly increased growth of tomato and cucumber seedlings, in terms of plant heights, leaf areas, shoot and root dry weights. According to Atiyeh et al. (2002), increased plant growth happened with increasing the concentrations of humic acids incorporated into the growing medium to a certain proportion, but this differed according to the plant species, the source of the vermicompost, and the nature of the container medium.

Table 7. Seedling length for *Opale* and *San Andrea* variety

Seedling length (cm)							
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	22.82±0.97	20.02±1.40	20.02±1.40	20.28±1.99	**20.78a**	3.28	4.98
2019	20.25±1.72	20.36±1.73	22.88±0.85	22.62±1.02	**21.53a**	2.64	4.00
2020	24.44±1.48	20.69±0.68	22.47±1.26	16.63±0.67	**21.06a**	1.86	2.82
AVG (%)	**22.50c**	**22.36bc**	**21.79ab**	**19.84a**			
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**LSD**	
						(α 0.05)	**(α 0.01)**
2018	16.60±1.28	14.62±0.99	18.02±0.55	16.81±1.10	**16.51a**	1.99	3.01
2019	17.45±1.08	16.96±0.59	19.48±1.29	19.47±0.68	**18.34b**	2.14	3.25
2020	16.53±1.72	16.05±0.91	18.85±0.54	15.34±1.82	**16.69a**	2.44	3.69
AVG (%)	**16.86ab**	**15.88a**	**18.78c**	**17.21b**			

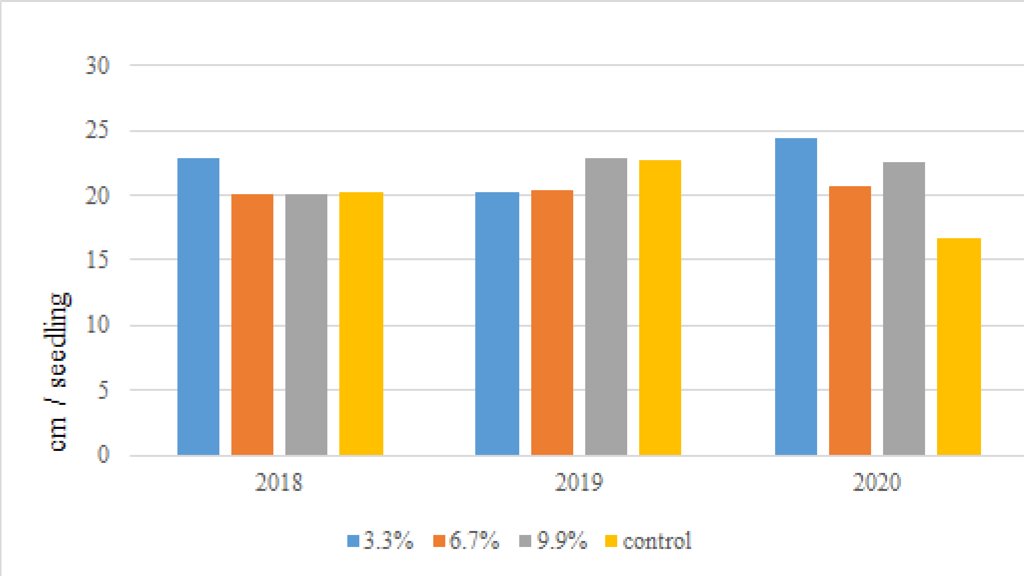

Figure 1. Seedling length for O*pale*

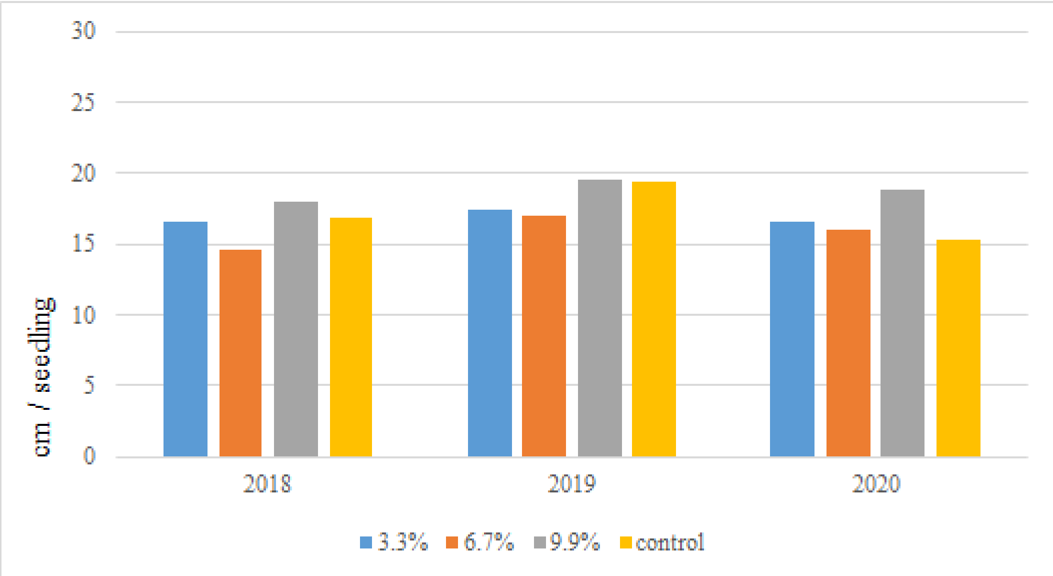

Figure 2. Seedling length for *San Andrea*

The statistical analysis for *Opale* variety did not show any significance among the treatments compared to the control in terms of dry matter. The analysis for dry matter showed statistical significance for *San Andrea* in 2018 for the 6.7% concentration (0.25 g) compared to the control (0.23 g).

Table 8. Dry matter for *Opale* and *San Andrea* variety

Dry matter (g)							
						LSD	
OPALE	**3.3%**	**6.7%**	**9.9%**	**Control**		**(α 0.05)**	**(α 0.01)**

					AVG (%)		
2018	0.18±0.02	0.18±0.01	0.18±0.02	0.18±0.02	**0.18a**	0.04	0.06
2019	0.18±0.01	0.18±0.02	0.15±0.01	0.18±0.01	**0.17a**	0.03	0.05
2020	0.18±0.01	0.20±0.01	0.20±0.01	0.20±0.01	**0.19b**	0.03	0.04
AVG (%)	**0.18a**	**0.19a**	**0.18a**	**0.19a**			
						LSD	
SAN ANDREA	**3.3%**	**6.7%**	**9.9%**	**Control**	**AVG (%)**	**(α 0.05)**	**(α 0.01)**
2018	0.23±0.01	0.25±0.01	0.23±0.01	0.23±0.01	**0.24a**	0.02	0.03
2019	0.23±0.01	0.25±0.01	0.23±0.01	0.25±0.02	**0.24ab**	0.02	0.03
2020	0.25±0.01	0.25±0.02	0.23±0.01	0.25±0.01	**0.25b**	0.03	0.04
AVG (%)	**0.24ab**	**0.25c**	**0.23a**	**0.24bc**			

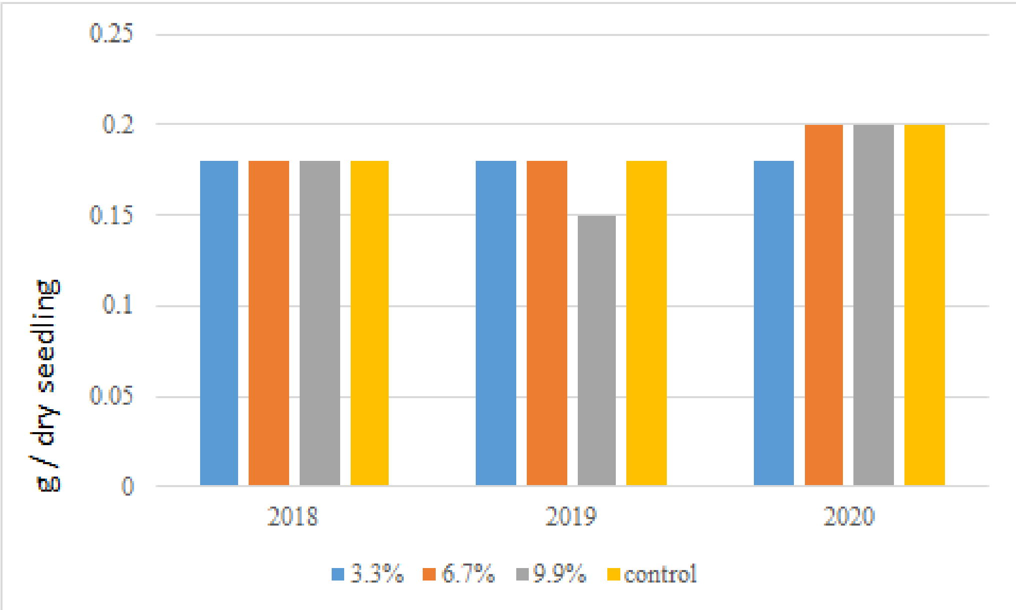

Figure 3. Dry matter for *Opale*

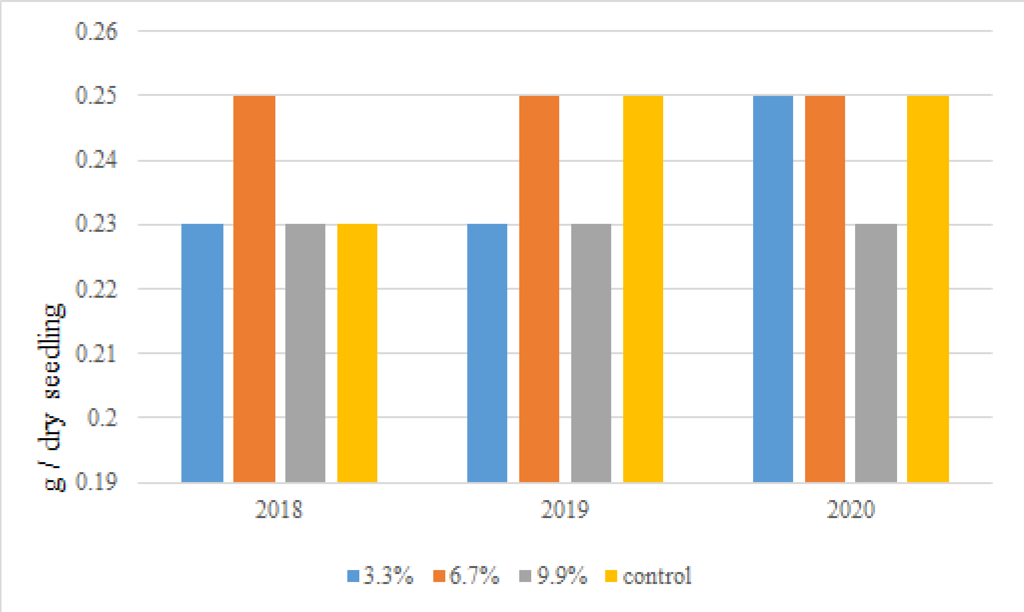

Figure 4. Dry matter for *San Andrea*

Conclusion

According to the results obtained from this study we can conclude that the Orgalife treatments had significant effect on the standard germination test only for the 2020 production year, which can be interpreted as a result of the seed age and the production conditions. Also here is present and visible the negative effect of the maximum concentration dose 9.9% for *San Andrea* variety, which means that different varieties behave differently in the same conditions. The Orgalife treatments did affect the other examined parameters such as the germination index, the early stages of growth and development expressed as seedling vigor index I and II, seedlings length and the total dry matter of the 14 days old seedlings. Based on the obtained results, we cannot recommend only one specific concentration because all of the treatments (3.3%, 6.7% and 9.9%) positively stimulated the germination and the seedling development expressing that effect through the different growing parameters. Although the highest concentration (9.9%) resulted in the highest seedling length and vigor indexes, it is not recommended concentration dose because it causes disbalance in the seedling growth by inhibiting the root development and overstimulating the shoot development. Moreover, this concentration significantly decreased the germination energy and the total germination.

The Orgalife fertilizer in the recommended dosage could be used for encouragement of the rice seedling development, mainly in controlled conditions, for example in seedling production.

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COMPARISON OF PYROXASULFONE'S BIOREMEDIATION WITH SOME SOIL BACTERIA BASED ON CHEMICAL OXYGEN DEMAND AND ITS EFFECT ON MORTALITY ON DAPHNIA MAGNA

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ABSTRACT

In this study, the environmental and toxic effects that may occur due to the use of herbicide with Pyroxasulfone active ingredient were minimized by bioremediation techniques, the removal rates of pollution were determined and the mortality (death rate) effect on Daphnia magna (water flea) was determined. In the study, removal experiments were carried out in solutions of herbicide containing Pyroxasulfone active ingredient prepared with some bacterial species in a liquid medium. The bacteria used were enriched and taken in required volumes and added to the solutions prepared based on the recommended concentrations of the herbicide with Pyroxasulfone active ingredient for use in agricultural areas. Chemical oxygen demand (COD) measurements were made in the solutions and the removal rates were compared on the basis of bacteria and mixtures of the pollutant. With the determination of the highest removal rates obtained, the death rates on Daphnia magna were determined with the samples taken. In the experimental studies, the COD removal percentages were determined over 80% and the reduction rates in the pollutant ratios in the medium differed according to the bacterial species in the prepared liquid medium. The bioremediation mechanism of the herbicide, which was monitored with important environmental parameters such as COD, decreased daily with some newly isolated bacteria and their mixtures, and mortality tests were performed with the environment where the highest reduction rate was observed for these parameters. In the environment where the most effective bioremediation rate was realized, a mortality effect of around 50% was detected on daphnia magna.

Keywords: Pyroxasulfone, Bioremediation, *Daphnia magna*, Chemical Oxygen Demand

INTRODUCTION

As a result of the increase in the use of pesticides since the middle of the twentieth century, many negative effects have emerged on the natural environment and human health (Kayhan, 2020; Karaismailoğlu, 2016). Under these conditions, it is necessary to get the highest yield from the unit area in order to feed the shrinking agricultural lands and the increasing human population. For this, the fight against diseases, pests and weeds that cause yield loss in agricultural areas becomes inevitable (Koc, 2020). Accordingly, plants and herbal products; it has been inevitable to use pesticides or pesticides in order to protect from the effects of pests, disease factors and weeds, and to obtain quality and abundant products. Pesticides are synthetic organic compounds used to destroy undesirable organisms in products (Altikat et al., 2009).

Pyroxasulfone is from a new class of chemicals but is primarily used to control annual weeds. However, it is also effective against broadleaf weeds (Hopkins, 2013). It has been registered for use in Japan, Australia, USA, Canada, Saudi Arabia and South Africa as of 2016 and is used in crops such as corn, soybean, wheat and cotton (Nakatani et al., 2016).

It is a necessity to use agricultural chemicals and especially pesticides in order to provide the necessary agricultural food production for the adequate and balanced nutrition of the growing population in our country and in the world. More research will be required in this area as the use of agrochemicals increases. While R&D investments in pesticides are decreasing in the EU, they are increasing in developing countries. This increase creates an opportunity for developing countries like us (Kaymak et al., 2014).

PESTICIDES

Pesticide definition and classification

As a term, pesticide means a substance used to kill harmful creatures, called "pest" in short. In general terms, pesticide can be defined as a tool, method or chemical used to kill unwanted animals and plants in foods offered for human use (Kekillioğlu et al., 2020).

The definition made by the United Nations Food and Agriculture Organization (FAO) for pesticides is as follows; “carrier of diseases that may occur in humans or animals; During the production, processing, transportation, storage and/or marketing of food, agricultural products, wood and wood products or animal feed, in order to prevent, destroy or control all kinds of pests that will adversely affect these practices, or to control the pests that may be present on animals or their bodies are substances used for the purpose (Altikat et al., 2009).

Pesticides are classified according to the targeted organism as follows.

- Acaricides (used against mites)
- Fungicides (used against fungi on the plant)
- Insecticides (used against insects)
- Rodenticides (used against mice and other rodents)
- Nematicides (used against nematodes)
- Herbicides (used against weeds) (Akturk, 2019).

Pesticide use in Turkey

The amount of herbicide usage in our country is below the world usage values. Especially in developed countries where herbicides are used intensively, herbicide usage rates are very high because labor costs are much higher than herbicide wages. In our country, labor is evaluated in areas where family labor is sufficient, but labor prices are increasing in our country, it is difficult to find workers and the use of herbicides is increasing (Demirkan, 2009, Figure 1).

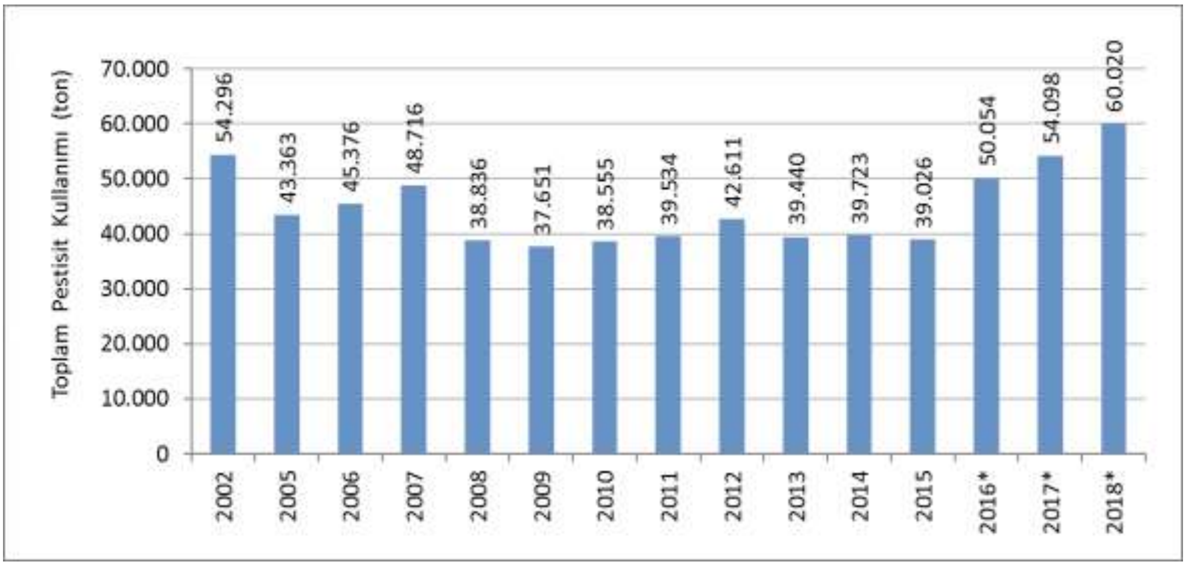

Figure 1. Total pesticide use in Turkey by years (URL-1)

The amount of pesticides used per unit area in Turkey is far behind the world and European Union countries average. Mediterranean (28%), Aegean (25%), Central Anatolia (16%) and Marmara (16%) regions generally have 85% of the country's pesticide use. More than half of the amount of pesticide use in Turkey occurs only in the Mediterranean and Aegean regions. For this reason, these two regions are the first to be investigated in terms of health problems that may arise due to pesticide use (Özpolat Cakar et al., 2020).

BIOREMEDIATION

The word bioremediation was coined by scientists in the early 1980s as a term to describe the use of micro-organisms to clean contaminated soils and waters. The prefix “bio” describes the biological process carried out by living organisms. The name of “remediation” is defined as cleaning the environment through complete degradation or removing toxic substances as a result of microbial activity (Akinci et al., 2016).

Bioremediation is a process that converts harmful substances into non-toxic compounds using microorganisms and is one of the promising techniques used for the treatment of chemical liquids and hazardous wastes (Ceyhan et al., 2012, Figure 2).

Bioremediation is a naturally occurring process; It is the process by which microorganisms fix or transform environmental pollution into a final product (Dindar et al., 2010).

Decomposition of herbicides also usually occurs with a large number of microorganisms. Each microorganism contributes to the bioremediation reactions on herbicides, but no examples of mineralization with a single strain have been described. For adequate bioremediation, different microorganisms must be present in the environment (Erguven et al., 2020).

Bacteria are widely used for bioremediation purposes. Recent studies have focused on the investigation of bacteria, consortia or biotransformation enzymes. Their rapid growth, ease of use and low cost make bacteria suitable for bioremediation. However, it also has some disadvantages such as bacterial biomass, pathogenicity, bioactivation and disposal. Bacteria can even be found in particles dispersed in the soil, water or air. Only a small fraction of bacteria (10% from soil) can be cultured under laboratory conditions. Therefore, the number of studies on pesticide biodegradation mechanisms is few and there is very little literature on biochemical mechanisms and enzymes (Erguven, 2019).

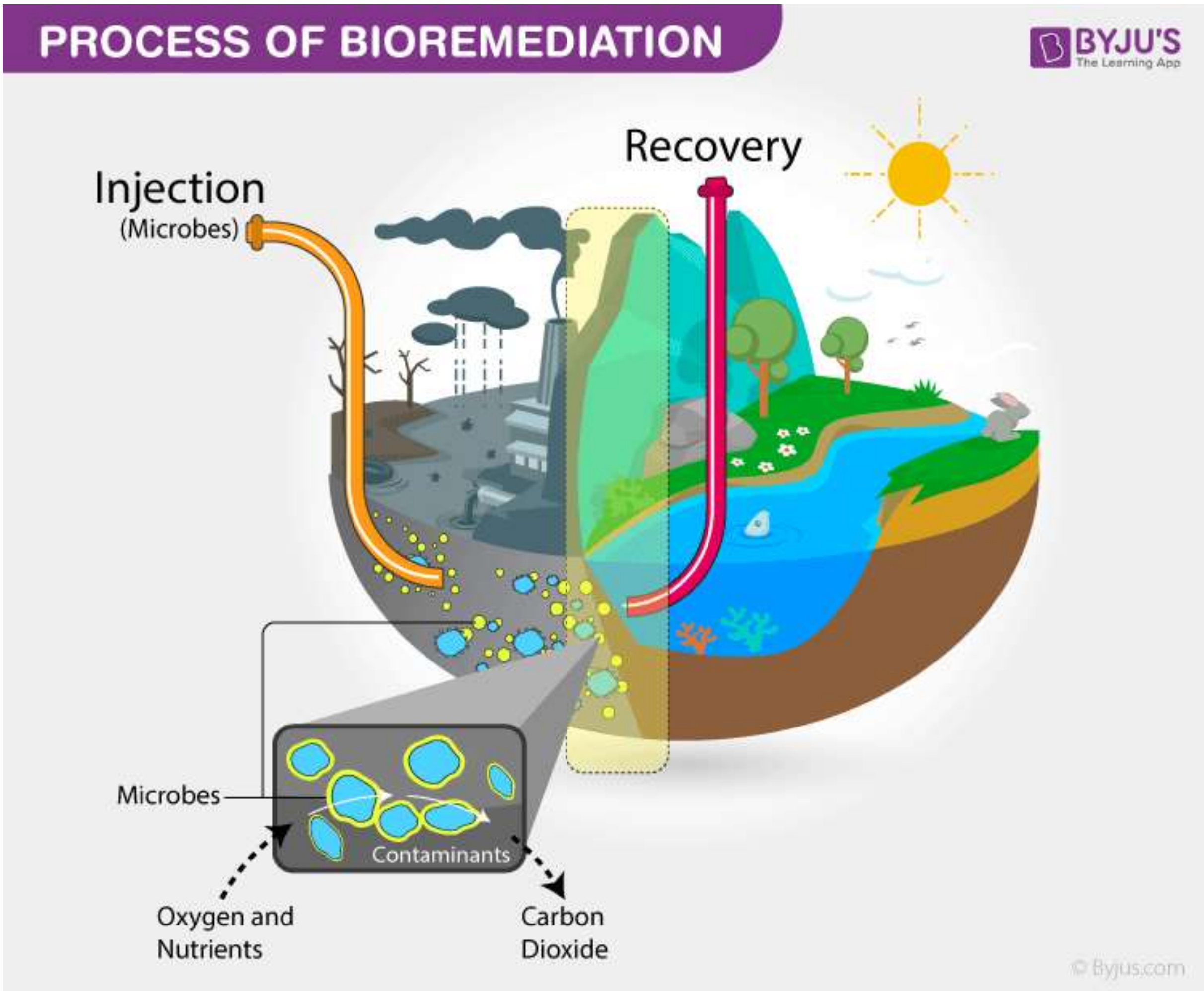

Figure 2. Bioremediation cycle (https://byjus.com/biology/bioremediation/)

Advantages of Bioremediation

• It is an environmentally friendly, ecologically safe and natural process
• Toxic compounds are converted into non-harmful CO_2, H_2O and fatty acids
• It can be applied directly in a restricted area
• It is cost-effective when compared to other traditional methods
• The equipment to be used in the application is simple and easily accessible
• The time required for surface remediation is less compared to other methods
• Waste product does not occur
• Following the biodegradation of the pollutant, there is a natural decrease in the microbial population, which increases in number depending on the presence of the pollutant
• It causes minimal disruption in the natural functioning of the bioremediation area
• Bioremediation efficiency can be increased by combining it with other remediation technologies
• Existing microorganisms increase if contaminants are present as a food source. When the contaminants decrease, the population decreases spontaneously
• In bioremediation, target chemicals are completely removed without transferring contaminants from one environmental medium to another

• Bioremediation is less expensive than the technologies often used for the disposal of hazardous waste.

Disadvantages of Bioremediation

• Environmental conditions required for microbial activity must be maintained continuously
• High concentrations of organic pollutants with low solubility have a toxic effect on the bioavailability of microorganisms
• While its applicability is higher in soils and channels with high permeability, it is not a suitable method for soils with low permeability
• Some limitations prevent the widespread use of bioremediation as a cleaning technology
• Before starting bioremediation, a very good research is required and a suitable bioremediation technology engineer is needed for the complex contaminant mixture and regions. Microbiologists are needed for the isolation of microorganisms from the soil, and biochemists are needed to determine the degradation pathway
• Cleaning with bioremediation takes a long time compared to burning or digging up the soil
• It is necessary to take precautions against the formation of some toxic by-products

Bioremediation techniques

The main purpose of bioremediation design is to provide optimal conditions for microbial growth and activity. Depending on the aeration and saturation degree of the area, different technologies can be used (Dindar et al., 2010).
Bioremediation is applied in two ways.

• By transferring nutrients to the area where the wastes are dumped, the bacteria already in the soil are activated according to the bacterial composition of the soil (in-situ disposal technologies). This method is used when pollutant concentrations are low.

• The soil is excavated and new bacteria capable of breaking down pollutants are added. Environmental conditions are controlled or conditions are changed to optimize the metabolic activities and growth of microorganisms. In the optimization of environmental conditions for bioremediation; Factors such as temperature, inorganic nutrients (primarily nitrogen and phosphorus), electron acceptors (oxygen, nitrate and sulfate) and pH are optimized (ex-situ disposal technologies) (Dindar et al., 2010).

DAPHNIA MAGNA

Daphnia sp. It is a freshwater crustacean invertebrate called the water flea because of its jumping like fleas. This creature has a wide distribution around the world. The most important member of the Daphniidae family, Daphnia sp, which is rich in protein and essential fatty acids, is the most important food source of freshwater fish in its natural environment. Although its nutritional value varies according to species and age, approximately 50% of its dry weight consists of protein (Sari, 2011).

Daphnia is a food source for some fish and invertebrates. Because of these features, Daphnia plays an important role by preparing the energy transfer worldwide (Korkmaz et al., 2021, Figure 3).

Figure 3. Daphnia magna

Water fleas, which are an important part of the food chain of the aquatic ecosystem, are frequently used in toxicological studies due to their rapid growth rate, high reproductive potential, short life cycle and indicator feature (Sari, 2011).

Daphnia is a planktonic crustacean that filters fresh water and plays a role in improving water quality by consuming algae (Tkaczyk et al, 2021). Water fleas show behavioral and physiological responses by being affected very quickly by environmental pollutants and chemical changes in their habitats, and these features have led them to be used as model organisms especially in toxicology studies (Barata et al, 2005, Ebert, 2005). The body size of the water flea provides a great advantage for laboratory studies because it is possible to use many individuals simultaneously in an experimental setup, and their high fecundity and parthenogenetic type breeding allow many water fleas to be tested in a short time. (Koivisto, 1995). In experiments with water fleas, topics such as lethal, reproductive, behavioral, physiological effects and changes in the biochemical properties of any chemical have been studied in general. Among these parameters, the determination of the lethal effect is the most preferred study.

Daphnia species, also called daphnia, are test organisms often used to investigate the toxic effects of numerous chemicals in the water system. When swimming behavior and mortality rates of water fleas are examined, these parameters become effective biomarkers in toxicity studies. Daphnia magna has an important role in aquatic ecosystems; There are many studies used to evaluate the toxic effects of pollutants (Korkmaz et al., 2021).

Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is the amount of oxygen required for chemical oxidation of oxidizable substances in water. One of the most important parameters used in determining the degree of pollution of domestic and industrial wastewater (especially industrial) is the chemical oxygen demand.

It also contains some substances that do not decompose by biological means. COD is an important and quick parameter in the study of river and industrial wastes.

Used Tool, Device/Materials

• Borosilicate digestion tube (with TFE cap)
• Thermoreactor
• Various glass materials (Pipette, Erlen)
Side Reagents (Reagents)
•Pure water

• Standard potassium dichromate solution (0.01667M) 4.903 gr., previously dried at 150 °C for two hours. The chemical K2Cr2O7, 167 mL H_2SO_4, 33.3 g $HgSO_4$ is dissolved in 500 mL distilled water, cooled to room temperature and diluted to 1000 mL.
• Sulfuric acid reagent is dissolved by adding 5.5 gr Ag_2SO_4 in crystal or powder form to 1 kg of concentrated sulfuric acid solution. In order for the silver sulfate to completely dissolve in the acid, it should be left for 1-2 days.
• Ferroin indicator solution is dissolved in 1.485 g of phenanthroline monohydrate and 695 mg of $FeSO_4$ .7H_2O in distilled water and made up to 100 mL. It is diluted 5 times.
• Standard iron ammonium sulfate solution (DAS): (0.1 M): 39.2 gr $Fe(NH_4)_2(SO_4)2.6H_2O$ (Iron ammonium sulfate) chemical is dissolved in distilled water. Add 20 mL of concentrated sulfuric acid, cool and make up to 1000 mL. This solution should be adjusted against the standard dichromate solution as follows each day of use
* Standardization: Add 5 mL of standard $K_2Cr_2O_7$ solution and 10 mL of distilled water to the beaker. Add 1-2 drops of ferroin indicator and titrate with iron ammonium sulfate (DAS). Thus, the standard DAS solution is adjusted for each day of use with the standard dichromate solution ($K_2Cr_2O_7$).
Different sample values such as 0.5 mL, 0.75 mL, 1 mL poured into the culture tube and 1.5 mL of digestion solution added to it and placed in the electric heater. At the end of 2 hours, the samples taken from the heater titrated with iron ammonium sulfate solution (DAS), whose normality is determined, by dropping ferroin (2-3 drops) after cooling, until the color of onion skin from green color is observed. Consumption recorded at the point where the color conversion occurs. DAS consumption will be calculated each week before titration.

$$\text{COD (mg/l)} = \frac{(A-B)*M*8000}{ml\ sample}$$

A: Iron ammonium sulfate consumption for the witness
B: Iron ammonium sulfate consumption for sample
M: Molarity of iron ammonium sulfate solution.

RESULTS

Some bacterial species were added to the solutions prepared on the basis of the concentrations of the pesticide with Pyroxasulfone active ingredient and the chemical oxygen demand (COD) measurements were compared with the removal rates of the pollutants. With the determination of the highest removal rate, the mortality rate on Daphnia magna was determined. In the experimental studies, the COD removal rate exceeded 80% and the reduction rate of the pollutants in the environment varied according to the bacterial species in the prepared liquid medium.

Pollution experiments using *D. magna* will provide very important data for environmental risk assessments of pesticides. The bioremediation mechanism of the herbicide, which is monitored with important environmental parameters such as COD, decreased daily with some newly isolated bacteria and their mixtures, and mortality tests were performed with the environment where the highest reduction rate was observed for these parameters. In the environment where the most effective bioremediation rate was realized, a mortality effect of around 50% was detected on *D. magna*.

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THE ROLE OF TUMOR MARKERS FOR EVALUATION THE COURSE OF CHEMOTHERAPY

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ABSTRACT
Gynecological tumors, including endometrial, cervical and ovarian cancer, have increased in incidence over time. The widespread introduction of screening programs and advances in diagnostic imaging methods has lead to a progressive increase in gynecological cancer detection. Accurate diagnosis and proper monitoring of disease remain the primary target for a successful treatment. In the last years, knowledge about cancer biomarkers has considerably increased providing great opportunities for improving cancer detection and treatment. In addition, in the last few years there has been an important development of imaging techniques. Nowadays, a multimodal approach including the evaluation of serum tumor biomarkers combined with imaging techniques, seems to be the best strategy for assessing tumor presence, spread, recurrence, and/or the response to treatment in female cancer patients In this review we provide an overview of the application of biomarkers combined with novel imaging methods and highlight their roles in female cancer diagnosis and follow-up.

Key words: gynecologic malignancy, tumor markers, chemotherapy

INTRODUCTION

Of the millions of new cases of cancer worldwide and deaths associated with cancer, a large number of them were breast and gynecological tumors (Molina R et al., 2005). Some tumor markers are fundamental to the workflow in diagnosis, control of therapy and the monitoring of advanced gynecological diseases (Sturgeon CM et al 2010). The biomarker should be absent in healthy people as well as in good conditions and it is released exclusively from specific tumor cells (Duffy MJ et al 2013). Tumor markers are soluble glycoproteins that are found in the blood, urine, or tissues of patients with certain types of cancer. They are typically produced by tumor cells, but in some cases they may be produced by the body in response to malignancy or to certain benign conditions. Tumor markers are not elevated in all cancer patients, particularly patients with early-stage cancer. The various tumor markers differ in their usefulness for screening, diagnosis, prognosis, assessing therapeutic response, and detecting recurrence. Normalization of tumor marker values may indicate cure despite radiographic evidence of persistent disease. In this situation, residual tumor is frequently nonviable. Sometimes, tumor marker values may rise after effective treatment (due to cell lysis), but the increase may not portend treatment failure. A consistent increase in a tumor marker value, combined with lack of clinical improvement, may indicate treatment failure. Residual elevation after definitive treatment usually indicates persistent disease. Many new tumor markers have been discovered since the development of monoclonal antibodies, and most tumor markers are now detected with them. No marker is completely specific. Therefore, diagnostic immunohistochemistry must be used in conjunction with morphologic and clinical findings. [2] Among asymptomatic persons, the biomarker should allow for the examination of early cancer or premalignant disease and in symptomatic patients the biomarker should help in the differential diagnosis of benign and malignant disease. After diagnosis, an ideal biomarker should also be used to estimate the prognosis and predict the most appropriate treatment. For patients receiving systemic therapy, the level of expression should correlate with therapeutic response and tumor burden (Duffy MJ et al 2015). A biomarker should contribute to improving beneficial clinical outcomes such as increased overall survival (OS), progression-free disease survival (PFS) or reduced cost of care (Di Gioia et al 2011, Lufter D et al 2000). CA125 is currently the most commonly used serological biomarker for the management of patients with some of the fallopian tubes or the vesicles. In recent years, the prognostic value of changes in serum levels of CA15-3 for breast cancer has gained a lot of attention (Ali HQ et al 2013). For multiple cancers, markers of serum tumors play important roles in early diagnosis, prognosis, and response for specific therapies, early detection of recurrence after curative surgery, and monitoring of therapy for patients with advanced disease (Tarhan MO et al., 2013). Tumor-like markers widely used for breast cancer are CA15-3 and CEA (Kim HS et al 2009). While the CA15-3 level is rarely elevated for patients with early stage or localized cancer, most metastatic breast cancer patients have demonstrated elevated serum levels of CA15- 3 (Nisman B et al., 2013). In the absence of measurable lesion (s), however, an increasing CA15-3 level can be used to indicate the failure of treatment (Zhang SJ et al 2013). More attention should be given to the interpretation of increased CA15-3 levels during the first 4-6 weeks of new therapy due to early premature growth (Thriveni K et al 2013). The temporary increase of CA15-3 that is affected by chemotherapy and followed by the decrease of CA15-3 (CA15-3 increases and decreases) may result in inappropriate early discontinuation or chemotherapy change. The vast majority of ovarian tumors are of epithelial origin and the 125 carbohydrate antigen (CA 125) is the most important marker of tumor. Increased levels depend on the histological type and stage of the disease (Sölétormos G et al 2016). Though it is sensitive in the early stages, CA 125 has high sensitivity and specificity in early dictation of the disease, especially in women and premenopause period (Castrillon DH et al 2002). Furthermore, some factors may cause high levels of CA 125, such as ethnicity, pregnancy, age, premenopausal postmenopausal period and menstrual cycle (Pauler DK et al 2001). In breast cancer, a combination of carcinoembryonic antigen (CEA) and CA 15-3 has prognostic potential in a preoperative environment (Huh JW et al 2010). While CEA and CA 15-3 are recommended for monitoring the therapy and early detection of disease recurrences they are also recommended for early diagnosis or screening due to their high sensitivity. In cervical cancer, the guidelines of the National Academy of Clinical Biochemistry (NACB) discuss (among the markers of other tumors such as the CEA and CA 125 squamous cell carcinoma antigen (SSCA) for predicting prognosis and preoperative prediction of metastases (Colombo N et al 2016) CEA has prognostic significance in colorectal cancer (Thirunavukarasu P et al 2011) but in cases where the origin of an uterine tumor is unclear, panel tumor markers are recommended including CEA (Zur B et al 2012, Haas M et al., 2013). The aim of the study is to monitor the alteration of tumour markers after chemotherapy.

MATERIAL AND METHODS

This is a prospective study performed during the period 2014-2016 with 107 patients diagnosed with breast, uterine and ovarian cancer in the hospital of Vlora district who also were treated with chemotherapy. For all patients, laboratory tests: biochemical, hematologic, tumor markers: CEA (mg / l) CA15.3 (U / ml) CA 125 (U / ml) were performed after the first and second cycle of chemotherapy. The reported data are expressed as mean ± standard deviation (SD). Wilcoxon test was used to compare the hematobiochemic and tumor markers between the 1st and 2nd cycle of chemotherapy. The receiver operating curve (ROC) curves for determining cut-off values and predictive parameters of tumor markers for Ca. A p-value ≤0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The mean age of patients was 57.1 (± 8.13) years and ranging from 38 to 70 years of age. Table 1 shows the study subjects' demographic characteristics and clinical history. By comparing the median values of hematobiochemic and tumor parameters after the 1st and 2nd cycle of chemotherapy, the statistically significant difference was found only for aspartate aminotransferase (AST) which showed decrease after the second cycle. Concerning the types of Ca, significant decrease of CA 15.3, CA 125 and CEA was observed in all three types of Ca following the second cycle of chemotherapy ($p<0.01$). The values of the three tumor markers are significantly higher in metastatic patients, as compared to metastasis-free patients ($p<0.01$) (table 2). No statistically significant difference was found between the ROC curves of the three tumor markers for the determination of Ca (figure 1). In predicting malignancy in ovarian tumor patients, besides history taking and physical examination, the use of tumor markers as a part of evaluation is also important.

Patients receiving first line chemotherapy for ovarian cancer are usually offered a minimum of 5 courses of chemotherapy. Unless there is evidence of clinical progression the first three courses will almost certainly be administered. If there is then evidence of inadequate response or progression when the patient attends for her fourth or subsequent course, there could be a change of therapy. A serial rise of tumor markers of 25% over three samples has been shown to indicate progression. Many doctors would consider a lesser rise or slight fall indicates poor response. It is essential that any decision is based on a baseline result and at least two further marker results with the second or subsequent marker results confirming the trend. To summarise: Obvious clinical improvement: continue planned therapy. Obvious clinical progression: change therapy. If patients not in above groups: Marker response (at least downward trend): continue planned therapy. Marker progression (>25% rise) chnage therapy. Our findings correspond to current guidelines such as NACB that recommend CA 15-3 in breast cancer - although not for diagnosis but for advanced disease monitoring and postoperative surveillance. Although the CEA application is still being discussed, various studies have shown its importance in, for example, anticipating and early detection of disease progression and metastasis (Stieber P et al 2015). While in the breast cancer analysis the our results show the high clinical performance of CA 15-3.

In ovarian cancer, the best diagnostic performance was achieved for CA 125. These results are in line with current recommendations, suggesting that CA 125 is of major importance in therapeutic monitoring, differential diagnosis for legal measures, recurrence and prognosis (Sölétormos, et al 2016) Serum Cancer Biomarker Cancer Antigen 125 (CA125) is proposed as an adjunct to non-invasive procedures in patients with advanced disease (Shao Y et al 2015; Wu et al 2014; Wang et al 2014). However, challenges remain on how to determine values in CA125 concentrations that allow an optimal interpretation that is vital for early diagnosis of tumor growth.

Table 1. Demographic characteristics and clinical history of study participants

Variables	N	%
Age of 1st menarche M (SD)	13.5 (2.4)	
Age of 1st sexual intercourse		
≤18	19	17.8
>18	88	82.2
Pregnant		
No	16	15.0
Yes	91	85.0
Parity		
Nullipara	16	15.0
Primipara	32	29.9
Multipara	59	55.1
Sectio Caesarea		
No	61	57.0
Yes	46	43.0

Abortions		
No	82	76.6
Yes	25	23.4
History for STI		
No	7	6.5
Yes	100	93.5
Family history for Ca		
No	103	96.3
Yes	4	3.7

Table 2. Mean values of tumor markers according to presence of metastasis

Metastasis	CEA (µg/l)	CA 15.3 (U/ml)	CA 125 (U/ml)
Yes	55.8 ± 25.9	122.3 ± 89.2	288.4 ± 206.7
No	13.2 ±218	78.6 ± 89.8	201.4 ± 197.1
P	<0.01	<0.01	<0.01

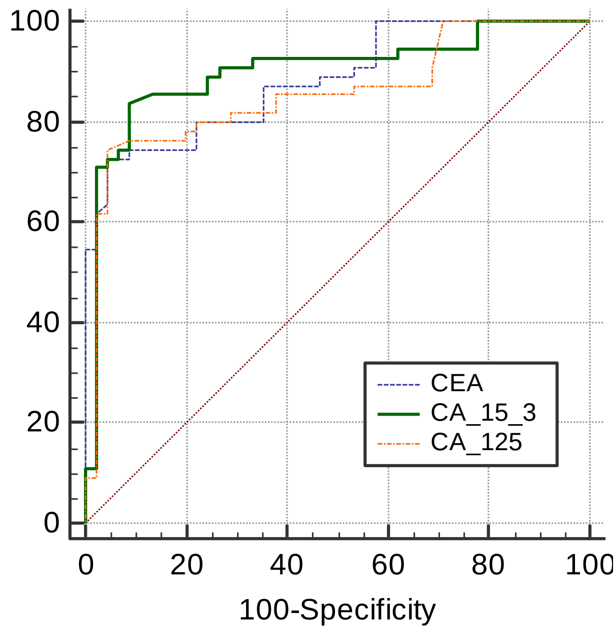

Figure 1. Comparison of ROC curves of tumor markers for the evaluation of Ca

CONCLUSIONS

The values of hematobiokimic parameters and tumor markers are important in identifying the course of therapy as well as predicting malignant conditions. Accurate determination of serum tumor marker levels is crucial, as their impact on diagnosis, prognosis, and therapy monitoring has been shown for many types of tumors.

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CHANGES IN BIOCHEMICAL PROPERTIES AND POSTHARVEST QUALITY OF POMEGRANATES CV. SILIFKE AŞISI UNDER MODIFIED ATMOSPHERE PACKAGING

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ABSTRACT

Importance of the pomegranate (*Punica granatum* L.) as a tropical and subtropical fruit has been increasing due to its premium nutrient quality and beneficial effects on human health with its rich flavanoids, fenolics and ascorbic acid contents. However, pomegranate fruit is susceptible to various postharvest quality problems including weight loss, decay and susceptibility to physiological disorders such as chilling injury and husk scald. The objective of this study was to determine the effects of different modified atmosphere packaging (MAP) on postharvest quality of 'Silifke Aşısı' sweet pomegranates during cold storage. Pomegranates were harvested at the commercial harvest stage and packed in two different types of MAP [low density polyethylene (LDPE) and polyvinyl chloride shrink film (PVC)]. After packaging, all fruit were stored at 5 °C and 90% RH for 90 days, then removed from storage for postharvest quality analyses. Weight loss, husk and aril color, soluble solids content (SSC), titratable acidity (TEA), total monomeric anthocyanin (TMA), ascorbic acid (AsA), total phenolic content (TPC) and total antioxidant activity (TAA) of pomegranates were examined. The results showed that MAP inhibited the increase in weight loss, delayed the decline of SSC, TEA and AsA, and improved TMA of pomegranates at 5 °C compared with the control. In general, the effect of LDPE was better than PVC on maintaining the postharvest quality of pomegranates. Considering the overall investigations, LDPE packaging could be recommended for controlling weight loss, maintaining visual and biochemical quality, delaying the changes in aril and husk color of 'Silifke Aşısı' pomegranate fruits during 90 days of cold storage.

Keywords: Pomegranate, storage, MAP, quality

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the oldest fruit species cultivated in the world, belonging to Punicaceae subfamily of Myrtifloare (Myrtales) order. Pomegranate, a perennial tree with multi-stem bush formed, dense branched habitus, is grown in many subtropical and tropical countries, especially in Mediterranean countries with mild climates (Artes et al, 2000).

The arils that make up the edible part of pomegranate fruits constitute approximately 55-60% of the total fruit weight and contain 80% water (Erkan and Doğan, 2018). The juice is rich in folic acid, potassium, phosphorus, iron, antioxidants, vitamin C, polyphenolic substances, alkaloids and flavonoid contents and fiber. Its antioxidant content is three times higher than green tea and it helps in increasing body resistance by strengthening cell renewal, growth and immune system. It has a protective effect against cardiovascular diseases, cough, constipation and heartburn (Holland et al., 2009; Viuda-Martos et al., 2010).

In the last decade, significant increases in pomegranate production have been recorded in the world and in Turkey due to the increasing awareness about nutrition and health. According to 2020 data, 600.021 tons of pomegranate production was recorded in 284.632 decares in Turkey (Anonymous, 2021). It is stated that ’Silifke Aşısı’ and ’Hicaznar’, among the most produced varieties in the world, are more preferred in European markets in recent years and have an important place in exports (Gündoğdu, 2011).

Pomegranate is a nonclimacteric fruit which is perishable after harvest. Weight loss, husk scald, aril color, acidity and vitamin C losses occur in harvested fruits. This causes decrease in both the nutritional and quality characteristics of the products. Studies indicated that postharvest heat applications, storage at appropriate temperature, modified atmosphere packaging, controlled atmosphere storage and surface coating applications give positive results in order to preserve the nutritional quality properties with bioactive components in pomegranates after harvest (Mphahlele et al., 2014).

Modified atmosphere packaging (MAP) is one of the postharvest techniques used to extend the storage and shelf life of fresh fruits and vegetables. In principle, MAP prolongs the storage and shelf life of fruits and vegetables by delaying the senescence of fruits and vegetables by decreasing the amount of O_2 and increasing the amount of CO_2 in the packaging. It also protects the quality of products by reducing water loss (Sandhya, 2010). Protection method in MAP results in prolongation of postharvest life by preserving the quality characteristics of the products without the need for the use of chemicals. This system can be used not only during storage but also during transportation and marketing. Due to the differences between the respiration rate of products and the ideal gas concentration between species and even varieties, packaging materials specifically designed for the product must be used for successful containment (Rennie ve Sunjka, 2018). The loss of water in the peel during cold storage in pomegranates causes weight loss and also a decrease in the amount of marketable products (Erkan and Doğan, 2018). Literature data confirmed that MAP or film coating reduced water loss and the resulting shriveling symptoms, crust burn and rotting, slowed down the changes in taste and aroma and provided quality protection opportunities in 'Hicaznar' (Bayram, 2007; Karaca and Şen, 2014; Selcuk and Erkan, 2015; Candır et al., 2019), 'Hicrannar' (Selcuk and Erkan, 2014), 'Wonderful' (Porat et al.2009), and 'Primosole' (D'Aquino et al., 2010) pomegranate varieties.

In this study, the effects of modified atmosphere packaging on 'Silifke Aşısı’ pomegranate variety on physical and biochemical features during cold storage were investigated.

MATERIAL AND METHOD

Pomegranates cv. Silifke Aşısı, harvested at commercial maturity in Karaman province Göksu Valley, were immediately transferred to the laboratory of the Department of Horticulture at Selcuk University. Pomegranates fruits were selected according to their uniformity in color, size and absence of damage. They were randomly divided into three equal groups for postharvest applications.

The first group of fruits were weighed and placed in polystyrene plates without using any packaging material and formed the control group. The second group of fruits were stored in low density polyethylene modified atmosphere packaging. For this purpose, all fruits weighed were placed in modified atmosphere packaging and placed in crates by sealing them (LDPE). Third group fruits were placed in polystyrene plates and covered with polyvinyl chloride shrink film (PVC). After the fruits were packaged, they were stored for 90 days in a cold storage at 5 °C and 90% relative humidity. Fruits were taken out of storage immediately after harvest and on the 30, 60 and 90th days, and their quality changes were determined by physical, chemical and biochemical analysis.

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 (%). CIE L * a * and b * values were read by using a CR 400 model Minolta brand color device on the opposite surfaces of all fruit samples taken from the storage in order to determine the color changes that occur on the skins of the fruits during storage. For the arils, the measurement process was carried out by placing approximately 100 g of samples in petri pots 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 by hand refractometer in fruit juices obtained by squeezing the fruits and the results are given as %. Titratable acidity (TEA) was titrated with 0.1 N NaOH until the pH was 8.1 and the results were expressed as % in citric acid. After juicing the preserved fruits were homogenized with 25 ml of methanol and kept at 4 °C for 16 hours and then centrifuged. This extraction was used for total phenol and total antioxidant analysis (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 placed in a balloon flask. Folin-Ciocalteu reagent was added to this mixture and shaken and kept at room temperature for 3 minutes. At the end of this period, saturated sodium carbonate solution was added and it was completed with distilled water. The solution which was incubated at 25 °C for 2 hours was read at 760 nm wave length in the spectrophotometer and the results were given as mg/g (Singleton et al., 1999).

Ferric Reducing Antioxidant Power (FRAP) method was used to determine the total antioxidant activity. 2850 µL of FRAP working solution was added onto 150 µL of sample extract and left in the dark for 30 minutes. At the end of this period, 593 nm wave length was read in the spectrophotometer. The values obtained were calculated with the standard curve of trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) prepared in 10-100 µmol/l concentrations and FRAP antioxidant activity was expressed as µmol/g fresh weight (Benzie and Strain , 1996).

In the total amount of anthocyanin, 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 (g/100 g) (Cheng and Breen, 1991).

Ascorbic acid 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 ($C_{12}H_6C_{l2}NO_2Na$) 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 was 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 Figure 1. The LDPE and PVC treatments effectively inhibited weight loss compared to the control. Weight loss increased during the storage and reached 10.21% in control, 2.06% in PVC and 0.42% in the LDPE treatments after 90 days at 5 °C. Between the MAP packagings, the weight loss was less when the fruit were packed with LDPE than with

PVC at cold storage. Selcuk and Erkan (2014) observed that after 120 days of storage at cold storage unpackaged control 'Hicrannar' pomegranate had a weight loss of 17.24%, while packaged fruit lost only 4.42% (MAP1) and 1.48% (MAP2).

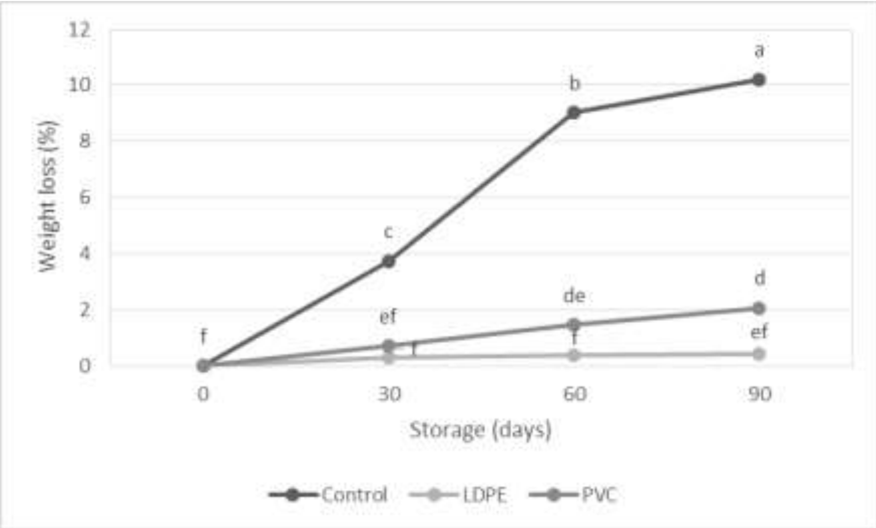

Figure 1. Effects of MAP on weight loss of pomegranates during storage.

Husk color is one of the most important visual attributes for pomegranates. L* values was always significantly higher in the pomegranate stored in LDPE and PVC than control. While the L* value declined during the storage for control pomegranates, it increased for 60 days in LDPE and PVC treatments and decreased on the 90th days. Fruit stored for 90 days at 5 °C, L* values decreased from an initial 67.62 at harvest to 66.30, 65.06 and 55.26 in LDPE, PVC and control, respectively (Table 1).

Changes in C* values of pomegranates during the storage were presented in Table 1. C* value of fruits decreased during storage but the differences between the treatments were statistically insignificant. The initial C* value of the fruits was 40.71, it decreased to 36.43, 35.15 and 33.89 for LDEP, PVC and control, respectively after 90 days of storage (Table 1).

LDPE and PVC treatments led slight increments in hº value of pomegranate husk during the 60 d storage. At the end of the storage, the highest change in hº value of husk was determined in control fruits, while the treatments showed lower changes.

Aril color is also an essential feature determining the market value of pomegranates. L*, C* and hº values across the samples displayed slight but insignificant decreases along with the prolonged storage duration.

SSC and TEA displayed a gradual decline during the whole storage period compared to the initial values at harvest of pomegranates at 5 °C. Treatment with LDPE or PVC delayed the decreases of SSC and TEA at varying degrees compared to the untreated fruits. But LDPE was more effective than PVC on delaying the SSC and TEA changes, except for the last values where both treatments had similar effects.

The initial AsA content of 15.68 mg/100 ml decreased during cold storage (Table 2). LDPE and PVC treatments significantly ($p \leq 0.05$) retarded the AsA loss, compared to the control. AsA content after 90 days of storage were 10.30 mg/100 ml, 7.66 mg/100 ml and 3.32 mg/100 ml for LDPE, PVC and control, respectively. Selcuk and Erkan (2016) reported a decrease in ascorbic acid content of pomegranates at cold storage and an extended storage period.

Table 1. Effects of MAP on husk color (L*, C* and h°) and aril color (L*, C* and h°) of pomegranates during storage.

Storage (days)	Treatments	Husk color			Aril color		
		L*	C*	h°	L*	C*	h°
0		67.62b	40.71	69.14cd	39.33	19.40	57.01
30	Control	65.12c	37.63	64.70ef	33.63	18.03	49.27
	LDPE	71.34a	40.78	76.13 a	38.57	18.57	53.18
	PVC	70.16a	38.68	74.44ab	36.18	18.36	51.47
60	Control	64.85c	37.48	69.29cd	32.63	17.45	45.87
	LDPE	70.07a	39.56	72.26bc	35.96	18.39	47.61
	PVC	69.99a	39.63	72.52bc	35.08	17.39	45.47
90	Control	55.26d	33.89	63.82f	31.64	17.41	40.43
	LDPE	66.30bc	36.43	70.91bcd	33.27	17.64	44.90
	PVC	65.06c	35.15	68.01de	33.46	17.07	43.08
	$LSD_{0.05}$	1.83	N.S.	3.56	N.S.	N.S.	N.S.

*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.

There were significant increases in TPC along with the storage time. Both of the treatment remarkably retarded the increase in TPC with greater effect of LDPE up to the 60 d. But, at the end of the storage, the lowest change in TPC was found in fruits treated with PVC.

As illustrated in Table 2, TAA of the pomegranates gradually decreased during the prolonged cold storage. However, either PVC or LDPE treatments significantly maintained the fruit TAA content in comparison to control fruits. Initial TAA content of pomegranates were 5.71 µmol/ml. At the end of the storage period, the highest TAA content was obtained from LDPE treatment (4.33 µmol/ml), followed by PVC treatment (3.98 µmol/ml). On the other hand, fruits of control treatment showed the lowest TAA content (3.59 µmol/ml). Previous studies pomegranate indicated that MAP have positive effects on preventing antioxidant activity loss (Selcuk and Erkan, 2014; Selcuk and Erkan, 2015).

TMA value showed slight fluctuations during the 60 d of storage duration. In general, PVC treatment was more effective than LDPE on maintaining the TMA during the storage, although obvious decreases were observed at the end of the storage time.

Table 2. Effects of MAP on SSC (%), TEA (%), AsA (mg/100 ml), TPC (mg/100ml, TAA (µmol/ml) and TMA (mg/l) of pomegranates during storage.

Storage (days)	Treatments	SSC (%)	TEA (%)	AsA (mg/100 ml)	TPC (mg/100ml)	TAA (µmol/ml)	TMA (mg/l)
0		15.93a*	0.82a	15.68ab	439.41g	5.71a	22.35ab
30	Control	15.53bc	0.68cd	14.20b	519.41de	3.50f	22.14ab
	LDPE	15.66b	0.70b	15.17ab	464.41fg	5.41a	22.04ab
	PVC	15.40cd	0.69c	16.14a	488.58ef	4.20cd	21.44abc
60	Control	14.86f	0.61f	7.85d	681.08b	3.74ef	17.03d
	LDPE	15.46bcd	0.67d	14.57ab	509.41e	4.52b	22.95a
	PVC	15.26de	0.64e	14.93ab	556.08cd	4.13cd	20.94abc
90	Control	14.86f	0.54h	3.32e	777.75a	3.59f	17.33d
	LDPE	15.60bc	0.57g	10.30c	652.75b	4.33bc	20.24bc
	PVC	15.06ef	0.57g	7.66d	589.41c	3.98de	19.24cd
	$LSD_{0.05}$	0.24	0.02	1.59	37.13	0.32	2.47

*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.

CONCLUSIONS

Pomegranate (*Punica granatum* L.) has been gained remarkable attention with its health rich flavanoid, fenolic and ascorbic acid contents. The present study determined the effects of different modified atmosphere packaging (MAP) on postharvest quality of 'Silifke Aşısı' sweet pomegranates during cold storage. The findings revealed that MAP delayed the increase of weight loss, decreased the change in SSC, TEA and AsA. MAP also improved TMA of pomegranates at 5 °C in comparison to untreated fruits. The beneficial effects of LDPE was greater than PVC in maintaining the postharvest quality of pomegranates. Therefore, LDPE packaging could be recommended for maintaining quality, biochemical compounds, initial aril and husk color of 'Silifke Aşısı' pomegranate fruits for 90 days in cold storage.

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CURRENT POSSIBILITIES OF MICRO MINERAL USE IN LAYING HEN DIETS: A REVIEW

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ABSTRACT

Today's laying hens have been bred for rapid growth, low feed conversion ratio and high egg production. In order to make progress in this framework, it was necessary to learn the nutritional needs of laying hens in more detail. Micro-minerals are also used to make the laying hens healthier and better performance r. In this context, micro-minerals such as Selenium (Se), Zinc (Zn), Copper (Cu), Iron (Fe) and Manganese (Mn), which have a small amount but a great effect on metabolism, are used in the nutrition of laying hens. These minerals play unique and comprehensive roles in important biological processes such as, oxidative stress protection, antimicrobial, antimutagenic, immune system regulation, and growth, respectively. The use of minerals with high bioavailability, especially during the egg-laying period, not only reduces oxidative stress but also increases the performance of layers. Micro mineral application with in ovo method also contributes positively to the embryonic period and post-incubation development of the chick. The aim of this review is to bring together general information about micro minerals and current literature on the use of micro minerals in laying hen diets in recent years.

Key Words: Bioavailability**,** egg yield, laying hen, metabolism, micro mineral, performance

INTRODUCTION

Micro minerals are of great importance in the diet of laying hens because of their various roles in metabolism, directly or indirectly. The main ones are; immune system, repair of various tissues, reproductive parameters, hormonal mechanisms, growth, egg production, enzymatic reactions, and nervous system. Egg yield and egg quality are among the most important parameters in laying hens. In particular, eggshell quality is an important issue in terms of marketable eggs. Shell quality problems are observed in 10-15% of eggs produced worldwide, which causes economic losses (Stefanello et al., 2014). Most studies on eggshell quality focus on dietary calcium and phosphorus contents (Neijat et. al. 2011). However, the effects of micro minerals on shell quality are also a matter of study. They act as activators or constituents of enzymes involved in eggshell synthesis and directly interact with calcium crystals during eggshell formation, affecting the quality of the eggshell (Fernandez et. al.2008, Xiao et al. 2014).

In addition to minerals from feedstuffs, mineral additives are prepared for laying hen diets. Because the micro minerals in feedstuffs are insufficient to meet the needs of laying hens. For this reason, the mineral pre-mixes that are being prepared are used to meet the macro and micro mineral needs. Generally, micro minerals used in mineral pre-mixes are copper (Cu), iodine (I), iron (Fe), manganese (Mn), selenium (Se),zinc (Zn), magnesium (Mg), respectively. The form of the minerals used in the preparation of mineral additives is of great importance in terms of mineral bioavailability. Because not all forms of the mineral are equally useful in metabolism. For example, the utilization of Fe(II)sulphate is high, while the utilization of Fe(II) oxide is very low. Therefore, minerals are used in different inorganic forms such as phosphate, sulphate, oxide and carbonate. In addition, organic forms of minerals are also used as an alternative to their inorganic forms (Londero et. al.2020). The use fulness of organic forms is higher than inorganic forms, but their higher cost than inorganic forms limits their use. The aim of this review is to bring to get her general in formation about micro minerals and current literature on the use of micro minerals in laying hen diets in recent years.

MANGANESE

Manganese (Mn) is an essential micro element in poultry nutrition due to its role in bone and eggshell formation, enzyme activity and nutrient metabolism. It is found in all tissues and is essential for the normal digestion of lipids, protein, and carbohydrates (Zhu and Richards,2017; Tufarelli and Laudadio, 2017). Mn is important for the enzyme Mn-superoxide dismutase (SOD) that is required for additional defense against oxidative stress caused by inflammatory responses to certain infections. Mn is characterized as a micro mineral related to improved immunity or immune-supporting activities where Junior et al. (2019) revealed that organic Mn was more efficient against *Salmonella enteritidis* vaccination than inorganic Mn.

Previous studies by Darvishi et al.(2020) also stated that organic Mn showed higher bioavailability compared with their inorganic sources when fed to laying hens, where the use of 50% (50mg/kg Mn) organic and inorganic minerals affects positively laying performance and egg quality trait. Cui et al. (2019) stated that Mn deficiency decreased egg production and egg quality of hens. Mn supplementation at a dose of 120 mg Mn/kg, either from Mn sulphate, Mn chelate of a protein hydrolysate, or Mn chelate of glycine enhanced SOD levels in the hens' blood which is recognized to be one of the first components of the antioxidant defense system. Furthermore, dietary levels of 40 mg/kg from Mn-amino acid complexes in Hy-Line Brown laying hens administered a corn-soybean meal based diet (21.95 mg/kg, Mn) for 23 to 46 weeks could increase egg production, egg weight, feed efficiency, and eggshell strength (Cui et al., 2019).

IRON

Iron (Fe) is classified as a micro mineral that is needed in small amounts in poultry metabolism. Approximately 60% of the Fe in metabolism is found in hemoglobin in the bloodstream. Although predictions about Fe have focused on its relationship with hemoglobin for many years, it has been found that it has a wider physiological significance by detecting its presence in the cytochrome oxidase enzyme group (Suttle, 2010).

Previous studies by Olomola et al. (2019) reported that the addition of vitamin C and vitamin D to laying hen rations had no effect on egg Fe accumulation. Qiu et al. (2020), found that the micro minerals (Fe, Cu, Mn, Zn) used in inorganic form (control) in laying hen rations at the end of the laying period, 1/3 less in mineral proteinate form (TRT) and inorganic form (ITM). The ITM group had lower egg production, eggshell resistance, serum estrogen, lutein, glycosaminoglycan concentration and carbonic anhydrase activity compared to the control group. Egg loss increased significantly in the ITM group compared to the control group. Also, the TRT group generally obtained similar results with the control group in all parameters. In the EFSA (2020) report, the effects of the combined use of Fe-lysine chelate, glutamic acid (Iron-LG) and Fe_2SO_4 on laying hens were investigated. It has been reported that egg production, egg weight and egg iron content increase in diets using 45-60-75 mg/kg Iron-LG. In blood parameters results, it was reported that the use of 45-60-75 mg/kg Iron-IG significantly increased the amount of red blood cells and hemoglobin. Also use of Iron-LG has a positive effect on antioxidant parameters.

COPPER

Copper (Cu) is an important micro mineral due to its presence in many enzymes in metabolism. These enzymes are also involved in the activity of many cofactors and reactive proteins. Because of these properties, Cu plays a major role in reproduction and bone development. In addition, they are found in the structure of tyrosinase and lysyl oxidase enzymes that play a role in pigmentation and tissue development, and in case of deficiency, the performance of poultry is adversely affected. It plays a key role in hemoglobin synthesis, Fe metabolism and erythrocyte formation. It also plays a role in the biosynthesis and cross-linking of elastin fibers and collagen and keratin and melanin synthesis (Suttle, 2010).

A previous study by Nyungen et al. (2020) compared the effects of using Cu hydroxychloride (CH) and Cu sulphate (CS) in broiler feeds. It was reported that the use of CH improved the feed efficiency, body weight gain, bone development as well as intestinal health and performances. Liao et al. (2020) reported that the use of high levels (220-330 mg / kg) of Cu caused autophagy in the kidneys and had negative effects on energy metabolism. Zhou et al. (2020). Investigated the effects of the amino acid Cu complex (Cu-Lys-Glu) at the level 0, 15, 75, 150 and 300 mg/kg in laying hens. It was reported that the use of high levels of Cu-Lys-Glu (300 mg / kg) negatively affected the ovulation rate. The use of 0, 15, 75 and 150 mg Cu / kg Cu-LysGlu does not cause a significant difference in hematological and serum biochemical parameters, organ indices and histopathological changes. However, the use of 300 mg Cu / kg Cu-LysGlu significantly increased mean corpuscular volume (MCV), albumin (ALB), total bilirubin (TBILI), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (UN) and creatinine (CRE) concentrations. It has also been reported to cause severe microscopic histopathological changes in the liver and kidney. It has been determined that the use of 150 mg / kg Cu-Lys-Glu is sufficient on performance and health parameters in laying hens.

IODINE

Iodine (I) is the component of thyroid hormones that has a vital role in metabolism. These hormones are known as thyroxine (T4) and triiodothyronine (T3). Thyroxine is the only hormone that contains an inorganic element. Thyroxine hormone is transferred from the thyroid gland to various tissues and regulates and controls the energy metabolism events in these cells (Suttle,2010).

Albdrani et. al. (2020) reported that potassium I (KI) application by in ovo method had no effect on hatchability and embryonic death, but it negatively affected hatching weight and the amount of cholesterol and triglyceride in blood. Serum Triiodothyronine (T3) value was negatively affected but had a positive effect on T4, and no effect on TSH was found. Sarlak et al.(2020) determined that performance and egg quality criteria were not affected by the use of 2 and 4 mg/kg organic or inorganic I. However, using 8 mg/kg of both I types negatively affected egg yield and feed intake. Especially the use of high doses of organic I reduced eggshell strength and Haugh units that caused the increase of abnormal eggs in parallel with the disruption of blood serum and egg yolk lipids. Supplemention 2, 4, and 8 mg/kg I linearly increased the amount of egg shell and egg particularly by addition of organic I. However after 12 week period of supplementation does not any affect of I content of egg.

SELENIUM

Oxidative stress is a serious damaging factor for cellular integrity through the continuous release of reactive oxygen species-mediated by various biotic (bacteria, viruses, fungi, etc.) and abiotic stressors. Micro elements such as selenium (Se)with strong antioxidant potential have wide applicability as feed additives to reduce oxidative stress in living systems. The specific biochemical role of Se was revealed by the discovery that glutathione peroxidase (GPX) is a selenoprotein (SeP) and correlations between GPX activity and Se uptake in tissues emerged. Most selenoproteins protect the tissue where they are formed from free radicals (ROS) and play important role since signaling and transcription. If peroxidation gets out of control, it can initiate chain reactions of ROS formation and cause tissue damage. Se has a close relationship with vitamin E. Both nutrients protect biological membranes from oxidative degeneration. Apart from that, they are found in the composition of proteins found in spermatozoa. It has a function in RNA as it can bind to purine and pyrimidine bases. It takes part in prostaglandin synthesis. It has various functions in the thyroid glands.

A previous study by Liu et al. (2020a) investigated the effect of using different Se sources (Sodiumselenite, Se yeast) at different rates (0.3-0.5 mg / kg) in laying hens. It was reported that different Se sources had no effect on egg Se content, blood biochemistry, egg weight and feed conversion rate. However, egg yield was positively affected by the use of 0.5 mg/kg sodium selenite. Liu et al. (2020b) and Liu (2020a) found the use of different Se sources positively affected the Se content of eggs with the improvement of the antioxidant capacity in chickens. Particularly The use of 0.5 ppm Se yeast is useful in obtaining high Se content eggs. Sun et al. (2020) investigated the effects of using earthworm with increased Se content in the diet of laying hens. As a result of the study, it was found the at earth worm powder containing 1mg/kg Se showed an increasing effect on total protein, albumin, glutathione peroxidase, superoxide dismutase, IgGand IL-2 values, but had a decreasing effect on triglyceride, total cholesterol, glucose and nitric oxide amounts as well as the antioxidant level. Zhao et al. (2021) investigated the effects of using Se as selenized glucose in organic form on laying hens which could increase glutathione peroxidase and total antioxidant activity in the spleen and ovaries. Zhou et al. (2020) reported that the use of glycine nano-Se in laying hens contributes to antioxidant activity, positively affects intestinal parameters, and has no effect on egg performance and quality.

ZINC

Zinc (Zn) is an essential micro mineral in metabolism. It exists a cofactor in more than 300 enzyme structures. It plays an important role in the metabolism of fat, carbohydrate, protein, nucleic acid and cell membranes. It is necessary for the continuity of feathering, growth, skeletal development, skin quality and reproductive parameters in poultry. However, it increase in diet, immune system activity is increase. It is involved in the carbonic anhydrase activity that ensures the improvement and continuity of the eggshell quality in laying hens (Suttle, 2010).

Neto et. al. (2020) reported that the use of Zn in combination with threonine has interaction effects on egg production and egg weight in laying hens. It has been reported that the use of Zn over 40 mg/kg in layer diets negatively affects the egg quality, nutrient digestibility and energy utilization. Li et al. (2021) reported that the use of Zn methionine under in vitro conditions increases intracellular Ca^{2+} concentration and can make an extra contribution on grow thin laying hens by improving mRNA metallothione in expressions. Yu et al. (2020) reported that the use of organic and inorganic Zn has no effect feed consumption, average egg weight and egg quality, but an increase observed in Zn content in egg and antioxidant capacity in laying hens.

CONCLUSION

In fact, microminerals are often overlooked, although they play very important roles in animal nutrition science. The main reason for this is that researches are mostly focused on macro minerals. However, it is clear that micro minerals perform irreplaceable functions in metabolism, production parameters, protection of health and product quality. On the other hand, since the micro-mineral content of the feed materials that make up the compound feed is limited, it usually does not meet the needs of the animals. For this reason, micro mineral forms and amounts used in diets are constantly updated. In this way, it helps to obtain clearer information about micro minerals. Studies on the bioavailability of different mineral forms in metabolism aim to find answers to the question marks on this subject. Because different mineral forms are evaluated at different rates in metabolism. The usability of especially nano-form minerals in poultry feeds is being investigated. In this way, it is aimed both to prevent environmental pollution and to achieve a more effective performance by using less minerals. Current research is still not able to clearly reveal the needs of micro minerals in poultry nutrition. Therefore, future needs should be identified and needs updated and clarified through more effective and environmentalist research.

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DETERMINATION OF THE CHANGE IN THE PROTEIN AND CELLULOSE CONTENT OF COTTON SEED MEAL FERMENTED WITH RUMEN LIQUID IN DIFFERENT ENVIRONMENTAL CONDITIONS

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ABSTRACT

In this study, the changes in nutrient composition were investigated by fermenting cottonseed meal with rumen liquid. The research was carried out on a total of 72 samples, with 3 different initial pH (4, 5 and 6), 3 different humidity (70, 80 and 90 %) and 4 different fermentation times (1, 3, 5 and 7 day), in a 3x3x4 factorial experiment design and in two replications in each group. A one-year-old ram was used for the rumen fluid sample. Cottonseed meal obtained from a local feed mill was ground to a fineness of 2 mm and placed in a pre-prepared medium with different pH and moisture content. An equal amount of urea was added to the fermentation medium in each group. 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, crude cellulose, ash, acid detergent fiber, neutral detergent fiber. At the end of the research, it was determined that the initial pH value could not affect the fermentation and could not have an affect on the nutrient composition. Different fermentation time and different moisture content parameters affected the fermentation and it was determined that they had affects on the nutrient composition. It was determined that the crude protein content of cottonseed meal fermented on the 5th and 7th days at 80% and 90% moisture content increased from 23.9% to 35% and 45%. It was determined that the cellulose content decreased from 32.62% to 25% and 30%. As a result, it was determined that the nutrient composition was improved by fermenting cottonseed meal using rumen liquid.

Key Words: Fermentation, rumen liquid, nutrient composition, cottonseed meal.

INTRODUCTION

The cotton plant, which is systematically included in the Gossypium (G) genus of the malvacea family, is grown in many regions of the world. The fact that the world population is constantly increasing, along with the foodstuffs, increases the importance of cotton day by day. About 6-12% of the original weight of the processed cotton seed are cotton fibers and 20-25% is the shell. Cotton seed contains 19-28% oil. After extracting the oil from the seed, the remaining 35-45% is used as cottonseed meal (CSM). The remaining 3-5% of the seed is considered as waste. The most important components of poultry diets are corn and soybean meal. There is no alternative to soybean meal used as a protein source. This is a subject worth researching in terms of nutrition science. Although other feedstuffs (Cottonseed meal, sunflower seed meal, hazelnut meal, etc.) are used instead of soybean meal, their use is limited.

There are factors limiting the use of cottonseed meal in poultry feeding. The most important of these is gossypol. Studies have shown that gossypol negatively affects performance in poultry, reduces amino acid availability and negatively affects egg yolk color (Henry et. al., 2001, Robinson et. al. 2001, Francis et. al. 2001, Pelitire et. al. 2014). In addition, the high cellulose content of cottonseed meal limits its consumption and digestion. (Salah and Esmail, 1997, Sterling et. al., 2002). At this point, some new feed processing methods (physical, chemical, biological or microbial processing methods) can give effective results in improving the nutritional composition of these plant protein sources. Otherwise, the use of cottonseed meal in feeds will be limited to a maximum of 5% (Kırkpınar and Ergül, 2003). Many physical, chemical and biological methods are applied to improve the nutritional composition of feedstuffs or wastes that can be used in animal feed and positive results are obtained in these studies (Altop et. al., 2019). In particular, the fermentation method using bacteria, yeast or fungal inoculants is more suitable, economical and effective for small farms than other methods (Altop et. al., 2018). In studies on fermentation, bacteria, fungi or their combinations are generally preferred as inoculants (Adeyemi et. al., 2008; Akinfemi, 2010; Ari et. al., 2012; Ari and Ayanwale, 2012). In addition to these, there are limited studies in which rumen liquids are used as inoculants.

The fact that the cellulolytic bacteria in the rumen content are higher in small ruminant, allowing them to digest cellulose better than other ruminant animals. For this reason, it is thought that the cellulose ratio in cottonseed meal may decrease with the liquids taken from the rumen of small ruminant. However, it is not sufficient to use the only cottonseed meal as a substrate for an effective fermentation. Appropriate temperature, pH, humidity and fermentation time are also important parameters for the growth of cellulolytic bacteria. No study was found in which sheep rumen liquid was used as a fermenter or suitable conditions for fermentation were determined.

In the present study, it was aimed to reduce the cellulose content of cottonseed meal, which limits its use in poultry diets, by using sheep rumen liquid. In addition, at the end of the study, it is aimed to determine the humidity, pH and fermentation time conditions in which the desired properties occur in the fermented cottonseed meal.

MATERIAL AND METHOD

The cottonseed meal used in the study was obtained from the feed factory operating in the Samsun region. Before the study, cottonseed meal was ground to 3 mm size and made ready for fermentation. The rumen liquid for fermentation was obtained from 8 months old and 45 kg weight sheep. The amount of roughage in the sheep diet was increased to 15 days before the rumen liquid was taken. In this way, it is aimed to increase the amount of cellulolytic bacteria in the rumen microflora. The rumen liquid obtained from the sheep was filtered into a preheated erlenmayer at 38°C.

The study was carried out on a total of 72 samples, with 3 different initial pH (4, 5 and 6), 3 different humidity (70, 80 and 90 %) and 4 different fermentation times (1, 3, 5 and 7 days), in a 3x3x4 factorial experiment design and in two replications in each group.

Before fermentation, cottonseed meal was sterilized at 121°C for 10 minutes. Afterwards, 1% rumen liquid was added to each 50 g of PTK under aseptic conditions. In addition, 40 g of urea per 1 kg was added to the fermentation medium to be used as a nitrogen source. The study was carried out in an anaerobic environment and at a temperature of 38 °C. The plan of the study is summarized in table 1.

Table 1. The plan of the study

	1. Day	3. Day	5. Day	7. Day
Ph 4-5	CSM-1-4-70-1	CSM-3-4-70-1	CSM-5-4-70-1	CSM-7-4-70-1
	CSM-1-4-70-2	CSM-3-4-70-2	CSM-5-4-70-2	CSM-7-4-70-2
	CSM-1-4-80-1	CSM-3-4-80-1	CSM-5-4-80-1	CSM-7-4-80-1
	CSM-1-4-80-2	CSM-3-4-80-2	CSM-5-4-80-2	CSM-7-4-80-2
	CSM-1-4-90-1	CSM-3-4-90-1	CSM-5-4-90-1	CSM-7-4-90-1
	CSM-1-4-90-2	CSM-3-4-90-2	CSM-5-4-90-2	CSM-7-4-90-2
Ph 5-6	CSM-1-5-70-1	CSM-3-5-70-1	CSM-5-5-70-1	CSM-7-5-70-1
	CSM-1-5-70-2	CSM-3-5-70-2	CSM-5-5-70-2	CSM-7-5-70-2
	CSM-1-5-80-1	CSM-3-5-80-1	CSM-5-5-80-1	CSM-7-5-80-1
	CSM-1-5-80-2	CSM-3-5-80-2	CSM-5-5-80-2	CSM-7-5-80-2
	CSM-1-5-90-1	CSM-3-5-90-1	CSM-5-5-90-1	CSM-7-5-90-1
	CSM-1-5-90-2	CSM-3-5-90-2	CSM-5-5-90-2	CSM-7-5-90-2
Ph 6-7	CSM-1-6-70-1	CSM-3-6-70-1	CSM-5-6-70-1	CSM-7-6-70-1
	CSM-1-6-70-2	CSM-3-6-70-2	CSM-5-6-70-2	CSM-7-6-70-2
	CSM-1-6-80-1	CSM-3-6-80-1	CSM-5-6-80-1	CSM-7-6-80-1
	CSM-1-6-80-2	CSM-3-6-80-2	CSM-5-6-80-2	CSM-7-6-80-2
	CSM-1-6-90-1	CSM-3-6-90-1	CSM-5-6-90-1	CSM-7-6-90-1
	CSM-1-6-90-2	CSM-3-6-90-2	CSM-5-6-90-2	CSM-7-6-90-2

After fermentation, dry matter, crude ash, crude protein, crude fiber, NDF (Neutral Detergent Fibre) and ADF (Acid Detergent Fibre) analyzes of the samples were carried out according to the Weende (Henneberg and Stohmann, 1858) and Van Soest (1991) analysis methods.

The data were analyzed by using the Windows version of SPSS 21.0 (SPSS Inc., NY, and USA) statistical package program. Duncan test was used to compare the differences between groups after ANOVA test was used for the variance of data.

RESULTS AND DISCUSSION

Nutrient changes in cottonseed meal at the end of fermentation are shown in the figures given below. As a result of the study, it was determined that the initial pH value had no effect on fermentation ($P>0.05$).

With the addition of urea to the fermentation medium, the amount of nitrogen in the medium increases. Depending on this increase, there was an increase in crude protein results. When the day-based results were examined, it was determined that the crude protein ratio on the 1st day was higher than the other days and the difference between the other days was statistically significant ($P<0.05$). However, this difference is due to the fact that urea nitrogen cannot be evaluated by bacteria in a 24-hour period. Because, in the following fermentation periods (3rd day and 5th day), there is a decrease in crude protein ratio and this is due to the make use of urea nitrogen by rumen bacteria. There was no statistical difference in crude protein values between the 3rd, 5th and 7th days ($P>0.05$). The humidity content of the medium is important for successful fermentation. Crude protein content were found to be higher in 70% and 80% humidity fermentation groups. There was no statistical difference between the 70% and 80% humidity environment groups ($P>0.05$). However, the difference between the two groups (70 and 80%) and the 90% humidity group was statistically significant ($P<0.05$).

Crude fiber analysis results are shown in figure 2. It was determined that the fermentation process caused an increase in the crude fiber content of cottonseed meal. These results are similar to Altop (2019). The reason for this increase at the end of fermentation may be due to the nutrient composition of the cottonseed meal, the microorganism type of the sample taken from the rumen liquid, or the movement of water-soluble nutrients from the cottonseed meal to the fermentation medium. In the study, it was determined that different ambient humidity had an effect on crude fiber. Especially the difference between 70% and 90% humidity content was found to be statistically significant ($P<0.05$), and there was no difference between 80% humidity and both humidity values.

The crude ash results are shown in figure 3. As a result of fermentation, a decrease in crude ash values was determined in all groups. According to these results, it can be said that the fermentation was successful. The reason for this may be that rumen bacteria produce organic matter by fermentation. It has been determined that the fermentation time has an effect on the crude ash values. There was no statistical difference in the amount of crude ash between the 3rd and 7th days ($P>0.05$). On the 5th day, the amount of crude ash was found to be higher than the 3rd and 7th days, and the difference between them was found to be statistically significant ($P<0.05$). In the study, it was determined that different ambient humidity had an effect on crude ash. Especially, the difference between 80% and 90% humidity content was found to be statistically significant ($P<0.05$), and there was no difference between 70% humidity and both humidity values.

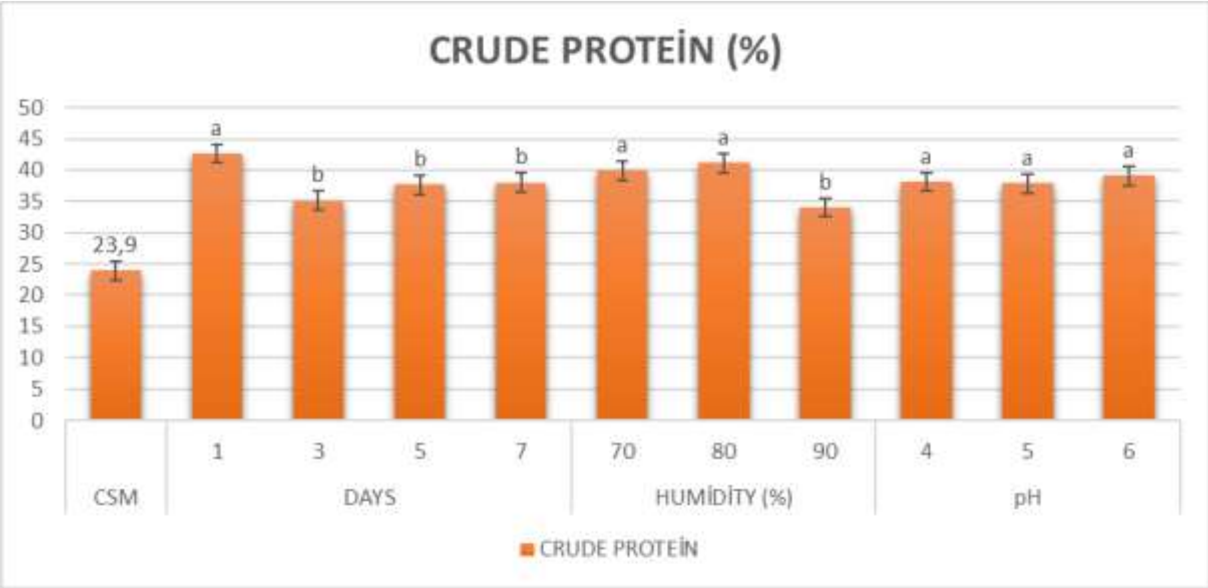

Figure 1. Crude protein amounts of cottonseed meal after fermentation

a-c; In the same column, the differences between the means of the relevant parameters with different letters are statistically significant ($P<0.05$).

The results of NDF and ADF analyzes are shown in figures 4 and 5. At the end of the study, it was determined that the NDF and ADF contents of fermented product increased. These results are similar to Altop (2019). These results may have occurred for reasons similar to the results of crude fiber analysis. Apart from the nutrient analysis, it is thought that more precise results will be obtained by analyzing the carbohydrates, amino acids and dissolved nutrients in the fermented product. It has been determined that the fermentation time and humidity content have a statistical effect on both parameters. It was determined that there was a statistically significant difference in NDF values between the 1st, 3rd and 7th days of fermentation. A statistical difference was found in NDF values in fermentation carried out in 80% and 90% humidity conditions. When the ADF values was determined that the 1st day and 5th day fermentation results were similar. When the ADF values was determined that the 1st day and 5th day fermentation results were similar. ADF results obtained by fermentation in a 90% humidity environment were statistically different from other humidity groups.

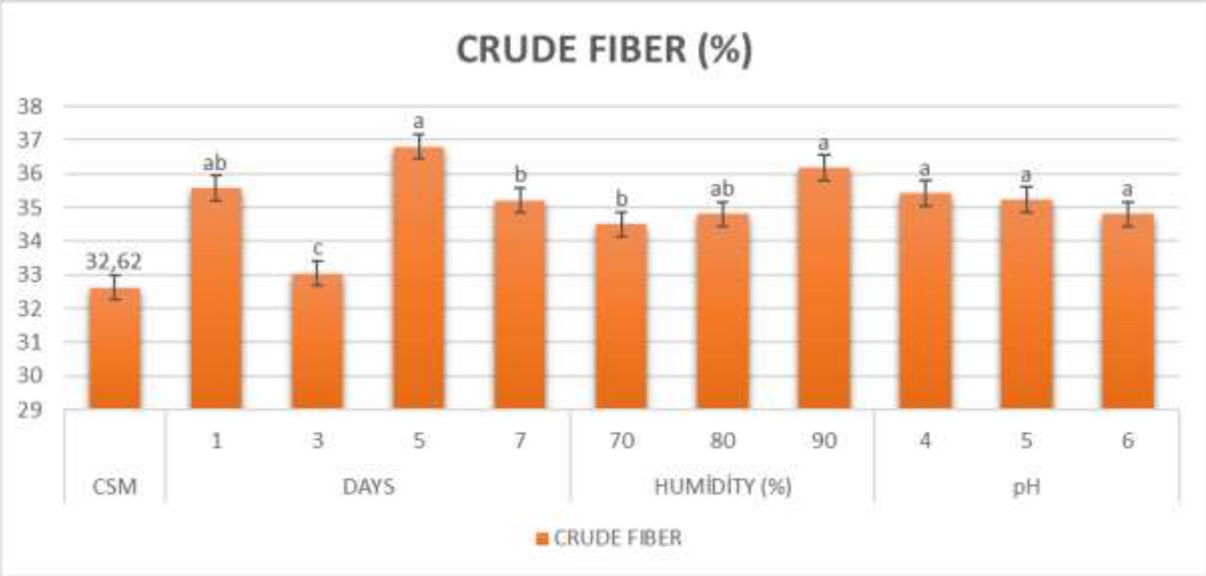

Figure2. Crude fiber amounts of cottonseed meal after fermentation.

a-c; In the same column, the differences between the means of the relevant parameters with different letters are statistically significant (P<0.05).

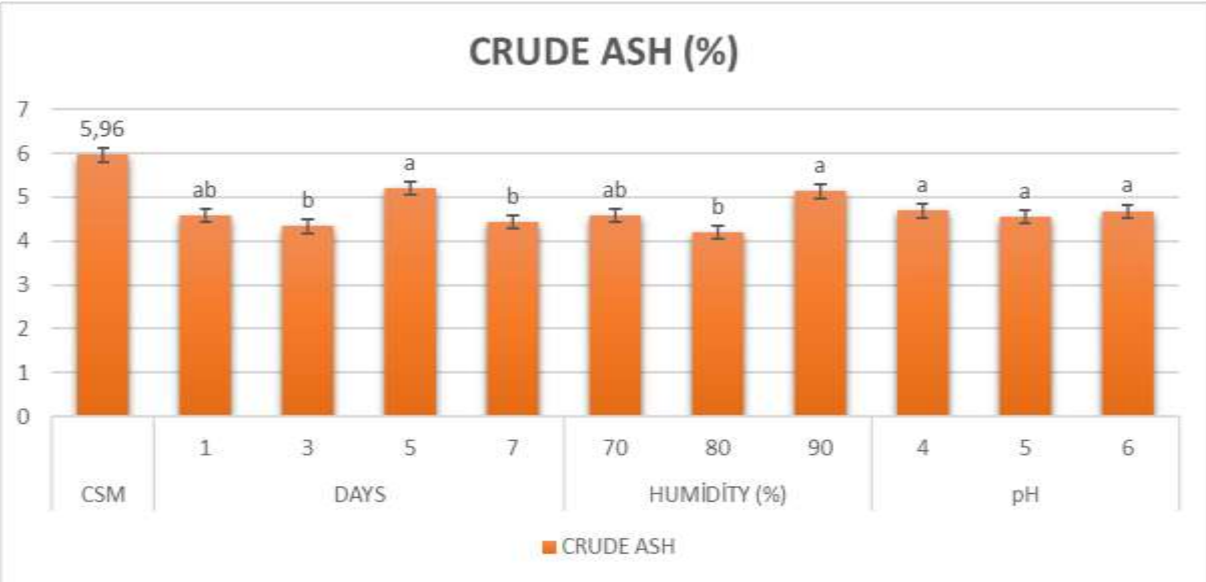

Figure 3. Crude ash amounts of cottonseed meal after fermentation.

a-c; In the same column, the differences between the means of the relevant parameters with different letters are statistically significant (P<0.05).

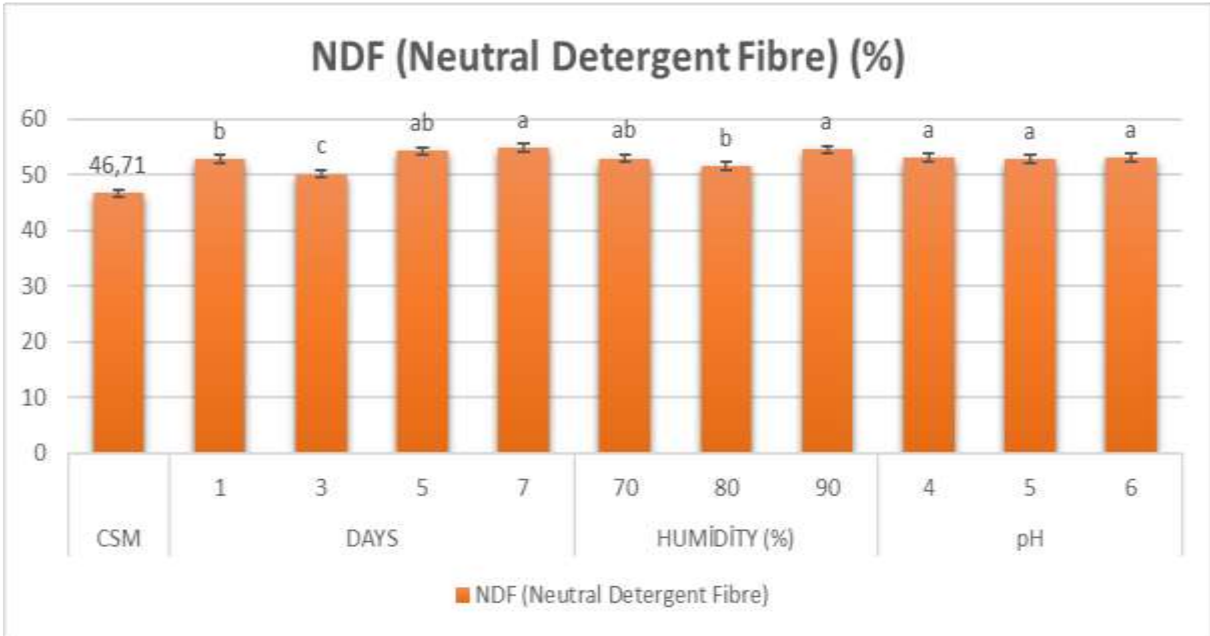

Figure 4. Neutral detergent fibre amounts of cottonseed meal after fermentation.

a-c; In the same column, the differences between the means of the relevant parameters with different letters are statistically significant (P<0.05).

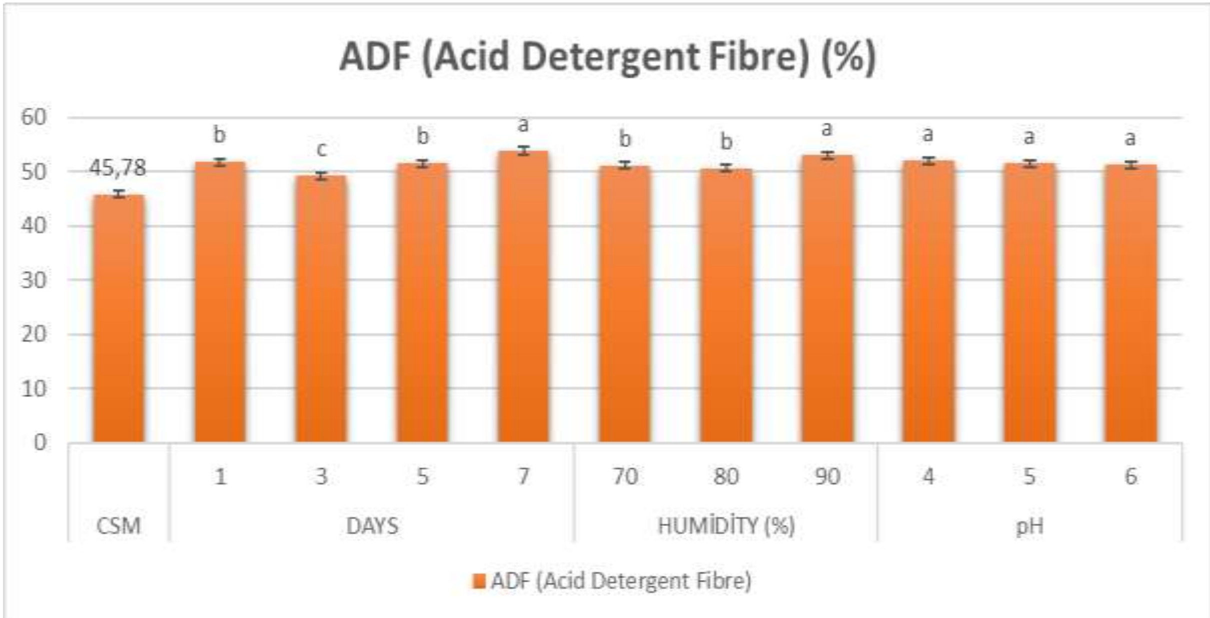

Figure 5. Acid detergent fibre amounts of cottonseed meal after fermentation.

a-c; In the same column, the differences between the means of the relevant parameters with different letters are statistically significant (P<0.05).

CONCLUSIONS

At the end of the study, it can be said that cottonseed meal fermented with rumen liquid has a positive effect especially with the addition of urea to the medium. The initial pH value was not found to be important for fermentation, but it was determined that the fermentation time and ambient humidity effected the fermentation. The results obtained from this study provide data for future studies. Research on this subject needs to be continued and progressed. When the results obtained in this study were examined, it was determined that 3 or 5 days and 80% humidity for optimum fermentation.

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USE OF VITAMINS IN LAYING HEN DIETS WITH CURRENT APPROACHES: A REVIEW

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ABSTRACT

Vitamins perform several functions in a reduction in the risk of disease, maintenance of good health and are essential for growth and metabolism. The vitamins are of two types, one is fat-soluble (vitamin A, D, E, and K) and the other is water-soluble (vitamin B complex group and vitamin C). Vitamins have a positive effect on the growth, development of reproductive organs, egg production and egg quality for laying hens. In addition to these basic roles, vitamins A, D, E play critical roles in immune system development, antioxidant effects and . have important role development and eggshell formation. This review is divided into sections on the different vitamins with a description of its role in laying hens. Then, there is a discussion of various studies applying different vitamin levels effects on production parameters, egg quality, the immune system of the laying hens.

Key words: egg production, egg quality, immunity, laying hens, vitamin

INTRODUCTION

Vitamins are organic substances that are not a group of carbohydrates, proteins, or lipids, and vitamins are also required in very small amounts (mg or µg) and they perform several functions in a reduction in the risk of disease, maintenance of good health and are essential for growth and metabolism (Akram et al. 2020). The vitamins are of two types, one is fat-soluble (vitamin A, D, E, and K) and the other is water-soluble (vitamin B complex group and vitamin C). Fat-soluble vitamins (A,D, E, and K) are digested and absorbed in the same way as fats. In the body, they are stored in fatty tissues, mainly the liver and adipose tissue, which acts as a reserve for the animal, but in excess is excreted in bile through the feces, but it canreach toxic levels especially vitamins A and D (Reddy and Jialal, 2020).

For laying hens, vitamins have a positive effect on the growth, development of reproductive organs, egg production and egg quality. Vitamin A and D can impact the gut microbiota by protecting intestinal barrier integrity and immune status, thus guarding the host from certain diseases (Riccio and Rossano,2018). Vitamin E is necessary for the cellulardefense system that plays an important role in scavenging free radicals, preventing lipids peroxidation, and protecting animals from the adverse effects caused by oxidative stress (Attia et al.,2018) and it is also essential to maintain fertility and hatchability in parent stocks (Shojadoost et al.,2021). Meanwhile, vitamin K has a vital role in the secretion of male sex hormones such as testosterone and calcium metabolism through activating the calcium-binding proteins that assistance to build and maintain calcium for bone as well as an eggshell (Katarzyna,2015; Elbossaty, 2018).

In laying hen diets, choline is needed for the formation of phosphatidylcholine that is the most abundant phospholipid in the body which is not only a major component of cellular membranes and but also needed for cell division and growth (Arias et al., 2020). Also, folic acid is important for methionine metabolism and plays a critical role in breeder performance and folic acid deficient embryos preserve methionine, rather than catabolize it to cysteine (Lu et al., 2021). Meanwhile, myo-inositol is needed to be involved in energy and lipid metabolism, bone and muscle formation, reproduction, general metabolic performance and glucose metabolism (Gonzalez-Uarquin et al., 2021). PABA is a cofactor and precursor in the synthesis of folic acid, purines and thymine in most species of bacteria, algae and higher plants (Krátký et al., 2019).

This review is divided into sections on thedifferent vitamins with a description of its role in laying hens. Then, there is a discussion of various studies applying different vitamin levels effect on production parameters, eggquality, the immune system of the laying hen.

VİTAMİN A

Vitamin A has importance in metabolic processes. Under conditions of adequate dietary vitamin A, the liver is the major site of vitamin A storage, with over 95% of the total neutral retinoid being present as retinyl esters, predominately retinyl palmitate, and stearate (Harrison,2019). Vitamin A binds to proteins in the cytosol and converts dietary vitamin A (retinol) to 11-cis retinaldehyde, which serves for the visual pigment rhodopsin that is important for sight, especially in poultry adapting to low-intensity light in intensive production. Vitamin A has important effects on bone growth and thus the development of young animals, the quantity and quality of semen produced, the growth and differentiationof epithelial tissues of the reproductive system, and the embryo, among others. Finally, vitamin A contributes to maintaining lysozyme stability inside the cells (Barroeta et al., 2012)

The recent studies on supplementation of vitaminA in laying hens diets under heat stress condition at the level of 0, 8000, and 16,000 IU/kg was conducted by El-Hack et al.(2016). They reported that dietary vitamin A up to 16,000 IU/kg diet significantly improved all productive traits without affected egg quality. Elsherif (2017) founded that dietary vitamin A before and during early egg production of laying hen had a beneficial effect on egg production and egg mass laying hens. Egg production at peak period was higher with supplementation of vitamin A at levels from 30,000 to 50,000 IU than either 10,000 or 20,000 IU Vitamin A. The other studies on supplementation of vitaminA levels in broiler breeder at 46 to 56 weeks of age was conducted by Chen et al. (2015) and results proved that the level of 5,400 IU/kg was improved egg production and egg mass. Another result reported by Yuan et al. (2014), supplementation in a high level of vitamin A (45,000 IU/kg) decreased the laying performance and excessive vitamin A also increased the concentrations in the yolk and liver. Therefore, the authors indicated a linearrelationship between dietary dosages used and vitamin A levels in the liver. According to Yuan et al. (2014) supplementation of vitamin A from 5,000 IU/kg to 20,000 IU/ kg appears to increase the Newcastle disease virus (NDV) antibody titer, but supplementation from 20,000 to 35,000 IU/kg decreased the NDV antibody titer.

VITAMIN D

Vitamin D is a group of ester compounds whose main function is to regulate the functions of genes essential for calcium absorption and phosphorus metabolism, bone mineralization and egg formation. Ergocalciferol (D2) is a compound of plant origin and is synthesized from ergosterol (plant steroid), whereas cholecalciferol (D3) is synthesized from precursor 7-dehydroxycholesterol, which is present exclusively in animal tissues. Hens reared in the closed house must have a supplementary of vitamin D due to insufficient endogenous synthesis (Swiatkiewicz et al., 2017).

Vitamin D3 is converted in the liver to become its primary circulating form of 25-hydroxycholecalciferol (25OHD) and dietary 69 μg/kg of 25OHD can increase in eggshell strengthen and lightness in high stocking density of laying hens compared to regular vitamin D supplementation (Wang et al., 2020). Supplementation of vitamin D2 in combination with vitamin D3 could result in an increase in Ca and P utilization, rate of laying, and egg mass in the late laying hen cycle (Adhikari et al.,2020; Attia et al., 2020). Also, the dietary vitamin D3 at 2,760 IU/kg with 25OHD at 2,760 IU(69µg/kg) increased the growth rate and bone size of pullets and also increased bone mineral deposition and improvement of structural during the early laying period at 18 to 60 weeks of age (Chen et al.,2020) which early bone development is very important before sexual maturity, and its prolonged effects on bone health during laying periods (Casey- Trott et al., 2017).

A previous study reported by Geng *et al.* (2018) that vitamin D_3 supplementation at 500, 1500, and 3000 IU/ kg decreased IgG and IgM in the vitaminD3-sufficient hens compared with the non supplemented hens under *Escherichia coli* lipopolysaccharide (LPS) challenge. Thus, they found that vitamin D3 supplementation could be beneficial to protect layer hens in preventing immunological stress chickens by induced splenic immunological stress through suppressing the transcription of NF-κB genes. Morris et al. (2015) found that a high dietary level of 25-OH-D3 (100 µg/kg) had a positive influence on immune response in layer chickens after the *coccidia* challenge.

VITAMIN E

Vitamin E activity is fat-soluble compounds consisting of α, β, γ, and δ-forms of tocopherols and tocotrienols. α-tocopherol is most abundant in animal tissues that have the highest activity (Yang et al., 2020). Vitamin E is necessary for the cellulardefense system in the face of oxidation. In poultry, vitamin E also has a crucial role in the prevention of nutritional encephalopathy and myopathies in chickens and turkeys (Klasing and Korver, 2020). Also, it is essential to maintain fertility and hatchability in parent stocks (Shojadoost et al.,2021).

In a recent study, the addition of 30 IU/kg vitamin E to the layer hen diet improved serum superoxide dismutase and glutathione peroxidase as antioxidant enzymes (Liu et al., 2019). Ding et al. (2021) also reported that the addition of 100 IU/kg vitamin E prevented aged corn-induced lipid peroxidation in laying hens, possibly via a direct increase in antioxidant enzyme activities and enhancing the relative expressing of antioxidant genes (SOD1mRNA) in the ovaries. The addition of vitamin E 60 IU/kg feed increased egg production yolk weight, albumen weight and vitelline membrane strength (Parolini et al., 2015) and similar result founded by Karadas et al. (2017) that the supplementation of vitamin E at 125 to 300mg/kg improved egg production and eggshell density. Additionally, vitamin E improves laying hens performance by reducing the negative effects of corticosterone hormone induced by heat (Li et al., 2020). Indeed, vitamin E protects male poultry reproductive systems against oxidative damage (Fouad et al., 2020). Dietary 200 mg of Vit E/kg diet increased semen quality and fertility rate (Zanussi et al., 2019). Vitamin E also reduced lipid peroxidation of the sperm membrane and enhanced the functions of sperm mitochondria (Asl et al., 2018).

VITAMIN K

Vitamin K has important physiological functions which relate to blood coagulation, bone turnover and strength, inhibition of arterial calcification, and anti-inflammatory (O'Sullivan et al., 2020). Vitamin K derived from plants is phylloquinone (K_1), vitamin K obtained from bacterial fermentation is menaquinone (K_2) and vitamin K_3 or menadione is produced through chemical synthesis and is the formnormally used for feeding animals (Walther and Chollet, 2017). Vitamin K plays important role in the activation of calcium-bound protein which responsible for transfer calcium into bones. Also, it increases the secretion of male sex hormones such as testosterone (Elbossaty, 2018).

In layıng hens, Fares et al. (2018) reported that the supplementation of up to 19 mg/kg vitamin K3 increased egg production, egg mass, egg weight, feed efficiency, eggshell weight percentage and shell thickness, but it significantly decreased embryonic mortalities during 15-21days of incubation. The other result reported by Souza et al. (2017) that supplementation of 17.86 mg/kg vitamin K3 and 1.4% Ca in the pullets phase presented greater levels of total serum Ca and it indicates that there is an increase in Ca binding proteins, which can lead to greater Ca absorption with a consequent increase in serum levels. These results suggest that these hens had better bone reserves for labile calcium and the formation of eggshells.

CONCLUSION

For laying hens, vitamins have a positive effect on the growth, development of reproductive organs, egg production and egg quality. Moreover, vitamins A, D, E play critical roles in immune system development and antioxidant. Also, vitamin D and vitamin K have important role in calcium and phosphorus metabolism that its well-documented involvement in bone development and eggshell formation. Nevertheless, some research has shown inconsistent results concerning dose–response relationships for some vitamins.

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EFFECTS OF POSTHARVEST UV-C TREATMENTS ON POSTHARVEST QUALITY DURING COLD STORAGE IN APRICOT CV. ROXANA

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ABSTRACT

UV-C irradiation treatment is one of the alternative methods to chemical applications in preventing postharvest pathogen-induced deterioration and increasing the storage life of horticultural products. Especially, UV-C treatments at 254 nm wavelength create a small amount of stress response in the product, increasing its resistance after harvest. In this study, the effects of UV-C irradiation applications at different times on the fruit quality of apricot cv. Roxana during cold storage were investigated. In the study, 8 UV lamps of 15 watts, 230V/50Hz were used. Fruits harvested at the stage when ¾ of the fruit surface turns straw yellow were brought to the laboratory in the Department of Horticulture, Faculty of Agriculture, Selcuk University. After the selection, the fruit samples were kept in a specially designed cabinet for this purpose at 4 different times (5, 10, 20 and 30 minutes) for UV-C irradiation application. Following the applications, the fruits were stored for 35 days in a cold storage containing 1 °C and 90% relative humidity. Weight loss, flesh firmness, skin color (hue angle), soluble solid content (SSC), titratable acidity (TEA), maturity index (SSC/TEA), total phenolic content, total antioxidant content, polygalactronase enzyme activity and visual quality were determined in the samples by removing them at 7 day intervals. At the end of the 35-day storage period, it was determined that UV-C applications were effective in maintaining quality losses when compared with control. Among the UV-C applications, 10 minute UV-C application was more effective than the other treatments. Considering the overall findings, it was determined that 10 minutes of UV-C application gave effective results in extending the storage period by preserving the quality properties of apricots during cold storage.

Key Words: Apricot, storage, UV-C, quality

INTRODUCTION

Apricot, belonging to the Prunus genus of the Rosaceae family (*Prunus armeniaca* L.), is a fruit that covers a wide geographical area including its homeland Central Asia and Western China (Bailey and Hough, 1979; Gülcan et al., 2001). According to 2019 data, 846.606 tons of apricot production was recorded in 131.178 hectares in Turkey. According to world production values, Turkey is the leader in apricot production, followed by Uzbekistan, Iran, Italy and Algeria, respectively (FAO, 2019).

About half of the apricots grown in our country are used for fresh consumption and half for drying. Apricot is one of the most popular fruits consumed in summer with its color, taste, aroma, high vitamin A and dietary fiber content (Asma et al., 2017). Apricot is a climacteric product that continues to mature after harvest. Since the quality of apricot depends on the distribution and marketing system, the prolongation of the distribution period reduces the marketing system and deteriorates the quality. Therefore, apricots should be harvested in early periods and kept at low temperatures to preserve their quality (Mencarelli et al., 2005). However, when harvested early, the fruits have low taste and aroma when they reach the consumer. The inadequacy of the cold chain system, storage and postharvest practices in the marketing stages of fresh apricots in Turkey reduces its quality. If good results are obtained in the cold chain, losses are reduced, quality is increased and profit is increased. In addition, these results increase consumption in the domestic market (Çalhan, 2010).

In order to control physiological disorders and pathological diseases that cause product loss after harvest, some physical and chemical applications are performed before and after harvest. In chemical applications, less harmful or harmless methods have been started to be researched in recent years due to the residues left on the product by fungicides, which are used as a reducing agent of decay factor in recent years (Klein et al., 1990; Wilson et al., 1994). Controlled and modified atmosphere storage, storage at hypobaric pressure, precooling, heat treatmentss, microwave applications, and ultraviolet light (UV) applications are physical methods used (Erkan et al., 2009; Turtoi, 2013; Usall et al., 2016).

UV-C light application is a tool approved for postharvest use in fresh fruits and vegetables by the United States Food and Drug Administration (FDA), and is used to control microorganisms in products and increase resistance to diseases, delay ripening and improve postharvest storage life method (Sen and Karaçalı, 2005; Pinheiro et al., 2015). When microorganisms on the surface of fruits and vegetables are exposed to UV rays, the DNA level is affected, which causes their death by damaging the reproliferation structures in the cells (Pinheiro et al., 2015). At the same time, these applications increase the production of phytoalexin compounds, which protect the products against subsequent infections (Sakaldas, 2012). With UV-C application, the activity of enzymes that are effective in defense increases, bacterial growth is prevented, and it causes gene expression related to resistance to diseases, it may consequently be effective in delaying ripening and aging in fruits and vegetables (Gil et al., 2009; Xu et al., 2016).

In this study, the effects of UV-C light applications at different times on the fruit quality of apricot cv. Roxana were investigated under cold storage and shelf life conditions.

MATERIAL AND METHOD

Apricot cv. Roxana harvested from a commercial orchard in Yalvaç (Isparta) region was used in the study. Apricot fruits harvested when ¾ of the fruit color turned orange were brought to the laboratory of Horticulture Faculty of Agriculture Selcuk University under suitable conditions. The damaged fruits were separated, a sample fruit was selected in terms of size and color, and divided into 6 equal parts for postharvest applications.

The first group of fruits were weighed in the plastic cups without any application and placed in the cold storage as the control group fruits in the open air. The second group of fruits were placed in plastic cups without any application, using Xtend® modified atmosphere bags specially produced for the species, and stored in cold storage. For the last four groups of fruits applied, 254 nm wavelength light was used in UV-C light applications. In the study, 8 UV lamps of 15 watts, 230V/50Hz were used. After the fruits were selected, they were arranged under and above the lamps for UV-C light application. The fruits were kept under UV-C lamp for 4 different times (5, 10, 20 and 30 minutes) and the application doses were determined in kJ/m^2 with a radiometer during the application. The treated fruits were placed in such a way that there were 18 fruits in each replication. Treated fruits were placed in modified atmosphere bags. Control fruits and treated fruits were stored for 35 days in a cold storage with 1 °C temperature and 90% relative humidity, and quality parameter analyzes were carried out by removing the fruits from the storage at 7 days intervals.

Weight loss was calculated by weighing the apricot fruits, whose weights were determined at the beginning of storage, during storage and the differences were calculated as percent weight loss (%). The fruit flesh firmness of the apricots was measured with a digital penetrometer from two mutual points using an 8 mm tip, and the results were determined as Newton (N). In order to determine the color changes in the skins of apricot fruits during storage, the fruit samples taken out of the storage were carried out by reading the CIE L* a* and b* values on their opposite surfaces using a CR 400 model Minolta brand color device. Hue angle (h°) values were calculated to determine the color changes (McGuire, 1992). The amount of soluble solid content (SSC) was measured by hand refractometer in the juice obtained by squeezing the apricot fruits in each application and the results were given as %. The titratable acidity (TEA) of the juice obtained was titrated with 0.1 N NaOH until the pH was 8.1 and the results were expressed as % malic acid. It was calculated by the ratio of the amount of SSC to the amount of titratable acid to determine maturity during storage (Cemeroğlu, 2007). Fruits were examined in terms of sensory analysis during the analysis periods. A scale of 1-9 in terms of external appearance, firmness and color in apricot fruits by the panelists were used [1: poor-not edible, 3: medium-consumable limit (material defects do not affect the marketability of the product), 5: good, marketable limit (small defects do not affect the marketability of the product), 7: Very good, 9: Excellent] (Bayindir, 2011; Hayta and Aday, 2015).

Fruit extracts for antioxidant and phenol analyses were prepared using method described by Thaipong et al. (2006) with certain modifications. Five grams toma-to tissue was homogenized in methanol using Ultra-Turrax homogenizer (IKA, T18 digital, Staufen, Ger-many) for 1 min. The homogenates were kept at 4°C for 14-16 h and then centrifuged at 8000 x g for 15 min at 5°C. The supernatants were recovered and stored at -20°C in dark color bottles until analysis. Total phenols were determined according to the method of Singleton et al. (1999) with slight modifications. The 0.1 ml extract, 6.0 ml distilled water and 0.5 ml Folin-Ciocalteu's reagent were mixed and then were vortexed. The mixture were incubate 3 min and then 20% sodium carbonate solution supplemented and volume was made up 10 ml distilled water. The solution was incubated at 25°C for 2 h and the absorbance was measured at 760 nm. The content of total phenols was calculate basis of the calibration curve of gallic acid and was expressed as mg/100 g FW.

Ferric Reducing Antioxidant Power (FRAP) method was used to determine the total antioxidant activity. 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. FRAP antioxidant activity was expressed as µmol/g/fresh weight by calculating the values obtained with the standard curve of trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) prepared at concentrations of 10-100 µmol l-1 (Benzie and Strain, 1996).

Polygalacturonase enzyme activity (Miller, 1959; Pathak and Sanwal, 1998) was made by performing some modifications to the DNS method. 100 µl of pectin solution was added to 20 µl of sample and it was kept in an oven at 30 °C for 10 minutes. Removed from the oven and added 120 µl of DNS. It was kept in a water bath at 96 °C for 4 minutes. It was then cooled in ice for 3 minutes. Reading was made in the spectrophotometer at a wavelength of 530 nm (Canan, 2012). The experiment was carried out according to the randomized design with three replications and each replication contained 18 fruits. The data obtained from the experiment were subjected to analysis of variance using the JMP package program and the differences between their averages were grouped according to Student's t-test multiple comparison test ($p<0.05$).

RESULTS AND DISCUSSION

Effects of postharvest UV-C treatments on fruit weight loss, flesh firmness and skin color during cold storage were presented in Table 1. Postharvest applications were effective in delaying the increase in weight loss that occurred as the cold storage period progressed, and this effect was statistically significant. At the end of the 35 day storage period, the highest weight loss occurred in the control group fruits (28.85%), followed by 30 min UV-C (2.90%), 20 min UV-C (2.78%), 10 min UV-C (2.67%). and 5 min UV-C (2.62%)

applications. The least weight loss occurred in fruits stored in MAP (2.61%). Five min UV-C (2.62%) and 10 min UV-C (2.67%) applications were statistically in the same group as MAP (2.61%). One of the most important problems encountered during the storage of fresh fruits and vegetables is the weight loss of the products with the prolongation of the storage period. Weight losses in products are due to the removal of water as a result of respiration. Water losses of more than 5% in many horticultural products cause them to look pale and shriveled, and as a result of severe water losses, a decrease in the fresh weight of the product will occur, and economic negativities are encountered (Koyuncu, 2017). Postharvest UV-C light application alone or in combination with different applications were reported to be effective in blackberry (Xu et al. 2016), cherry (Kocak and Bal, 2017; Durmaz, 2019), strawberry (Sabir et al., 2018) and grape (Sabir et al., 2020) for reducing weight loss. The findings of the present study about the effect of postharvest UV-C application on weight loss in apricot cv. Roxana were similar to those of the mentioned researchers.

The effect of postharvest UV-C applications on fruit flesh firmness was statistically significant. The firmness value of the fruit, which was determined as 57.99 N at the beginning of storage, decreased with the progression of the storage period, and it was determined that postharvest applications were effective in delaying such decrease. At the end of the 35 day storage period, the highest fruit flesh firmness value was measured in the fruits treated with 10 minutes of UV-C (26.43 N) with a loss of firmness of 54.42% compared to the initial value. The lowest firmness was determined in the control fruits (3.99 N), a firmness loss of 93.12% occurred in this group compared to the initial value.

Changes in skin color during ripening and storage in fruits are an important quality criterion. Among the color features, the hue angle value of the skin is an important color parameter in monitoring this change in color, and with maturation, a decrease in the hue angle value occurs due to the darkening of the skin color. The change in hue angle value was found to be statistically significant in fruits stored in cold temperatures for 35 day. The hue angle value, which was 80.44° at the beginning of storage, was determined at the highest value in 10-minute UV-C application (79.96°) at the end of the 35-day storage period, while the lowest value was detected in the control group fruits (68.45°).

The SSC value, which was measured as 10.53%, showed fluctuations at different rates in all applications with the progression of the storage time (Table 2). While the highest increase occurred in the control group fruits during the storage period (14.73%) at the end of the storage period. The lowest SSC value was found in 10 min UV-C (9.73%) application. The TEA value of the fruits decreased with the progression of the storage period. It was determined that postharvest applications during the 35 day storage period were generally effective in slowing down the change in TEA value, but this effect was not statistically significant. At the end of the 35 day storage period, the lowest TEA value was measured in fruits treated with 5 min of UV-C (1.05%), while the highest value was measured in fruits stored in MAP (1.26%). While the maturity index value increased significantly in apricot fruits stored for 35 day in cold storage (Table 2). The maturity index value of apricots stored in the cold changed according to the postharvest applications.

Table 1. Effects of postharvest UV-C treatments on fruit weight loss (%), flesh firmness (N) and skin color (h°) during cold storage

Treatments	**Storage (days)**					
	0	**7**	**14**	**21**	**28**	**35**
Weight loss (%)						
Control	0.00 p	5.68 e	11.57 d	18.02 c	22.79 b	28.85 a
MAP		0.99 no	1.27 l-o	1.61 i-n	2.21 f-j	2.61 fgh
5 min UV-C		0.87 o	1.21 mno	1.67 i-n	2.00 h-l	2.62 fgh
10 min UV-C		0.83 o	1.38 k-o	1.57 i-o	2.07 g-k	2.67 fgh
20 min UV-C		1.10 no	1.48 j-o	1.66 i-n	2.06 g-k	2.78 fg
30 min UV-C		1.11 no	1.46 k-o	1.86 i-m	2.27 f-i	2.90 f
Firmness (N)						
Control	57.99 ab	47.49 e	31.70 ij	24.09 n	12.81 r	3.99 s
MAP		50.33 d	34.52 gh	29.53 jk	27.67 klm	16.04 q
5 min UV-C		56.06 bc	36.54 g	33.37 hi	28.82 kl	17.07 pq
10 min UV-C		59.82 a	42.58 f	32.16 hij	27.66 klm	26.43 lmn
20 min UV-C		53.88 c	42.17 f	25.67 mn	25.79 mn	20.95 o
30 min UV-C		43.35 f	28.04 klm	30.04 jk	19.11 op	10.35 r
Skin color (h°)						
Control	80.44 abc	74.16 fgh	75.29 efg	73.05 gh	69.57 ij	68.45 j
MAP		80.22 abc	80.04 abc	80.25 abc	75.83 efg	73.08 gh
5 min UV-C		80.25 abc	76.46 def	74.54 fg	73.45 fgh	68.79 j
10 min UV-C		81.39 ab	78.02 cde	79.79 abc	75.25 efg	79.96 abc
20 min UV-C		79.44 a-d	81.75 ab	82.71 a	72.87 ghi	72.69 ghi
30 min UV-C		79.15 bcd	75.86 efg	74.42 fg	71.07 hij	73.42 fgh
LSD for Weight loss: 0.74, Firmness: 2.71, Skin color: 3.30						

For each quality feature, the values significantly different at $p<0.05$ are indicated by different letters according to Student’s t-test.

Table 2. Effects of different postharvest UV-C treatments on SSC, TEA (%) and SSC/TEA in apricot cv. Roxana during cold storage

Treatments	**Storage (days)**					
	0	**7**	**14**	**21**	**28**	**35**
SSC (%)						
Control	10.53 de	10.53 de	11.87 c	12.60 bc	13.40 b	14.73 a
MAP		10.47 de	10.27 def	10.13 d-h	10.13 d-h	10.20 d-g
5 min UV-C		9.87 d-i	10.00 d-i	10.27 def	10.73 d	11.73 c
10 min UV-C		9.20 i	9.33 ghi	9.53 f-i	9.73 e-i	9.73 e-i
20 min UV-C		9.27 hi	9.27 hi	9.33 ghi	9.33 ghi	10.27 def
30 min UV-C		10.60 de	10.07 d-i	10.13 d-h	10.20 d-g	10.47 de
TEA (%)						
Control	2.013	1.981	1.800	1.533	1.518	1.127
MAP		1.894	1.682	1.459	1.422	1.264
5 min UV-C		1.903	1.791	1.549	1.543	1.052
10 min UV-C		2.022	1.833	1.562	1.421	1.221
20 min UV-C		1.911	1.771	1.433	1.527	1.087
30 min UV-C		1.807	1.893	1.462	1.447	1.148
SSC/TEA						
Control	5.23 m-p	5.32 m-p	6.59 h-k	8.24 de	8.94 cde	13.07 a
MAP		5.53 l-p	6.12 h-n	6.96 ghi	7.14 f-i	8.12 ef
5 min UV-C		5.20 m-p	5.60 k-o	6.63 hij	6.97 ghi	11.19 b
10 min UV-C		5.10 nop	4.56 p	6.12 i-n	6.95 ghi	7.97 efg
20 min UV-C		4.85 op	5.24 m-p	6.55 h-l	6.18 h-m	9.46 c
30 min UV-C		5.87 j-o	5.33 m-p	6.95 ghi	7.14 fgh	9.21 cd
LSD for SSC: 0.89, TEA: N.S., SSC/TEA: 1.03						

For each quality feature, the values significantly different at $p<0.05$ are indicated by different letters according to Student's t-test. N.S: Not significant

While the initial maturity index value was measured as 5.23, it varied between 7.97 (10 min UV-C) and 13.07 (control) after 35 days. It has been determined that 10 minutes of UV-C application during the whole storage period (except the 28th day) was more effective than other applications in preserving the maturity index value.

During the 35 day storage period, a decrease occurred with the prolonged of the storage in the visual quality evaluated by the panelists using the 1-9 scale and this change was found to be statistically significant (Figure 1). At the end of the 35 day storage period in apricot fruits, which was initially 9.0 points, the control group fruits received a value below the marketable limit of 5 (2.33). The highest evaluation score was 6.78 for 10 minutes of UV-C application, followed by 5 minutes of UV-C (6.00), 20 minutes of UV-C (5.89), MAP (5.67) and 30 minutes of UV-C (5.56) applications. Panelists also stated that apricots stored in the cold resulted in darkening and browning in fruit color, wrinkling and softening as the storage period progressed.

The postharvest treatments were effective in maintaining the total phenolic substance content of the fruits with the progression of the storage period (Table 3). The amount of total phenolic substance in apricot fruits stored in cold changed with the progressed storage period. At the end of the 35-day storage period, the highest value was determined in the control group (71.41 mg/100g), followed by fruits stored in MAP. The lowest total amount of phenolic substance was detected in the 30-minute UV-C application (20.95 mg/100g).

The change on the total antioxidant activity amount in fruits during cold storage was found to be statistically significant (Table 3). As the cold storage period progressed, there was a general decrease in the amount of TAA. The value measured as 51.11 µmol/g at the beginning of storage ranged from 14.24 µmol/g (control) to 38.51 µmol/g (10 min UV-C) at the end of the 35 day storage period. As the cold storage period progressed, an increase in polygalacturonase enzyme activity occurred and it was determined that postharvest applications were effective in delaying this increase. At the end of the 35 day storage period, the highest polygalacturonase enzyme activity was detected in the control group fruits (1.28 mmol/kg/h). The lowest enzyme activity was determined as 1.11 mmol/kg/s in 5 minutes of UV-C and 10 minutes of UV-C applications.

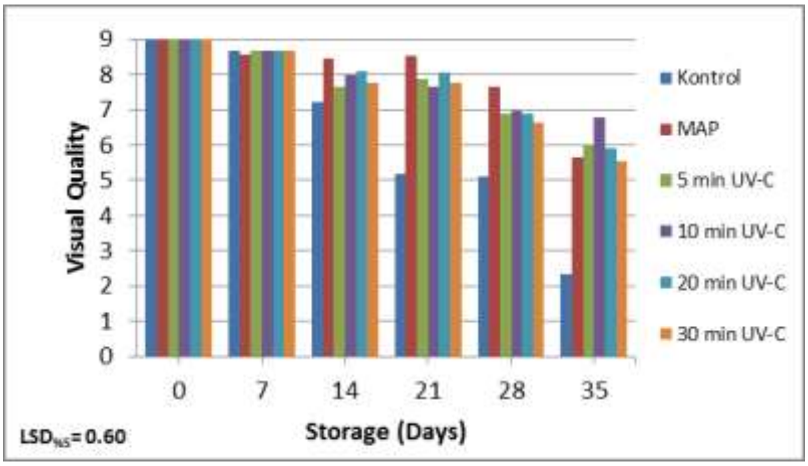

Figure 1. Effects of different postharvest UV-C treatments on visual quality analysis in apricot cv. Roxana during cold storage

Table 3. Effects of different postharvest UV-C treatments on total phenolic substance content, total antioxidant activity amount and polygalacturonase enzyme activity in apricot cv. Roxana during cold storage.

Treatments	**Storage (days)**					
	0	**7**	**14**	**21**	**28**	**35**
Total Phenolic Substance Amount (mg/100g)						
Control	54.74bcd	55.10 bcd	51.23 b-g	56.36 bc	61.41 ab	71.41 a
MAP		55.64 bc	52.40 b-f	51.32 b-g	51.05 b-g	61.14 ab
5 min UV-C		52.58 b-f	48.61 c-h	42.13 f-j	39.97 hij	44.47 d-i
10 min UV-C		51.68 b-g	48.61 c-h	46.90 c-i	45.82 c-i	42.94 e-j
20 min UV-C		53.66 b-e	40.95 g-j	32.13 jkl	28.70 klm	37.45 ijk
30 min UV-C		47.62 c-i	27.99 klm	41.85 f-j	24.01 lm	20.95 m
Total Antioxidant Activity (µmol/g)						
Control	51.11 abc	42.48 e-h	36.70 h-k	29.30 lm	15.49 n	14.24 n
MAP		54.80 a	55.54 a	51.19 a-d	39.77 e-j	37.42 g-k
5 min UV-C		41.81 e-i	36.74 h-k	38.10 g-k	31.80 klm	35.16 i-l
10 min UV-C		49.96 a-d	50.70 a-d	44.09 d-g	45.02c-f	38.51 f-j
20 min UV-C		51.92 ab	53.54 a	45.31 b-e	40.38 e-j	34.57 jkl
30 min UV-C		50.64 a-d	51.81 ab	51.65 abc	38.95 e-j	26.86 m
Polygalacturonase Enzyme Activity (mmol/kg/h)						
Control	0.58 m	1.07 e-i	1.00 h-k	1.23 abc	1.20 a-d	1.28 a
MAP		0.94 jkl	1.15 b-g	1.19 a-d	1.17 a-e	1.13 c-g
5 min UV-C		0.83 l	1.00 h-k	1.10 d-h	1.14 c-g	1.11 c-h
10 min UV-C		0.91 kl	0.94 jkl	0.95 i-l	1.21 a-d	1.11 c-h
20 min UV-C		0.93 jkl	1.04 f-j	1.04 f-j	1.17 a-f	1.17 a-f
30 min UV-C		1.10 d-h	1.03 g-k	1.14 c-g	1.21 a-d	1.27 ab
LSD for Phenol: 10.95, Antioxidant: 6.69, Polygalacturonase enzyme: 0.12						

For each quality feature, the values significantly different at $p<0.05$ are indicated by different letters according to Student's t-test.

CONCLUSIONS

Investigations revealed that UV-C applications were effective in reducing weight loss, protecting firmness value and fruit appearance, and slowing down biochemical changes in apricot cv. Roxana during 35 days of storage at 1 °C compared to control. Among the UV-C application durations, 10 minutes exposure time were more effective on the quality maintenance of apricot fruits. While 5 minutes of UV-C application was not sufficient to protect many quality parameters, 30 minutes of UV-C exposure was not an appropriate time because it caused darkening on the fruit surface. As a result, it was determined that 10 minutes of UV-C application in apricot cv. Roxana gave promising results in extending the storage period by preserving the quality characteristics of the fruits during cold storage.

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EFFECTIVE ORGANO-MINERAL FERTILIZERS OF PROLONGED ACTION FROM THE PROCESSED ORGANO-CONTAINING WASTES OF VARIOUS ORIGIN

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ABSTRACT

Nowadays, the use of organo-mineral fertilizers in agricultural production is one of the main measures to address the environmental and food security of the country.

Our research suggests that excess sludge from the aeration station and processed vegetable waste in the OMF are valuable raw materials for the creation of granular agrochemically effective fertilizers to improve the mineral nutrition of crops, including phosphorus. The loose structure of such fertilizers promotes adsorption and retention of soil moisture, which allows to inhibit the processes of weathering and soil erosion. The high adsorption properties of the new OMFs prevent the chemical binding of phosphorus to the soil and promote its mobilization from hard-to-reach soil phosphates and fertilizers. Phosphorus removal rates by plants in the experiment increased by 51-79% with the use of OMF.

The results of field research in 2018-2019 with Moskito corn hybrid (*Zea mays L.*) and Sumico sunflower hybrid (*Helianthus annuus L.*) on dark-gray podzolic soil confirmed the high agrochemical efficiency of our proposed fertilizers. This affected a significant increase in the yield of these crops (by 5.1 and 0.7 t/ha, respectively) and improved grain quality indicators. Namely, the protein content in corn grain increased by 20%. Determination of the after-effect (1st year) of OMF after corn in the sunflower feeding system confirms the effect of prolonged fertilizer action (increase in grain productivity of the plant by 21%). The use of such fertilizers is characterized by a complex effect on soil processes and soil bioproductivity, which ensures the preservation of soil organic matter.

Keywords: Organo-mineral fertiliser, Phosphoric nutrition, Plant waste, Corn, Sunflower, Crop yields, Quality

INTRODUCTION

At the present stage of development of society around the world, awareness of the effects of environmental pollution and depletion of natural resources has contributed to the fact that the global direction has been the development of approaches to disposal and reuse of waste as a secondary material resource. Recycling of waste generated by society is one of the indicators of its sustainability. Therefore, in most developed countries, waste disposal and disposal have become a priority in the economy (Puzik et al., 2014).

According to the Food and Agriculture Organization of the United Nations (FAO), 1.3 billion tons of food waste annually litter the environment. In the developed world, their number per capita is from 95 to 115 kg, in EU member states - 280-300 kg throughout the food chain. The value of a similar indicator in the countries of Southeast Asia is 6-11 kg per year. Every year, about 30% of the world's food is turned into waste, which is estimated at almost $ 1 trillion in monetary terms (Food waste et al., 2021; Food wastage et al., 2021; Keeney, 2014; Lin et al., 2013).

Our modern agro-industrial production leads to the destruction and depletion of soil cover and land potential in general. Agriculture has become a source of pollution of land and drinking water, and through them - and agricultural products - a number of agrochemicals, which, in turn, has led to an increase in morbidity in both rural and urban areas. According to experts, the "contribution" of the agro-industrial complex of Ukraine in pollution and degradation of the environment reaches 40%, including - land resources - more than 50%. The agro-industrial complex (AIC) is one of the most significant factors influencing the environment. With the current level of development of science and technology, optimally using secondary raw materials, you can get an additional 2 million kD, which will increase meat production by 160 thousand tons per year. In addition, 800-900 thousand tons of limestone and granular organo-mineral fertilizers, tens of thousands of tons of various varieties of vegetable oil, pectin and other products can be produced annually from unused processing waste (Food wastage et al., 2021; Keeney, 2014; Lin et al., 2013).

In today's high-tech and dynamic economy, innovative products made from waste from the industrial processing of agricultural raw materials are becoming increasingly important. Such innovations should become the basis for ensuring leading positions in the production of organo-mineral fertilizers, feeds, which are aimed at its technological and organizational modernization and competitiveness (Shyryaeva, 2014).

To date, it is scientifically substantiated and proven in practice that organo-mineral fertilizers (OMF), in contrast to mineral fertilizers, have high agrochemical efficiency and mobilizing ability against sparingly soluble phosphates, provide access to basic nutrients for plants. The realities of today are extremely low doses of organic, mineral, including phosphorus, fertilizers, unstable weather conditions, lack of nutrients in the soils of Ukraine, so the cultivation of leading cereals, the use of agrochemical-effective long-acting OMF, created from waste economic significance. The use of wastes and substances of humic nature as organic fertilizers (manure, lignin, various composts, etc.) is inefficient even at high application rates - 20-60 and more tons per hectare. Some increase in yield, accumulation of humus does not always recoup the cost of preparation and application of compost, high doses of manure and lignin. A huge amount of waste from food, oil and fat, alcohol, sugar, wine, woodworking, animal husbandry and household origin can be involved in the production of organo-mineral fertilizers. Nowadays, due to high prices for fertilizers, agrochemical scientists face a problem in determining the most economically and agrochemically efficient fertilizer system (types of fertilizers, methods and rates of application). Over the last decade, a significant reduction in the amount of fertilizers applied, including phosphorus, has led to an annual negative balance of phosphorus and almost zero - humus - in Ukrainian agriculture. This, in turn, affected the deterioration of agricultural land productivity and plant resistance to adverse weather conditions. Drought, desertification and systemic soil depletion annually leads to huge crop losses in agriculture (Khrushchev and Remezova, 2018). Therefore, leading domestic and global companies are focused on creating effective anti-stress compounds against drought and other environmental stressors, agrochemical and effective long-acting fertilizers to improve conditions for growth, crop development and environmentally friendly products of high quality. In this regard, we have proposed the use of deposited sewage sludge (BSA) with a high content of nutrients macro- and micronutrients and more than 35% organic matter after thermal disinfection in a complex granular OMF with the addition of humic preparation, processed plant waste biophilic plant silicon, potassium).

It should be noted that silicon is also an extremely important nutrient in plant nutrition. Studies by leading scientists have shown that silica-containing fertilizers help optimize nitrogen-phosphorus nutrition, reduce the mobility of potassium compounds and its loss from the arable soil layer due to its high adsorption capacity. It is also known that silicon compounds enhance the humification of organic waste and increase the population of ammonifiers, which intensifies nitrification processes. It has been experimentally proven that silicon fertilizers in the soil ensure the absorption of phosphorus by plants from hard-to-reach forms of phosphates, their use reduces the chemical binding of phosphorus to the soil, has a positive stimulating effect on plant root development, growth rate, productivity and stress resistance (Lyakina et al., 2010; Bocharnikova et al., 2011; Matichenkov et al., 2001; et al., 2004; Epstein, 2009; Pashkevich and Kiryushin, 2008; Kulikova, 2012; Kozlov et al., 2015).

The basis and justification for the possibility of creating agrochemically effective organo-mineral fertilizers were the results of our previous vegetation studies, which showed significant contribution to new organo-mineral compositions based on deposited sewage sludge with biophilic silicon, potassium and nutrients on the formation of wheat plants more developed root system, improvement of its morphological parameters, increase of its mass by 28-33%, total adsorption surface - up to 80%, working absorption - up to 50% (Kashkovsky et al., 2018). Such changes in the root system increase the area of root contact with the soil, the volume of rhizosphere soil, where the most intense chemical (including the interaction of root exudates with soil mineral and organic phosphates) and microbiological processes.

Thus, with the help of the proposed utilization of deposited sewage sludge, vegetable waste, which is difficult to dispose of, in agrochemically effective fertilizer, we can solve such problems as reducing the amount of accumulated sludge at aeration stations, rice and sunflower husks with a valuable source calcium, phosphorus and some nutrients in such essential products for modern agricultural production.

MATERIAL AND METHOD

Field research was carried out in the experimental field in the Horodyzhe village t of Boryspil district of Kyiv region on dark gray podzolic light loam soil with average content of mobile compounds of basic macronutrients. The experiments were performed on 4 repetitions. The area of the sown area was 1300 m^2, the accounting area was 1200 m^2. Placement of options - systematic. Seed sowing rate – 80 thousand similar corn’s seeds per ha. The predecessor in the experiment was corn. Agrotechnics is typical for the Forest-Steppe zone of the Left Bank and adapted to farm conditions.

The scheme of the experiment with Moskito corn hybrid (*Zea mays L.*) provides the following doses of mineral fertilizers: before sowing was applied OMF-I at a dose of 0.2 and 0.5 t per ha on the background of nitroammophos fertilizer 32:32:32 (200 kg/ha) before sowing, liquid complex fertilizers (ammonium polyphosphate (APP)) ($N_{16}P_{55}$) at a dose of 150 kg/ha, urea ammonium nitrate (UAN-32) N_{96} (300 kg/ha) and timely nitrogen fertilization with ammonium sulfate (100 kg/ha) N_{21}, in certain phases of the growing season. Control – background ($N_{165}P_{87}K_{32}$), without the introduction of OMF.

1. - Background (control) – $N_{165}P_{87}K_{32}$;
2. - Background + OMF-I – 0.2 t/ha;
3. - Background + OMF-I – 0.5 t/ha;

Direct sowing of corn seeds was carried out with a Super Walter W1770 seeder. According to climatic conditions, the growing season of 2018 year was marked by variability and contrast of the first and second half of the corn growing season. The first half was characterized by waterlogging, and the second - high temperatures and heat (temperature 25-30 ^{o}C and above). Due to the sufficient moisture content in the soil, the corn plants grew well and developed to a state of milk-wax ripeness, in which the harvest was recorded and plant samples were taken according to the standard method - GOST-27262.

Harvesting and accounting were performed manually from each plot by the weight method according to the method of B.O. Dospekhov (Dospekhov, 1985). Corn grain moisture, grain yield from cob harvest and conditioned grain crop yield were determined in cob samples (50 pcs.), which were taken during harvesting separately at each accounting plot. Crop grain yield was calculated at a moisture content of 14%. Selection and preparation of plant samples was performed according to standard methods (Hrytsaienko et al., 2003). The content of nitrogen and phosphorus after wet ashing in dry corn plants samples were determined by the method of K. Ginsburg with the following determination: nitrogen - photometric method using Nessler's reagent, phosphorus - photometrically by the method of Denizhe in modification of A. Levitsky. The inorganic components of plant sample ash were determined using Expert 3L XRF (INAM, Ukraine).

Field experiment in 2019 was carried out to determine the after-effect (1st year) of our organo-mineral fertilizers - OMF-1 in the area where these were applied when sowing Moskito corn hybrid in 2018. The experimental crop was Sumico F1 sunflower hybrid (*Helianthus annuus L.*). Seed sowing rate – 80 thousand similar seeds per ha. The fertilizer system in the experiment involved the application of 200 kg of fertilizer mixture (4:12:12) scattered with the salary in the fall; in the spring, under closing of moisture – 150 kg of UAN-32, at sowing of sunflower – APP of 50 kg and UAN – 100 kg. In the phase of growth and development of sunflower plants - bud stage, carried out foliar fertilization (spraying) of plants with an aqueous solution of microfertilizer BOR-Ekoorganika with a dose of 1 l/ha.

Harvesting and accounting of sunflower harvest were carried out in the phase of full ripeness of grain manually from each part of the experiment by weight. The yield of conditioned seeds was determined in samples of heads (50 pcs.) which were taken during harvesting separately at each accounting site. Biochemical assessment of sunflower seed quality was determined by the index of fat content by extraction method, its removal from seeds by ethyl ether (Soxhlet extractor), according to GOST 10857-64 (GOST, 2010). Determination of grain quality was performed by infrared spectroscopy Infratec 1241 Grain Analyser.

RESULTS AND DISCUSSION

One of the important elements of corn growing technology is the fertilizer system. Obtaining a high yield of corn grain is possible only with the use of scientifically substantiated technologies for its cultivation at a high level of resource provision. To optimize the mineral, in particular, phosphorus nutrition of corn on dark-grey podzolic soil in the fertilizer system were used OMF-I in the seed application at a dose of 0.2 and 0.5 t/ha. One of the advantages of such OMFs is that ammonium nitrogen is bound to organic matter. This contributes to its slow dissolution and migration in the arable layer of the soil, which is a critical area for the absorption of nutrients by cultivated plants. Humic acids, active silicon, sulfur in the OMF in the experiment contribute to the mobilization of sparingly soluble phosphates of fertilizers and soil. Also these improve the phosphorus nutrition of corn plants. Under the use of fertilizers (OMF-I) the plants formed a stronger root system with improved morphological parameters, increased absorption surface. The weight of the roots exceeded the control samples by 43% (Figure 1). Such changes in the performance of the root system have led to a significant increase in the volume of roots and rhizosphere of the soil, where there are numerous chemical and biological transformations, sorption, desorption, absorption. Namely, the imbalance of elements in the food system leads to extremely negative consequences - a decrease in crop productivity and deterioration of quality.

Figure 1. The roots of some corn at the field study

Our structural analysis (Table 1) shows that all elements of the structure are involved in crop formation and change depending on the level of supply of corn plants with nitrogen, phosphorus, potassium, trace elements and weather conditions during the plant vegetation.

Table 1. Elements of the yield structure and productivity of Moskito corn hybrid, 2018.

#	Treatment	Cob diameter, cm	Cob length, cm	Cob weight, g	Number of rows, pcs	The number of grains per row, pcs	The number of grains per cob, pcs	1000 grains weight, g
1	**$N_{165}P_{87}K_{32}$** - background	4.5±0.03	18.2± 0.6	206± 10.6	13,9± 0,41	39.6± 1.6	534± 22.9	278± 11.9
2	Background + OMF-I (0.2 t/ha)	4.6±0.04	20.6± 0.8	214± 10.1	14,2± 0,39	44.9± 1.5	637± 28.7	317± 13.7
3	Background + OMF-I (0.5 t/ha)	5.1±0.04	22.8± 0.7	331± 15.1	14.7± 0.40	48.0± 1.9	682± 29.5	375± 14.8

Cob diameter under the influence of fertilizer variants in the experiment increased by 13% (Figure 2). The largest cobs of the plant were formed with the application of 0.5 t/ha OMF-1 – 22.8 cm. Quantitative analysis of such cob indicators as a number of rows and grains per row shows a significant increase in these indicators relative to control. The content of grains per row increased by 21% with the introduction of OMF-1.

One of the important indicators of crop structure, which is the most prerequisite for increasing yields is grains weight, which is formed on the cob. The largest weight of grain in the cob was established – when applying OMF at a dose of 0.5 t/ha – 761 g (+42%). The 1000 grains weight, as an indicator of the grain size formed on the cobs, was the highest – 375 g in the variant where OMF was applied at a dose of 0.5 t/ha and exceeded the control indicator by 34%.

Data from the determination of grain productivity of the 1 plant were positively correlated with the grain yield in the respective experimental plots. With the additional application of nutrients to the soil in the form of OMF-I, on the background $N_{165}P_{87}K_{32}$ received a significant increase in yield relative to control. The norm of 0.5 t/ha of OMF-I proved to be quite effective, while the grain growth was – 5.1 t/ha (Table 2). It is known that the yield of corn is formed due to precipitation, solar radiation and air temperature. Therefore, in our opinion, very favourable climatic conditions in July-August had a significant impact on the increase in yield in the experiment. During this period, more than 155 mm of precipitation fell compared to the same months in 2017, which contributed to the improvement of mineral nutrition, growth and development of corn plants in the critical period for moisture. With optimal moisture, the roots of the experimental plants of corn penetrated the granules, absorbing nutrients not only from the soil solution but also from the created new fertilizers OMF-I, during which the yields increased significantly.

Figure 2. The cobs of some corn at the field study

Table 2. Yield and quality of Moskito corn hybrid, 2018.

#	Treatment	Crop yields, t/ha				Average	Yield increase		Content,%		
		replication									
		1	2	3	4		t	%	starch	fat	albumen
1	**$N_{165}P_{87}K_{32}$** - background	10.8	10.5	9.9	11.2	10.6	-	-	73.3	3.3	7.0
2	Background + OMF-I (0.2 t/ha)	12.3	13.5	12.2	13.2	12.8	1.8	20.8	73.2	3.3	7.2
3	Background + OMF-I (0.5 t/ha)	16.4	16.3	14.7	15.4	15.7	5.1	48.1	72.1	3.3	8.3
4	HIP_{05}	0.53	0.64	0.79	0.75	0.77					

It was found that the fertilizer system had different effects on the main indicators of corn grain quality (Table 2). The protein content in the experiment was distinguished by the variant with the introduction of OMF-I at a dose of 0.5 t/ha. Namely, a more balanced diet with the introduction of 0.5 t/ha OMF-1 in the system of corn fertilizer and favourable weather conditions was a prerequisite for the formation of grain with high protein content – 8.3% compared to the control – 7.0%. The starch content in the grain was inversely proportional to the processes of protein accumulation. This is probably due to the fact that the increase in protein content inhibited the deposition of carbohydrates in grains. The fertilizer system did not affect the starch content in the grain.

Among cereals, corn has the highest yield and absorption rate of macro- and microelements from the soil. Traditionally, this culture is considered an "indicator" of the content of trace elements in the soil. Corn is sensitive to their use, especially zinc (Zn), manganese (Mn), copper (Cu) and boron (B). Lack of which inhibits the growth and development of plants reduces crop productivity (Mudry, 2005; Huriev et al., 1992). X-ray fluorescence analysis of Mosquito corn grain (Table 3) confirms that the content of heavy metals in plant samples and grain using OMF in the field experiment did not exceed the maximum allowable values (DSTU, 2009), and therefore it may be suitable for food, feed, technical needs.

Table 3. Composition (%mass) of Moskito corn hybrid ash using OMF-1 in the field experiment, 2018 (XRF)

Treatment		CaO	Fe_2O_3	Cl	K_2O	P_2O_5	MnO	SiO_2	Al_2O_3	TiO_2	ZnO	Cr_2O_3	SrO
$N_{165}P_{87}K_{32}$ - background	leaf	16.63	0.393	-	19.332	3.924	0.381	43.748	-	0.090	ppm 93	-	ppm 139
	stalk	10.93	0.181	-	43.398	5.882	0.170	30.104	-	-	-	-	ppm 197
	roots	2.619	3.234	-	18.079	1.727	-	62.427	-	0.831	ppm 93	-	-
	grain	-	0.265	-	42.51	44.921	-	1.2	-	-	0.117	-	-
Background + OMF-I (0.2 t/ha)	leaf	18.321	0.398	-	25.311	5.938	0.318	43.132	-	-	-	-	-
	stalk	7.318	0.286	-	44.277	6.019	0.179	29.487	-	-	-	-	-
	roots	2.006	2.973	2.114	21.983	2.798	0.082	51.338	-	-	-	-	-
	grain	-	0.272	-	42.006	48.1	-	0.057	-	-	-	-	-
Background + OMF-I (0.5 t/ha)	leaf	17.732	0.385	0.144	26.517	9.032	0.263	42.513	-	0,106	ppm 216	-	-
	stalk	6.678	0.322	0.768	52.8	10.1	0.193	28.75	-	0.104	-	-	ppm 178
	roots	2.054	2.481	1.319	22.433	3.009	0.094	53.51	0.149	0.624	0.068	-	ppm 117
	grain	-	0.316	-	51.62	48.337	-	-	-	-	0.194	-	-

The increase in phosphorus uptake by plants using experimental OMF can be explained by additional phosphorus uptake by the root system with an increased surface area, uptake activity and accelerated mobilization of absorbed compounds. (Figure 3.).

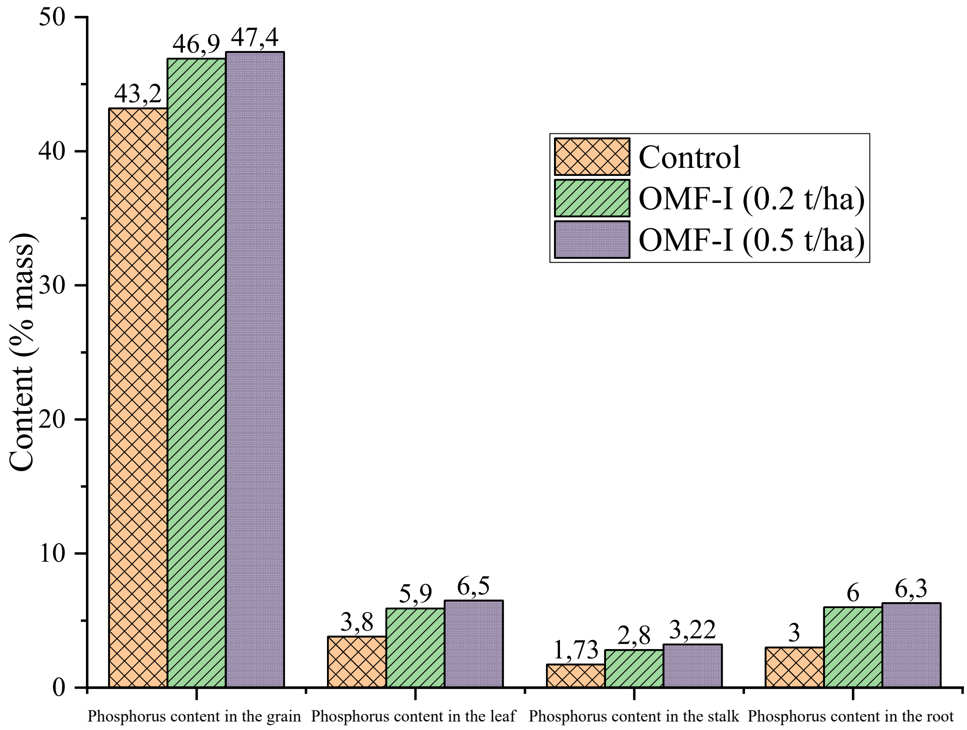

Figure 3. The phosphorus content in the Moskito corn hybrid ash using OMF-1 in the field experiment, 2018

In the second field experiment with Sumico F1 sunflower hybrid, using the mineral fertilizers against the background of the after-effect of OMF-I had a positive influence in the dry 2019. Thus, the data of structural analysis show (Table 4) that in the experiment the fertilizer system had a positive effect on all elements of the structure. Against the background of the after-effect of long-acting fertilizer OMF-I, there was a 26% increase in head diameter of sunflower plant, the 1000 seed weight – by 20% compared to control plants (background). The fullest seed (72.0 g) was obtained in the experimental version (after-effect for the introduction of OMF-I under corn in 2018 in the system of its fertilizer). This had a positive effect on such an important indicator, as the grain yield of one plant, there was an increase in this indicator compared to the control by 21%.

Table 4. Elements of the yield structure of Sumico F1 sunflower hybrid (the 1st year after-effect of OMF-1) 2019

Treatment	Head diameter, cm	Grains per head, pcs	1000 seed weight, g	Total mass of the seeds of the head, g
$N_{90}P_{52}K_{24}$ – background	11.4±0.3	1321±59	60±2.5	79.5±3.3
Background + after-effect ща OMF-I (0.5 t/ha)	14.4±0.4	1349±57	72±2.7	96.5±4.8

The presence of mineral nutrients in the soil in optimal proportions helps to increase plant productivity, improve seed quality. The aftereffect of the 1st year of the organo-mineral fertilizer system in the experiment with corn using OMF-I (normally 0.5 t/ha) provided an increase in the content of mineral phosphates in the dark-grey podzolic soil relative to control (+ 9.9% in arable land layers). The nature of phosphorus compounds in the soil is related to their negative charge and ability to chemically react with a negatively charged soil absorption complex. This enhances the active fixation of phosphoric acid anion in the soil using the phosphorus compounds in the composition of organo-mineral fertilizers. Studies of soil phosphate conversion processes in the experiment showed that the application of OMF-I at a dose of 0.5 t/ha increases the content of mobile phosphorus compounds compared to the control by 18.2 mg/kg (soil layer 20 cm). As a result of the application of OMF-I for corn in 2018, the nitrogen content of alkaline hydrolyzed compounds increased compared to the control – by 9.5 mg/kg, mobile potassium – by 19.3 mg/kg, pH – by 0.2 units. The high content of Ca indicates high levels of neutralizing ability of our fertilizer and the feasibility of its use as an ameliorant on lands with an acid reaction of the soil solution. Reducing the acidity of the soil solution by 0.2 units, increasing the mobile compounds of the main macronutrients indicates a positive effect of OMF-I on the nutrient regime of the experimental dark-grey podzolic light loam soil. The increase in the content of mobile compounds of the main nutrients in the soil (layer 0-20 cm) with the introduction of OMF-I contributed to increasing in sunflower yield by 13.0% compared with the control (Table 5). In many oilseeds on the background of phosphorus-potassium fertilizers at moderate doses of nitrogen, the oil content in the seeds increases. The aftereffect of OMF-I fertilizers helped to improve the nutritional conditions of experimental sunflower plants.

Table 5. Yield and quality of Sumico F1 sunflower hybrid (the 1st year after-effect of OMF-1) 2019

Treatment	Yield, t/ha replication				Average	Yield increase		Content, %
	1	2	3	4		t	%	fat
$N_{90}P_{52}K_{24}$ – background	3.6	4.2	4.1	3.7	3.9	-		40.27
Background + after-effect of OMF-I (0.5 t/ha)	4.4	4.8	4.7	4.5	4.6	0.6	13.04	43.92
HIP_{05}	0.20	0.19	0.20	0.22	0.21			

Oil and protein contents in seeds are varietal characteristics. It was found that the maximum oil content was formed in the seeds of experimental samples (43.9 %) on the background of the after-effects of OMF-I at a dose of 0.5 t/ha, compared to the control – (40.3 %) (Figure 4).

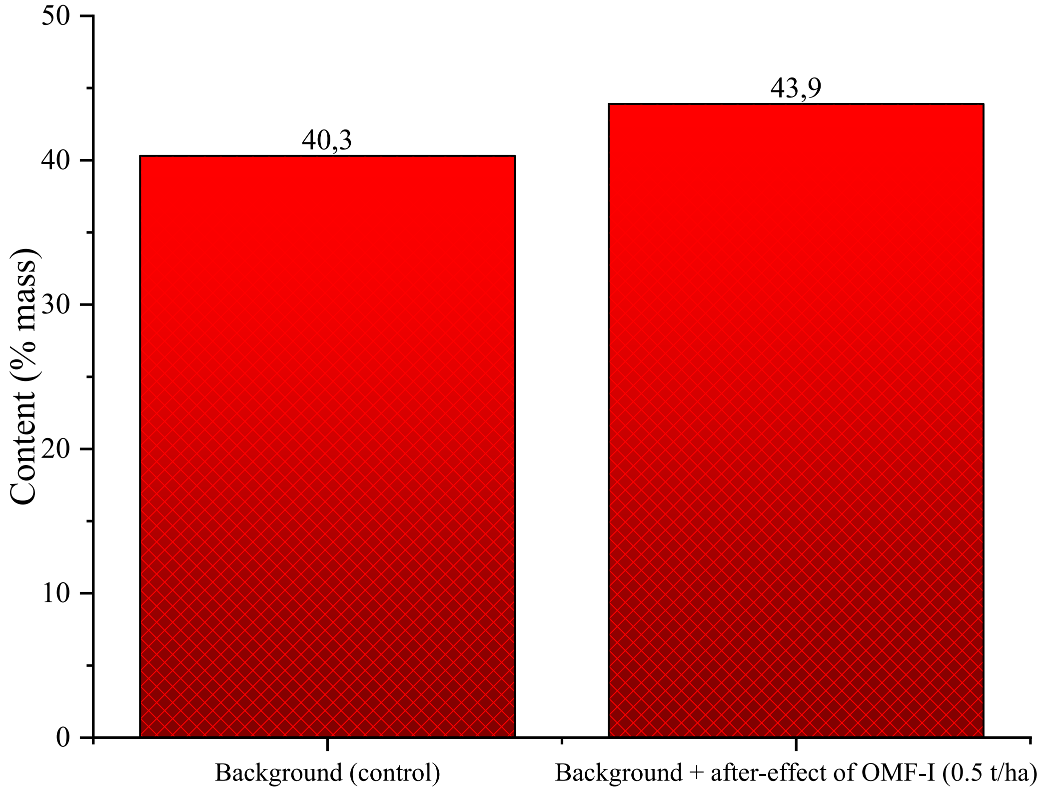

Figure 4. The oil content of in Sumico F1 sunflower hybrid using the 1st year after-effect of OMF-1, compared to the control 2019

CONCLUSIONS

The study of transformation processes in the dark-grey podzolic soil showed that the aftereffects of the created OMF have a positive effect on the improvement of its physical and chemical parameters, so the content of basic nutrients increases significantly. Due to the application of such fertilizers in the root layer of the soil, comfort zones are created for plants with a high concentration of nutrients, favourable conditions for the preservation of the ammonia form of nitrogen, as well as for the vital activity of beneficial microflora.

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EFFECT OF HUMIC ACID APPLICATIONS ON TUBER QUALITY IN POTATO (Solanum tuberosum L.)

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ABSTRACT

There is some factors affect tuber yield and quality such as cultivars, environmental conditions and cultural practices. One of the most important cultural practices is fertilizer application which has positive effect on yield and quality parameters. Recently, organic fertilizers are preferred instead of chemical fertilizer. Humic acid is one of the organic fertilizer which is mostly used by farmers due to source of nutrition element for plant growth and improve quality of some soil types. Soils comprise of high pH and low organic matter in Central Anatolia where potato production is carried out intensively. It provides sustainability of soil and promote growth and development of plants. This study was conducted to investigate the effect of different humic acid doses and application methods on tuber quality parameters of potato at Konaklı small town, Nigde in 2019. The field experiments were laid out in the randomized complete block design with tree replication, five different doses of humic acid (HA0: 0 kg HA/da, HA1: 2 kg HA/da, HA2: 4 kg HA/da, HA3: 6 kg HA/da, HA4: 8 kg HA/da) soil + foliar application (half of each doses applied to soil before planting and half of each doses applied to foliar). Starch content, internal darkening, chips and french-fired quality were determined in this study. Results indicated that different doses of humic acid and application methods had various effects on quality parameters. Soil + foliar application of 4-6 kg HA/da^{-1} (1/2 soil+1/2 foliar) and 4-6 kg HA/da^{-1} foliar application were determined as an optimum doses for investigated quality parameters.

Keywords: *Solanum tuberosum*, Fertilization, Humic acid, Chips, French fry

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important crops in terms of agriculture owing to wide adaptability and ranked as the world's fourth most important food crop after rice, wheat, and maize (FAO, 2021). It is a vital food source as consisting of protein, carbohydrates, vitamins and minerals and the countries that have lack of *food availability and* for human diet. It is considered as main or second crop production in large areas (Çalışkan et al., 2010) with a production area of approximately 17.3 million hectares and a production amount of 370 million tons in the world (FAO, 2021), and approximately 148 thousand hectares of production area and 5.2 million tons of production in Turkey (TÜİK, 2021). China, India, Russia, Ukraine and USA is the first five countries that is the biggest potato producers while Turkey is currently ranks 16 nd in production quantity and one of the major potato-producing country as well (FAO, 2021). The provinces of greatest production are Niğde, Konya, Afyonkarahisar and İzmir where comprise 55% of total potato production. On the other hand, the highest production is occured in Nigde region where provide 13.3% of total potato production in Turkey (TÜİK, 2021). Potato can be characterized as critical crops in terms of nutritional value as it is today and future due to the growth of world and Turkey population, global warming and drought situtation. Although it has produced in large areas in high quantities, the yield and quality of potato is affected by many factors. These factors can catagorized within three main groups such as cultivars , environmental conditions and cultural practices. One of cultural practice is that fertilizer applications are important parameter due to effect on yield and quality. The fertilizers are classified as chemical and organic. Both types of fertilizer provide various nutritions for plants. Besides the potential benefit of chemical fertilizers, chemical fertilizers cause degredations such as soil salinity, soil pH and soil texture (Turhan, 2005). In order to avoid these adverse effects of chemical fertilizer, organic fertilizers have been used to enable nutritional supplement in recent years. Organic fertilizers are derived from plant and animal resudies and contain varied amount of plant nutritions based on source. Green manure, *farm* yard *manure* and composts are commercially available for producer (Anonim, 2021). Humic acid contains naturally occuring organic substances. Recently, producer prefer to use humic acid due to enhancing soil fertility and soil structure recovery. Humic acid increase organic content of soil and it helps to recover physically (relative water content, aeration), chemical (pH, salinity) and biological properties of soil (Özkan, 2007). Humic acid increased yield and quality by effecting different factors such as microorganism activitiy in soil, root deveopment, plant growth and photosynthesis (Özdemir, 2011). In addition, Humic acid uptake is increased in plants to overcome stress conditions.

Humic acid having organic substances that provide sustainability of soil and promote growth and development of plants is intensively applied due to soil properties of study area (high pH, low organic matter and heavy soil type). It is known that humic acid increase yield and quality prameters as well. For this reason, current study aimed to determine the effect of different humic acid doses and application methods on quality parameters of potato such as chips, french fried, starch and crips color.

MATERIAL AND METHOD

The research was carried out in 2019 in Misli Plain, Konaklı, Niğde, where mass potato cultivation is common. Humic acid which is a natural organic soil conditioner product with a total humic+fulvic content of 21% w/w, a total organic matter of 20% w/w and a K_2O ratio of 3% (pH range of 1-3), is used extensively by farmers and was preferred for this study. Five different humic acid concentrations (0, 2, 4, 6, 8 kg HA/da^{-1}) were used and 9 different treatments as “Soil + Leaf” (half of the humic acid was applied to the soil (S) and the other half to the leaf (L) as top fertilizer by spraying) and "Leaf" (all of the humic acid coming from the leaves), were tested. In the experiment, Belmando cultivar was used as plant material, which is mid-early, with characteristics growth shape and white flower color. Tubers of which are 35-45 mm were selected particularly. The experiment was designed as randomized block design with 3 replications. Plot was 5.1 m long, 2.8 m wide, and tubers were planted with a semi-automatic potato planting machine to plant 68 tubers per plot, with a density of 70 cm * 30 cm and a depth of 15-18 cm. Sprinkler system was used for irrigation and the plants were irrigated at 5-7 days intervals depending on the condition for soil moisture. The soil was loamy and had neutral pH (7.5). The organic matter content (0.7462%) in soil was also very low however the available phosphorus (P_2O_5) and total potassium content were found to be high.

The harvest was accomplished by potato harvest machine and twenty tuber samples were collected from each plot after harvest to determine tuber quality parameters. Tuber specific gravity and dry matter percentage were measured by PW-2050 Digital Hydrometer as three repeats from each plot, and tuber starch ratio was determined by using tuber specific gravity value according to formula of Haase, 2003. Randomly selected six tubers were cut into three and waited for 30 to observe tuber darkening according to the darkening scale (1=V-shaped darkening, 2=Significant darkening, 3=Slight darkening, 4=Local darkening, 5=No darkening). For the quality measurements, French fries, 9mm*9mm, and chips, 2 mm, were cut from tubers and the tuber was peeled, later fried for 3 min at 180^{o}C (Palazoglu and Gökmen, 2008). Fried samples were grouped by the USDA color scale (Chip color: 1=No chips, 2=Risky, 3=Moderate, 4=Good 5=Very good;

Roast color: 000-00-0=Very good, 1=Good, 2=Medium-Good, 3=Moderate (30% maximum), 4=Low (10% maximum) and the quality was also tested by using Colorimeter (Konica Minolta- CR700) device and L, a, b values were obtained. Measurements were repeated 10 times for each plot.

The data was analyzed using the SAS statistical program and the significance of their means were tested according to the LSD test.

RESULTS AND DISCUSSION

In the study, the effects of different humic acid applications on starch ratio and the effects of interactions between applications were found to be statistically insignificant (Table 1). In the study, the starch ratios varied between 12.74% and 14.51%. In addition, when all applications were analyzed, the highest starch ratio was obtained from T3+Y3 and T4+Y4 applications with 14.51%, and the lowest starch ratio was obtained from the control (HA0) application. In the study conducted by Oğurlu (2019), humic acid applications did not cause any change in starch ratio and Suh et al. (2014), in the same way, it was determined that the amount of humic acid did not have any effect on the starch ratio. However, Rajendiran and Purakayastha (2014) reported that singly humic acid applications did not have a significant effect on tuber quality. Therefore, the findings obtained in this study support previous studies. The effect of the humic acid doses on darkening values ranged from 3 (slight darkening) to 5 (no darkening) in the study.

Table 1. Average values for the effects of different Humic acid treatments on potato starch and darkening.

Application (HA/da^{-1})	Starch (%)	Darkening of the inside of the tuber (1-5 scale)
S1+L1	13.84 ab	5
S2+L2	14.09 a	5
S3+L3	14.51 a	5
S4+L4	14.51 a	3
L2	13.35 ab	5
L4	13.35 ab	4
L6	13.90 ab	4
L8	14.15 a	5
Control	12.74 b	5
Average	13.83	-
LSD (%5)	1.29	-
Replication	3.59*	-
Application	1.84	-
Error	0.55	-
CV (%)	5.39	-

*: $p < 0.05$, **: $p < 0.01$

The highest darkening score was observed in the tubers obtained from S1+L1, S2+L2, S3+L3, L2, L8 and control applications, while the lowest darkening score was observed in tubers obtained from S4+L4 application. It was determined that the humic acid applications given from the soil did not have any effect on the darkening of the tuber, except for the S4+L4 application. The results obtained from foliar application were to be same with the results of the control applications, except for the L4 and L6 application.

Table 2. Average values of L, a, b and USDA scale data obtained in terms of the effects of different Humic acid applications on the quality of french fries.

Application (HA/da^{-1})	L	a	b	USDA color scale score
S1+L1	66.56 b	2.99 b	24.63 a	1
S2+L2	68.75 a	1.69 bc	24.45 a	00
S3+L3	66.88 b	1.28 cd	22.62 abc	0
S4+L4	66.48 b	3.11 ab	25.33 a	1
L2	67.24 b	2.31 bc	24.19 a	0
L4	66.25 b	4.70 a	25.29 a	0
L6	64.05 c	1.60 bc	19.78 c	0
L8	64.16 c	1.38 cd	22.63 ab	1
Control	65.98 b	-0.15 d	20.07 bc	1
Average	66.30	2.10	23.22	-
LSD (%5)	1.35	1.58	2.84	-
Replication	3.57*	2.78	0.66	-
Application	10.57**	6.80**	4.94**	-
Error	0.60	0.84	2.70	-
CV (%)	1.17	43.61	7.08	-

*: $p < 0.05$, **: $p < 0.01$

The effects of different humic acid applications given from leaves and soil on the quality of french fries and chips were statistically significant. When Tables 2 and 3 are examined, it is seen that L, a, b values are divided into different groups. In terms of french fries quality, the highest value in terms of "L" was obtained from the S2+L2 application with 68.75, while the lowest value was obtained from the Y6 application with 64.05. In terms of "a", the highest value is L4 application with 4.70, the lowest value is control application with -0.15, the highest value in terms of "b" value is S4+L4 with 25.33, and the lowest value is L6 application with 19.78 (Table 2).

L, a and b values are measured with the CIE Lab (a color space based system) system. At the “L” value, 0-50 is considered a dark color and 51-100 is considered a light color. In the CIE Lab system, a positive number is red and a negative number is green; If the value is “b”, the positive number indicates yellow, and the negative number indicates blue (Hunterlab, 2012). Color is an important quality characteristic of french fries. The color of the potatoes is golden yellow, which is preferred by the consumer. It has been stated that the color of the tuber after frying in potatoes is related to the amount of reducing sugar in its content (Asghari, 2015).

When the "L" value was examined in terms of chips quality, the highest value was obtained from the control application with 63.40, and the lowest value was obtained from the S1+L1 application with 56.53. In addition, in terms of "a", the highest value was 7.22 for L4 application, the lowest value was 2.26 for control application, for "b" the highest value was 21.22 control and the lowest value was S1+L1 application with 17.05 (Table 3). USDA color scale scores also differed between applications in terms of both quality characteristics. However, similar results were obtained from all scale scores in terms of french fries and chips quality.

Table 3. Average values of L, a, b and USDA scale data obtained in terms of the effects of different Humic acid applications on the quality of potato chips.

Application (HA/da)	L	a	b	USDA color scale score
S1+L1	56.53 c	5.70 ab	17.05 b	1
S2+L2	58.17 bc	4.59 bcd	17.35 b	00
S3+L3	61.99 ab	3.31 de	19.18 ab	0
S4+L4	57.57 c	5.41 abc	17.77 ab	1
L2	59.83 abc	3.31 ed	19.13 ab	0
L4	57.33 c	7.22 a	17.48 b	0
L6	57.94 bc	3.44 dec	17.94 ab	0
L8	59.28 abc	3.84 bcde	17.95 ab	1
Control	63.40 a	2.26 e	21.22 a	1
Average	59.11	4.34	18.34	-
LSD (%5)	4.24	2.02	3.45	-
Replication	1.89	1.64	1.84	-
Application	2.60**	5.19**	1.28	-
Error	6.01	1.36	3.98	-
CV (%)	4.14	26.88	10.88	-

*: $p < 0.05$, **: $p < 0.01$

In different humic acid applications, the color L value of the chips was more than 50 and the color of the fried potatoes was found to be yellow. Hassanpanah (2011), reported that the factors affecting the chips color in tubers are the variety and the amount of reducing sugar. In the same way, the color of chips can change in potato according to the cultural practices and storage conditions during its production, although it is a variety characteristic. The amount of reducing sugar in the tuber varies according to the cultural treatments, fertilization (Yıldırım, 2019), growing conditions, storage, stress conditions, variety, frying temperature and slice thickness of potatoes (Çalışkan et al., 2011). It has been reported by Khan et al., (2012) that reducing sugar accumulation in tubers is one of the causes of undesirable dark color in potato chips.

CONCLUSIONS

In our study, the effect of application methods and doses of humic acid on tuber quality of potato was determined. Results indicated that different doses of humic acid and application methods had various effects on quality parameters. As a result, Soil + foliar application of 4-6 kg HA/da^{-1} (1/2 soil+1/2 foliar) and 4-6 kg HA/da^{-1} foliar application were determined as an optimum doses for investigated quality parameters.

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USE OF THIDIAZURON IN TISSUE CULTURE STUDIES IN VEGETABLES

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ABSTRACT

Thidiazuron (TDZ; N-phenyl-1, 2, 3-thidiazole-5ylurea) is a plant growth regulator with both auxin and cytokinin-like behavior with the chemical formula $C_9H_8N_4OS$. The plant growth regulator *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (thidiazuron, TDZ) originally registered as a cotton defoliant in 1967 by Schering AG (Berlin, Germany) was first described to have cytokinin activity in 1982. In recent years, the use of TDZ in plant tissue culture propagation has been increasing. The optimum concentration of TDZ in nutrient media; varies depending on vegetable species, explant type, and explant exposure time to TDZ. In this study, the studies conducted between 2000 and 2020 on the use of thidiazuron (TDZ) *in vitro* plant tissue culture studies in vegetable species and the results obtained are summarized. As a result of the researches, it has been determined that TDZ is mostly used for *in vitro* shoot regeneration and proliferation in plant tissue culture, as well as for callus production, stimulation of somatic embryogenesis, androgenesis, and gynogenesis.

Keywords: Callus, Shoot regeneration, Somatic embryogenesis, Thidiazuron, Vegetable species

INTRODUCTION

Thidiazuron (TDZ; N-phenyl-1, 2, 3-thidiazole-5ylurea) is a plant growth regulator with both auxin and cytokinin-like behavior with the chemical formula $C_9H_8N_4OS$. The plant growth regulator *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea (thidiazuron, TDZ) originally registered as a cotton defoliant (Arndt et al., 1976) in 1967 by Schering AG (Berlin, Germany) was first described to have cytokinin activity in 1982.

Thidiazuron (TDZ), in plant tissue culture studies in vegetable species; It is used in many areas such as shoot regeneration, callus formation, stimulation of gynogenesis, and andrgenesis, somatic embryogenesis, extension of shoot length, and adventitious bud formation (Dinani et al., 2018). The optimum concentration of TDZ in nutrient media; varies depending on vegetable species, explant type, and explant exposure time to TDZ (Deepa et al., 2018).

In this review, the studies conducted between 2000 and 2020 on the use of TDZ *in vitro* micropropagation of vegetable species and the results obtained from these studies are summarized.

SHOOT REGENERATION AND ELONGATION

TDZ's CK-like activity has also shown to be useful for the development of shoot buds and shoot proliferation/multiplication in plants. Studies on the use of TDZ *in vitro* shoot regeneration and shoot elongation in different vegetable species between 2000 and 2020 are summarized in Table 1. The doses indicated in Table 1 are the optimum TDZ doses for shoot regeneration. For this purpose, a total of 21 studies were conducted in the Solanaceae family, 6 studies in the Cucurbitaceae family, 5 studies in the Leguminosae family, 13 studies in the Cruciferae family, 1 study in the Amaryllidaceae family, 2 studies in the Malvaceae family, and 1 study in the Apiaceae family (Table 1). MS (Murashige and Skoog, 1962) nutrient medium was used in all *in vitro* shoot regeneration studies. It has been determined that the most used explant type is hypocotyl and cotyledon explants derived from sterile plantlets derived *in vitro* conditions, as well as leaf, petiole, shoot tip, meristem, node, internode, callus and root parts. In the studies, the optimum dose of TDZ differed according to the species and the type of explant used (Table 1). In studies conducted on different vegetable species, TDZ can be added alone to the MS (Murashige and Skoog, 1962) nutrient medium to ensure shoot regeneration (Khan et al., 2006; Peddaboina et al., 2006; Sharma et al., 2006; Pal et al., 2007; You et al., 2007; Ranjan et al., 2010; Ashakiran et al., 2011; Badr-Elden et al., 2012; Sujatha et al., 2013; Ugandhar et al., 2018; Farouk Omar, 2019), TDZ' used together with auxin group hormones such as Indole-3-butyric acid (IBA), Indole-3-acetic acid (IAA), and 1-Naphthaleneacetic acid (NAA) (Thiruvengadam et al., 2010; Ugandhar et al., 2011; Zhang et al., 2011; Vinoth et al., 2012; Salim and Rashid, 2014; Gambhir and Srivastava, 2015; Jamous and Abu-Qaoud, 2015; Kumar et al., 2018) or with cytokinin group hormones such as Benzyl adenine (BA), and Benzyl adenine purine (BAP) (Khan et al., 2011; Mookkan, 2015). (Table 1).

SOMATIC EMBRYOGENESIS

TDZ is a substitute for the auxin/CK requirement that is needed during somatic embryogenesis, thereby increasing the number of formed somatic embryos (Visser et al., 1992). It has been determined that there are 2 studies in pepper (Khan et al., 2006; Ugandhar et al., 2011) and 1 in melon (Stipp et al., 2001) on the use of TDZ in vegetable species to stimulate somatic embryogenesis between 2000 and 2020 (Table 1). In a study using shoot tip and stem explants in pepper to stimulate somatic embryogenesis, 0.5 µM TDZ dose in MS medium (Khan et al., 2006) and in another study using leaf explants MS + TDZ (0.5 mg/l) + 2,4- D (3 mg/l) combination was the most successful PGR combination (Ugandhar et al., 2011). In the study performed on melon, it was determined that the combination of MS + 5 mg/l 2,4-D + 1 mg/l TDZ was effective (Stipp et al., 2001). In order to stimulate somatic embryogenesis, TDZ can be added to the MS nutrient medium alone (Khan et al., 2006), or it can be used together with 2,4-D, an auxin group hormone. (Stipp et al., 2001)

STIMULATION OF CALLUS FORMATION

TDZ is also added to nutrient media in order to induce in vitro callus formation from different explant types in vegetable species. In tomato (*L. esculentum* Mill), from anther explants MS + 0.022 mg/l TDZ + 1 mg/l IAA medium (Abate et al., 2004), *S. lycopersicum* L. var. *cerasiforme*, callus formation was reported from hypocotyl explants with the combination of MS + 1-2 mg/l TDZ (Farouk Omar, 2019). In bitter melon (*Momordica charantia* L.), callus was obtain from leaf explants on MS + B5 vitamins + 7.7 µM NAA + 2.2 µM TDZ (Thiruvengadam et al., 2010), from internode explants MS salts + B5 vitamins + 5 µM 2,4- D + 2 µM TDZ (Thiruvengadam et al., 2012) combination. In *Vigna unguiculata* L., both shoot and callus were obtained from shoot tip explants in MS + 0.25 mg/l TDZ + 3 g/l activated charcoal + 2 mg/l yeast extract medium (Aasim et al., 2009). In non-heading Chinese cabbage from hypocotyl explants, MS + 2 mg/l TDZ + 0.1 mg/l NAA + 9 mg/l $AgNO_3$ (Kamal et al., 2020), in okra (*Abelmoschus esculentus* L.), it was stated that callus was obtained from the combination of MS salts + B5 vitamins + 2 mg / l NAA + 0.5 mg / l TDZ (Anisuzzaman et al., 2008).

Table 1. Use of TDZ *in vitro* micropropagation of vegetable species

Species	Explant type	Protocol (Optimum TDZ dose)	Result	Reference
Solanaceae				
Tomato				
*L. esculentum*Mill	Anter	MS + 0.022 mg/l TDZ + 1 mg/l IAA	C	Abate et al., 2004
L. esculentum Mill.	Cotyledon	MS + 0.5 mg/l TDZ + 0.5 mg/l BAP MS + 3 mg/l TDZ	SR SR	Osman et al. 2010
L. esculentum	Cotyledon	MS + 3.40 µM TDZ	SR	Ashakiran et al, 2011
L. esculentum L.	Cotyledon	MS+1.5 mg/l TDZ+1.5 mg/l IBA	SR	Vinoth et al., 2012
S. lycopersicum L.	Cotyledon Hypocotyl	MS + 2 mg/l TDZ MS+1.5 mg/l TDZ+0.5 µM IAA	SR SR	Sharma and Srivastava, 2014
L. esculentum Mill.	Leaf	MS + 4 µM TDZ +2.7 µM NAA	SR	Jamous and Abu-Qaoud, 2015
S. *lycopersicum* Mill	Shoot tip	MS + 2 mg/l TDZ MS + 2.5 mg/l TDZ	SR ShE	Ugandhar et al., 2018
S. lycopersicum L. var. *cerasiforme*	Hypocotyl Leaf	MS + 1-2 mg/l TDZ MS + 2 mg/l TDZ	C SR	Farouk Omar, 2019
L. esculentum L.	Leaf	MS+1.5 mg/l picloram+1.0 mg/l TDZ+80 mg/l casein hydrolysate	SR	Vinoth et al., 2019
Pepper				
C. annuum L.	Cotyledon	MS + 1-3 mg/l TDZ	SR	Venkataiah et al., 2003
C. annuum *C. baccatum* *C. frutescens* *C. praetermissum*	Meristem	MS + 4.54 µM/l TDZ MS + 9.18 µM/l TDZ MS + 4.54 µM/l TDZ MS + 4.54 µM/l TDZ	SR	Peddaboina et al., 2006
C. annuum L.	Hypocotyl	MS + 0.75 mg/l TDZ	SR	Sharma et al., 2006
C. annuum L.	Stem Shoot tip	MS + 0.5 µM TDZ	SE	Khan et al., 2006
C. annuum L.	Nodal	MS + 1 µM TDZ	SR	Ahmad et al., 2006
C. annuum L.	Cotyledon	MS + 1.5 mg/l TDZ MS + 1 mg/l TDZ	SR	Ranjan et al., 2010
C. annuum L.	Leaf	MS + 3 mg/l 2,4-D + 0.5 mg/l TDZ MS + 3 mg/l TDZ +0.5 mg/l IAA	SE SR	Ugandhar et al., 2011
C. annuum L.	Internode derived callus	MS + 5 µM BA + 2.5 µM TDZ	SR	Khan et al., 2011

C: Callogenesis, SR: Shoot regeneration, ShE: Shoot elongation, SE: Somatic embryogenesis

Table 1. (Continued)

Species	Explant type	Protocol (Optimum TDZ dose)	Result	Reference
Pepper				
C. annuum L.	Cotyledon	MS + 44.38 µM BAP or 9 µM TDZ +5.77 µM GA_3 + 14.7 µM PAA MS + 8.87 µM BA or 0.45 µM TDZ +5.77 µM GA_3 + 14.7 µM PAA	SR ShE	Mythili et al., 2017
C. annuum L.	Hypocotyl drived callus	MS + 1.5 mg/l TDZ	SR	Ranjan et al., 2018
Eggplant				
S. melongena L.	15 d-old root	MS + 0.45 µM TDZ + 13.3 µM BA	SR	Franklin et al., 2004
S. melongena L.	Hypocotyl	MS + 0.5 mg/l TDZ	SR	Mallaya and Ravishankar, 2013
S.melongena x S. aethiopicum	Leaf	MS + 0.05 mg/l TDZ	SR	Calvo-Asensio et al., 2014
S. melongena L.	Cotyledon Leaf	MS + 2 mg/l TDZ + 0.5 mg/l BAP + 0.5 mg/l NAA	SR	Taghipour et al., 2015
Cucurbitaceae				
***Luffa* spp.**				
Luffa cylindrica L.	Leaf and nodal	MS + 0.5 mg/l TDZ	SR	Sujatha et al., 2013
Squash				
Cucurbita pepo L.	Hypocotyl	MS + 0.5 mg/l TDZ	SR	Pal et al., 2007
Cucurbita pepo L.	Cotyledon	MS + B5 vitamins + 1 mg/l BAP + 0.05 mg/l TDZ	SR	Mookkan, 2015
Cucumber				
Cucumis sativus L.	Unpollinated ovules	CBM + 0.02 mg/l TDZ	G	Gemes-Juhasz et al., 2002
Cucumis sativus L.	Unpollinated ovules	CBM + 0.03-0.07 mg dm^{-3} TDZ	G	Li et al., 2013
Cucumis sativus L.	Unpollinated ovules	IMC + 30 g/l sucrose + 200 mg/l ampicillin + 0.2 mg/l TDZ	ELS	Domblides et al., 2019
Watermelon				
Citrulus lanatus L.	Axillary shoot-tip	MS + 0.5 mg/l TDZ	SR	Badr-Elden et al., 2012
Melon				

Cucumis melo var. *inodorus*	Cotyledon segments and leaf discs	MS + 5 mg/l 2,4-D + 1 mg/l TDZ	SE	Stipp et al., 2001

SR: Shoot regeneration, ShE: Shoot elongation, SE: Somatic embryogenesis, ELS: embryo-like structures, G: Gynogenesis

Table 1. (Continued)

Species	Explant type	Protocol (Optimum TDZ dose)	Result	Reference
Melon				
Cucumis melo L.	Unpollinated ovaries	MS + 0.02-0.04 mg/l TDZ	G	Malik et al., 2011
Bitter melon				
Momordica charantia L.	Mature and immature leaf	MS + B5 vitamins + 7.7 µM NAA + 2.2 µM TDZ MS + 5.5 µM TDZ + 2.2 µM NAA + 3.3 µM $AgNO_3$	C SR	Thiruvengadam et al., 2010
Momordica charantia L.	Stem derived callus	MS + 0.2 mg/l IBA + 0.1 mg/l TDZ	ABF	Tang et al., 2011
Momordica charantia L.	Internodal explants	MS salts + B5 vitamins + 5 µM 2,4-D + 2 µM TDZ MS + 4 µM TDZ + 1.5 µM 2,4-D + 0.07 mM L-glutamine	C SR	Thiruvengadam et al., 2012
Leguminosae				
Cowpea				
Vigna unguiculata L.	Shoot tip	MS + 0.25 mg/l TDZ + 3 g/l activated charcoal + 2 mg/l yeast extract	C, SR	Aasim et al., 2009
Pea				

Pisum sativum L.	Root	MS + 2 mg/l TDZ	SR	Sharma et al., 2017
Bean				
Phaseolus vulgaris L.	Stem	MS + 2 mg/l TDZ + 0.1 mg/l 24-Epi	SR	Taha et al., 2014
Mung Bean				
Vigna radiata L.	Cotyledon hypocotyl	MS + 0.9/0.09 µM TDZ	SR	Amutha et al., 2006
Soybean				
Glycine max L.	Cotyledon petiole	B5 + 0.5 mg/l TDZ + 0.05 mg/l NAA + 5.0 mg/l $AgNO_3$	SR	Zhang et al., 2011
Cruciferae				
Cabbage				
B. oleracea L. var. *capitata*	Leaf Petiole	MS + 0.22 mg/l TDZ + 0.02 mg/l NAA MS + 0.33 mg/l TDZ + 0.02 mg/l NAA	SR	Gambhir and Srivastava, 2015
B. oleracea cv. Pride	Cotyledon Hypocotyl	MS + 0.330 mg/l TDZ + 79.70 mg/l Adenine MS + 0.220 mg/l TDZ + 0.088 mg/l IAA	SR	Gambhir et al., 2017

C: Callogenesis, SR: Shoot regeneration, G: Gynogenesis ABF: Adventitious buds formation

Table 1. (Continued)

Species	Explant type	Protocol (Optimum TDZ dose)	Result	Reference
B. oleracea L. var. *capitata*	Hypocotyl	MS + 2.27 µM TDZ	SR	Ravanfar et al., 2014
B. oleracea	Cotyledon	MS + 1 µM TDZ	SR	Cheng et al., 2001

B. oleracea L. var. *capitata*	Cotyledon	MS + 0.5 mg/l TDZ + 0.1 mg/l NAA	SR	Salim and Rashid, 2014
Broccoli				
B. oleracea L. var. *italica*	Cotyledon	MS + 0.1 mg/l TDZ + 0.1 mg/l NAA MS + 0.5 mg/l TDZ + 0.1 mg/l NAA	SR	Salim and Rashid, 2014
B. oleracea L. var. *italica*	Cotyledon	MS + 0.1 mg/l TDZ + 0.1mg/l NAA	SR	Ravanfar et al., 2014
B. oleracea L. var. *italica*	Hypocotyl Cotyledon	MS + 2 μM TDZ + 0.5 μM IAA MS + 2 μM TDZ +0.59 mM adenine	SR	Kumar and Srivastava, 2015
B. oleracea L. var. *italica*	Hypocotyl Petiole Leaf Cotyledon	MS + 2 μM TDZ + 0.5 μM IAA MS + 2 μM TDZ + 0.107 μM NAA MS + 1 μM TDZ + 0.107 μM NAA MS + 2 μM TDZ + 0.59 adenine	SR	Kumar et al., 2018
Turnip				
B. rapa. var. *turnip*	Seed derived calli	MS + 1 mg/l TDZ + 1 mg/l BA	SR	Kakar et al., 2014
Chinese cabbage				
Chinese cabbage	Cotyledon	MS + 1 mg/l TDZ + 0.5 mg/l NAA + 5 mg/l $AgNO_3$	SR	Fan et al., 2005
B. rapa. var. *parachinenis*	Microspore suspension	NLN-13 + 0.10/0.50 mg/l TDZ	MDE	Jia et al., 2019

Non-heading Chinese cabbage	Hypocotyl Embryo	MS + 2 mg/l TDZ + 0.1 mg/l NAA + 9 mg/l $AgNO_3$ MS + 3 mg/l TDZ + 1 mg/l NAA + 9 mg/l $AgNO_3$	C R	Kamal et al., 2020
Cauliflower				
B. oleracea var. *botrysis*	Hypocotyl Cotyledon	MS salts + B5 vitamins + 0.2 mg/l TDZ + 0.01 mg/l NAA	SR	Yu et al., 2010
B. oleracea var. *botrysis*	Hypocotyl Cotyledon	MS + 0.44 mg/l TDZ + 0.08 mg/l IAA MS + 0.77 mg/l TDZ + 79.7 mg/l adenine	SR	Gaur and Srivastava, 2017

C: Callogenesis, SR: Shoot regeneration, MDE: microspore derived embryo, , R: Root regeneration

Table 1. (Continued)

Amaryllidaceae				
Shallot, Garlic				
Allium ascaloncum L.	Shoot tip	BDS + 0.1 mg/l picloram + 1 mg/l TDZ	SR	Hidayat, 2004
Malvaceae				
Okra				
Abelmoschus esculentus L.	Hypocotyl	MS salts + B5 vitamins + 2 mg/l NAA + 0.5 mg/l TDZ	C	Anisuzzaman et al., 2008
Abelmoschus esculentus L.	Cotyledonary node meristem Hypocotyl Cotyledon	MS salts + B5 vitamins + 1mg/l BAP + 1 mg/l NAA + 0.04 mg/l TDZ	SR R C	Mallela et al., 2009
Abelmoschus esculentus L.	Cotyledonary node	MS + 0.044 µM TDZ	SR	Kabir et. al., 2016

Apiaceae				
Celery				
Apium graveolens L.	Seed	MS + 1 mg/l TDZ	SR	You et al., 2007

C: Callogenesis, SR: Shoot regeneration, R: Root regeneration, BR: Bulb regeneration

STIMULATION OF GYNOGENESIS AND ANDROGENESIS

TDZ added to nutrient media at different rates can stimulate *in vitro* gynogenesis and androgenesis. In cucumber (*Cucumis sativus* L.), gynogenesis formation was stimulated from unpolinated ovules explants at doses of 0.02 mg/l TDZ (Gemes-Juhasz et al., 2002) and 0.03-0.07 mg/l TDZ (Li et al., 2013) added to CBM nutrient medium. In another study, gynogenesis was induced from a dose of 0.2 mg/l TDZ added to IMC nutrient medium (Domblides et al., 2019) (Table 1). In a study on melon; positive results were obtained from the 0.02-0.04 mg/l TDZ dose added to the MS medium (Malik et al., 2011). In *B. rapa* var. *parachinensis*, when the microspore suspension was cultured in NLN-13 medium supplemented with 0.10/0.50 mg/l TDZ, it was reported that androgenesis was stimulated and microspore-derived embryos (MDE) were obtained (Jia et al., 2019).

CONCLUSIONS

In conclusion, in this review, the uses of TDZ in *in vitro* plant tissue cultures in different vegetable species are summarized. As a result of the researches, it has been determined that TDZ is mostly used for in vitro shoot regeneration in plant tissue culture studies, as well as for stimulation of callus formation, stimulation of somatic embryogenesis, androgenesis and gynogenesis.

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FATTY ACID COMPOSITION AND DIVERSITY OF GRAPE SEEDS

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ABSTRACT

Horticulture plants including grapes has gained more popularity during last two decades due to increasing interest to plant-derived natural products. In this context food industry, consumers, and public health organizations etc. has great attention to health benefits of horticulture plants. In addition, attention is focused on grape by-products identification and bioactivity evaluation. In this study, we attempted to determine fatty acid profile of black grapes including three clones of Karaerik grape variety, one national variety (Okuzgozu) and one international variety (Cabernet Sauvignon). A total of 10 fatty acids were determined in seeds of grape varieties and clones. Results indicated that fatty acid profile of varieties and clones differs each other. Linoleic acid (18:2) were found to be predominant for all varieties and clones ranged from 52.33 to 64.21%, and followed by oleic acid (18:1) in range of 17.83-23.14%, palmitic acid (16:0) between 8.64–13.36%, and stearic acid (18:0) between 4.04-6.11%, respectively. Grape varieties and clones had more polyunsaturated fatty acids (PUFA) than saturated fatty acids (SFA) in their seed oils. ΣPUFA/ΣSFA were in range of 2.58 and 4.88 among varieties. Results indicated that there was diversity among Karaerik grape clones for fatty acid profiles. Fatty acid richness of Karaerik clones comparable with Okuzgozu and Cabernet Sauvignon as well.

Keywords: Grape, by product, fatty acids, metabolic profile

INTRODUCTION

Grapes (*Vitis* spp.) is widely grown almost all continents over the world from USA to China and from New Zealand to Russia with annual world production of nearly 79 M tones (FAOSTAT, 2020). Among the grape producer countries, China was the largest producer of grapes, accounting for 16.9% of global production and followed by Italy with 10.8%, and the United States (8.7%), Spain (8.4%), France (7.8%) and Turkey (4.9%), respectively. Half of the global grape production processed into wine, and the rest mainly used as fresh and processed into dried grapes (raisins) (Bordiga et al., 2019).

Mediterranean region is famous for wine production and particularly Italy, France and Spain are leading the world wine production accounting half of the world production (http://www.oiv.int). As a member of Mediterranean countries, wine production of Turkey is lower than the other Mediterranean countries and USA and instead of wine, juice (şıra) and molasses production is placed the most in industry in the country. In Turkey, winery and grape juice and molasses industries produce large amount of by products (grape pomace). In fact, grape pomace corresponds to the 20% w/w of grape berry weight and grape seeds constitute up to 52% (w/w) of grape pomace (Silva et al., 2018; Pasini et al., 2019). The grape pomace as by-product have been neglected in grape producing countries for many years and only used as traditionally to produce some distillates, or as animal feed and fertilizer (Bordiga et al., 2019). However, this figure has changed over the past decade and alternative ways to valorize grape pomace have emerged (Lucarni et al., 2018).

Grape seeds not only rich in lipids (e.g., linoleic acid) but also it shows richness of proteins, carbohydrates, vitamin E, phytochemicals, polyphenols and natural antioxidants (Shinagawa et al., 2015; Aljuhaimi and Ozcan, 2017; Durante et al., 2017; Mirbagheri et al., 2018). In particular rich unsaturated fatty acid profile and antioxidant components in grape seed oil make the product very significant and beneficial for human health. Thus, more recently attention has been paid on grape seed oil in nutraceutical market with a focus on its health and wellness properties (Doshi et al., 2015). It is used for formulations of dietary supplements, and food additives. In fact, centuries grape seed oil is known and in Mediterranean cuisine primarily used as a gourmet oil and in salad dressings due to its nutty and light flavor (Aksoy et al., 2021). Another aspect of grape seed oil is that due to its natural structure and it can be a good raw material alternative for "eco cosmetic" products. They also have moisturizing properties for the skin (Da Porto and Nunez, 2017).

In Turkey the studies on grape seed oil metabolomic profiling is limited. Thus, the aim of this study to determine seed oil fatty acid profile of black grape varieties/clones including Cabernet Sauvignon, Okuzgozu and Karaerik.

MATERIAL AND METHODS

Plant material

Grapes in particular black skin colored are renowned for their excellent eating quality and full colour, enjoying worldwide popularity. In present study we used black grape varieties. Grape seeds obtained from 3 clones belongs to Karaerik variety. Clones showed different berry shapes (round, oblate, ovoid). One national variety (Okuzgozu) and one international variety (Cabernet Sauvignon) are also included the study. After harvest the seeds separated from berries and also removed of foreign substances.

Oil extraction

Automatic soxhlet device was used for total oil (lipid) extractions. Approximately one hundred fifty grams of dried grape seeds were used for oil extraction. Hexane used as solvent.

Determination of fatty acids

The fatty acids were analyzed by a GC ((Perkin Elmer, Shelton, USA). Chromatographic separation was done using a (30 m×0.25 mm) column equipped with a flame ionization detector (FID). The oven temperature was 120 ºC for 2 min, raised to 5 ºC/min to 220 ºC, which was held for 10 min, while the injector and the detector temperatures were set at 280 ºC and 260 ºC, respectively. The results were expressed in GC area % as a mean value and ± standard deviation.

Statistical analysis

All data were analyzed using SPSS software and procedures. Analysis of variance tables were constructed using the Least Significant Difference (LSD) method at $p<0.05$.

RESULTS AND DISCUSSION

The fatty acid profiling of seeds of three grape clones belongs to Karaerik variety and one national variety, Okuzgozu and also one international variety, Cabernet Sauvignon are shown in Table 1. Same Table also included ΣSFA (total saturated fatty acids) ΣMUFA (total monounsaturated fatty acids), ΣPUFA (total polyunsaturated fatty acids) and ΣPUFA/ ΣSFA ratio of clones and varieties.

As listed in Table 1, we determined ten different fatty acids in analyzed oils and among them 5 were belongs to saturated fatty acid groups (C14:0, C:16:0, C17:0, C18:0 and C20:0), 3 were within monounsaturated fatty acid group (C16:1, C18:1 and C20:1) and 2 were within polyunsaturated fatty acid group (C18:2 and C18:3), respectively. The effects of variety and clones were significant in the case of palmitic acid, stearic acid, oleic acid, gadoleic acid and linoleic acid (Table 1).

Table 1. Fatty acids in grape seeds (% of Σ FAME)

	Karaerik 1	Karaerik 2	Karaerik 3	Okuzgozu	Cabernet Sauvignon
Myristic acid (C14:0)	0.20±0.01^{NS}	0.24±0.01	0.27±0.02	0.33±0.01	0.14±0.01
Palmitic acid (C16:0)	9.93±0.3b	10.33±0.2a	8.64±0.2b	13.36±0.2a	8.81±0.09b
Margaric acid (C17:0)	0.10±0.01^{NS}	0.14±0.01	0.10±0.01	0.10±0.04	0.06±0.00
Stearic acid (C18:0)	5.25±0.06b	4.39±0.04bc	5.70±0.04ab	6.11±0.09a	4.04±0.05c
Arachidic acid (C20:0)	0.50±0.01^{NS}	0.41±0.01	0.50±0.01	0.60±0.01	0.20±0.01
Palmitoleic acid (C16:1)	1.34±0.02^{NS}	1.24±0.02	1.30±0.01	1.10±0.04	0.56±0.04
Oleic acid (C18:1)	20.22±0.3c	21.13±0.2b	23.14±0.2a	21.10±0.2b	17.83±0.1d
Gadoleic acid (C20:1)	0.90±0.01ab	1.02±0.02a	0.80±0.01ab	0.70±0.01ab	0.40±0.01b
Linoleic acid (C18:2)	59.20±0.4b	58.44±0.3c	57.11±0.5d	52.33±0.4e	64.21±0.7a
α-Linolenic acid (C18:3)	0.35±0.01^{NS}	0.40±0.01	0.44±0.01	0.50±0.02	0.41±0.01
ΣSFA	15.98±0.02b	15.51±0.02b	15.21±0.02b	20.50±0.02a	13.25±0.01c
ΣMUFA	21.45±0.02b	23.39±0.02ab	24.44±0.02a	22.90±0.02ab	18.79±0.02c
ΣPUFA	59.55±0.06ab	58.84±0.06b	57.91±0.03ab	52.83±0.06c	64.62±0.05a
ΣPUFA/ΣSFA	3.73	3.78	3.81	2.58	4.88

Data ex expressed as the mean ± standard deviation, $n = 3$. *Different letters in the same row indicate significant difference ($p<0.05$). FAME, fatty acid methyl esters; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. NS: Non significant

Moreover ΣSFA (Total saturated fatty acids), ΣMUFA (Total monounsaturated fatty acids) and ΣPUFA (Total polyunsaturated fatty acids) in seeds of clones and varieties were found significant each other at 0.05 level.

Palmitic and stearic acid were the major saturated fatty acids in all used varieties and clones and myristic, margaric and arachidic acid content were negligible (Table 1).

Oleic acid was dominant monounsaturated fatty acids among used materials and were found between 17.83 and 23.14%, respectively. The Karaerik clone 3 had an increased oleic acid content and differed from the rest of varieties and clones (Table 1).

The differences in the content of this major saturated fatty acid were then reflected in the difference in total SFAs (Table 1).

On the other hand, all varieties and clones were characterized by high linoleic acid (polyunsaturated fatty acid) content which were between 52.33% (cv. Okuzgozu) and 64.21% (cv. Cabernet Sauvignon) (Table 1).

Kamel et al. (1985) studied on fatty acid profile of grape seeds belongs to varieties and found that major fatty acid in grape seeds was linoleic acid. In western Turkey, Uslu and Dardeniz (2009) used seeds of 12 grape varieties and found that seed oil of varieties had linoleic acid between 72.50-77.59%, oleic acid between 11.62-16.10%, palmitic acid between 6.51-8.40%, stearic acid between 3.07-3.86%, linolenic acid between 0.11-0.46% and arachidic acid between 0.10-0.68%, respectively indicating similarities with our results. Aljuhaimi and Özcan (2017) used seeds of 17 grape seeds in Turkey and they obtained oils by the cold press method and reported that the main fatty acids were of the ranges 60.7-68.5% linoleic, 16.1-23.4% oleic and 8.0-10.2% palmitic acid. They also found that the highest percentages of linoleic acid (68.5%) was determined in Siyah pekmezlik seed oil. Ariturk et al. (2021) studied on fatty acid profile of two important black grapes and found that fatty acids vary according to grape varieties. They found that major fatty acids were linoleic acid varied from 47.56 to 54.75%, and followed by palmitic acid between 15.26 and 20.45% and oleic acid between 17.90 to 19.40%, respectively which in agreement with our present study. In Turkey, Sevindik and Selli (2016) and Islek (2018) reported that in grape seeds belongs to different varieties, main fatty acids were linoleic, oleic, palmitic and stearic acid and they implied that the differences between grape seed fatty acid profile of varieties were found to be statistically significant. Izzo and Muratore (1993) studied on red and white grape varieties seed oils in Italy and found that linoleic (65.9–62.2%), oleic (18.6-16.9%), palmitic (11.6–10.7%) and stearic acid (3.8–3.4%) were dominant. Han Chul et al. (2001) used grape seeds in Korea and reported that predominant fatty acid was linoleic acid (66.15%), and oleic, palmitic and stearic acids were also found as major in grape seeds. Dabetic et al. (2020) used six grape varieties to determine seed fatty acid composition in Serbia and found major fatty acids was linoleic acid contributing between 62.8% and 67.4% of total fatty acids, followed by oleic acid (15.3 to 18.9%) with in coincide with our study. Fernandes et al. (2013) used seeds of ten grape varieties in Portuguese and revealed that SFA were in range of 11.64-14.94%, MUFA were 14.19-21.29%, and PUFA were between 63.64-73.53% that supports our findings. Lutterodt et al. (2011) found that in grape seeds PUFA were changed from 66.3 to 75.8%, MUFA changed from 14.5 to 22.2% and SFA changed from 9.66 to 12.6%. In another study Tangolar et al. (2009) reported PUFA between 62.88 and 69.49% MUFA between 18.19 and 23.29% and SFA between 12.01-15.10% in grape seeds belong to a number of varieties. Nutritionally, C18:1 is considered as the most important MUFA (Reddy and Katan, 2004). Karaerik 3 clone was found to contain significantly higher MUFA ($p < 0.05$) (Table 1).

Considering general classification of fatty acid, sequence was PUFA>MUFA>SFA in seeds of investigated black grape varieties. We found PUFA/SFA ratio between 2.58 for Okuzgozu and 4.88 for Cabernet Sauvignon which shows good values for nutritional perspective as dietary fat by British Department of Health (Anon. 1994). Obtained PUFA/SFA values were similar to those previously report by Dabetic et al. (2020).

The studies revealed that fatty acid profile in grape seeds were strongly affected by genotype, environment in particular altitude, maturation stage, soil composition and type, harvest period etc. (Baydar and Akkurt, 2001; Han Chul et al., 2001; Demirtas et al., 2013; Islek, 2018).

CONCLUSIONS

In literature, very limited studies so far have been concerned with fatty acid composition of oils obtained from local grape varieties and clones in Turkey. This implied the importance of the present study. Results also indicate that local Karaerik variety and its clones was outstanding with one of the lowest contents of SFA and clonal variation among them on fatty acid profiles was evident. Taking all these facts into account, Karaerik variety and clones can be recommended for human consumption due to comparable fatty acid profile with the other investigated grape varieties.

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CHARACTERISTICS OF LIVESTOCK FARMERS AND TRADERS PARTICIPATING IN LIVESTOCK MARKETS: A CASE STUDY FROM THE REPUBLIC OF BENIN

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Abstract

Livestock farming system in Africa is mostly extensive (traditional). Most of the West African countries have a pasture-based livestock system. Livestock farming in Benin is mainly family production system and practiced by pastoralists and agro-pastoralists. It is applied with a minimum monetary investment. Farmers market their animals in different type of livestock markets. In this study, primary data were collected from face-to-face surveys of a random sample of 600 respondents consisting of livestock farmers (300) and livestock traders (300) participating in self-managed livestock markets (MBA) and traditional livestock markets (MT). MT livestock markets are the oldest livestock trading system in the study areas. The MBA are the modern livestock markets where transactions are under strict control. In both livestock markets, cattle and small ruminants (sheep and goat) are traded live. This study was conducted to describe characteristics of the participants in the MBA and MT livestock markets and to compare the two marketing systems. The results showed that in both markets, 97.67% and 98% of farmers and traders respectively were men and only 2.33% and 2% of farmers and traders respectively were women. Of the farmers, 45.33% are between 41-60 years old, and 48.33% of traders are between 21-40 years old. Most farmers and traders in both markets had completed primary education. 40% and 38.6% of farmers have a family size of less than 3 members and between 4-7 members respectively and 42% of traders have a family size between 4-7 members. Of the farmers, 49.67% have more than 21 years of experience in livestock farming and 29.66% traders have between 6-10 years in livestock marketing.

Keywords: self-managed livestock markets, traditional livestock markets, Republic of Benin, pastoralists, agro-pastoralists, marketing systems

Introduction

Located in the intertropical zone, Benin is a coastal country in West Africa covering an area of 114,763 km2 with a population of just over 10 million and an estimated current Gross Domestic Product (GDP) of $8.4 billion in 2015 (PADDSA, 2017). The economy is oriented towards agriculture and the processing of agricultural products, sectors largely based on the two export products of cotton and cashew nuts, and towards trade (Fourcaut, 2020). It is the main production activity for rural livelihoods in Benin (Akouegnonhou and Demirbas, 2019). Agriculture sector contributes 32% of GDP and employs 70% of the workforce (FAO, 2018).

Livestock occupies an important place in the production system of the agricultural sector. It ranks second after crop production in terms of potentially exploitable natural resources that can be converted into foreign currency (ANOPER, 2014; Fabien, 2019).

Livestock farming is traditional in Benin. It is a semi-sedentary production system with the practice of a small transhumance during the crop season (to avoid damage to crops) and a large transhumance during the dry season (ANOPER, 2014).

The populations that practice livestock activities are mainly pastoralists and more recently agro-pastoralists who combine livestock with agriculture for reasons of animal traction, transport, manure, etc. (Onibon, 2004; INSAE, 2018). For pastoral and agro-pastoral populations, livestock is the main source of income on which they depend for survival. To convert their livestock into cash, they go to the livestock markets.

The livestock markets are mainly run by pastoralists, agro-pastoralists, and livestock traders (Hauglustaine and Mees, 2015). They come from many places to sell and buy animals. Some for pure commerce, others for family needs.

In this study, traditional livestock markets (MT) and self-managed or modern livestock markets (MBA) are the two types of livestock markets studied in the field surveys. Livestock farmers and traders appear under several characteristics that are described in this paper.

MBA are the new model for the animal marketing system. This type of market is a farmer's innovation of development, initiated by farmers of the North-Benin. It is a strategy that has the merit of involving all stakeholders in its operation, and is based on a willingness to learn, organize and train new leaders, and the rise of local entrepreneurs (Onibon, 2004). It concerns the chain of large (cattle) and small ruminants (sheep and goats) that farmers, butchers, livestock traders and other actors learn to self-organize among themselves, to build this new model of livestock market management that differs from the old traditional mode that is not suitable for local development (Onibon, 2004)..

MT are the old livestock marketing system that is widespread throughout Africa (Djedjebi, 2009). This system is particularly characterized by long marketing chains managed by traders and brokers (Hauglustaine and Mees, 2015).

The objective of this study is to describe the characteristics of livestock farmers and traders participating in livestock markets. This will provide important information about the stakeholders involved in each livestock market.

Material and Method

This research was carried out in the Republic of Benin. Two different livestock markets were concerned in the study area. These are traditional livestock markets (MT) and self-managed livestock markets (MBA). MT are the oldest livestock trading system in the local areas. The marketing systems in this type of market is traditional and they are somewhat further away from city centres. The MBA are the modern livestock markets where the transaction controls are more rigorous. The trading system in the MBA market is much more organized and modernized compared to MT markets.

The main material for this study was obtained from face-to-face surveys with 600 respondents consisting of livestock farmers (300) and livestock traders (300), randomly selected from livestock markets of Gogounou, Nikki, Bassila, Matéri, Savè and Iwoyé (Kétou). Descriptive statistics such as percentage calculations were used to evaluate the data in accordance with the purpose of the study. Obtained data are shown with tables.

Result and Discussion

Livestock farming system in the Republic Benin

Livestock farming system in Africa is mostly extensive (traditional). The intensive system is still not much developed. Most of the West African countries have a grazing system. Livestock production in Benin is conducted predominantly as family production system. It is applied with a minimum monetary investment. It is currently evolving into a mixed crop-livestock system under the influence of cotton cultivation and the introduction of certain food products (Chabi, 2016).

The main actors in the livestock marketing system: pastoralists, agro-pastoralists, transhumant, traders, butchers.

Livestock farmers

They are composed of pastoralists and agro-pastoralists mostly from the Peulh and Gando ethnic groups who own large herds of cattle in Benin. They mainly raise cattle and practice a semi-sedentary livestock farming. In addition to these pastoralists, there are farmers who also raise livestock for agricultural services such as animal traction and sometimes transport of agricultural products. Finally, it is important to mention the presence of transhumant farmers who come from neighbouring countries (Onibon, 2004; Djedjebi, 2009; Chabi, 2016).

Livestock traders

Collectors are generally small traders. Also known as rural assemblers, they usually collect / assemble animals from their locality and remote markets and supply to big and small-scale traders in livestock markets (Girma and Abebaw, 2012). They play on prices to earn small margins compared to the margins of other traders in the marketing channel.

Wholesalers are large traders of livestock. They may be natives or foreigners from several neighbouring countries. They are permanently known for purchasing large numbers of animals from a variety of sources in order to supply their customers (Ayele et al., 2017).

Slaughterhouse / butchery are retailers. The retailers are mostly butchers, restaurant managers and kebab sellers.

Intermediaries: The intermediaries also called brokers or « Dilalï » (Haoussa name) are a traditional institution in livestock markets. They still exist in MT markets and are key players in the marketing chain, but in MBA markets they are converted into witnesses and their role is limited to witnessing the transaction and certifying animals. The system of managing the livestock market through intermediaries does not allow all actors to be involved in the market management, nor does it promote a price policy in favour of the farmers, as required by the pastoral policy in Benin. It is a system that is contrary to the principles of self-promotion of livestock markets and therefore does not constitute an appropriate approach to local democracy and the current local development context (Onibon, 2004; Djedjebi, 2009).

General characteristics of livestock farmers in MBA and MT livestock markets

Based on the Table 1, most of the farmers interviewed in the study area (97.67%) in both markets were men and only 2.33% were women. Men are generally head of the households and owners of the herd; their sons are shepherds; their wife has the right to milk the herd and sell it (Chabi, 2016). This could be due to the fact that livestock marketing involves physical activities such as trekking, chasing and restraining the animal, hence men are more capable due to their masculine nature. Another reason might be the customs of the traditional livestock keeping societies as in the societies almost all livestock belong to men while women only own some animals through inheritance (Mapunda, 2007; Chabi, 2016).

Of the farmers, 45.33% were between 41-60 years old, and 43.34% were between 21-40 years old. The results indicate that livestock farming is dominated by people with advanced age. This is due to the fact that among pastoralists in Africa especially, it is the older ones who own the livestock (Montshwe, 2006).

The efficiency of livestock production and marketing can be improved through the education of its actors. The largest group (76%) of the farmers in both markets had a primary education level. Farmers in MBA market had the highest education level. This may be due to the young educated agro-pastoralists and pastoralists who are now interested in the new system of self-managed livestock markets, which is also their full-time job. But the situation is the opposite with the farmers of the MT markets where people are older. According to Mcfalls (2003) a population comprised of old and low levels of education, tends to resist change and lack initiatives. Of the farmers, 30% have a farmland between 3-4 ha and 27% have a farmland less than 2 ha.

The family size of 40% of the farmers is less than or equal to 3 members, followed by those (38.6%) who have a family size between 4-7 members. Traditional livestock keeping societies practice polygamy and hence tend to have big families. It also depends on their ethnic group. Normally as herd grows, the owner (man) marries another woman in order to get more children to take care of the growing herd. Because of the vital role played by children, especially sons, as herd men, the size of the household is usually large (Rupindo, 2009).

Of the farmers, 49.67% have more than 21 years of experience in livestock farming. Most of the farmers have been involved in animal husbandry since childhood.

Table 1. Descriptive statistics results for the livestock farmers

	MBA		MT		General	
	Number	%	Number	%	Number	%
Gender						
Male	147	98.0	146	97.3	293	97.67
Female	3	2.0	4	2.7	7	2.33
Total	150	100.0	150	100.0	300	100.0
Age						
21-40	90	60.0	40	26.7	130	43.34
41-60	48	32.0	88	58.7	136	45.33
>61	12	8.0	22	14.7	34	11.33
Total	150	100.0	150	100.0	300	100.0
Education (year)						
Uneducated (<1)	3	2	63	42	66	22
Primary school (1-6)	142	94.7	86	57.3	228	76

College (7-10)	5	3.3	1	0.7	6	2
Total	150	100.0	150	100.0	300	100
Land size (ha)						
<2	63	42.0	18	12.0	81	27
3-4	60	40.0	30	20.0	90	30
5-6	25	16.7	48	32.0	73	24.33
>7	2	1.3	54	36.0	56	18.67
Total	150	100.0	150	100.0	300	100
Household Size						
≤3	94	62.7	26	17.3	120	40
4-7	44	29.3	72	48.0	116	38.67
8-11	12	8.0	51	34.0	63	21
≥12	-	-	1	0.7	1	0.33
Total	150	100.0	150	100.0	300	100
Experience (year)						
≤5	5	3.3	8	5.3	13	4.33
6-10	25	16.7	33	22.0	58	19.33
11-20	41	27.3	39	26.0	80	26.67
≥21	79	52.7	70	46.7	149	49.67
Total	150	100.0	150	100.0	300	100

General characteristics of livestock traders in MBA and MT livestock markets

Cattle traders are composed of several ethnic groups. The most dominant are the *Hausa*, *Zerma*, and *Peulh*. Traders of live animals represent the second group of actors after livestock farmers. They are composed of collectors, wholesalers and retailers. Traders buy animals, usually with the help of intermediaries in the MT markets but directly from farmers in the MBA markets. According to Table 2, most of the traders interviewed (98%) in the study area in both markets were men and only 2% were women. The women traders only market the small ruminants (sheep and goat). 48.33% of livestock traders were between 21- 40 years and old 43.67% were between 41-60 years old. The livestock marketing system is dominated by young traders, especially because of the MBA markets. MBA markets are more attracted by young traders from both inside and outside the country (Onibon, 2004). The largest group (62%) of the traders in both markets have a primary education level and some of them (1.3%) have high education level.

Of the traders, 42% of have a family size between 4-7 members, followed by those (25.3% and 25.0%) who have a family size between 8-11 and <3 members respectively. 45% of traders have 6-10 years of experience in livestock marketing, followed by those (30.7%) who have 11-20 years of experience. This is due to the recent interest in animal trading as well as the MBA that have become the focal point for livestock traders (DDC, 2017).

Table 2. Descriptive statistics results for the livestock traders

	MBA		MT		General	
	Number	%	Number	%	Number	%
Gender						
Male	146	97.3	148	98.7	294	98
Female	4	2.7	2	1.3	6	2
Total	150	100.0	150	100.0	300	100
Age						
21-40	89	59.4	56	37.3	145	48.33
41-60	54	36.0	77	51.3	131	43.67
>61	7	4.7	17	11.3	24	8
Total	150	100.0	150	100	300	100
Education (year)						
Uneducated (<1)	13	8.7	90	60.0	103	34.3
Primary school (1-6)	127	84.7	59	39.3	186	62
College (7-10)	4	1.3	1	0.3	5	1.7
High school (11-13)	2	0.7	0	0.0	2	0.7
University undergraduate (14-17)	4	1.3	0	0.0	4	1.3
Total	150	100.0	150	100.0	300	100.0
Household Size						
≤3	55	36.7	20	13.3	75	25.0
4-7	56	37.3	70	46.7	126	42.0
8-11	29	19.3	47	31.3	76	25.3
≥12	10	6.7	13	8.7	23	7.7
Total	150	100.0	150	100.0	300	100.0
Experience (year)						
≤5	25	16.7	26	17.3	51	17.0
6-10	71	47.3	64	42.7	135	45.0
11-20	45	30.0	47	31.3	92	30.7
≥21	9	6.0	13	8.7	22	7.3
Total	150	100.0	150	100.0	300	100.0

Conclusion

The results of the study showed that participants in the MBA and MT markets in Benin are predominantly men, with 97.67% of farmers and 98% of livestock traders participating in both types of markets. The number of women involved in livestock markets is much lower. Women are more involved in other activities such as catering, selling condiments, etc. in the livestock markets. Most farmers and livestock traders are under the age of 60. The majority of households are large, with 4-7 members. The large size of their households allows them to have the necessary labour to carry out their farm and domestic activities (Chabi, 2016). The majority of farmers and traders had a primary education. Farmers have a lot of experience in livestock farming since they have been involved in it from a young age. Livestock traders have less experience in livestock marketing because the livestock marketing system has been recently established in these rural areas.

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THE DEVELOPMENT OF AQUACULTURE PRODUCTION AND SOME ARGUMENTS IN FISH GENETICS AND BIOTECHNOLOGY STUDIES IN ALBANIA

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ABSTRACT

The expansion of the species structure, the application of intensive forms along with the semi-intensive ones and the inclusion of biotechnological practices in the Albanian aquaculture of the last three decades has created a new vision for this economic activity. In Albania the aquaculture economy, initially realized in semi-intensive systems, has started with rearing of common carp (beginning of the'60s of the last century) and has enriched species structure at the beginning of the '70s the involvement in the production of Chinese cyprinids, such as grass carp (*C.idella*), silver carp (*H.molitrix*), big-head carp (*A.nobilis)* and Wuchan bream (*M.amblycephala)*. Prior to the 1990s, more than 25 cypriniculture farms were put into operation in Albania, mainly in the central and coastal regions of Albania, with a total area of over 800 ha. Intensive aquaculture started in Albania in the mid-1970s with the introduction of rainbow trout (*O.mykiss*). At the end of 2002 and during 2003, significant investments were made for the construction of a hatchery for production of fingerlings of Ohrid trout (*S.letnica*) in Lin (Pogradec). Beginning from the mid-1990s, in a large part of the country, numerous private plants were put into operation, mainly with family administration for the cultivation of rainbow trout. Semi-intensive mariculture in Albania has its beginnings in the mid-90s with the Kavaja farm for the cultivation of the sea shrimp of the species *Marsupenaeus japonicus*. Not too long ago, starting from Bay of Valona to the southern edge of the Albanian coast several intensive swimming plants (about ten private enterprises with a total area of 8000 m^2) have been put into operation for the cultivation of marine ichthyc species, sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). The topic of studies in the fields of fish genetics and biotechnology has been generally related to specific problems of aquaculture in Albania. The species included in the studies were carp, rainbow trout and alloctonic cyprinids, the reproduction of which is realized by applying hypophysation. It is worth mentioning topics such as: a. Evaluations on triploid hybrids, obtained by crossing the "rainbow" trout with the "wild" trout (*S.trutta fario*); b. Biotechnological interventions aimed at increasing the efficiency of hypophysation during hormonal stimulation of reproduction in alloctonic cyprinides; c.Analysis of the impact of geographical variability of environmental factors on the variability of meristematic and morphometric features of carp (C.carpio); d. Genetic determination of reductive evolution for carp scaly cover; e. The role of the moment of realization of hormonal stimulation on the duration of the latency time to reproducers of grass carp (*C.idella*). This theme is realized by the research staffs of the Department of Biotechnology at the University of Tirana and by the Department of Aquaculture and Fisheries at the Agricultural University of Tirana.

Keywords: Albanian aquaculture, Chinese cyprinids, rainbow trout, sea shrimp, marine ichthyc species, fısh genetıcs, bıotechnology studıes.

INTRODUCTION

Aquaculture has a very important role in guaranteeing human nutrition. The development of this economic activity takes on special importance in terms of reducing ichthyotic natural reserves as a result of the application of irrational fishing, overfishing and destructive practices of catches (Stiles, LMet al. 2010; FAO , 2020). Aquaculture in Albania was developed for two purposes; providing protein sources for human food and repopulation of inland natural waters in which professional fishing activity took place (Flloko, A.2005). Being a weak economy, Albania oriented the initial phase of aquaculture towards extensive and semi-intensive forms of cypriniculture (Spaho, V.and A. Floko, 1994; SpahoV., A. Floko and Sh.Selfo 1997; Berni, P.et al., 2004). This aquaculture was characterized by limited yields, but also for products that were produced at minimal cost and that supplied not only the food market but also the canning industry. Albanian aquaculture, with the inclusion in the production of phytophage alloctone cyprinids, rainbow trout, shrimps and bivalve mollusks guaranteed the diversification of ichthyotic products by affecting domestic consumer nutritional behaviours as well as increasing exports.
Studies in the fields of genetics and biotechnology contribute to the modernization of production technologies in aquaculture, guarantee the rapid and efficient racial improvement of species and lines cultivated in aquaculture systems and integrate new elements into artificial reproduction practices. Biotechnology allows scientists to identify and combine traits in fish and shellfish to increase productivity and improve quality. Genetic modification and biotechnology also holds tremendous potential to improve the quality and quantity of fish reared in aquaculture (Mayekar, T.S.et al.1991). Research on genetics and biotechnology of fish farmed in Albania is in its infancy, although the first study on the breeding of trout by creating triploid hybrids was conducted in 1989 (Memia, Sh., Spaho, V.and Jero, J.1989). This study, conducted at the experimental level and not on a commercial scale, had its impact on the expansion of trout culture in our country (Flloko, A.2005). Other studies in the field of aquaculture genetics, which have been conducted in the conditions of Albanian cyprinoculture plants (Sadikaj, R.et al., 2011; Spaho, V.and V. Shermadhi 2014; Shermadhi, V.et al., 2014) were based on the aims and methodologies of previous studies conducted by foreign authors who had worked in the field of genetics of qualitative carp traits (Lindsey,C.C.1988; Kirpicnikov,V.S.1999; Nicolescu, C.2004; Sfakianakis, D.et al., 2011; Casas,L.et al.2013).

MATERIAL AND METHOD

This paper is referential and consequently, its formulation is based on the collection and evaluation of publications that have been made over the years by Albanian authors in the fields of history of aquaculture development in Albania and the evolution of species structure and forms of production. This paper presents the achievements of several studies conducted by Albanian authors in the field of genetics and biotechnology of aquaculture. Most of the selected publications are articles published in domestic and foreign scientific journals. It is worth noting that the works done in the fields of genetics and aquaculture have been published in 3 foreign scientific journals and in two scientific journals of the country.

RESULTS AND DISCUSSION

The expansion of the species structure, the application of intensive forms and the inclusion of biotechnological methods in the Albanian aquaculture of the last three decades has created a new vision for this economic activity. (Berni,P.et al 2004;Flloko,A.2005). Experiences in semi-intensive and intensive aquaculture in Albania start from the beginning of the 60s of the last century. The first plant for artificial reproduction of common carp (*Cyprinus carpio*) to produce fingerlings was created in Saranda while in Pogradec was put into operation a hatchery for production of larvae and fingerings of ohrid endemic trout (*Salmo letnica*). These two plants produced seedlings in the framework of repopulation programs; the first for the carp population of natural and artificial lakes and the second for the repopulation of Lake Ohrid with Ohrid trout (Spaho V.and A.Flloko 1994;Spaho,V.et al.,1997;Spaho,E.2013).

Figure 1. Technical structure in Albanian farms of carp-culture

In 1959 and 1969, four allotonic species (allocton or alien species) were imported from the Chinese Republic that were part of the Cyprinidae family such as silver carp (*Hypophthalmichthys molitrix*), big-head carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*) and Wuchan bream (*Megalobrama amblycephala*) (Rakaj,N.and A.Flloko 1995; Spaho,V.et al.,1997;Spaho,V.2007).

In 1972, aquaculture farm for rearing of cyprinid fishes in Bregu i Lumit near Tirana became the first plant in Albania where artificial reproduction was done by hypophysation stimulation of ictic Chinese alloctonic species. The experience of this activity, which spread to all carp-cultivation plants in our country, served to expand the cultivation of cyprinids on farms and enabled the implementation of repopulation programs of different categories of inland waters, guaranteeing the improvement of their fish productivity values.

Figure 2. Common carp family fish that reared in the Albanian farms of freshwater aquaculture.

Before the 90s of the last century in Albania were put into operation more than 25 cultivation plants of cypriniculture, mainly in the central and coastal regions of Albania, with a total area of over 800 ha (Kavaja 200 ha, Durres 200 ha, Vlora 150 ha, Shkodra 100 ha, Elbasan 30 ha etc.) (Flloko,A.2005).

Figure 3. Technical structure in Albanian plants of trout culture.

In 1978, rainbow trout (Oncorhynchus mykiss) was imported for the first time from Italy and in 1980 was put into operation the first farm in Saranda with concrete tanks and ponds of 7 ha for rearing and production of this fish. This plant achieved an average annual production of 200 tons of trout for the food market and about 1 million seedlings. At the end of 2002 and during 2003, significant investments were made for the construction of a modern incubator for laying Ohrid trout eggs in Lin (Pogradec). Starting from the mid-1990s, in a large part of the country, numerous private plants were put into operation, mainly with family administration (250-1500 m^2), for the cultivation of rainbow trout. (Flloko,A.2005).

In the ’80s cultivation of Mediterranean mussel (*Mytilus galloprovincialis*) was carried out in 80 concrete structures of panel type installed on Butrint Lake. The farm of this mollusc guaranteed an average annual production of 2000 tons, with the maximum reached during 1989 of 5000 tons. With the cessation of the export of live molluscs, in the early '90s this activity suffered a gradual reduction. Currently the mussel farm is privatized but only a part of the panels are in operation. There are attempts by private individuals to apply in Butrint Lake the cultivation of molluscs in “long line” swimming plants

Figure 4. Technical structure for the cultivation and depuration of mussels (M.galloprovincialis) in Butrint Lake.

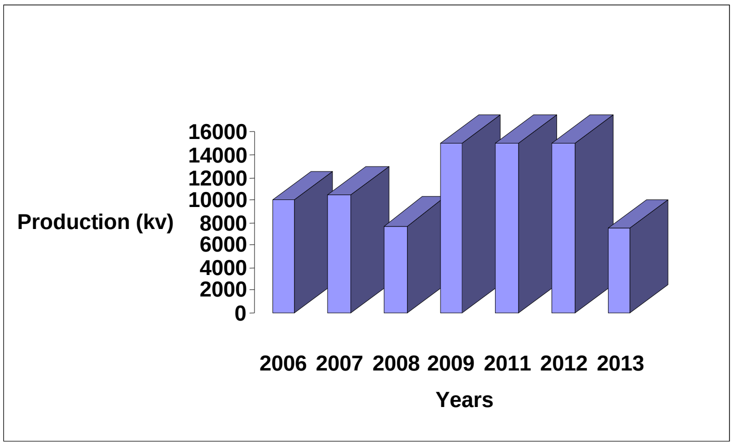

Figure 5. Quantities of mussels (kv) that have been produced in the plants of Butrint Lake for the period 2006-2013 (according to the "Project for the mussel-Butrint agricultural cooperation company").

The first intensive mariculture started operating in the mid-1990s at the Kavaja plant for the cultivation of the sea shrimp of the species Marsupenaeus japonicus (kuruma shrimp). The joint Italian-Albanian economic initiative, KAP-Kavaja operated for more than ten years, transforming the incubation and cultivation system from specialization for the production of freshwater species to specialization for shrimp reproduction, production of post-larvae and shrimp breeding for the food market. This was achieved thanks to the investments for the improvement of technology in this important direction of aquaculture (Arapi, D. et al., 2009). Not too long ago starting from the Gulf of Vlora to the southern part of the Albanian coast, several swimming plants (about ten private enterprises with a total area of about 8000 m^2) have been put into operation for the cultivation of ictic marine fish like sea bream (Sparus aurata) and sea bass (Dicentrarchus labrax) (Flloko,A.2005).

Figure 6. Floating cages for the cultivation of marine fish in the Albanian coast of Adriatic sea and Ion sea.

The first production in 2001 was about 20 tons. The Ionian coast has suitable areas for the development of mariculture and there are no negative interactions with other activities that take place there. The results achieved by Albanian mariculture in recent years are promising and have stimulated the interest of domestic and foreign business for the expansion of this activity in other areas of the coast.

The evolution of aquaculture forms and technological progress of this economy in Albania has been accompanied by scientific research activities which have been carried out by specialized institutions, such as the Institute of Aquaculture and Fisheries Research (Durres) and institutions of higher education, such as the Department of Aquaculture and Fisheries in the Agricultural University (Tirana) and the Department of Biotechnology at the University of Tirana. Studies conducted in the fields of genetics and biotechnology have mainly included ictic species cultivated by the Albanian inland aquaculture, namely the common carp (Cyprinus carpio typicus) and the rainbow trout (Oncorhynchus mykiss). The main applied goals of these studies have been the optimization of the connections between the ictic form (genotype / phenotype) produced in the aquaculture system and the technology (semi-intensive / intensive) applied as well as the racial improvement of the respective aquaculture species by practicing interspecies hybridization.

Aiming to inform this forum with some of the achievements of research activity in the mentioned fields, we will focus on some arguments that constitute the main models of studies that have been conducted by Albanian aquaculture researchers.

The earliest study conducted in the field of genetics applied in aquaculture was valuable triploid hybrids, obtained by crossing the "rainbow" trout with the "wild" trout (Memia, Sh et al., 1989). The aim of this study was to create triploid hybrids (3n), fertilizing the eggs of the rainbow trout (O.mykiss) with the sperm of the brown trout (Salmo trutta fario), which was found as a wild form (not cultivated) in the river of Bistrica (South of Albania). A group of fertilized eggs were subjected to "thermal shock", in order to perform induced gynogenesis through this procedure, ensuring the retention of the second polar body. From the counting of chromosomes in triploid F1 hybrids between rainbow trout (female breeders) and wild brown trout (male breeders) it resulted that the total fund was 3n = 100 chromosomes (Figure 7b). The triploid number of chromosomes in gynogenetic offspring was caused by the fact that the ovules, as a result of the action of thermal shock, kept the diploid polar body, maintaining the number of 58 chromosomes. Other chromosomes, with haploid status (n = 42), belonged to the sperm of the male parent. In this way, the application of thermal shock-induced gynogenesis led to the union of diploid ovules with haploid sperm, generating subsequent triploid hybrid trout (3n = 100 chromosomes).

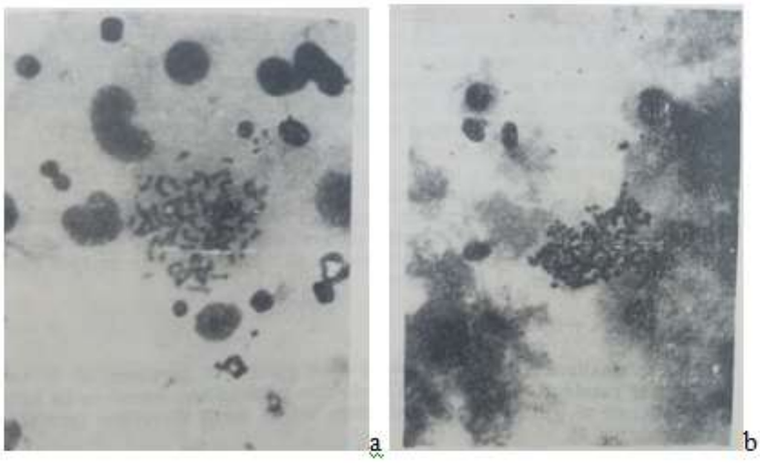

Figure 7.a. Kariogram of rainbow trout (*O.mykis*,ex.*Salmo gairdneri*),2n = 58 chromosomes;b.Kariogram of hybrid triploids ,3n = 100 chromosomes , obtained from crossbreeding O.mykis (♀) x S.trutta fario (♂), after application of thermal shock induced induction gynogenesis to fertilized eggs (Memia,Sh.et al.,1989).

It was found that the fertilized eggs belonging to the control group, which underwent heat shock experienced gynogenesis (ie contained triploid hybrid embryos), manifested a slower larval hatching rate compared to the diploid hybrid egg group. However, during periods P1 and P2 embryonic and larval mortality for triploid hybrid trout (3n = 100) resulted in lower values compared to the same indicators that were estimated for diploid hybrid trout (2n = 71). Regarding growth rates, the authors of the study had made comparisons between hybrid gynogenetic offspring (F1) (3n = 100 chromosomes) and pure diploid offspring of rainbow trout (F1) (2n = 58 chromosomes), for two performance traits, growth according to zoological length (Lcm) and growth by total weight (Wg). The calculations made by the researchers had pointed out the fact that at the age of 160 pdi (after the day of placement for incubation) the average weight of triploid hybrid trout was W_{mes} = 16.8 g while the average weight of pure rainbow trout was W_{mes} = 3.5 g, so 4.8 times lower.

The other study we thought to comment on had the argument: "Some phenomena that accompany the reduction of the scaly cover in carp (Cyprinus carpio L.) with a “mirror” phenotype (Spaho, V. and Shermadhi, V., 2014). The authors aimed to determine the existence of a possible regularity in the process of gradual reduction of scales that occurred during the transition from the strands of the "mirror" phenotype to the "naked" phenotype.

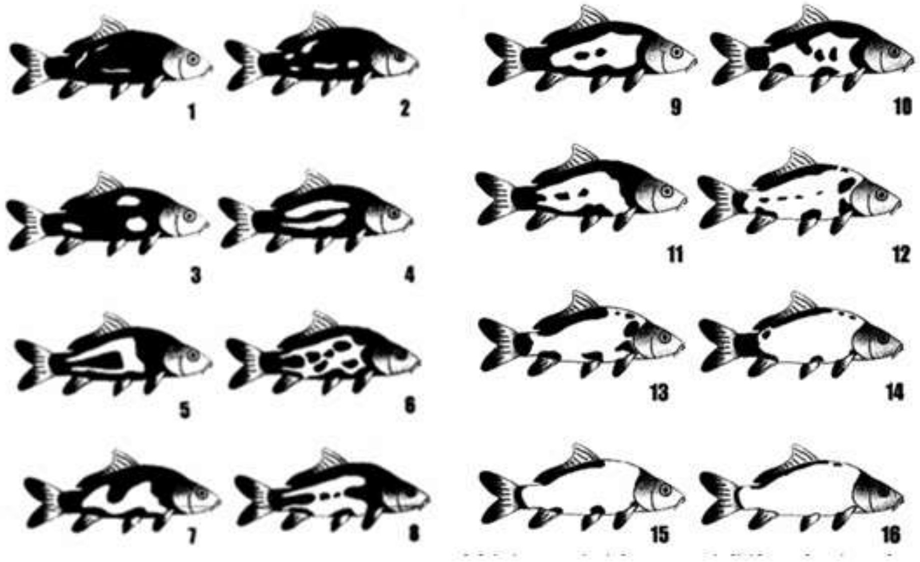

Figure 8. Progressive reduction of scales in carp individuals that have intermediate phenotypes between the "scattered scale carp" phenotype and the "nude" phenotype. Dark spots indicate areas of the body covered by scales; colorless sketches show areas of the body where scales are lost (Spaho, V. and V. Shermadhi, 2014).

The analysis of the significance of the changes for the mean values of the naked surface of the body, during the comparisons made between successive phenotypes had proved the existence of a gradation defined in the rhythm of scaling reduction. In the early stages, the reduction of scale number was high (see Figure 8 diagram for the interval between phenotypic variants 1 and 4). From phenotype 1 to phenotype 4 the bare surface on the right side of the body was extended 13.8 times. From sketch 4 to sketch 6 there are three different phenotypic variants while the reduction of scales in this interval has been negligible. Comparison of the mean values of the naked fraction of the body surface, for phenotypic variants 6 and 7 showed significance of the change of these values ($tD = 2.37$; $P < 0.05$; $n = 10$). This fact proved the existence of another phase in the process of reducing scales. From sketch 7 to sketch 10 the authors have listed four phenotypic variants distinct from each other, but the reduction of scales at this stage has been moderate (the bare surface has increased only 1.2 times or 17.16%). Significant difference between the mean values of the naked surface of the body was also experienced when comparing phenotypes 11 and 13 ($tD = 2.35$; $P < 0.05$; $n = 10$). Phenotypic variants from 13 to 16 presented small changes in terms of manner of the distribution of scales as well as the degree of their reduction.

Based on the analysis of the morphology of the scaly cover in 16 phenotypic variants and the results of biometric processing of digital data, the authors defined three phases of the most intensive reduction of the number of scales; the phase that characterized the transition from phenotype 1 to phenotype 4, the phase between phenotypes 6 and 7 and the phase between phenotypes 11 and 13.

Sketches positioned from number 1 to number 16 demonstrate the increase in the value of the naked fraction (or "mirror") on the body surface. According to the authors of the study, there is a regularity in the development of bare areas while initially the reduction of scales occurs in small spaces located on both sides of the lateral line, more near the abdominal line and less in the back area (sketches 1- 3). Furthermore, the "mirror" areas are fused to form two bands that are positioned on both sides of the lateral line, one along the back area and the other above the abdominal area (figure 4). These two bands had different widths, but in most cases the "abdominal" band appeared wider. In another phase the "mirror" area spread to the front 1/3 of the body causing the fusion of two longitudinal scales without scales (Figure 5).

Further evolution of the scaling reduction process (Figures 6-7) led to the creation of the typical "mirror" phenotype (Figure 8, Figure 8). This phenotype is distinguished by the existence of a full or dashed row of scales surrounding the body and a full or dashed row of scales placed on the lateral line. The scales have never been the same size. Sketches 9-11 demonstrated the gradual elimination of scales that were placed on the sideline. The carp belonging to the phenotype shown in Figure 11 were distinguished by a very small number or by the complete absence of scales in the lateral line, while the other scales were positioned as in the typical "mirror" phenotype. This way of positioning the scales is known as the "frame" phenotype, which is in fact a variant of the "mirror" phenotype. In all cases represented by sketch 16 (5 individuals in total) the study authors had found a longitudinal row with small scales along the base of the dorsal fin. Of the 208 individuals who were analyzed in the study we commented on in no case was the complete disappearance of the scales found.

Spaho,V.and V.Shermadhi (2014) had formulated these conclusions as important achievements of their work:

- The analysis of the significance of the changes, for the average values of the naked surface of the body, during the comparisons performed between 16 consecutive phenotypic variants showed that there was a gradation defined in the rhythm of the reduction of the scales..

-Following the order of reduction of scales in the intermediate phenotypic variants, which lie between the "scattered scales" phenotype with representation of "mirror" areas and the "naked" phenotype it was proved that this process follows a defined pattern and takes place in three stages..

- The morphological characterization of carp that are classified as "naked" showed that the evolution of this phenotype has as a distinctive feature the extreme reduction of scales but not their complete elimination.

A study that was conducted in the years 2018-2020 by Sadikaj, R. and D.Arapi (2020) had as object biotechnological interventions which aimed to increase the efficiency of hypophysation during hormonal stimulation of reproduction in alloctonic cyprinids. The authors investigated the effects of different concentrations of pituitary extract on response time (latency period) and egg survival during the incubation period. In fact, the control of the necessary time interval, which starts with the administration of stimulant hormone to the reproducers and ends with the delivery of gametes by them, is of great importance to determine the volume of work required by the farm to achieve artificial reproduction. (El Gamal,A.A.,2006).

It was found that the correlation between the concentration of pituitary extract (CPE, mg / kg) and the extension in minutes of the response interval or latent period (I, min) was strong (r = 0.991) but negative; consequence of the increase in the concentration of pituitary dust, in the first injection of females of grass carp (C.idella), was the shortening of the time interval of egg release. Specifically, the duration of the response interval, after the second treatment of female reproducers, was: for the third variant of the test 365 ± 17 minutes, for the second variant 404 ± 11 minutes and for the first variant 427 ± 8 minutes. Thus, the response time from the moment of injection to the moment of starting the egg release was reduced 1.2 times while the concentration of pituitary emulsion was increased 2.1 times.

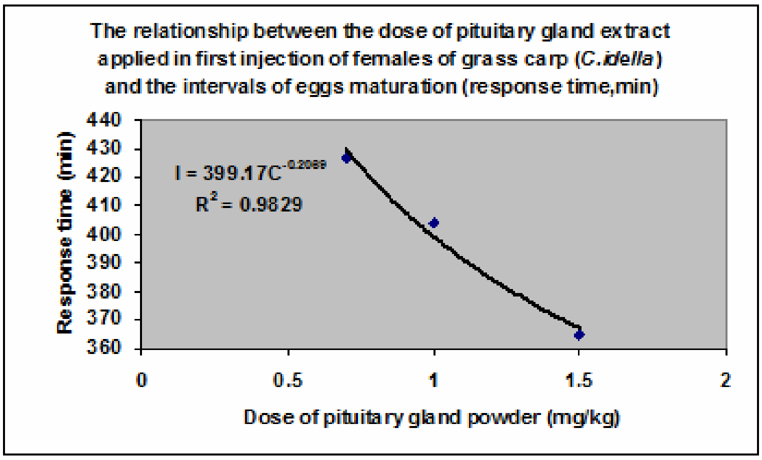

Figure 9. Negative correlation between dose of pituitary extract (C, mg / kg) in the first injection of female reproductive of grass carp and response time interval (I,min) (R^2 = 0.983 ; r = 0.991) (Sadikaj,R.and D.Arapi,2020).

The correlation between the indicator "dose of pituitary gland emulsion" (C, mg / kg) and the indicator "response interval" (I.min) resulted in a function in effect (y = a x b) (Figure 9). In this function "y" represented the value of the parameter I, min while "x" was the value of the parameter C, mg / kg, "a" was the angular coefficient of the regression line and "b" was the intercept of the axis "y". The negative values of the variable "b" (b = - 0.2088) confirmed the existence of a negative correlation between the two parameters that were included in the analysis.

The other study selected for comment was focused on the field of quantitative trait analysis for some geographically isolated carp populations (*C.carpio*) [Shermadhi,V.and V.Spaho (2014)].

Quantitative estimates did not show significant differences in morphometric and meristional traits between the sampled individuals belonging to the three populations included in the study. But the values calculated for the coefficient of variability (CV) of these traits, in the carp population of Lake Belsh, showed more pronounced variability compared to the other two populations. The differences were most noticeable when analyzing particular morphometric characters. This situation was proven, in particular, when comparing the carp population of Lake Shkodra with the carp population of Lake Prespa. The populations of these two basins consisted of individuals who had smaller body sizes, for the same age groups, compared to the population of Lake Belsh.

Based on the nature of the information which is guaranteed by the implementation of the connection system schemes, the relationships between direct measurements for some primary morphometric characters and standard length (SL) were analyzed. It is very likely that the variability of values for the ratios that were calculated was caused by the action of two factors, geographical isolation and hybridization. The first factor seems to have had the same impact on the three populations included in the analysis while the second factor may have had a greater impact on the population of Lake Shkodra. This finding is based on the fact that the carp population of Shkodra has been in a permanent impact of repopulation programs, which have distributed to the lake significant amounts of genetically enhanced fishery material.

Comparison of the differences that existed between the three populations analyzed in this study showed that the variability of morphometric parameters was greater compared to the variability of meristematic parameters. Shermadhe, V. and V.Spaho (2014) emphasize that temperature is the main environmental factor that turns out to be related to altitude above sea level and that has a primary impact on development rates from the beginning of egg segmentation until hatching. larves. The length of embryonic development intervals is considered as a factor that determines the quantitative aspects of morphometric and meristematic features.

CONCLUSIONS

-Experiences in semi-intensive and intensive aquaculture in Albania start from the beginning of the 60s of the last century. The first objects of Albanian aquaculture were the common carp and the cyprinids of the Far East.

- Currently, intensive aquaculture in Albania is represented by salmoniculture [cultivation of rainbow trout (O.mykiss) and the endemic form known as Ohrid trout (Salmo letnica)] as well as mariculture. Other Albanian aquaculture activities are the cultivation of mussels (Mytilus galloprovincialis) and sea shrimp (Marsupenaeus japonicus).

-The evolution of aquaculture forms and technological progress of this economy in Albania has been accompanied by scientific research activities which have been carried out by specialized institutions, such as the Institute of Aquaculture and Fisheries Research (Durres) and institutions of higher education, as the Department of Aquaculture and Fisheries in the Agricultural University (Tirana) and the Department of Biotechnology at the University of Tirana.

-The main research conducted in the fields of genetics and biotechnology of aquaculture has had as its main goals the optimization of the connections between the ictic form (genotype / phenotype) produced in the aquaculture system and the technology (semi-intensive / intensive) applied, the racial improvement of aquaculture by practicing gynogenesis, cross-breeding and triploidine hybridization as well as standardization of biotechnological elements that guarantee the success of artificial reproduction of alloctonic cyprinides, with the application of hypophysation.

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SWELLING AND EROSION OF NATURAL MATRIX TABLETS

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ABSTRACT

The present study aimed to elaborate matrix tablets from powder mixture (2:1 ratio) of Algerian date (*Phoenix dactylifera* L.) fruit and lyophilized berries (LB) (*Arbutus unedo* L.), using the direct compression technique. In a first part, the physicochemical properties, including the X-ray diffraction, of individual powders and their mixture were determined. In the second step, the swelling, erosion and in vitro release rate characteristics of tablets were studied. Taking into account the nutritional and physiological potentials of the basic components of the analyzed powder, the obtained tablets may be successfully used as dietary supplement and/or as excipient in the pharmaceutical industry.

Keywords: Arbutus berries, date fruit, tablet, dissolution, swelling, erosion.

INTRODUCTION

The use of wild plants as food source is well known in all countries of the world, particularly during the periods of war and /or drought. Currently, the research results highlight the significance of the wild edible plants as cheap source of nutrients **[1, 2]** which can explain the interest for using these species as dietary supplements **[3]**. However, many in vivo studies have evaluated the beneficial effect of the plants on the human health.

Wild edible fruits were found to have significant amounts of bioactive compounds, including polyphenols and flavonoids **[4]**. The high quantities of anthocyanins and natural pigments make them potential candidates for functional food statute **[5]**. In this context, some wild fruits in India have been identified to have better nutritional value than cultivated fruits **[6]**. The richness of the wild fruit in valuable ingredients as iron, sodium, potassium, zinc and calcium indicate the scope of using wild edible fruits for dietary supplement **[7, 8]**.

The Mediterranean region is rich of locally grown, wild and semi-wild edible fruit plants. The strawberry tree (*Arbutus unedo* L.) is one of the typical Mediterranean wild trees, growing in mountains, heavy clay and dry soils, on siliceous and decarbonated substrata **[9]**. In fresh form, its berry fruits are always incorporated into yogurts and used as confectionaries for pie, pastry fillings and cereal products **[10]**. After processing, they are also employed for the production of alcoholic beverages, jams, jellies and marmalades **[10, 11]**. Like other plants which are fitted with wonderful defense system assured by various biopharmaceuticals **[12]**, the berries are also known to be used in folk medicine as antiseptic, diuretic and laxative and against cardiovascular pathologies **[9]**. Wild fruit of Arbutus unedo L. is rich in numerous nutriments specially calcium, phosphorus and potassium **[13**Its sugar content is from 42% to 52% of the total weight of dry fruits **[14,15].** Sacchararose (87.7 ± 0.6 g per kg of dry fruit) and fructose (208 ± 2 g per kg of dry fruit) are the major carbohydrates in the unripe and ripe stage, respectively [14].

Strawberry tree fruits are a good source of antioxidants **[16]**, including carotenoids, flavonoids, anthocyanin and ellagic acid. The proanthocyanidins accounting for more than 80 % of the total flavonoid in arbutus **[14-17]**. On the other hand, Rodríguez et al. **[18]** have earlier reported that the higher antioxidant potential of the arbutus berries may be due to the activity of various bioactive components, including vitamin C. In this context, the reducing power of the strawberry tree fruits was found to be one of the highest, among 27 Algerian fruits **[19]**. So, considering the dietary ingredient, any herbal or botanical material containing vitamins and minerals **[20]**, arbutus berries may be listed as a dietary supplement.

The date palm, *Phoenix dactylifera* L., is one of most important plants of arid, desert area of the Middle East, Southern Asia and Northern Africa. Date fruits assume great importance in human nutrition owing to their rich content of essential nutrients, bioactive compounds and good antioxidant properties **[21]**. In addition, according to the water content, three date varieties are listed: soft, semi-soft and dried corresponding to the moisture contents of above 30, 26-30 and below 26% dry basis (d.b) respectively **[22-24].** Mech-Degla date variety is the vegetable material studied in the present work because of its availability and long shelf-life due to its low moisture content (14-16%) **[25].** In previous works, some technologic and physicochemical characteristics as well as drying ability of three common (dried) date varieties were reported **[26-29].**

Most of the carbohydrates in dates are in the form of reduced sugars, mainly fructose and glucose **[30].** It is a high source of energy, as 100 g of flesh can provide an average of 314 kca **[31].** Consequently, the date transformed products can positively substitute the ordinary refined sugar. Although several forms of dates such as fresh dates, date paste, date syrup are used for different applications, date powder would be highly beneficial in improving shelf-life, ease of handling, and blendability with various foods prepared.

In the last decade, many food products are processed and commercialized in powder form, whereas scientific investigations of various foods powder properties remain insufficient despite their importance in the engineering field **[32]**. In addition, studies about tableting properties of whole fruits are very scarce : date (*Phoenix dactylifera* L.) **[33]**, guava and pitaya **[34]**, chebula **[35] and** baobab **[36]** and mango **[37]**.

The main objective of the present study concerns the tabletting ability of the powder mixture (2:1 ratio) of Algerian date (*Phoenix dactylifera* L.) fruit and lyophilized berries (*Arbutus unedo* L.) (LB), using the direct compression technique. Some preliminary results have been already communicated [**38,39]**.

MATERIAL AND METHODS

Fruits and fruit powders:

Fully ripe berries were randomly picked at various trees in Kabylie region (North Algeria) during the winter 2017. The fruit was submitted to freeze drying at -64 °C under vacuum (4.5 Pa) during 48 h, using lyophilizer Type (Christ Alpha1-4LD), provided with vacuum pump (RZ 6, max pressure 0.04 Pa). The dried product is ground, sieved (sieve of type Euromatest-Sintoo, NFX11-501) to obtain powder with particle diameters (200 ≤ Ø ≤ 400 μm) and then kept in closed glass flask at 4 °C.

Mech-Degla date fruits were purchased from Boumerdes city (50 km east Algiers). The dates were first cleaned, pitted and cut in small size pieces that were dried at (40 ± 1) °C in laboratory oven (type MELAG 405) until a constant weight was reached. The date powder (DP) (200 ≤ Ø ≤ 400) μm is kept into a hermitic glass at 4 °C.

The Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) of LB, DP and LB/DP mixture were investigated using ~~Malvern,~~ FTIR Spectra 2000 (Perkin Elmer) and diffractometer (Panalytical Xpert Pro ®.) respectively.

Physicochemical properties of powders:

The three powder types DP, LB and DP/LB (2:1 ratio) mixture were characterized first for their bulk (ρ_{bulk}) and tapped (ρ_{tab}) densities, according to the European Pharmacopoeia **[40]** by means of an apparatus of type ERWEKA (Engelsmann, Germany).

The Carr index (CI) **[41]** and the Hausner ratio (HR) **[42]** were calculated using the following equations:

$$\boldsymbol{CI}(\%) = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}} . \mathbf{100} \quad \textbf{(1)}$$

$$\mathrm{HR} = \frac{\rho_{tap}}{\rho_{bulk}} \quad \textbf{(2)}$$

The flow rate (g/s) was determined by measuring the time (t) of passage of 100 g of powder (W) through a standardized glass funnel as described by the European Pharmacopoeia **[28]**:

$$\text{Flow rate (g/s)} = \frac{W}{t} \quad \textbf{(3)}$$

At the same time, the angle of repose (θ) formed by the cone of the powder onto the flat surface at the exit of funnel was expressed by arctan (θ).

Tablet preparation and basic physical–chemical characterization:

Tablets from DP and LB mixture (2:1 ratio) were obtained by compacting the powder using semi alternative tableting press (Mark ED Frogerai. type OA 307).

Tablets processing and their physical characterization were performed in laboratory of CRD/SAIDAL (Algiers) according to the European Pharmacopoeia **[40]**.

- The hardness of 10 tablets was determined with an automatic hardness tester type PHARMA TEST.

- The friability of the tablets was evaluated with a friabilator of type ERWEKA TA. For weight uniformity test, 20 tablets were randomly selected, weighed and weight variation (%) was calculated.

- The disintegration time was evaluated on 6 tablets using a disintegrator ERWEKA control ZT 2. The disintegration is considered achieved when 6 tablets are completely disintegrated.

-The swelling ability was quantified through liquid uptake by the tablets which were placed in 3 different liquid mediums (distilled water, 0.1 N HCl and phosphate buffer pH 6.8) heated at 37 °C.

- The erosion test was immediately performed after the swelling and consistedof the determination of the dried weight of wet tablet by drying at 50 °C during 24 h according to The morphological examination of tablets during their immersion in distilled water was carried out using a digital camera (Sony®DCR-SX65E).

Statistical analysis

All measurements were performed in triplicate. The statistical analysis of the experimental data was performed using Origin software version 8.

RESULTS AND DISCUSSION

Powder properties:

The physical properties of DP, LB and their mixture DP/LB (2:1 ratio) from which tableting is carried out, are summarized in **Table 1**.

Table 1: Properties of formulation ingredients LB, DP and mixed DP and LB (2:1) powder

Parameters	Ingredients		
	LB	DP	LB/DP
Bulk Density (g/cm^3)	0.431 ± 0.002	0.535 ± 0.003	0.438 ± 0.003
Tapped Density (g/cm^3)	0.481 ± 0.004	0.562 ± 0.005	0.512 ± 0.003
Carr's index (%)	10.395 ± 0.005	4.850 ± 0.014	14.452 ± 0.005
Hausner's ratio	1.116 ± 0.006	1.050 ± 0.015	1.169 ± 0.007
Angle of repose (°)	26.400 ± 0.782	29.93 ± 0.51	24.740 ± 0.182
The flow rate (g/s)	10.291 ± 0.193	9.511 ± 0.053	8.960 ± 0.061

Date are represented as mean ± SD (n=3).

The results show that the LB, DP and their mixture possess excellent flowness ability. However, all powders present CI, HR and angle of repose values below the required limits i.e. 15 %, 1.25 and 40 °, respectively **[40,43]** which facilitate the die cavity filling and tableting process **[44]**. At the same time, the HR values of DP, LB and DP/LB are lower than those of maize (1.57) and wheat (1.81) starch which were experienced as pharmaceutical excipients in tablet formulation **[41]**, but the found values were comparable to that of *Azadirachta indica* fruit powder (~ 1.41) reported by Hindustan et al. **[45]** which have also found an angle of repose of 39.55°, higher than that determined in the present work.

The FTIR spectroscopy is known to be a method commonly applied for characterizing food powders **[46, 47]**. The spectra of LB, DP and (DP/LB) mixtures **(Fig. 1)** attest that there is no appearance or disappearance of peaks in the powder mixture which confirm the absence of any chemical interaction between DP and LB, thus indicating that these powders are compatible **[48]**. The FTIR spectra also revealed characteristic signals corresponding to various broad and intense bands; in particular, five specific peaks can be mentioned. The intense band in the 3400-3500 cm^{-1} region is assigned to stretching (ν) vibrations of hydroxyl (-OH) group (free and intermolecular hydrogen band), whereas that around 2930 cm^{-1} is attributed to (C-H) absorption and include -CH, $-CH_2$, and $-CH_3$ stretching and bending vibrations. The peaks in the region (1630 - 1061 cm^{-1}) are due to vibrations of the carboxylate (-COO-) group and stretching vibrations of C-O-C bonds in ethers or related compounds respectively **[49]**. In addition, the FTIR spectra shows another specific peak at around 600 cm^{-1} which could be assigned to stretching (ν) of ($-CH_2$) and (-CH) groups as previously suggested by Kamil et al. **[50]** for tomato products.

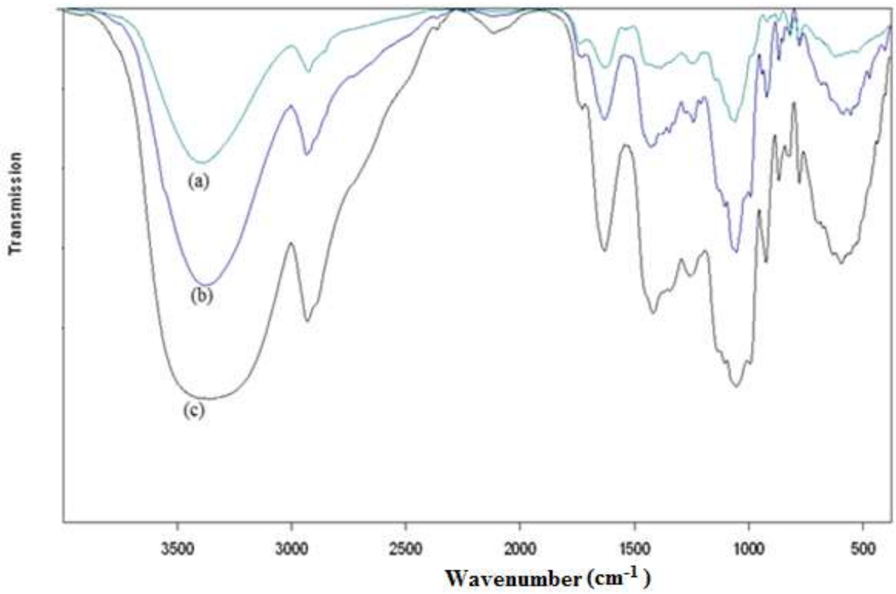

Fig 1: FTIR Spectrum of powders: (a) DP; (b) LB; (c) LB/DP

The XRD patterns of DP, LB and the mixture of powders (DP/LB) are presented in **Fig. 2.** A very broad band with very weak peaks, characteristic of amorphous forms, is observed in the spectrum of LB (**Fig. 3a**). On the contrary, in the case of DP, the diffraction peaks are intense, indicating the presence of a crystalline structure (**Fig. 3b**). We think that the high sugar content of DP may be the principal responsible of the enhanced crystallinity. The XRD pattern of DP/LB mixture (**Fig. 3c**) exhibited peaks corresponding almost perfectly to those of DP. In opposite, LB picks are not clear, being probably masked by the high DP peaks. On top of that, the effects of compression and sample high hygroscopicity probably alter the XRD analysis as reported by Ledur Alles et al. **[51]** on saccharides powder from
yakon roots (Smallanthus sonchifolius). Furthermore, the amorphous characteristics are clearly reported on different dried mango powders **[52]** and fluidize-dried gum extracted from the fresh fruits of Abelmoschus esculentus **[53]**. However, Niimura et al. **[54]** have shown that strawberry flesh has low-crystallinity cellulose I. According to these results, it can be concluded that the cristallinity depends on both the nature of the extract and composition of fruits.

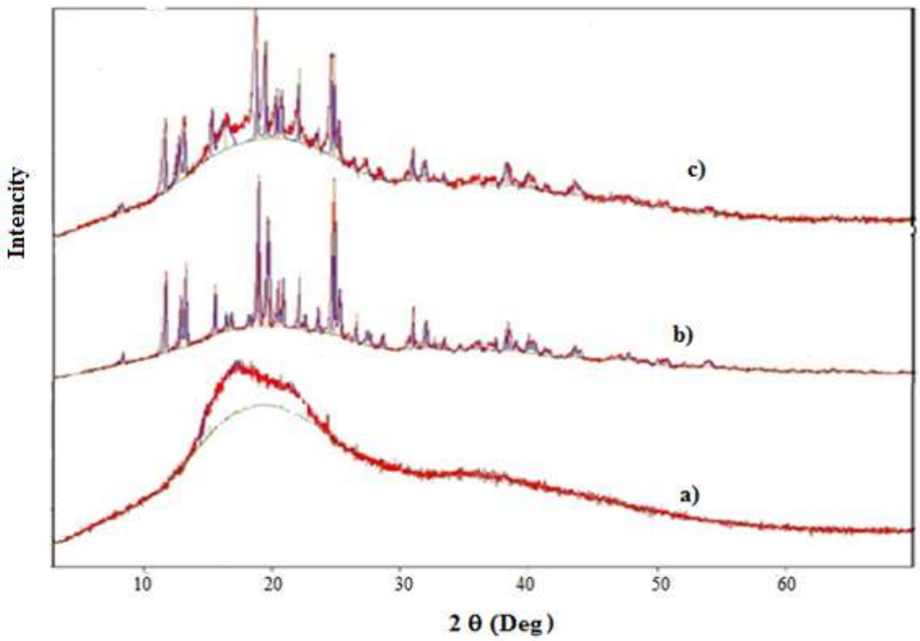

Fig 2: X-ray diffraction patterns of powders: (a) DP; (b) LB; (c) LB/DP

Some physical-chemical characteristics of tablets:

The physical characteristics of prepared tablets are presented in **Table 2**. Crushing strength test demonstrates the ability of tablets to withstand pressure or stress during handling, packaging and transport. Furthermore, the mechanical strength of tablets determines the disintegration time and the dissolution rate.

Table 2: Technological characterization attributes of the prepared tablet (mixture LB/PD)

Properties	Value
Hardness (kp)	9.670 ± 0.810
Friability (%)	0.102 ± 0.003
Weight (g)	0.502 ± 0.002
Time disintegration (min)	25.00 ± 3
Diameter (cm)	1.260 ± 0.010
Thickness (mm)	2.000 ± 0.005
Humidity (%)	1.75 ± 0.020

Date are represented as mean ± SD

The hardness and friability of obtained LB/DP tablets met the minimum requirement to be within the Pharmacopoeial limits (> 4 kp and < 1% respectively), thus confirming the ability of LB/DP powder mixture (2:1 ratio) for: i) tabletting application without adding any chemical blinder, and ii) withstanding the mechanical shocks during their handling and transport.

The disintegration time (25 min) of the matrix is comparable to that (24 min) found by Tavakoli et al. **[55]** about the disintegration of tablets from noni (Morinda Citrifolia, L.) fruit extract added with maltodextrin as subcoating material, and it is less than (> 30 min) that found by Ngwuluka et al. **[56]** regarding the disintegration of paracetamol tablets added with the dried fruit of date (Phoenix dactylifera L.) as an excipient.

Dissolution properties of obtained tablets:

Tablet swelling and erosion studies

Tablet swelling and erosion is a valuable test to better understand the mechanisms of release and the relative importance of participating parameters **[57]**.

Matrix tablet erosion and swelling kinetics **(Figs. 3 and 4)** demonstrated a linear increase of both parameters up to 20 min. According to the immersion liquid, the following order of evolution can be established for erosion and swelling intensities over the dissolution process: distilled water > phosphate buffer > HCl and phosphate buffer > HCl > ditilled water respectively. The tablets showed a maximum degree of swelling at 24 min whatever the surrounding media. This swelling phenomenon is governed by the osmotic effect whose mechanism is correlated to that already described by Costa et al. **[58]**; the water migration takes place from less concentrated to the more concentrated solution. Khan et al. **[59]** suggesting that the swelling kinetics of the matrices were an important determinant of drug release. At the same time, the tablets had the highest erosion rate in phosphate buffer which is in correlation with the swelling results. In all media, the dissolution is a balance between swelling and erosion which determines the release process of ions from the fruit tablets. These findings are in agreement with works of Adiba et al. **[33]** and Zea et al. **[34]** concerning food tablets prepared with date (*Phoenix dactylifera* L.)/spirulina (*Spirulina* sp.) and guava/pitaya powders respectively.

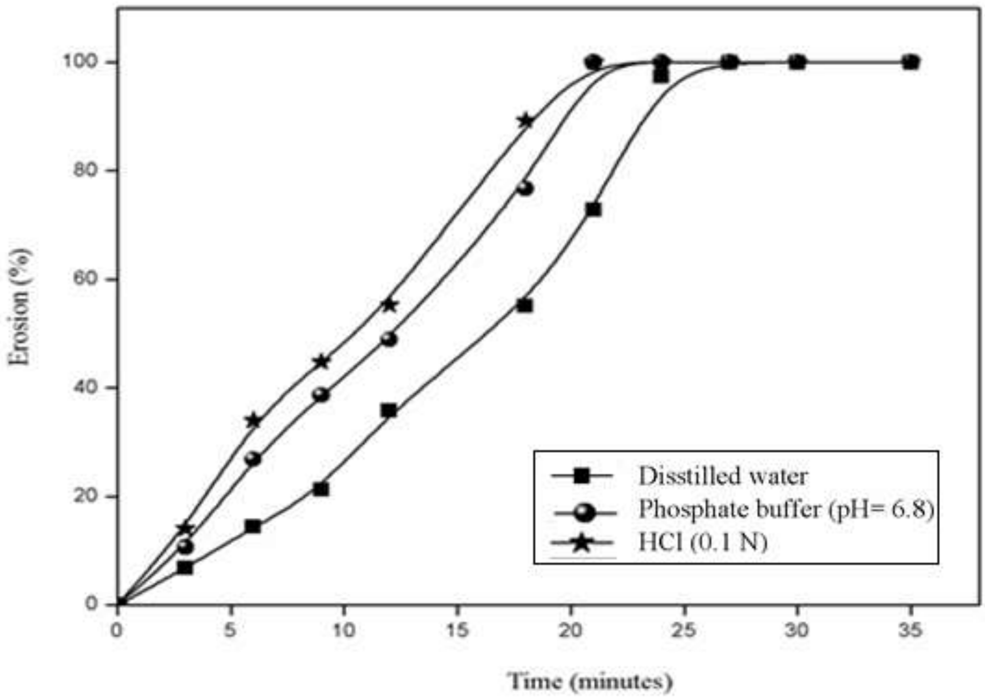

Fig 3: Erosion (%) versus immersion time of formulated DP/LB-tablets at 37 °

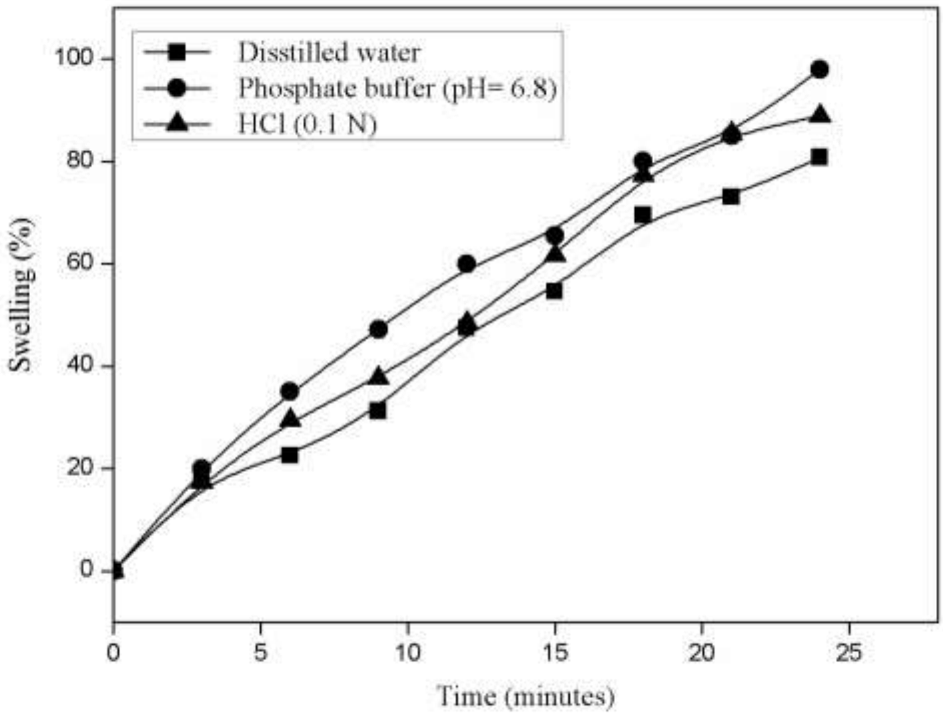

Fig 4: Swelling (%) versus immersion time of formulated tablets at 37 °C.

CONCLUSION

Powders from Strawberry tree (*Arbutus unedo* L.) fruits, date (*Phoenix dactylifera* L.) fruits and their mixture with 1/2 ratio were first investigated for some of their physical properties. Based on X-ray diffraction analysis, the powder mixture shows a crystalline structure, similar to that observed for date fruit powder. The results also revealed the good ability of the powder mixture for tableting by direct compression. Taking into account the nutritional and physiological potentials of the basic components of the analyzed powder mixture, the obtained tablets may be used as dietary supplement and/or as excipient in the pharmaceutical industry. Additionally, the physicochemical characterizations (toxic elements, behavior of tablets in various solutions simulating the physiological mediums, photochemical screening) are in the process of finalization and the results will be communicated in a future paper.

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OPTİMİSATİON OF THE COAGULATİON-FLOCCULATİON PROCESS BY BENTONİTE AND ALUMİNUM SULPHATE

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ABSTRACT

Aluminum sulphate (AS) was applied in the coagulation process and bentonite in the flocculation process to remove the turbidity of the wastewater. The objectif of this study is to follow of parameters and the optimization of the process in the wastewater treatment plant of KARMA (ORAN) town, Five independent factors have been studied; pH, dose of aluminum sulphate, agitation rate and coagulation time and flocculation rate that affect turbidity removal efficiency [5,6]. An experimental design was used to study the effect of independent factors and interaction factors on the elimination of turbidity (%) .The results obtained by the experimental design method show that all the factors have a significant effect on the elimination of turbidity (%). In addition the order of significance of the main effects is: temperature > Agitation speed of flocculation> coagulant dose> pH> Agitation speed of coagulation.. As well as among the most important factors had the strongest effect on the elimination of turbidity (%), are the pH * dose of the coagulant.

Keywords: Coagulation-flocculation; Used water ; aluminum sulphate; experimental design.

Introduction

The El Karma wastewater treatment plant is the largest sewage treatment plant in Algeria, which produces fertilizers for agriculture and gas (methane) for the production of its own electricity. It was operational since 2009 in the wilaya of Oran. This station has a processing capacity of 350000 m^3 / day. It can support the wastewater treatment needs of a population of about 1.5 million. It was managed by an Austrian company for two years. Then a Spanish company (Sior) took over with an Algerian staff trained in highly sophisticated equipment. The construction of the El-Karma wastewater treatment plant serves the dual purpose of eliminating wastewater from the entire Oran wilaya and protecting the groundwater in the region [2]. In order to improve the clarity of the water at the EL-KARMA wastewater treatment plant, coagulation treatment with flocculation has been proposed by adding a coagulant to the wastewater.

Our work will consist of process optimization and monitoring parameters that influence coagulation / flocculation such as speed and clotting time, aluminum sulphate dose, speed and flocculation time. The experimental design approach is a robust tool for understanding and

optimizing experimental parameters. It allows a rational study of their influences on the chosen answers, with a considerable reduction of the number of tests, so it is possible to obtain a maximum of information in a minimum of experiences and time, with a reduction of the cost . They also make it possible to study a very large number of factors, to detect possible interactions and to precisely model the results obtained and to organize optimally experiments. Experimental plans are therefore useful when we look for the link between a quantity of interest y and explanatory variables xi, also called factors. The factors are quantitative and the values taken by these factors are called levels [3].
No documentation has been reported on the application of experimental designs to determine the effect of coagulation-flocculation factors from an aqueous solution using aluminum sulphate and bentonite. In this study, five independent factors, namely pH, dose of aluminum sulfate, agitation rate and coagulation time and flocculation rate, that affect the removal efficiency of the turbidity [1], were taken into account to determine their effects on coagulation-flocculation using a 2^{5-1} fractional factorial design.

MATERIAL AND METHODS

Effluent

The water withdrawal was carried out at the EL KARMA sewage treatment plant after the secondary treatment, because the water remained arrogant and filled with non-decomposable colloid particles. Its physico-chemical characteristics are presented in table1

TABLE I. Physicochemical characteristics of wastewater.

parameters	**value**
Temperature	25.3°C
pH	7.68 à 23.7°C
Conductivity	2061 µS/cm à 25.4°C
COD	135mg O_2/l
BOD_5	72mg O_2/l
MES	59 mg/l
Total phosphorus	4.485 mg/l
orthophosphate	8.154 mg/l

Preparation of the coagulant

In our tests, we used a solution (10g / l) of $Al_2(SO_4)_3.18H_2O$ aluminum sulphate hydrate as a coagulant which is a product manufactured by Panreac (Spanish companion), and bentonite as a flocculant. [7]

Experimental design

All the coagulation-flocculation tests are carried out in a stirred reactor of parallelepiped shape with a capacity of 2 liters. However, in order to standardize the tests, the stirring time for flocculation is 20 minutes, the coagulation stirring time and speed, the flocculation stirring speed, the aluminum sulfate dose and the pH according to design. Then the flocs are allowed to settle for 30 minutes. When, the pH of the sewage was controlled by adding hydrochloric acid or soda introduced after the addition of aluminum sulphate. The turbidity of the water is determined by a HANNA LP 2000 turbidimeter.

Coagulation-flocculation tests

In this study, five factors were taken into account to study their effects on turbidity removal (%): dose of coagulant, pH, coagulation and flocculation agitation speed. using a factorial design Fractional 2^{5-1}.Table 1 shows the factors and levels to be used for experiments. 22 tests were carried out. Factor levels were coded as -1 (low level) and +1 (high level); While 0 indicates the center point. Center points have been added for curvature. The results of the experiment plan are analyzed with the Minitab 14 statistical software to evaluate the effects as well as the statistical parameters. The response was analyzed using ANOVA based on p value with a 95% confidence level.

TABLE II. Experience Plan Matrix.

Run Order	pH	Dose coagulation	température	agitation speed coag	agitation speed floc	Rend %
1	5.8	1	20	200	60	97.25
2	7.2	1	20	200	40	95.62
3	5.8	2	20	200	40	87.84
4	7.2	2	20	200	60	70.09
5	5.8	1	40	200	40	91.65
6	7.2	1	40	200	60	68.98
7	5.8	2	40	200	60	73.25
8	7.2	2	40	200	40	92.54
9	5.8	1	20	300	40	99.84
10	7.2	1	20	300	60	60.33
11	5.8	2	20	300	60	80.33
12	7.2	2	20	300	40	63.88
13	5.8	1	40	300	60	93.67

14	7.2	1	40	300	40	90.98
15	5.8	2	40	300	40	99.18
16	7.2	2	40	300	60	96.27
17	6.5	1.5	30	250	50	99.03
18	6.5	1.5	30	250	50	99.14
19	6.5	1.5	30	250	50	99.12
20	6.5	1.5	30	250	50	98.99
21	6.5	1.5	30	250	50	98.38
22	6.5	1.5	30	250	50	99.46

TABLE III. . Experimental domain of each factor

Factors	variables	Levels		
		(-1)	(0)	(+1)
pH	X1	5.8	6.5	7.2
Dose of coagulant (g/l)	X2	1	1.5	2
Temperature (°C)	X3	20	40	30
Agitation speed of coagulation (tr/min)	X4	200	250	300
Agitation speed of flocculation (tr/min)	X5	40	60	50

RESULTS AND DİSCUSSİONS

Standardized effects graph

The importance of each factor for the elimination of turbidity (%) was evaluated using a normal probability plot of normalized effect with α = 0.05, as Graph 1 shows for insignificant factors , their effects are normally distributed with a mean zero and a variance in which they are usually distributed along the line. in addition the interaction factors or they are deviated from the right imply that there are significant effects on the elimination of turbidity (%). The results show that all factors are significant. As the factors deviate more from the right, the effects are greater. The order of significance is: temperature > Agitation speed of flocculation> coagulant dose> pH> Agitation speed of coagulation.

It is also noted that the effects of first-order dose of coagulant-pH (X1X2) and dose of coagulant- Agitation speed of coagulation (X2X4) interactions is most important on colloid elimination (%) where it is most deflected from the right.

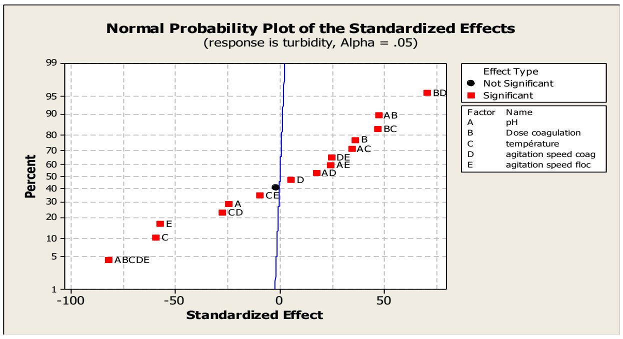


Fig. 1. : Standardized effects graph for turbidity removal (%).

Fig. 2. Main Effects and Interaction Effects of Factors

The plots of the main effects and interaction effects consist of average response values at different factor levels are shown in fig 2 and 3 respectively. The slope of the graph indicates the relative strength for the effect of the factors. Adding a center point to the plane shows that there is a detected curvature between levels. At the focal point of the factors, the rate of elimination of turbidity (%) is good.
In fig 2, a maximum elimination of turbidity (%) is obtained at a dose of 1.5g / L; But it diminishes afterwards. This is attributed to the colloid which requires a certain amount of coagulant to destabilize the colloidal particles during coagulation. Further addition of the assay causes the resuspension of destabilized flocs in the supernatant. It is also observed that at a low flocculation stirring speed give a good elimination of the turbidity (%) but decreases when passing from a low level of flocculation stirring speed to a high level. This is due to the breaking of the flocs formed at a high shear rate, where the high shear breaks the flocs back into the supernatant.
The result also shows that the elimination of turbidity (%) increases from pH 5.8 to 6.5 and then falls. Where beyond pH 6.5. This explains by a weak interaction between hydrolyzed forms of aluminum and colloids. So the effluent has recovered its turbid appearance.
fig 3 shows that there is a strong first-order interaction between coagulant dose and pH, but there is no interaction between the other factors.

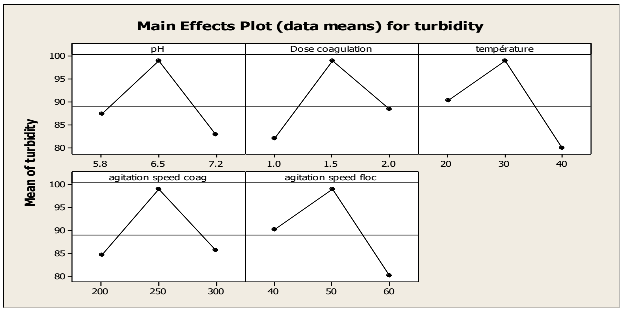
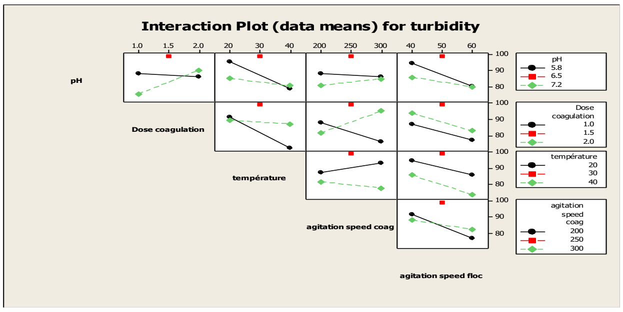


Fig. 3. Main Effects Chart for Turbidity Elimination (%).



interaction effect graph on turbidity removal (%).

Analysis of variance (ANOVA)

Table 4 presents the ANOVA analysis for turbidity removal (%) using SA. The p-value of the main effects is less than 0.05, which means that the independent factors are significant. It is necessary to examine all the important interactions in the analysis of the experimental design; double interactions show their importance at a 95% confidence level. Remarkable turbidity (%) observed at the midpoint for all the factors of the previous stage indicates that a significant curvature was detected at the location of the optimal condition. Therefore, a higher regression model, e.g. quadratic model is necessary to adapt the response for the elimination of turbidity (%).

Table 4. ANOVA analysis of turbidity removal (%).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	5	1102.41	1102.41	220.483	1753.76	0.000
2-Way Interactions	10	1622.12	1622.12	162.212	1290.26	0.000
5-Way Interactions	1	844.77	844.77	844.767	6719.43	0.000
Residual Error	5	0.63	0.63	0.126		
Pure Error	5	0.63	0.63	0.126		
Total	21	3569.92				

Conclusions

The results obtained by the experimental design method show that all the factors have a significant effect on the elimination of the turbidity (%) In addition The order of significance of the main effects is: temperature > Agitation speed of flocculation> coagulant dose> pH> Agitation speed of coagulation. Given the strong pH-dose interaction of coagulant, we notice in graph 3 that coagulation of effluents with a low pH requires a low dose of coagulant. The results show that the experimental design is appropriate for studying the effect of a large number of factors with a minimum number of experiments. Finally, the additional treatment of the treated wastewater at the EL-KARMA wastewater treatment plant showed a significant reduction in turbidity, which is a very important parameter during treatment with aging lagoons for reuse in the water. 'irrigation. In perspective of this work, we suggest the improvement of the biological treatment in the aeration basin by adding the nitrification-denitrification stage in order to exploit the treated water without rejecting it in the sebkha.

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ASSESSMENT OF IRRIGATION WATER QUALITY OF THE DAM LAKES LOCATED IN THE NORTH – WEST PART OF MARMARA REGION (TURKEY)

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ABSTRACT

Thrace Region is located in the north – west part of Marmara Region of Turkey and has a great agricultural potential because of its fertile soil and many freshwater resources. Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes, which were constructed by DSİ (State Water Works) in order to provide irrigation and drinking water and flood protection, are located in the Thrace Region. The aim of this research was to assess the water quality of these reservoirs in terms of irrigation by using Sodium Adsorption Rate (SAR), Sodium Percentage (Na%), Magnesium Rate (MR) and Kelly Index (KI). Water samples were collected in spring (rainy) season of 2018 from 15 selected stations and Na, K, Mg and Ca levels were investigated by using an ICP-MS. According to the results of applied irrigation water quality assessment indices, although it is known that almost all the aquatic habitats in the Thrace Region are exposed to agricultural pollution pressure, the investigated reservoirs were found as suitable for use as irrigation water, in general.

Keywords: Thrace Region Reservoirs, Irrigation Water Quality, Indices based evaluation

INTRODUCTION

Irrigation water quality is significantly effective on soils and crops. It has to be remembered that high quality and healthy crops can only be produced by using high – quality irrigation water. As a result of anthropogenic activities, freshwater resources have been faced with an intensive threat of pollution. Therefore, assessment of water quality in terms of irrigation and monitoring of physical – chemical changes in water bodies has a great importance for a sustainable water management (Çiçek et al., 2019; Özer and Köklü, 2019; Ustaoğlu and Tepe; 2019; Köse et al., 2020; Varol and Balcı, 2020).

Thrace Region covers about a 3% part of Turkey, but 15% of the total population of the country lives in this region. Increase of the population in the region caused significant environmental problems mainly polluting the soil and water resources (Tokatlı and Baştatlı, 2016; Tokatlı, 2017; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021). Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes are the most significant reservoirs of the Thrace Region and they are being effected from especially agricultural contamination.

The aim of this research was to determine the sodium, potassium, magnesium and calcium levels in water of Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes and evaluate the water quality in terms of irrigation water supply by using Sodium Adsorption Rate (SAR), Sodium Percentage (Na%), Magnesium Rate (MR) and Kelly Index (KI).

MATERIALS AND METHODS

Study Area and Collection of Samples

Water samples were collected from 15 stations selected on the reservoirs in rainy (spring) season of 2018. Topographic map of Thrace Region and Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes and selected stations on the reservoirs are given in Figure 1.

Trace Element Analysis

For determination of Na, K, Mg and Ca concentrations in water, water samples of one liter were adjusted to pH 2 by adding 2 ml of HNO_3 into each. Afterwards, all the samples were filtered (cellulose nitrate, 0.45 µm) in such a way as to make their volumes to 50 ml with ultra-pure water. The element levels in water samples were determined by using the "Agilent 7700 xx" branded Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) device in Trakya University Technology Research and Development Application and Research Center (TÜTAGEM). The center has an international accreditation certificate within the scope of TS EN / ISO IEC 17025 issued by TÜRKAK (representative of the World Accreditation Authority in Turkey). The element analyses were recorded as means triplicate measurements (APHA, 1992; EPA, 2001).

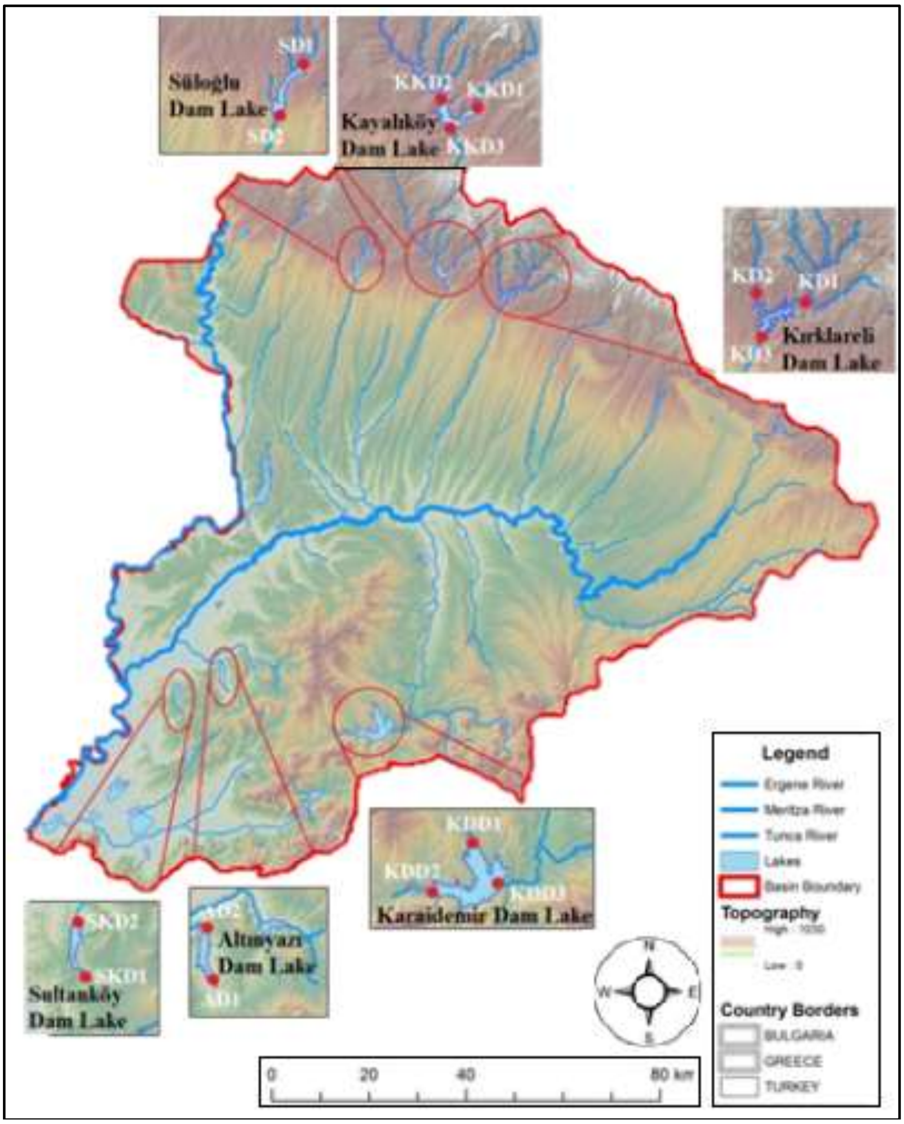

Figure 1. Study area and selected stations

Irrigation Water Quality Assessment Indices

The formulas of the applied Sodium Adsorption Rate (SAR) (Richards, 1954), Sodium Percentage (Na%) (Wilcox, 1955), Magnesium Rate (MR) (Raghunath, 1987) and Kelly Index (KI) (Kelly, 1963) are given below and evaluation scales are given in Table 1.

$$SAR = \frac{Na}{\sqrt{\frac{(Ca+Mg)}{2}}} \tag{1}$$

$$Na\% = \left(\frac{Na+K}{Na+K+Mg+Ca}\right) * 100 \tag{2}$$

$$MR = \left(\frac{Mg}{Mg+Ca}\right) * 100 \tag{3}$$

$$KI = \frac{Na}{Mg+Ca} \tag{4}$$

Table 1. Evaluation scales of the applied indices

Name of Index	Evaluation Scale	Name of Index	Evaluation Scale
Sodium Adsorption Rate	< 20 Excellent 20 – 40 Good 40 – 60 Permissible 60 – 80 Doubtful > 80 Not applicable	Magnesium Rate	< 50 Suitable > 50 Not suitable
Sodium Percentage	< 20 Excellent 20 – 40 Good 40 – 60 Permissible 60 – 80 Doubtful > 80 Not applicable	Kelly Index	< 1 Suitable > 1 Not suitable

RESULTS AND DISCUSSION

In the present investigations, Sodium Adsorption Rate (SAR), Sodium Percentage (Na%), Magnesium Rate (MR) and Kelly Index (KI), which are among of the most widely used irrigation water quality assessment tools, are applied to detected data to evaluate the water quality of Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes in terms of irrigation water supply. The results of applied indices are given in Figure 2.

According to detected data, all the locations selected on the investigated dam lakes were found to be as "Excellent (< 20)" and "Suitable (< 50)" in terms of SAR and MR values, respectively. In terms of applied Na%, Kırklareli Dam Lake was found to be as "Good (20 – 40)" quality, while the other dam lakes were found to be as "Permissible (40 – 60)" quality. In terms of applied KI, except for the input station (SD1) of the Süloğlu Dam and the input and output stations (SKD1 and SKD2) Sultanköy Dam (> 1), all the other investigated stations on the reservoirs were found as suitable to be used as irrigation water (< 1).

Thrace Region of Turkey is known as an “agricultural city”, because of its quite productive soil and quite rich freshwater resources. Therefore, agricultural runoff originated from intensive cultivation applications conducted almost all around the region is reported as one of the main pollution factors for the reservoirs located in the Thrace Region (Tokatlı, 2019; 2020; 2021). It is thought that the detected quite high Na% values in the water of almost all the reservoirs except Kırklareli Dam Lake are due to the agricultural activities carried out in the region.

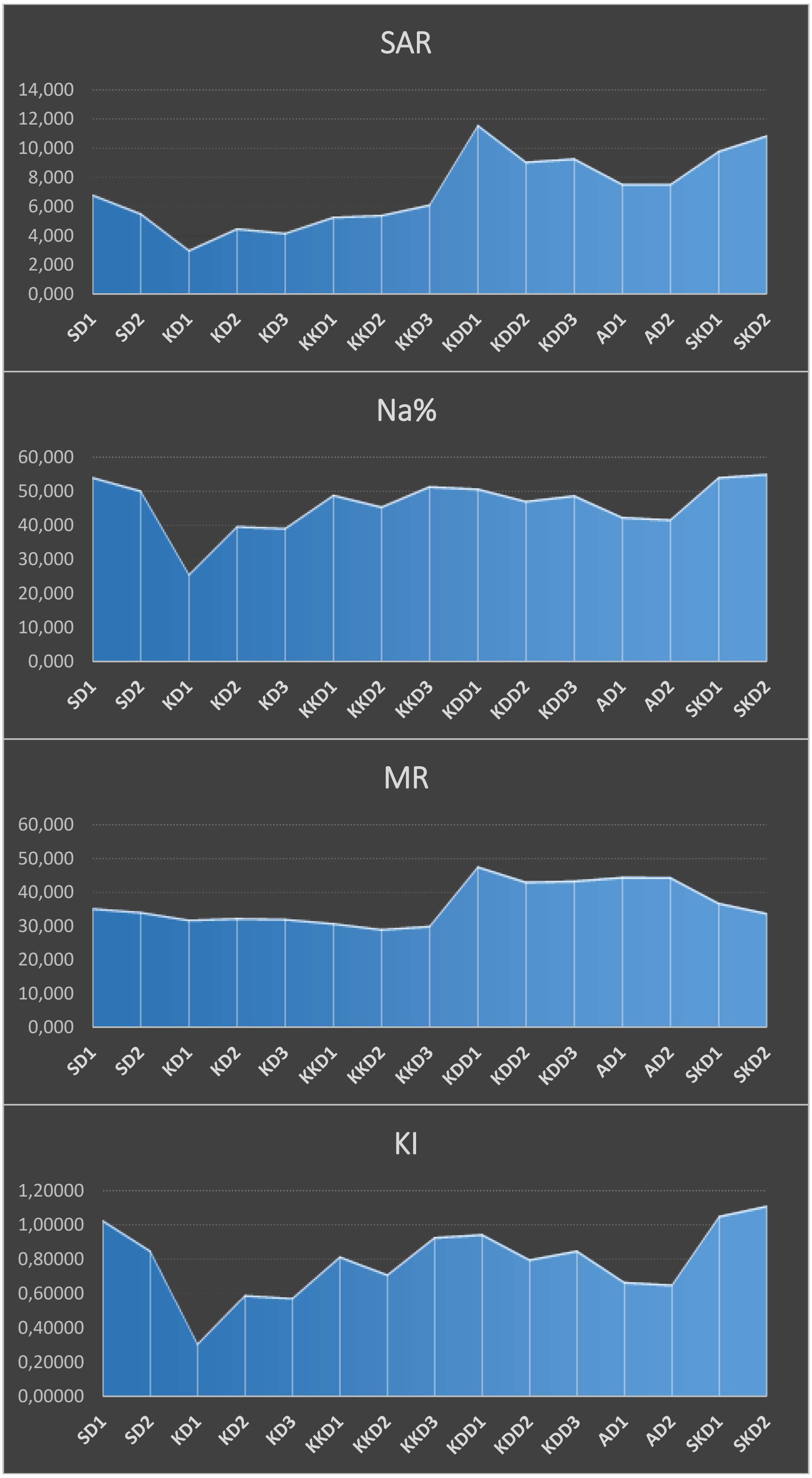

Figure 2. SAR, Na%, MR and KI scores of reservoirs

CONCLUSIONS

In the present investigation, irrigation water quality of Altınyazı, Karaidemir, Kayalıköy, Kırklareli, Sultanköy and Süloğlu Dam Lakes were evaluated by using 4 widely used irrigation water quality assessment indices including SAR, Na%, MR and KI. As a result of this research, although the Sultanköy Dam Lake was found as the riskiest reservoir in terms of irrigation water supply among the investigated lentic habitats and use of this reservoir as irrigation water was found to be quite risky in terms of applied KI, the investigated all the reservoirs located in the Thrace Region were found as suitable for use as irrigation water in terms of SAR, Na% and MR, in general. The data of the present research also clearly reflects the importance of the use of irrigation water quality assessment indices.

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ASSESSMENT OF ERGENE RIVER BASIN IN TERMS OF IRRIGATION WATER SUPPLY: APPLICATION OF TRACE ELEMENTS BASED INDICES

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ABSTRACT

Ergene River Basin is the main lotic ecosystem of Thrace Part of Marmara Region and the system has a great importance in terms of irrigation water supply for the region. The aim of this research was to assess the water quality of Ergene River Basin in terms of irrigation by using 4 commonly used macro element evaluation indices. Surface water samples were collected in spring (rainy) season of 2018 from 21 selected stations (2 of them were on the Meriç River, 9 of them were on the Ergene River and 10 of them were on the most significant tributaries) located on the basin and Na, K, Mg and Ca levels were investigated by using an ICP-MS. Sodium Adsorption Rate (SAR), Sodium Percentage (Na%), Magnesium Rate (MR) and Kelly Index (KI) were applied to detected data in order to evaluate the water quality in terms of irrigation. As a result of this research, it was determined that the investigated tributaries (except Çorlu Stream) and Meriç River were found as suitable for use as irrigation water, while the Ergene River and Çorlu Stream were found as not suitable for use as irrigation water, in general.

Keywords: Ergene River Basin, Irrigation Water Quality, Trace Elements Based Indices

INTRODUCTION

As a result of anthropogenic activities, water resources have been faced with the threat of pollution in recent years, especially because they are used as receiving environments. Continuous monitoring and evaluation of physical and chemical changes in water bodies is important for a sustainable water management and the use of these resources for drinking and irrigation purposes (Çiçek et al., 2019; Özer and Köklü, 2019; Ustaoğlu and Tepe; 2019; Köse et al., 2020; Varol and Balcı, 2020; Tokatlı et al., 2021).

Ergene River Basin that is located in the Thrace Region of Turkey is the most important irrigation water source for the region. Besides this great importance, the system is unfortunately known to be exposed to pollution due to intensive agricultural and industrial applications conducted around the watershed (Tokatlı, 2017; 2018; 2019; 2021; Tokatlı and Baştatlı, 2016; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021).

The aim of this study was to determine the sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) accumulations in water of Ergene River Basin and evaluate the water quality in terms of irrigation water supply by using Sodium Adsorption Rate (SAR), Sodium Percentage (Na%), Magnesium Rate (MR) and Kelly Index (KI).

MATERIALS AND METHODS

Study Area and Collection of Samples

21 stations were selected on the Ergene River Basin (2 of them were on the Meriç River, 9 of them were on the Ergene River and 10 of them were on the most significant tributaries). Map of study area and selected stations are given in Figure 1. Water samples were collected from the middle of the rivers (over the bridges) in spring (rainy) season of 2018, when the precipitation and surface runoff have increased significantly in the region. Water samples were collected 0.5 m below the water surface in 1 L pre-cleaned glass bottles and kept at 4 ^{0}C until the element analysis.

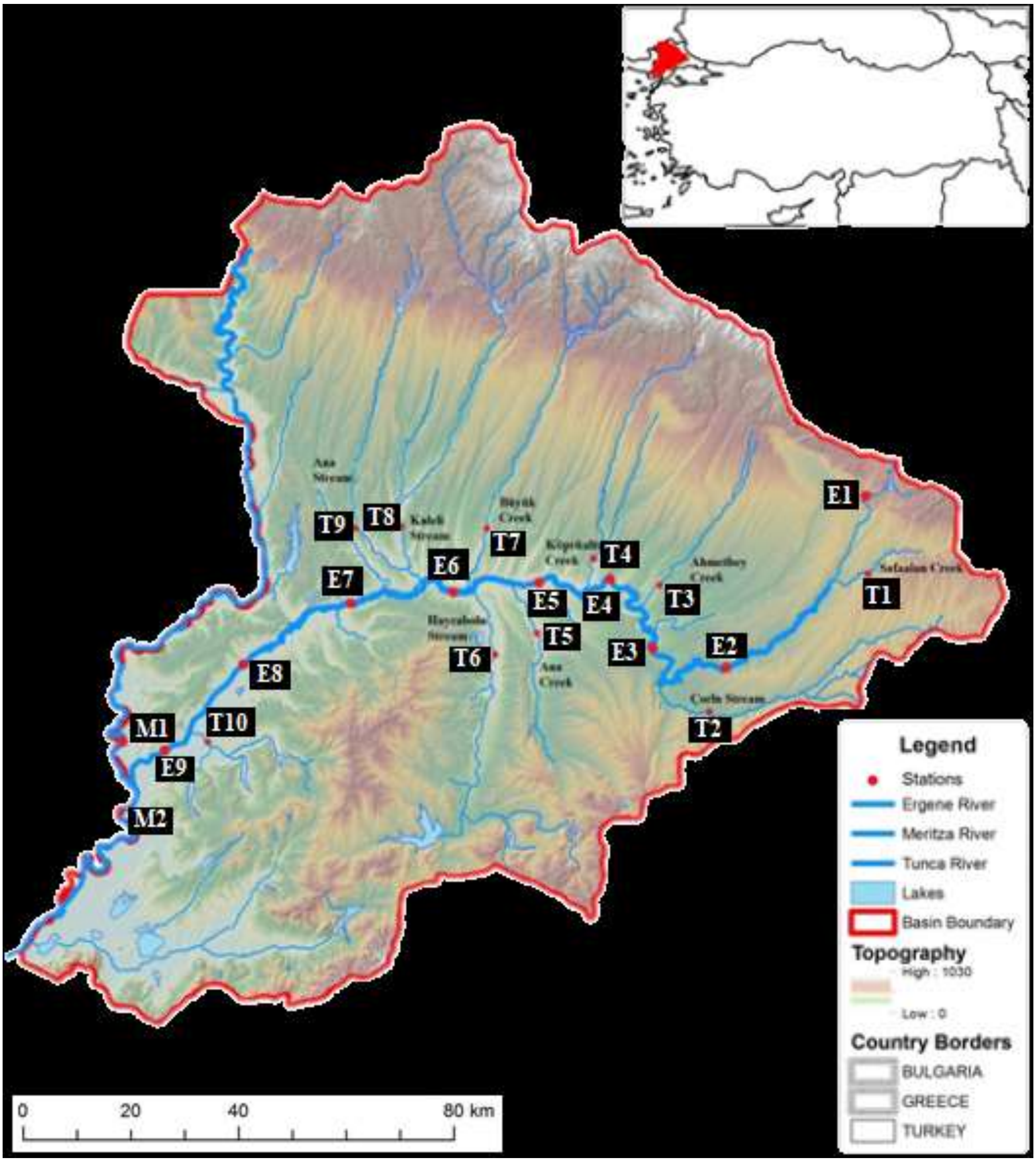

Figure 1. Study area and selected stations

Chemical Analysis

For determination of Na, K, Mg and Ca concentrations in water, water samples of one liter were adjusted to pH 2 by adding 2 ml of HNO_3 into each. Afterwards, all the samples were filtered (cellulose nitrate, 0.45 µm) in such a way as to make their volumes to 50 ml with ultra-pure water. The element levels in water samples were determined by using the "Agilent 7700 xx" branded Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) device in Trakya University Technology Research and Development Application and Research Center (TÜTAGEM). The center has an international accreditation certificate within the scope of TS EN / ISO IEC 17025 issued by TÜRKAK (representative of the World Accreditation Authority in Turkey). The element analyses were recorded as means triplicate measurements (APHA, 1992; EPA, 2001).

Irrigation Water Quality Assessment

The formulas and evaluation scales of the applied macro element evaluation indices are given below as a Table (Table 1).

Table 1. Irrigation water evaluation indices

Name of Index	Formula	Evaluation Scale
Sodium Adsorption Rate (SAR) (Richards, 1954)	$SAR = \frac{Na}{\sqrt{\frac{(Ca+Mg)}{2}}}$	< 20 Excellent 20 – 40 Good 40 – 60 Permissible 60 – 80 Doubtful > 80 Not applicable
Sodium Percentage (Na%) (Wilcox, 1955)	$Na\% = (\frac{Na+K}{Na+K+Mg+Ca}) * 100$	< 20 Excellent 20 – 40 Good 40 – 60 Permissible 60 – 80 Doubtful > 80 Not applicable
Magnesium Rate (MR) (Raghunath, 1987)	$MR = (\frac{Mg}{Mg+Ca}) * 100$	< 50 Suitable > 50 Not suitable
Kelly Index (KI) (Kelly, 1963)	$KI = \frac{Na}{Mg+ Ca}$	< 1 Suitable > 1 Not suitable

RESULTS AND DISCUSSION

In the present investigations, Sodium Adsorption Rate (SAR), Sodium Percentage (Na%), Magnesium Rate (MR) and Kelly Index (KI), which are among of the most widely used irrigation water quality assessment tools, are applied to detected macro elemental data in order to evaluate the water quality of Ergene River Basin in terms of irrigation water supply. The results of applied irrigation water quality assessment indices are given in Figure 2.

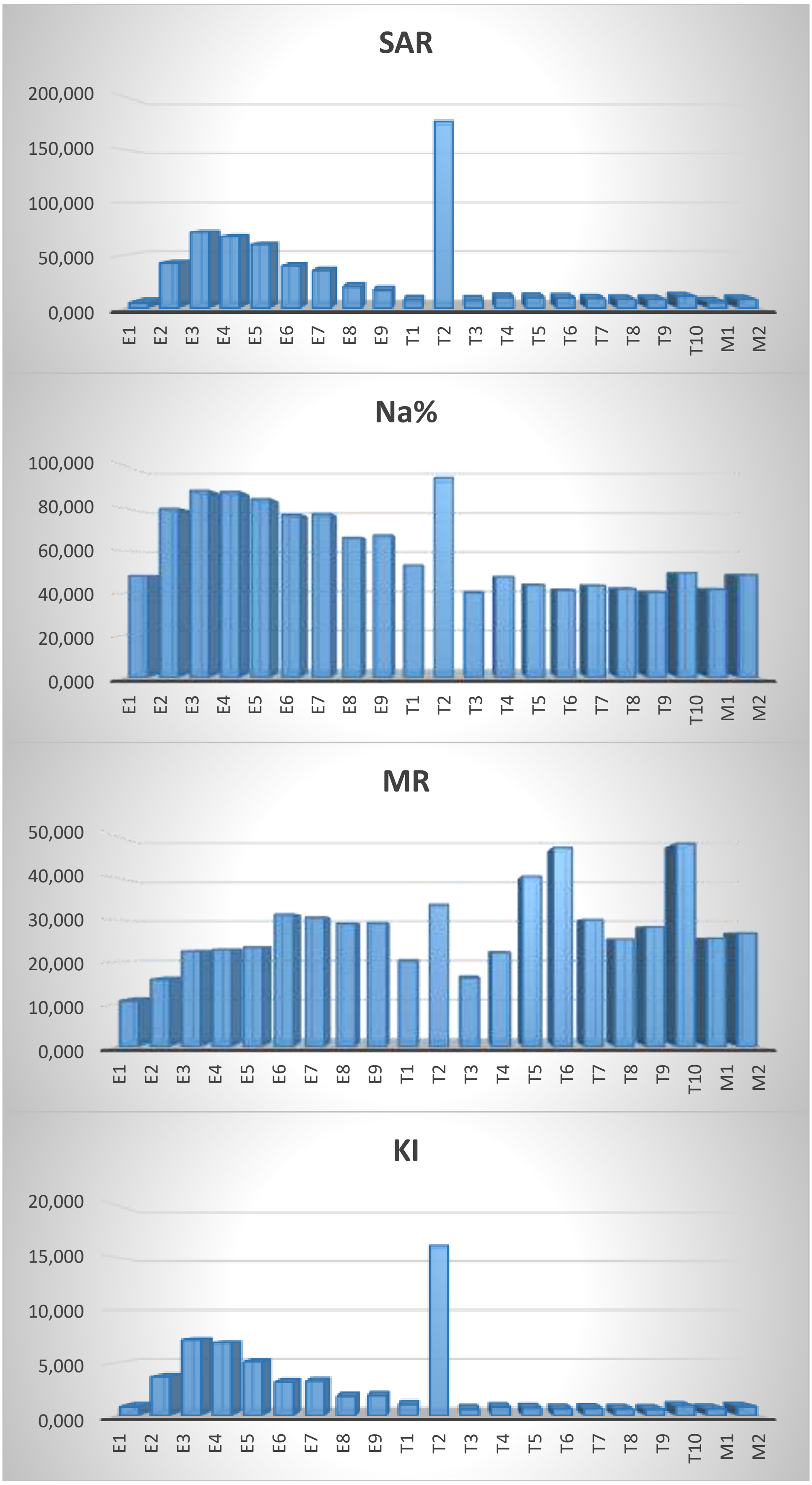

Figure 2. SAR, Na%, MR and KI scores of investigated locations

According to the results of applied SAR, except the middlestream of Ergene River (E3, E4 and E5 stations) and Çorlu Stream (T2 station), SAR coefficients were below the permissible limit value of 60 in all the other investigated locations. E3, E4 and E5 stations were recorded as "Doubtful (60 – 80)" in terms of irrigation water, while T2 station was recorded as "Not applicable (< 80)".

The Na% values detected in all Ergene River stations except the source area (E1 station) and Çorlu Stream (T2) were quite above the permissible limit value (< 60). In addition, while it was recorded that all the basin waters were suitable for irrigation water use in terms of applied MR (< 50), it was determined that the use of Ergene River (excluding E1), Safaalan (T1) and Çorlu (T2) Streams as irrigation water were risky in terms of applied KI (> 1).

In addition to the intensive agricultural applications, Thrace Region is also known as a significant industry zone. There are many industrial enterprises on the region within the borders of Ergene River Basin mainly in the Çorlu, Çerkezköy, Muratlı and Lüleburgaz districts. Therefore, Ergene River and Çorlu Stream are being exposed to an intensive industrial pressure (Tokatlı, 2017; 2018; 2019; 2021; Tokatlı and Baştatlı, 2016; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021). As a result of applied macro elements based indices, Ergene River and Çorlu Stream were found as the riskiest habitats in terms of irrigation water supply among the investigated basin components.

Although it was determined that water quality has decreased slightly from upstream to downstream and the detected SAR, Na%, MR and KI scores in the downstream increased by 41%, 17%, 5% and 35%, respectively, both the up (M1) and downstream (M2) stations of Meriç River were recorded as having "Excellent (<20)", "Permissible (40 – 60)", "Suitable (<50)" and "Suitable (<1)" water quality characteristics in terms of applied SAR, Na%, MR and KI scores, respectively. The decrease in water quality in terms of irrigation water supply in the downstream of the Meriç River is thought to be due to the Ergene River, because the M2 station was located after the area where the Ergene River flows into the Meriç River.

CONCLUSIONS

In this study, water quality of Ergene River Basin was evaluated in terms of irrigation water supply by using SAR, Na%, MR and KI.

According to the results of applied irrigation water quality assessment indices, Ergene River and Çorlu Stream were found as the riskiest habitats in terms of irrigation water supply among the investigated basin components and use of these aquatic habitats as irrigation water were found to be quite risky, while the investigated other tributaries and Meriç River were found as suitable for use as irrigation water, in general.

The data of the present research also reflects the importance, applicability and necessity of the use of different irrigation water quality assessment indices together on evaluation of surface water ecosystems in especially rural lands.

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SEASONAL CARBON EMISSIONS AND SEQUESTRATION IN ORGANIC AGROECOSYSTEMS IN CENTRAL LITHUANIA

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ABSTRACT

The growth rate of atmospheric carbon dioxide (CO_2) concentrations since industrialization in Anthropocene is characterized by large variability, mostly resulting from variability in CO_2 uptake by terrestrial ecosystems including agroecosystems. Various crops emit different rates of CO_2 into atmosphere. Investigations of seasonal carbon exchange in agroecosystems were carried out at the Training Farm of Agriculture Academy in 2014–2016. The aim was to investigate and compare carbon exchange rate of different crops of ecological farming. This study involved carbon exchange rate of agroecosystems including measurement of emitted and absorbed CO_2 fluxes by applying closed chamber method.

Plant (Ra) and soil respiration (Rs) varied between crops and during growth stages. However total respiration compose less than 30% of total carbon exchange in agroecosystems. Main drivers of mean plant and soil respiration were meteorological conditions, crop species, vegetation period and growth stage. Generally, respiration emissions were completely recovered by atmospheric carbon rates sequestered in crops gross primary production (GPP). Therefore, the ecosystems biota was acting as atmospheric CO_2 sink. Photosynthetically assimilated mean CO_2 rates ranged between 10.148 µmol m^{-2} s^{-1} in vetch+oat mixture and 11.923 µmol m^{-2} s^{-1} in ley and exceeded mean respirational emissions by 72 %. The differences in photosynthetically assimilated CO_2 rates were significantly interacted and correlated with leaf area index (LAI) (r=0.4-0.8, p=0.01-0.04), specific leaf area (SLA) (r=0.3-0.8, p=0.01-0.03) and dry biomass (r=0.4-0.6, p=0.03-0.05). Between ecological crops, the highest mean net ecosystems production (NEP) was sequestered in biomass of ley and wheat and ranged between 9.931 and 9.199 µmol m^{-2} s^{-1}, respectively. These crops might be considered the most environmental sustainable between crops.

Key words : CO_2 fluxes, bio-parameters, environment, crops

Introduction

Croplands represent near 12% of the Earth's surface (Smith et al., 2007) and one third of the land surface in Europe (FAO, 2003). Therefore agroecosystems plays a significant role in generation of anthropogenic emissions which accounts for 10–12% of total anthropogenic emissions of greenhouse gas (GHG) (*Schlesinger & Bernhardt 2013*). EU produces 9.78% of agricultural emissions, out of which 4.94% are emissions from the soil (*EEA 2010*). Agriculture produces near 21.4% of total emissions in Lithuania (*LR 2012*).

Photosynthesis process assimilates atmospheric CO_2 and creates a huge organic C pool in terrestrial vegetation, including crops biomass (*Schlesinger & Bernhardt 2013*), which is usually estimated by multiplying total plant biomass by a corresponding biomass C conversion factor, i.e., the C content (*Thomas & Martin 2012, Zang et al. 2014*). The most widely employed C content in plants is near 50% in the regional and global vegetation C stock estimations (*Lambers et al. 2008, Johnson et al. 2007*). Farming system, growth period duration and water content are the main factors that determine photosynthesis process, C fluxes in agroecosystems (*Domingo et al. 2011*), CO_2 fluxes and exchange in the system atmosphere-plant-soil (*Aertsens* *et al. 2013*). Strong correlation between CO_2 emission temperature and precipitation (r=0.7) was found during the summer season in organic and conventional agroecosystems (*Baležentienė & Kusta, 2012*). The conventional farming is aimed toward productivity for greater profits rather than to maintenance of sustainable environment. Thus, conventional farming has a significant negative impact on the long-term soil productivity and sustainability entire agroecosystem. Numerous studies revealed that control of yield, environment and carbon sequestration becomes possible if appropriate farming system, growing technology and crops have chosen (*Janssens et al. 2003, Wood et al. 2000*). Ecological point needs to be sustainably managed to reduce environmental impacts of agrothechnologies, while production quality, and profitability augmented (*Huisingh et al. 2015*). The relationship between the rates of carbon fluxes and bio-parameters of plants stands important for the evaluation of the possibility for the climate change mitigation and the development of sustainable agriculture. Evidence of the crop NEP would improve the understanding of the factors and mechanisms that influence carbon emissions and sequestration, and thus optimally regulate these processes reducing CO_2 emissions and predicting their changes (*EC 2019*).

The main objectives of this study were to assess the potential of atmospheric carbon assimilation and accumulation in biomass during growth period in organic farming agroecosystems of ley, winter wheat, vetch + oat mixture and barley + ley undercrop; to determine seasonal respiration fluxes and the rates of assimilated carbon. In order to explain carbon exchange, the photosynthesis parameters (crop density, leaf area index, productivity) were investigated at different plant' growth stages.

Material and Methods

Measurement object and location

Investigations of seasonal C exchange of organic farming ley (L), wheat (W), vetch + oat mixture and barley + ley undercrop (B) were carried out during growth period in 2014–2016 at the Training farm of Vytautas Magnus University (former Aleksandras Stulginskis University, 54°52' N, 23°49' E), Kaunas district (Table 1). The cropland soil types were *Hapli-Epihypogleyic Luvisol, LVg-p-w-ha*, or *Albi-Epihypogleyic Luvisol, LVg-p-w-ab*) (*FAO 2015*).

For evaluation of crop photosynthetic surface, the crop density (plant m^{-2}) and leaf surface area (LAI, m^2 m^{-2} was determined for the plots of 0.25 m^{-2} (0.5 m x 0.5 m) in six replications (*Breda 2003*). Fresh plant biomass (FM, g m^{-2}) and dry matter content (DM, g m^{-2}) were determined by the weighting method. Dry matter content was determined by drying plant samples (80^{o}C thermostat (Tritec HANNOVER, Germany).

Table 1. Agroecosystem parameters

Farming type	Agroecosystem	Area, ha	Crop fertilising
Ecological	Ley	8.75	-
	Wheat	8.75	Manure, 36 t ha^{-1}
	Vetch + oat mixture	12.48	-
	Barley + ley undercrop	12.48	-

Carbon footprint investigation

Agroecosystems' seasonal C exchange was investigated applying static chamber method

(Smith et al., 2013) using LCpro system (ADC Bioscientific LTD, UK). The rate of gross primary production (GPP, µmol m^{-2} s^{-1}) and respiration emissions of soil and autotrophs (Rs+a, µmol m^{-2} s^{-1}) were measured every 7–10 days with regard to environmental conditions and plant growth stages (BBCH-scale) (*Meier 2001*). Carbon exchange of each agroecosystem was evaluated by net ecosystem production (NEP, µmol m^{-2} s^{-1}) (*Leith & Whittaker 2008*) which was calculated by the formula: NEP=GPP- Ra+s (*Amthor & Baldocchi 2001*).

Meteorological conditions. The vegetation period suitability for plant growth is expressed as a ratio of humidity and temperature, or hydrothermal coefficient (HTK).

2014 March HTK = 1.83 shows the extent moisture, and in April HTK = 0.78 - too dry conditions for plant growth, or 2 times drier than in March. May HTK = 2.03 June HTK = 1.13, July HTK = 0.82, August HTK = 2.02, September HTK = 1.98 (Fig. 1). October HTK = 1.61, or nearly optimal humidity, while November HTK = 4.15 indicates 3 times more moisture than in October.

2015 spring was excessively wet for plant growth, since March HTK = 3.2, April HTK = 2.2, May HTK = 1.22, but June was too dry with HTK = 0.41, July HTK = 1.34, and in August, there was no rain all with HTK = 0.11, September HTK = 1.32, October HTK = 1.96.

Summarizing, 2015 growing season was warmer but drier in comparison with 2014 and multi-annual averages. This is confirmed by the average hydrothermal coefficient (HTK), which was 1.81 in 2014 and 1.47 in 2015. Fluctuations and differences in weather conditions could affect not only autotrophs biometric parameters, but also photosynthesis and respiration processes.

Results and Discussion

C exchange and plant growth are closely related with meteorological conditions during growth period and soil chemical properties. Average air temperatures seasonally ranged depending on the season and month. It was found strong positive correlation (r_a=0.6 and r_s=0.8; p=0.02, respectively) between plant and soil respiration (R_a and R_s) and air temperature in OF agroecosystems (Fig. 1,2). During vegetation period mean soil respiration varied between 0.236 and 2.065 µmol m^{-2} s^{-1} since the smallest values obtained in early spring and late autumn when soil moisture surplus in (and in spring) determined unfavourable anaerobic conditions for soil biota, and thus decreased soil respiration Rs in our temperate climate. However, summer precipitation deficiency also reduced soil respiration R_s rates even though air temperature increased. Mean plant respiration (R_a) varied from 0.354 in March to 1.704 µmol m^{-2} s^{-1} in June (Fig. 2). Plants respiration increased in conjunction with their growth intensity up to flowering stage and decreased at plant maturity stage. Plant respiration subject to LAI values (Fig. 3).

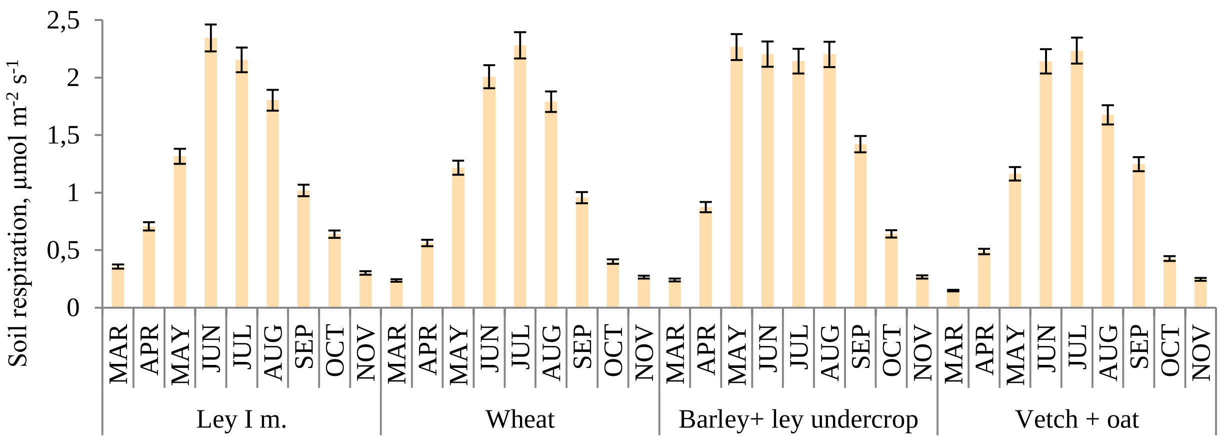

Fig. 1. Soil respiration (Rs) in organic crops, 2014-2016 (mean±SE)

These plant and soil respiration emissions (Ra+Rs) ranged between 2.519 and 3.517 µmol $m^{-2}s^{-1}$ however composed insignificant part in total carbon exchange. Moreover, respirational emissions were completely compensated by the plant photosynthesis when atmosphere CO_2 has been absorbed and assimilated. Short-living vetch+oat mixture and barley+ley undercrop exhibited the maximal Ra+ Rs of 3.037 µmol $m^{-2}s^{-1}$ and 3.517 µmol $m^{-2}s^{-1}$ respectively if compared to minimal respiration in ley and wheat agroecosystems. These results correspond to previous findings that more intensive agrotechnologies applied in rotational systems with short-living crops increased negative impact on environment (*Goglio et al. 2018*).

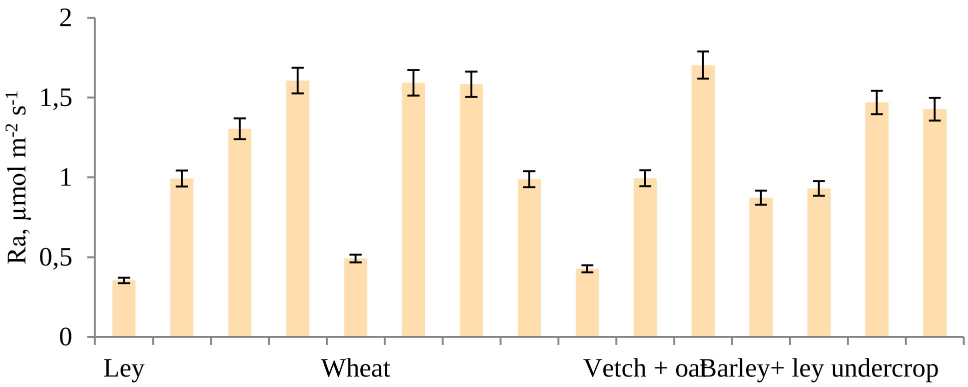

Fig. 2. Plant respiration (Ra) in organic agroecosystems, 2014-2016 (mean±SE)

Leaf area formation (LAI) and growth occurred during plant intensive growth stages until flowering stage. The plant growth and their bio-parameters also varied seasonally in dependence on meteorological and agrotechnical conditions. LAI is the main structure involved in photosynthesis process when plants assimilated and sequestered CO_2 in GPP (Fig. 4). The maximal mean LAI of 0.625 m^2 m^{-2} and 0.661 m^2 m^{-2} was recorded for ley and wheat, respectively. nonetheless, the minimal LAI of 0.348 m^2 m^{-2} and 0.466 m^2 m^{-2} was documented for vetch+oat mixture and barley+ley undercrop due to their small habitat and short vegetation period. Specific leaf area (SLA) is another important trait regulating and controlling plant functions such as carbon assimilation and carbon allocation (*Smith et al. 2007*). SLA increased together with plant maturity from 0.005 to 0.016 m^2 kg^{-1} since LAI decreased due to withering of bottom leaf.

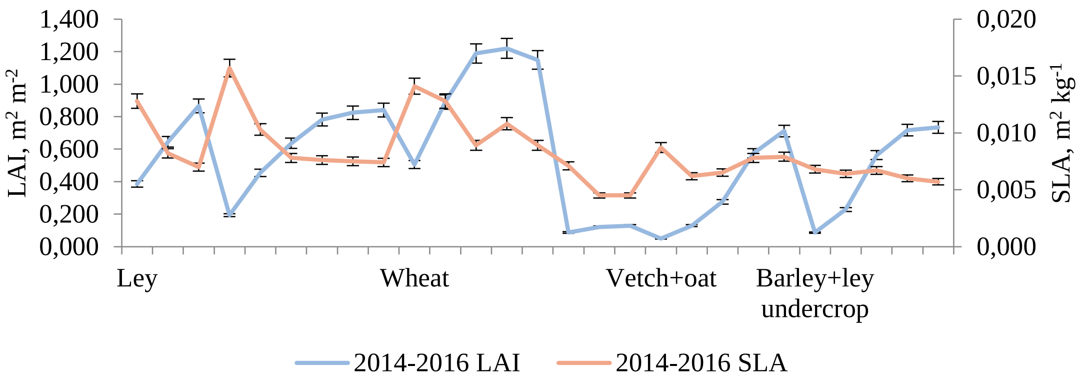

Fig. 3. LAI and SLA in crops agroecosystems (mean±SE)

LAI and SLA determined the photosynthetic surface and volume of assimilated CO_2 (GPP and NEP) in agroecosystems.

Significantly strong correlation between temperature and GPP (r=0.7, p=0.001) confirmed that seasonal temperature fluctuations resulted the average seasonal GPP alteration in agroecosystems. Seasonal changes in the rates of assimilated CO_2 (GPP) can be attributed to seasonal variation of LAI and biomass at different growth stages. This was confirmed by strong correlation between GPP and LAI (r = 0.8) in assessed agroecosystems. Maximal mean GPP rate of 11.9 µmol $m^{-2}s^{-1}$ was recorded in ley and minimal of 10.1 $m^{-2}s^{-1}$ – in vetch+oat mixture due to different bio-parameters and growth period.

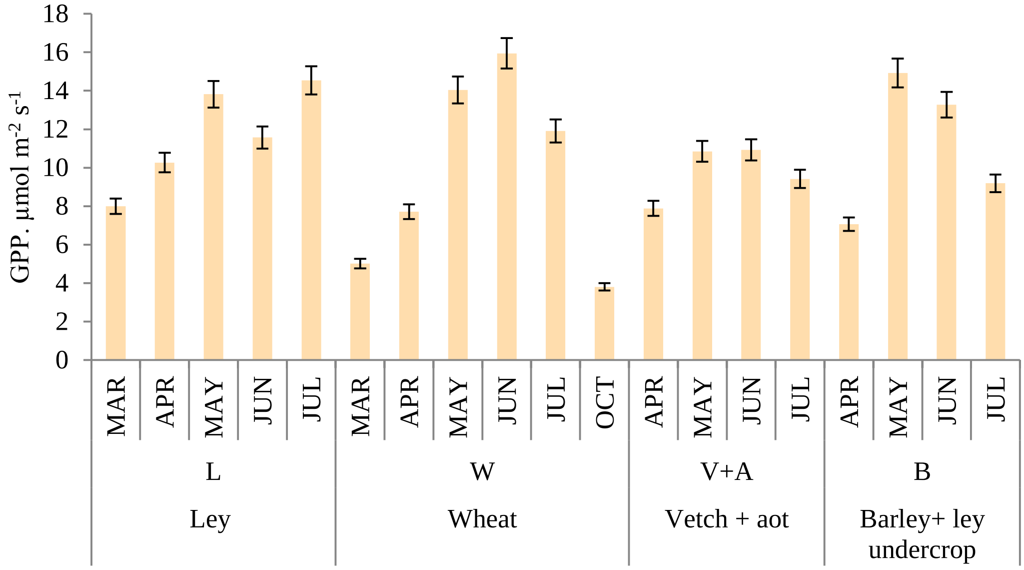

Fig. 4. Atmospheric CO_2 sequestration in crops GPP (mean±SE)

Net ecosystem production (NEP) shows the CO_2 content of assimilated and converted to biomass. NEP was different in agroecosystems depending on plant species and longevity of growth period (Fig. 5). NEP varied correspondingly to environmental conditions and bio-parameters, particularly to LAI (r=0.8), during the growth period. The mean NEP values responded to seasonal climate. The highest NEP was found in the ley and wheat agroecosystems and mean value rated 9.936 µmol $m^{-2}s^{-1}$ and 9.199 µmol $m^{-2}s^{-1}$ respectively. These crops sank and assimilated the highest amount of atmospheric CO_2, which was accumulated in the biomass. Therefore, they may significantly contribute to increase in sustainability of agriculture due to decrease in CO_2 concentration in the atmosphere and consequent climate change mitigation. This result obtained due to their the longest vegetation period between assessed crops. Subsequently the minimal NEP was of short vegetation crops, i.e. vetch+oat mixture and barley+ley undercrop.

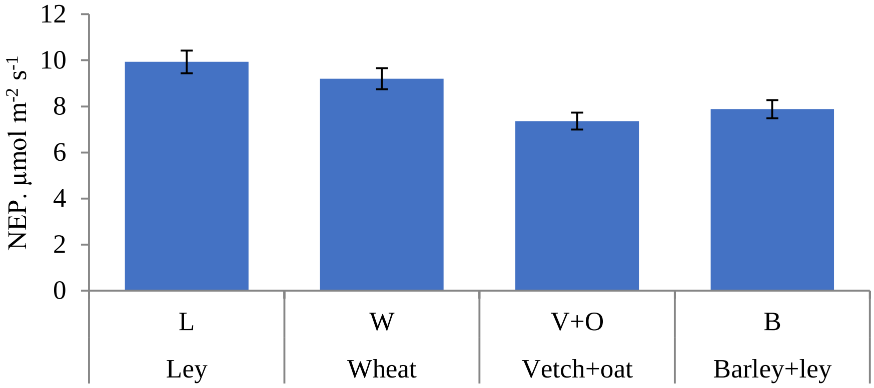

Fig. 5. Net ecosystem production (NEP) in organic agroecosystems (mean±SE)

The total amount of carbon sink by plants (NEP) exceeded total respiration rate several times, thus agroecosystems significantly reduced atmospheric CO2 concentration.

Conclusion

Mean soil respiration advanced plant respiration by 18%, thus promoting atmospheric CO_2. Nonetheless, the investigated agroecosystems sequestered greater rates of atmospheric CO_2 than they emitted during respiration. Total respirational CO_2 emissions were higher in short-living vetch+oat mixture and barley+ley undercrop than that in ley and winter wheat agroecosystems. Alteration of respiration CO_2 emissions strongly depended on meteorological conditions, i.e. air temperature (r_s=0.6 and r_a=0.8). Precipitation exhibited negative correlation to both soil (r_s=-0.6) and plant respiration (r_a=-0.7). Differences in CO_2 exchange throughout photosynthesis and respiration among investigated agroecosystems strongly correlated with the leaf area index (r = 0.8). Crop's leaf area index determined the photosynthetic surface, and thus rates of assimilated CO_2 (GPP and NEP) in assessed agroecosystems. Among the agroecosystems analysed, ley and wheat exhibited the highest CO_2 assimilation capacity. Therefore, they may significantly contribute to enhance the sustainability of crop rotation by reason of decrease in CO_2 concentration in the atmosphere. The results also revealed, that appropriate choice of plant species as well as their growing area in crop rotation may reduce CO_2 emissions and their impact on the environment and climate change.

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PHOSPHOMOLYBDATE TEST METHOD FOR ANTIOXIDANT ACTIVITY IN EXTRACTS OF ANIMAL FEED

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ABSTRACT

Antioxidant activity in feed from plant sources are pharmacologically potent sources and have little or no effects on the health of livestock and through its diet and at humans. For this aim used method to reduction of Mo (VI) in Mo (V), by samples of animal feed. Extracts obtained with methanol + ethanol (0.6M H_2SO_4, 28mM Na_2PO_4 and 4M $(NH_4)_2$ MoO_4, Soxlet method: Green complexes formed at acidic pH value are spectrophotometric in the UV range at wavelength $\lambda = 695$ nm. The values of milk primers are compared with respect to the calibration curve of IUPAC (3,4,5-Trihydroxybenzoic acid) or gallic acid. Extracted samples of feed tested for antioxidant activity than make statistics in Excel. Measurements were made on samples of animal feed natural plant as alfalfa, silage and concentrate. The results of the concentration of nine feed samples range c = 3,25 – 18,0 / (μg / ml).The highest value of antioxidant activity is in alfalfa versus concentrate, which is result of quantity of vitamin C. It was concluded that the total antioxidant activity in natural plant food extracts is higher than concentrates that have antioxidant supplements.

Key words: antioxidants, antioxidant activity, animal feed, alfalfa, concentrate.

1. Introduction

Antioxidant activity

Antioxidants can be used to prevent the oxidation of nutrients in animal feed. The choice of antioxidant is important for the effective prevention of oxidation in the nutrients of animal feed, especially if fats are added to it to improve the energy value. When using certain antioxidants in the diet of farm animals, the requirements of FAO (1978) should be met. Many researchers (De Souza, et al., 2014), evaluate that by examining the chemical composition of animal feed, bioactive compounds are also identified. The term "bioactive" comes from the words: bio - from the Greek (βío), which refers to life, and active from the Latin "activus", which means dynamic, full of energy that includes activity (Bernard G. and Dromar A., 2011). Antioxidant activity represents all phenomena that manifest a form of life, function, or process (Alain, Le Robert, 1994). In scientific terms, the term "bioactive" is an alternative term to "biologically active". A bioactive compound is a simple substance that has biological activity (Online Etymology Dictionary, 2013). Bioactive compounds and their antioxidant activity as well as identification of minerals: potassium, calcium, magnesium, iron, copper, manganese, and zinc in crushed tropical fruit peel, mango, papaya, melon and pineapple have also been studied by the authors (Sabino, LBS, et al., 2015). Due to the large variations observed between different food samples (Carlsen H. M., et al., 2010), clinical and epidemiological studies emphasize the importance of using a comprehensive database and their combination leads to a detailed registration system. of food.

The current antioxidant database is an essential research tool to further explain the potential health effects of phytochemical antioxidants in the diet. According to research (Kothari V., et al., 2014) for the extraction of seeds from five different plants prepared in water, methanol and ethanol, using five different extraction methods: Soxlet method, ultrasonic bath, continuous shaking extraction at room temperature and microwave extraction, with and without alternating cooling, showed the Soxlet method to be the best. A strong positive linear correlation between extraction efficiency and overall antioxidant and antibacterial activity has been demonstrated in plant seed extracts (Kothari V., 2010). Therefore, there is a need to select an appropriate extraction methodology, when different methods are applied to the same plant material with the same solvent, the extraction efficiency may vary.

The selective separation of the target components from the sample to obtain the maximum amount, to reduce and eliminate the obstacles during the extraction processes (Badarinath A.V., et al., 2010) is the main goal to achieve the widest possible range of phytochemicals. Since research has been done on animal feed, which is composed of parts of plants, such as leaves, flowers, fruits, seeds or whole plants, the methods used are the same or similar to the methods most commonly used in the science of testing. of antioxidant capacity in medicines produced from plant parts or in parts and fruits of fruits, which are rich in antioxidant activity (Carlsen HM, et al., 2010).

1. Material and methods

To test the total antioxidant activity, 9 samples of animal feed were used, namely concentrates (1 and 2), alfalfa hay and wheat hay from 3 farms A, B, C in the following order:

1. Concentrate 1 from farm B; 2. Concentrate 2 used on the same farm B; 3. Concentrate 1 used on farm A; 4. Concentrate 2 used on the same farm A; 5. Concentrate 1 from farm B; 6. Alfalfa hay used on farm A; 7. Wheat straw used on the same farm A; 8. Alfalfa hay used on farm B; 9. Alfalfa hay used on farm C.

The molybdate test, the Phosphomolybdate method, is based on the reduction of the molybdenum element Mo (VI) to Mo (V) from the sample and the subsequent formation of a green phosphate Mo (V) complex at acid pH (Alam N., et al., 2013 ; Prieto et al., 1999). As a material it has been tested in samples of animal feed extracts: straw, alfalfa, concentrates from 3 farms. The reaction performed in a test tube, 1 ml of test extract and 1 ml of reagent (0.6 M Sulfuric acid 95%, AnalaR Normapur, VWR Chemicals, 28 mM sodium phosphate, Merck and 4 mM ammonium molybdate, Merck). Samples incubated in aqueous bath at 95^0C for 90 minutes, then cool the mixture to room temperature and measure the absorption at a wavelength of 695 nm on a spectrophotometer (Spectroquant Pharo 300 Merck) relative to the blank water sample.

The standard curve is prepared with known concentrations (0.2-14 μg / ml) Galic acid, Cayman Chemical Company. The antioxidant capacity of the extracts expressed as the ratio of the gallic acid equivalent per gram of dry extract (m GAE / g).

The test were use according to the research of Prieto et al. (1999). Ascorbic acid (2 mM) as a positive control, corresponding to the value of 30.80 mMGAE, the basic solution of gallic acid CH_6 (OH) OOCOOH or ($C_7H_6O_5$) the same prepared with Mt = 170.02 g / mol or 1 M solution and the procedure and reading of standard solutions were the same as for test samples.

The total antioxidant capacity of the feed samples was determined using gallic acid as standard (measuring range: 0.00 - 14 μg / ml, y = 0.0344x + 0.0519, R2 = 0.9709). The results were express as mean ± standard deviation and the statistical significance of the differences was determined using one-way analysis of variance, student t-test. Differences are considered significant if $p < 0.05$. The values are displayed as mean ± SD (n = 3).

2. Results and Discusion

The results of the examination of the antioxidant activity in the feed extracts by the Phosphomolybdate method read from a calibration curve. The first read the standard gallic acid calibration solutions and construct a standard gallic acid curve. The absorbed readings from the samples were apply to the standard curve and the concentrations of the samples were reading.

Standard curve of gallic acid

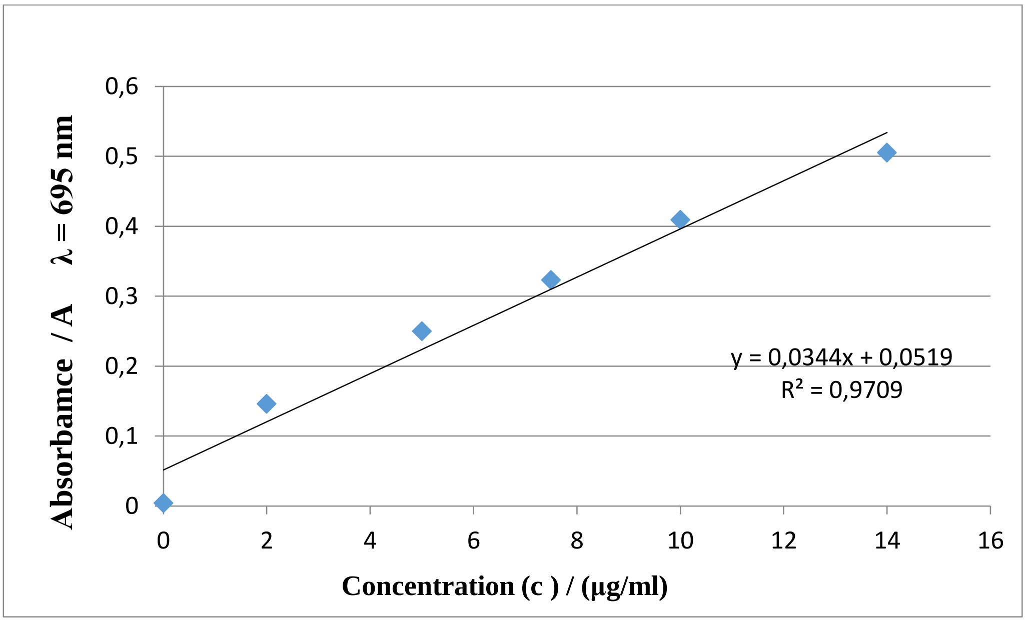
Concentrations of reduced Mo (VI) valence in Mo (V) valence in feed extracts are read on the standard gallic acid curve, Table 1, in the measuring range (0.00 to 14.00 μg / ml, y = 0.0344 + 0.0519, R2 = 0.9709). The values of the concentrations graphically shown in Graph 1.

Table 3 shows the concentration and absorption of reduced molybdenum Mo (VI) to Mo (V) in feed sample samples (read from the standard curve in Graph 2). The mean values of the absorbance, at wavelength λ = 695 nm of reduced molybdate in the samples of feed extracts, their deviations from the mean value, as well as the standard deviation are shown in Graph 3.

Concentration (c) / (μg/ml)	**Absorbance (A) λ = 695 nm**
0	0.004
2	0.146
5	0.25
7.5	0.323
10	0.409
14	0.505

Table 1: Standard gallic acid curve

Graph 1 shows the absorptions and concentrations of gallic acid, which read the corresponding concentrations of reduced molybdenum Mo (V) for all samples, as well as for feed extracts.



Graph 1: Standard gallic acid curve

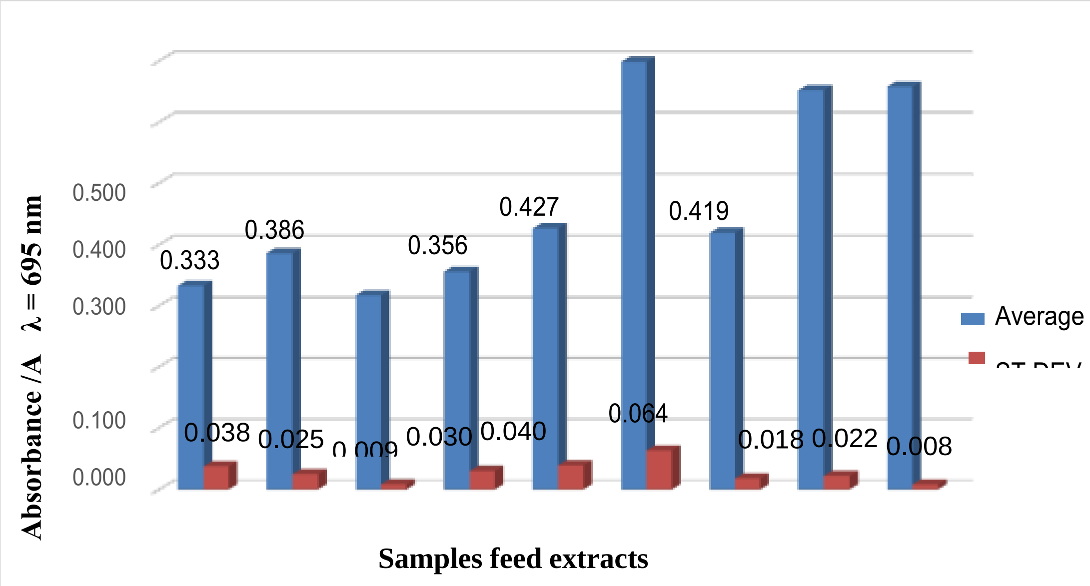
Table 2 shows the absorbance A values for the nine samples of animal feed extracts, statistics in Excel, the mean value $\bar{x}$, the standard deviation s or (SD), and the relative standard deviation RSD or coefficient of variation (CV).

No. Measurements	**No. of samples of feed extracts**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	0.348	0.394	0.329	0.348	0.431	0.73	0.454	0.636	0.661
2	0.35	0.394	0.329	0.349	0.431	0.731	0.457	0.638	0.664
3	0.359	0.35	0.321	0.395	0.361	0.73	0.411	0.639	0.641
4	0.362	0.352	0.319	0.398	0.361	0.577	0.412	0.636	0.644
5	0.275	0.42	0.309	0.319	0.461	0.577	0.427	0.638	0.664
6	0.377	0.42	0.308	0.315	0.462	0.725	0.421	0.682	0.665
7	0.277	0.39	0.320	0.395	0.435	0.725	0.454	0.682	0.66
8	0.35	0.392	0.321	0.348	0.436	0.737	0.423	0.686	0.659
9	0.34	0.359	0.308	0.345	0.462	0.73	0.421	0.639	0.66
10	0.289	0.39	0.307	0.348	0,430	0.723	0.42	0.64	0.662
n = 10									
$\bar{x}$	0.333	0.386	0.317	0.356	0.427	0.699	0.419	0.652	0.658
s	0.038	0.025	0.009	0.030	0.040	0.064	0.018	0.022	0.008
RSD / %	11.309	6.512	2.698	8.488	9.262	9.185	4.164	3.370	1.282

Table 2: Statistical analysis of the values of reduced Mo (VI) to Mo (V) in the samples of feed extracts

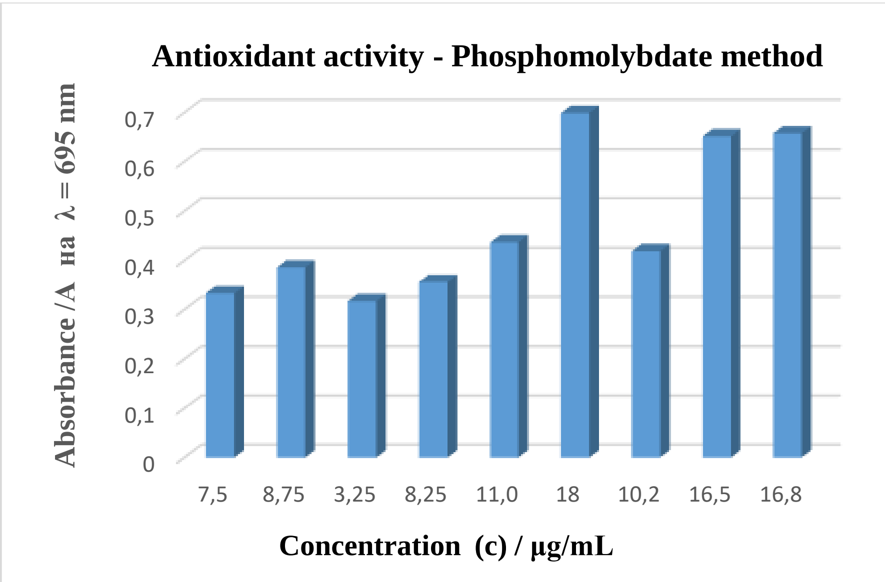
No. samples	Absorbance (A) λ = 695 nm	Concentration (c) / (μg/ml)
1	0.333	7,5
2	0.386	8,75
3	0.317	3,25
4	0.356	8,25
5	0.437	11,0
6	0.699	18
7	0.419	10,2
8	0.652	16,5
9	0.658	16,8

Table 3: Concentration and absorption of reduced molybdate in animal feed extracts



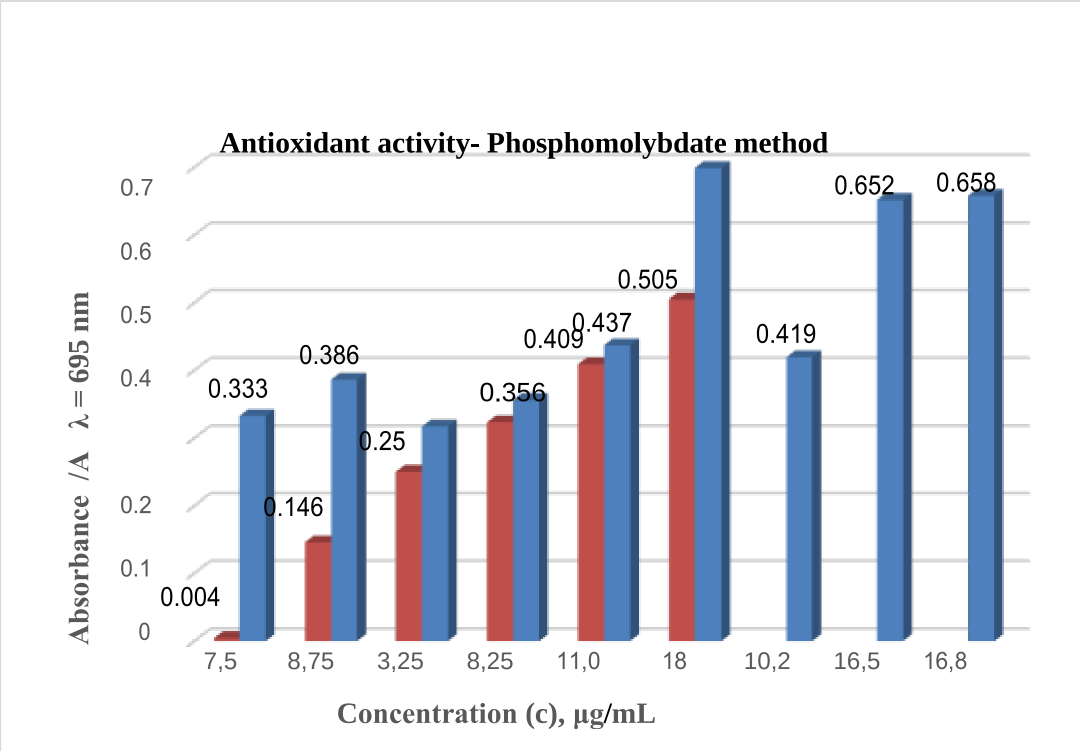
Graph 2: Statistical analysis of the absorption of reduced Mo (VI) to Mo (V) in feed extracts

The concentration of reduced molybdenum from (VI) to (V) in the samples of animal feed extracts shown in Graph 3. Through the values obtained by the reduction reaction of molybdenum, the antioxidant activity expressed in μg / mL at wavelength λ = 695nm is read.



Graph 3: Antioxidant activity in animal feed extracts

The concentration of reduced molybdenum from (VI) to (V) in the feed extracts, read on the standard gallic acid curve shown in Graph 4, and that concentration corresponds to the antioxidant activity of the samples in relation to gallic acid.



Graph 4: Antioxidant activity of animal feed extracts relative to the standard curve

To obtain high extraction efficiency and high phenol content (and its antioxidant activity) the Soxlet method may be considering the best option (Sudjaroen Y., et al., 2005). According to their research, in which they used several methods of extraction at room temperature, the degree of superiority of the extraction according to the Soxlet method compared to that with the microwave oven, in terms of antioxidant activity of anthraquinones. However, this method has shown better efficiency in the extraction of antioxidant compounds. This method has been applied to a large number of different specimens and has been compared to several room temperature extraction methods using methanol. In this research, the same Soxlet method was used at room temperature and solvents methanol and ethanol in a ratio of 1: 1, of which methanol proved to be the most interesting compared to other solvents. It has been shown to be better at extracting phenols, flavonoids, antioxidant metabolites and antibacterial phytochemicals than water and ethanol (50% applied). Better suitability of methanol (compared to ethanol and water) for the observation and isolation of antimicrobial therapy compounds has been seen in previous research by other researchers (Eloff J.N. 1998); (Parekh J., 2005) and others. According to Gupta P. et al., (2017), research has shown that alcohol extract has excellent antioxidant activity or activity for the removal of free radicals, has antiglycemic properties and inhibits the accumulation of sorbitol and the enzyme alkaline phosphate, which may be due to the presence of different types of ingredients, especially phenolic and flavonoid contents. Our research on the total antioxidant activity in animal feed by reducing molybdenum in addition to the presence of vitamins A, E and C, and the chemical composition and certain minerals that act as cofactors, but mostly the presence of the same compounds, phenols and flavonoids.

Although alfalfa is a rich source of flavonoids and other antioxidants (Stochmal A. and Oleszek W., 2007) and phenolic compounds that have anti-inflammatory activity (Choi et al., 2013), alfalfa also It also contains vitamins A, E, K and B vitamins which due to lack of opportunities, conditions and equipment, are not analyzed in this research. Antioxidant activity thought to increase with the presence of antioxidants (A, E and C) which are substances that inhibit oxidation and especially used as substances to prevent the breakdown of canned foods as well as additives in concentrates. Antioxidant activity affects the removal of potentially harmful oxidizing agents in the livestock organism. Most studies do not mention the purity of phytochemicals, which may mask their activity. Several articles show extracts that are not easily soluble in water, so they are dissolve in organic solvents, such as methanol, acetate, ethanol, chloroform, etc., which are powerful purifiers of the OH group. Many publications indicate that molar and millimolar concentrations may often be irrelevant due to the condition of the sample as a whole. Plant foods show not only antioxidant properties, but also other biological properties, which are relevant for further research. Currently, a number of highly specialized techniques are used in testing antioxidant properties, and the results often depend on the techniques used (Blekić M. et al., 2011). This test is an in vitro method for determining the antioxidant activity of various plant extracts (animal feed extracts). Free radicals often generated as a by-product of biological reactions or derived from exogenous factors. Although in vitro antioxidant tests have been perform on a number of medicinal plants, information on in vivo testing is lacking. Therefore, collaborative studies needed to standardize these methods.

As a result, more detail studies needed to elucidate the mechanism of the prooxidant effect and to determine its relevance in vivo. The active compounds of many plant extracts that possess antioxidant activity have not identified yet. In our case, ascorbic acid used as the standard and the total antioxidant capacity expressed as the equivalent of ascorbic acid.

3. Conclusion

Total antioxidant activity in feed extracts by the method of reduction of molybdate (VI) to (V) valence, shown on the standard gallic acid curve at $\lambda = 695$ nm, in the measuring range with concentration (from 0.00 to 14.00 μg / ml) with y = 0.0344 +0.0519, $R^2 = 0.9709$, is corresponding. This method shows that the highest value for the concentration of reduced molybdenum is in the alfalfa content of farm 1, and the lowest concentration is in the feed mixture- concentrate 1 from farm B. A similar concentration was also measure in concentrate1 from farm A. The highest total antioxidant activity in all foods used together on the three farms in animal feed applied in Farm A. In other farms we have lower values due to the fact that the number and quantities of food used in farms B and C are smaller where we have 3 and 2 types of food compared to farm A where 4 types of food are used. The high antioxidant activity of the total food from farm A is due to the presence of vitamins A, E mostly from vitamin C, which according to our research in farm A is 91.0 μg / mL, in contrast to farm B which is 79.2 μg /

mL and in farm B of 57.3 µg / mL vitamin C. From the studies on the antioxidant activity of animal feed, it can be conclude that alfalfa hay is a natural food and is rich in antioxidants, and shows high antioxidant activity compared to concentrates.

On the other hand, we conclude that the highest antioxidant activity is obtain in concentrates that we believe are the result of nutrient supplements in addition to vitamin C, antioxidants may be present: butyl hydroxy anisole, butyl hydroxy toluene, alpha tocopherol and others, which can increase antioxidant activity. Comparing the highest values obtained with the analyzes for the antioxidant activity of the three farms, it is concluded that it is low in concentrates 1 and 2 of farm A in relation to the application of feed alfalfa hay and straw. The highest value for the total antioxidant activity in animal feed is obtain from alfalfa hay on farm A. The end conclusion is the use of several types of food such as alfalfa, two types of concentrates and straw, proves the dependence of antioxidant activity on the impact of nutrients, their type and quantity.

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COMPARISONS OF HIGH YIELDING GENETICALLY DIVERSE WHEAT LINES IN CROATIA

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ABSTRACT

Wheat is one of the most common and important cereals in Croatia. Therefore, high selection pressure at wheat breeding program is continually placed on disease, drought and lodging resistance, heading date and end-use quality. The usage of genetically resistant wheat lines to abiotic and abiotic stresses can be useful in controlling of wheat diseases and food contamination. The aim of the current study was to evaluate the wheat grain productivity and quality, as well as response of wheat seedlings to drought. In the current study, five lines of winter wheat with referent variety were used for evaluation of desired traits. In general, investigated wheat lines had such good characters as early maturity, high yield, but they were smaller in test weight and protein content, but with higher sedimentation value, dough energy and extensibility, compared to referent variety Kraljica. Overall, lines Osk.4.330/6-18, Osk.3.530/59-18, Osk.4.354/12-18 out yielded the referent check with regard to the grain yield. According to the results collected in this research, wheat lines differences in germination energy, and seedling growth affected by drought were obtained. As planting conditions are not always ideal, all lines could offer farmers tolerance to mild drought during sowing, and will achieve high yields. Nevertheless, stability and drought tolerance of investigated winter wheat lines at different environments needs to be checked at multi-location trials.

Keywords: Drought, Yield performance, Quality traits, Wheat

INTRODUCTION

Increased biotic and abiotic stresses occurring as a consequence of climate change are a threat to the already decreasing wheat productivity (Suzuki et al., 2014). According to Liu et al. (2016) for every 1°C increase in temperature, global wheat yields are predicted to decline by 4.1–6.4%. By 2050 increase of more than 60% in global grain production will be needed, as a consequence of food demand by population growth (Godfray et al., 2010). Therefore, the main targets of wheat breeding programs should be for increasing grain production limits. The main objectives of the winter wheat breeding program at Agricultural Institute Osijek are good quality and high yields, early maturity including some other various characteristics such as disease and drought resistance. The major threat in yield production is various pests and pathogens which cause considerable losses every year where fungal diseases cause the highest damage. The genetic basis of defence mechanisms to biotic stress is stored in the plant's genetic code (Singla and Krattinger, 2016). Despite the fact that the most important is the resistance of the genotype, most of wheat varieties were produced with high inputs of fungicides for plant defense against biotic stress.

Besides biotic stress, the concurrent occurrence of abiotic stresses such as drought and heat is more and more common due to global warming (Mittler, 2006) where increasing drought also threatens agricultural productivity and food security (FAO, 2018). Hence, these climatic changes will have a dramatic effect on agriculture especially at the regions with water shortage and high temperatures (Rinaldi 2009). Nevertheless, drought is a global problem, which leads to osmotic stress with huge impacts on global wheat production (Daryanto et al., 2016). Therefore, the development of new wheat varieties with high water use efficiency is crucial for producing optimum yield under marginal rainfall conditions. According to Mickky and Aldesuquy (2017) in wheat certain developmental stages appear to be more sensitive to osmotic damage. However, it was demonstrated that wheat genotypes that showed drought tolerance at the germination stage exhibited the same tolerance to water deficit under field conditions (Khakwani et al., 2011).

In order to obtain correct levels of yield and quality, investigated winter wheat lines and referent variety were screened without usage of fungicide to find the best suited varieties for disease stressed conditions, as well as seedling test for drought resistance was performed in controlled conditions.

MATERIAL AND METHOD

Plant material and field experiment

Five winter wheat lines with referent variety (Table 1) have been studied in the field trial in vegetative season 2019/2020 at Osijek (45°27' N, 18°48' E) in Croatia, where soil type is eutric cambisol. The plants were sown in October in the experimental plot area of 7.56 m2 in four replications. The average annual precipitation in vegetative period in 2019/2020 was 408.6 mm and the average annual temperature was 11.1˚C. Insecticides and herbicides were applied as needed, without usage of fungicides. In the field trial, traits as heading date, resistance to lodging and plant height were evaluated. After harvest at the beginning of July, grain yield (t ha^{-1}), test weight (kg hl^{-1}) and 1000 kernel weight (g) were obtained.

Table 1. Investigated five winter wheat lines and referent variety Kraljica

No.	Variety/Line
1	Kraljica
2	Osk.4.324/5-18
3	Osk.4.312/10-18
4	Osk.4.330/6-18
5	Osk.3.530/59-18
6	Osk.4.354/12-18

Technological and rheological quality

The quality tests described in this section are standardized testing procedures commonly used for quality control purposes. After milling the grain samples protein and wet gluten content, gluten index, zeleny sedimentation volume and falling number were obtained by ICC method No. 155, 116/1 and 107/1, respectively. Farinograms were obtained using method for using the Brabender Farinograph (HRN ISO 5530-1:1999). Extensogram test was conducted using method for using the Brabender Extensograph (HRN ISO 5530-2:1999).

Experiment with drought on seedlings

The growth chamber experiments were conducted to evaluate drought resistance in seedlings stage by usage of polyethylene glycol-6000 (PEG6000) solutions as the moisture stress inducing media. Seedlings were grown in trays and water stressed up to seven days with 16 h light period at 25˚C and 8 h dark period at 20˚C, with constant relative humidity of 60%. Drought-exposed plants were watered daily with 10 ml of 10 and 20% PEG solution, while in the control treatment only distilled water was applied. After seven days germination energy, growth parameters, as well as relative water content (RWC) were calculated. Germination energy was defined as the percentage by number of seeds in a given sample which germinate within 7 days. Growth parameters (root and shoot length) were recorded in mm with a ruler after seven days of different treatments. RWC was calculated by the formula: RWC (%) = (FW-DW)/(TW-DW)*100 where FW was a fresh weight of leaf tissue which upon weighting was submerged in distilled water for 24 h to reach turgid weight (TW). Dry weight (DW) of tissue was obtained by drying leaf discs at 105°C for 24 h.

Statistical analysis

All recorded values for agronomical and quality parameters represent the means of the results of four replications. For drought experiment, 15 replicated seedlings were taken for investigated traits as a mean value.

RESULTS AND DISCUSSION

The investigated winter wheat lines were selected by the pedigree method over few years of selection and tested in varietal experiments in trial in vegetative season 2019/2020 at one location. Climate changes enhance the importance of drought stress in wheat growing regions of the world where drought stress can lead to a high yield decline in recent years. We want to create drought-tolerant varieties and therefore, it is essential to primarily understand response of wheat plants in water-deficient conditions at different stages of growth.

Agronomical and morphological traits

The highest grain yield had line Osk.3.530/59-18 (12.75 t ha^{-1}). The lowest grain yield was recorded for line Osk.4.312/10-18 (11.55 t ha^{-1}) with the lowest test weight. Test weights ranged between 78.5 (Osk.4.312/10-18) to 82.2 kg hl^{-1} (Kraljica). The tallest line Osk.4.354/12-18 had the highest 1000 kernel weight (43 g). Currently, referent variety such as Kraljica (a compromise between yield and proteins, with good baking quality) perform excellent in Croatia, Slovenia, Bosnia and Herzegovina, Slovakia, Kosovo, Macedonia and Romania. Although referent variety Kraljica had the highest test weight, it did not give the highest yields, where the lowest resistance to lodging could be one of the reasons for not performing the best yields. Feng et al. (2019) reported that changing weather patterns such rain, wind, and hail storms have made the current varieties more susceptible to lodging, leading up to 80% yield losses. Test weight as the specific volume was in good range in all wheat lines. According to Mecha et al. (2017) by selecting for this trait together with grain filling period, number of productive tillers per plant, spike length, number of spikelets per spike, number of kernels per spike, 1000 kernel weight, biomass yield per plot and harvest index, there is also possibility to increase grain yield of bread wheat.

Five wheat lines had the similar heading date as Kraljica, except line Osk.4.330/6-18 with three days later heading date. Investigated five lines developed were an early maturing, similar as Kraljica, making it well suited to short seasons. It is more and more important to grow winter wheat varieties that are not late-maturing as harvest delays or terminal heat stresses can often occur (Mondal at al., 2016). Among wheat lines, the maximum plant height (96 cm) was recorded in line Osk.4.354/12-18, and minimum plant height (85 cm) was recorded in line Osk.4.312/10-18, the same as for referent variety Kraljica. All lines together with Kraljica had red coloured seed (Table 2). Plant height was measured from soil surface to the base of the ear head of main shoot at maturity stage. It should be in relation to the plant architecture, lodging resistance and yield performance (Wang et al. 2017). In the current research, only one wheat line was taller than 90 cm, which potentially could be resource of feed for ruminants.

Table 2. Agronomical and morphological traits of five winter wheat lines and referent variety Kraljica

Variety/Line	GY*	TW	1000KW	HD	LOD	PH	SC
Osk.3.530/59-18	12.75	79.5	40	5.05.2020.	1	86	Red
Osk.4.330/6-18	12.62	81.6	39	8.05.2020.	0	89	Red
Osk.4.354/12-18	12.62	81.9	43	5.05.2020.	0	96	Red
Kraljica	12.54	82.2	40	5.05.2020.	1,5	85	Red
Osk.4.324/5-18	12.52	80.1	37	5.05.2020.	0	89	Red
Osk.4.312/10-18	11.55	78.5	37	6.05.2020.	0	85	Red

*GY-grain yield in t ha^{-1}, TW-test weight in kg hl^{-1}, 1000KW-1000 kernel weight in g, HD-heading date, LOD-lodging evaluated as 1-9 (0-no lodging, 9-fully lodged plants at the plot), PH-plant height in cm, SC-seed colour (soaked in 1 M NaOH)

Table 3. Technological and rheological parameters of of five winter wheat lines and referent variety Kraljica

Variety/Line	P	SED	VG	GI	FN	WA	QG	E	Ext
Osk.3.530/59-18	13.2	49	26.6	97	463	55.5	B2	90	155
Osk.4.330/6-18	13.1	42	26.9	93	369	56.1	B2	-	-
Osk.4.354/12-18	14.3	46	24.6	97	376	58.2	A1	98	159
Kraljica	14.5	44	28.7	99	450	58.3	A2	86	155
Osk.4.324/5-18	12.8	49	22.9	99	354	51.2	B1	96	160
Osk.4.312/10-18	13.2	48	24.4	99	374	53.1	B2	91	175

*P-protein content in %, SED-sedimentation value in ml, VG-wet gluten content in %, GI-gluten index, FN-falling number in s, WA-water absorption in %, QG-quality group (ranking A1-C1), E-energy in cm2, Ext-extensibility in mm

Technological and rheological quality

To meet specifications of mill and bakery industry in grain and flour quality testing is necessary to evaluate dough and gluten strength properties. The farinograph test is used to measure the resistance of dough to mixing, while extensograph test is used to measure the resistance of dough to stretching. Results from these tests have a direct relationship to finished product quality. In the current research, the lowest protein content and wet gluten content were recorded for line Osk.4.324/5-18 (12.8 and 22.9%), while referent variety Kraljica had the highest values of those two parameters (14.5 and 28.7%). The same line Osk.4.324/5-18, together with line Osk.3.530/59-18 had the highest sedimentation value (49 ml). Gluten index ranged between 93 (Osk.4.330/6-18) to 99 (Kraljica, Osk.4.324/5-18 and Osk.4.312/10-18). The highest falling number had line Osk.3.530/59-18 (463 s), followed by Kraljica (450 s).

Line Osk.4.354/12-18 had a high yield potential and also maintained a higher grain protein content than other lines with similar yield potential. Four wheat lines had very good sedimentation values, compared to referent variety, thus showing good gas retention in dough stability and baking volume. Usually, sedimentation value of flour depends on the wheat protein composition and is mostly correlated to the protein content (Hrušková and Faměra, 2003), which was not the case in the current study. It was observed that referent variety Kraljica had the highest wet gluten content. It was previously concluded that the higher a flour's protein content, the higher the gluten formation (Baslar and Ertugay, 2011). All tested wheat lines were selected for low pre-harvest sprouting, where falling number as the level of alpha amylase activity, was within acceptable limits. Values below 300 seconds are indicatives of pre-harvest sprouting (grain may start to germinate before harvest) which will result in poor quality (Kiszonas et al., 2018).

Knowing the technological properties of the flour is also not enough to fully characterize end-use quality of wheat. Therefore, it is useful to obtain rheological properties of the dough where in the current study we obtained water absorption and quality group from farinograph measurements, as well as energy value and dough extensibility from extensograph tests. The best water absorption had Kraljica (58.3%) and line Osk.4.354/12-18 (58.2%), belonging to quality groups A2 and A1 thus showing good end-use quality properties. Line Osk.4.330/6-18 exhibited the lowest dough energy and extensibility. The highest dough energy had line Osk.4.354/12-18, and the best extensibility was obtained in line Osk.4.312/10-18 (Table 3). The flour with good bread-making properties has higher water absorption, takes longer to mix and is more tolerant of over-mixing than poor-quality bread flour (William, 2001). All lines, except Osk.4.330/6-18, had higher energy values showing the greater gas holding capacity and fermentation tolerance of the dough where no difficulties in bread making should be obtained. Similar behaviour of those lines was obtained for dough extensibility. According to Anderssen et al. (2004) rheological parameters can be used to determine the processability of wheat to different products.

Drought-related traits in seedling

During germination wheat plants are very sensitive and drought stress can delay or inhibit germination processes, leading to potential yield loss due to reduced cropping density (Almansouri et al., 2001). In general, both solutions (PEG10 and 20%) significantly affected germination energy and seedling growth parameters (shoot and root lengths) compared to controlled treatment. Relative water content (RWC) was influenced in lesser extent.

The highest germination energy had Kraljica and Osk.4.330/6-18 (91.25%) in controlled treatment, Kraljica in PEG10 treatment (92.5%) and line Osk.4.330/6-18 in PEG20 treatment (81.25%) (Figure 1). Germination energy which is used to determine the speed of germination at two various concentrations of PEG differed among wheat lines, where Osk.4.530/6-18 showed the lowest value in PEG10% treatment. The highest germination energy in PEG20% treatment had line Osk.4.330/6-18, compared to other lines. According to the results of Duan et al. (2017) germination of wheat seeds could be inhibited by drought stress, where germination energy significantly decreased with the increase of drought degree in most wheat lines. In the current research the germination energy of Osk.4.330./6-18 and Kraljica was stronger compared to other lines.

The highest reduction of shoots in 10% PEG solution, compared to controlled treatment, was recorded in line Osk.4.354/12-18 (13.04%) (Figure 2), while the length of the roots was increased in all wheat varieties, where line Osk.3.530/59-18 was showing the highest increase among other varieties (67.33%), followed by line Osk.4.324/5-18 (58.36%) (Figure 3). The highest reduction of shoots was recorded in line Osk.3.530/59-18 (37.54%) in 20% PEG treatment, while the highest reduction of the roots had line Osk.4.354/12-18 in 20% PEG treatment (27.5%). Lines Osk.4.330/6-18 and Osk.3.530/59-18, as well as Kraljica increased length of the roots in 20% PEG treatment (17.13, 14.41 and 1.39%, respectively), compared to seedling in controlled conditions. Liu et al. (2013) reported that wheat seedling growth indices decreased under drought stress. In the current research it was different where lines Osk.4.330/6-18, Osk.3.530/59-18 and Osk.4.324/5-18 showed the increase of shoots length in PEG10% treatment, with the highest reduction of shoots in PEG20% treatment, compared to controlled treatment. The same lines had the highest increase of root length in PEG10% treatment with lower increase in PEG20% treatment, except Osk.4.324/5-18 line which showed root reduction in PEG20% treatment. According to Xu et al. (2015) alterations in root system architecture aids in short-term adaptation to water deficit. Root length at the seedling stages of the plant is a key genetic trait for increasing yield under drought conditions (Shahbazi et al., 2012).

Relative water content (RWC) ranged between 88.0% (Osk.4.324/5-18) to 104.7% (Osk.4.312/10-18) in controlled treatment, while in 10% PEG treatment that range was from 82.4 (Osk.4.354/12-18) to 89.6% (Osk.4.312/10-18), and in 20% PEG solution it ranged from 70.02 (Osk.4.324/5-18) to 95.5% (Kraljica) (Figure 4). The line Osk.4.312/10-18 (with the lowest yield) had the highest RWC in controlled and PEG10% treatment, but not in PEG20% treatment thus showing that increased drought stress will significantly influence RWC. Almeselmani et al. (2011) concluded that RWC is a good criterion for the selection of drought-tolerant wheat varieties at the seedling stage. Datta et al. (2011) applied both normal and water-deficient conditions to wheat genotypes and observed that genotypes performed better under environments which had optimum RWC and root and shoot length, which were considered drought-tolerant genotypes. In the current research all varieties retained good RWC when grown under mild drought conditions (PEG10%). Similar results were obtained by Tahara et al. (1990) in winter wheat varieties as the high-yield selections maintained a significantly higher RWC than the low-yield selections.

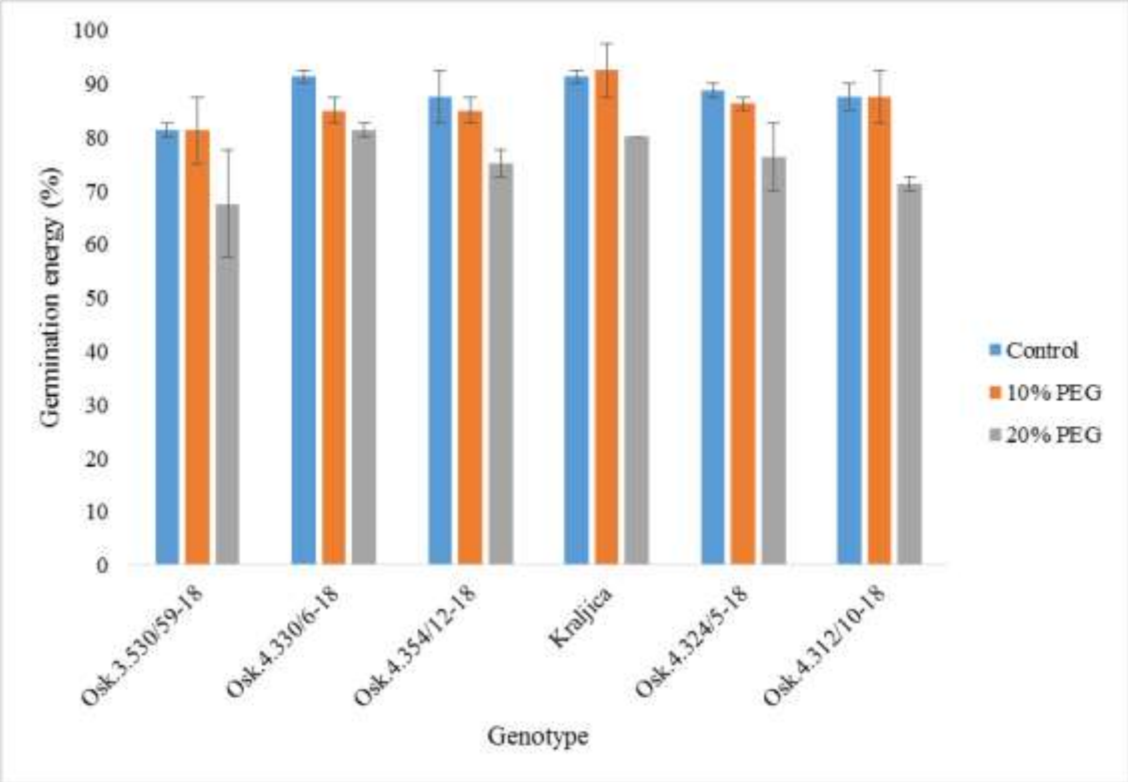

Figure 1. Germination energy of five winter wheat lines and referent variety Kraljica

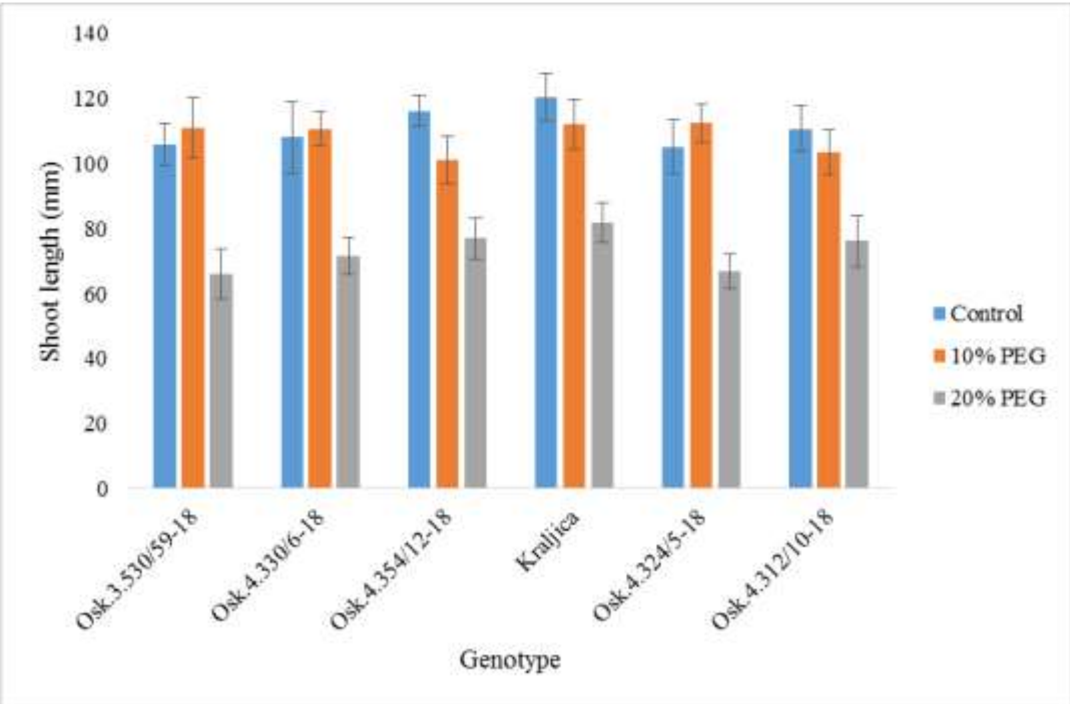

Figure 2. Shoot length of five winter wheat lines and referent variety Kraljica at 7th day of experiment

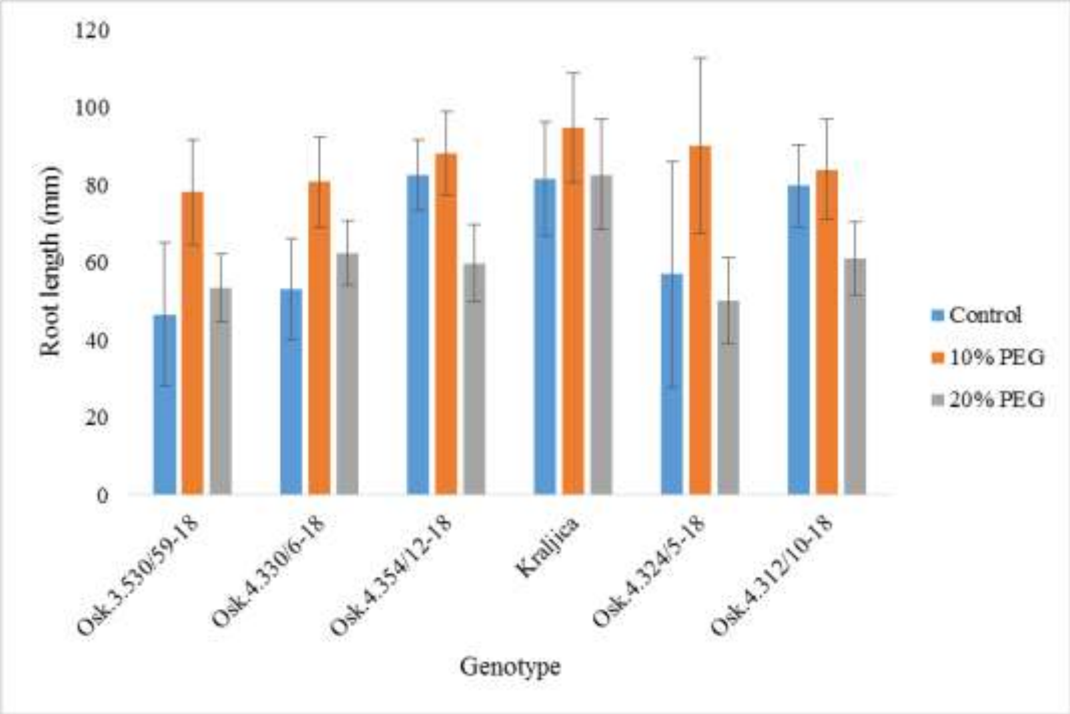

Figure 3. Root length of five winter wheat lines and referent variety Kraljica at 7th day of experiment

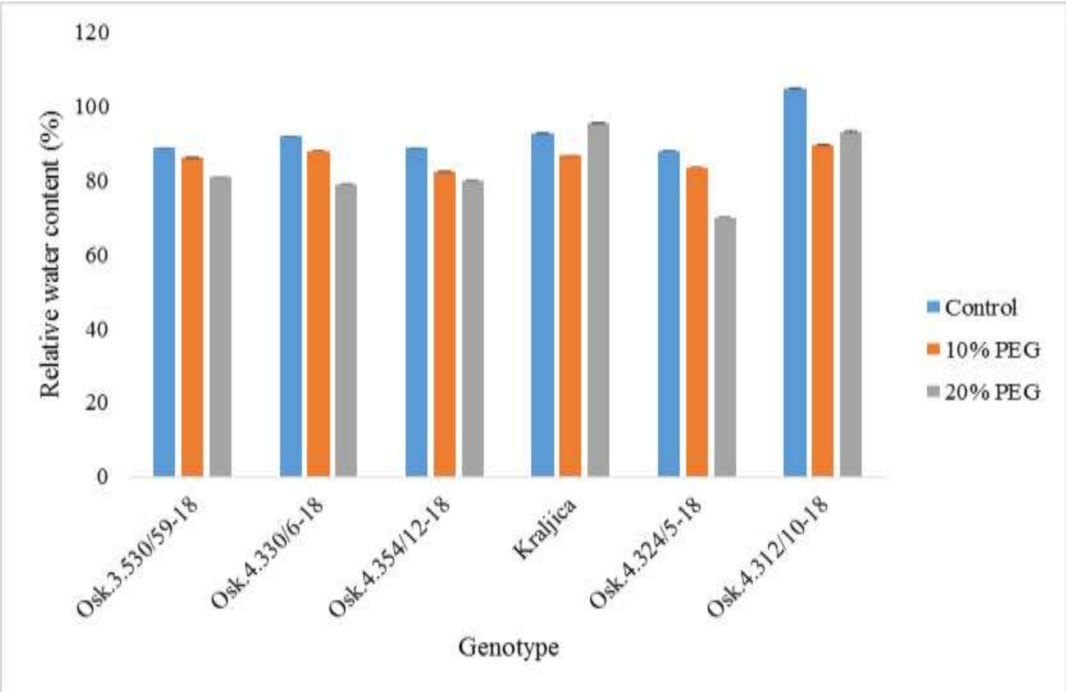

Figure 4. Relative water content of five winter wheat lines and referent variety Kraljica

Although wheat plants with early maturity can contribute to drought escape and could be suitable for environments subjected to late season drought stress, the line with the latest heading date exhibited the best germination energy in PEG20% treatment. These results indicate that breeding efforts to produce drought-tolerant wheat can benefit current climate change adaptation. According to Bernier et al. (2008), there is the possibility to combine high yield potential and good yield under drought successfully. Nevertheless, in the current research all tested lines having good characteristic for grain yield and quality still need to be checked how they are broadly adapted.

CONCLUSIONS

Although, created winter wheat lines have been tested for production in Croatia, it is desired and anticipated that those lines have good yield and quality performance at different countries. Therefore, multi-location trials need to be set up in next seasons. Multi-location testing of those lines will allow a better understanding of the genotype×environment (G×E) interactions related to grain yield. The current results demonstrated that advanced winter wheat lines perform well compared to referent variety with good levels of yield and ground cover; baking quality data are good up till now, but have yet to be completed this year. Continued emphasis has been placed on selecting breeding lines with superior quality and disease resistance where line Osk.4.354/12-18 showed good performance. We expect that most of the lines are high yielding, by showing good emergence in more dry soils.

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ASSOCIATION OF YIELD AND YIELD COMPONENT IN DURUM WHEAT (Triticum durum Desf.) GENOTYPES UNDER RAINFED CONDITIONS

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ABSTRACT

This study aimed at investigating genotypic differences in the leaf area duration of durum wheat genotypes and their relation to grain yield under rainfed conditions. The research was carried out at Trakya Region, Turkey, in 2016-2017 growing years with 30 genotypes in randomized completely blocks design with 4 replications. Data on grain yield, flag leaf area, days of heading, plant height, peduncle length, spike length, spikelet per spike, number of kernel per spike, and relationships among these characters were investigated. The analysis of variance revealed highly significant differences ($p<0.01$) among the genotypes for all parameters investigated. The mean grain yield of the genotypes was 6496 kg ha^{-1}. The highest yields were obtained in the G25 line with 7466 kg ha^{-1} and followed by genotypes C1252, G6, and G13 had the highest grain yield. The mean values of the genotypes were varied between 74.8 to 92.8 cm for plant height, 26.1 to 35.3 cm for peduncle length, 7.0 to 9.5 cm for spike length, 16.6 to 23.4 for spikelet number per spike, 30.2 to 69.5 for kernel number per spike and 16.4 to 28.2 cm^2 for flag leaf area. Correlation coefficients based on the investigated parameters were determined by Pearson's correlation analysis. Flag leaf area positively correlated with grain yield and kernel number per spike. Days of heading were positively significantly correlated with spike length (r=0.562**) and spikelet number per spike (r=0.749**). A significant positive correlation was determined between plant height with peduncle length (r=0.412*), and spike length (r=0.498**). A negative correlation was observed between the flag leaf area with plant height (r=-0.442*). Spikelet number per spike was negatively correlated with peduncle length (r=-0.488**) and positively correlated with spike length (r=0.477**). The correlations among agronomic parameters revealed that flag leaf can be used as selection criteria to improve grain yield in durum wheat.

Keywords: Durum wheat, Genotypes, Yield, Yield component

INTRODUCTION

Grain yield of durum wheat (*Triticum turgidum* L. var. *durum*) under Mediterranean conditions is frequently limited by both high temperature and drought during grain growth. Grain yield under cooler conditions was mostly determined by kernel weight, whereas the number of spikes per square meter predominantly influenced grain production in warmer environments. The number of kernels per spike had a significant contribution to grain yield, especially under drought stress conditions. Compensatory effects among yield components were almost absent in the cooler environments, probably due to the relative availability of water and N during the critical phases of plant development. Contrarily, under warmer conditions, negative effects of the number of spikes per square meter were registered on both the number of kernels per spike and kernel weight (Garcia del Moral et al., 2003). Periods at such high temperatures occur frequently during grain filling in both the Mediterranean and continental climates, and such extremes may be more frequent throughout Europe in future climates of warmer mean temperatures (Barrow and Hulme, 1996). Drought is one of the main constraints to rainfed wheat production in the Mediterranean region, where durum wheat (*Triticum turgidum* L. var *durum* Desf.) is extensively grown. Achieving genetic increases in yield in these environments has been recognized to be a difficult challenge for plant breeders while progress in yield gains has been much higher in favourable environments (Richards et al. 2002). Yield components develop sequentially, with later-developing components under the control of earlier-developing ones and interacting in compensatory patterns, particularly under stressful environments (Simane et al.1993; Moragues et al. 2006).

It has been shown by several authors that grain yield in wheat is more closely correlated to grain number than to grain mass (Zamski and Grunberger 1995, Beharav et al. 1998). Thus, selecting for a high number of spikes per m^2 and a high number of grains per spike has to result in an improved yield. Nevertheless, as grains have to be filled with carbohydrates produced by photosynthetic organs, both source and sink of carbohydrates have to be taken into account when breeding for drought-stressed environments. In this sense, the role of architecture, distribution and size of leaf area has been studied and discussed by several authors (Berdahl et al. 1972, Canevara et al. 1994).

In winter wheat, high-temperature episodes occurring near to anthesis can reduce the number of grains per ear and the subsequent rate of increase in harvest index, resulting in smaller grain yields (Wheeler et al., 1996). Drought stress reduces grain yield of wheat through negative affecting the yield components, such as number of plants per unit area, number of spikes and grains per plant, or unit area and single grain weight, which are determined at different stages of plant development (Francia et al., 2013; Hossain et al., 2012; Farooq et al., 2009). Effects of flag leaf area and flag leaf area duration on spike grain yield is positive but insignificant when drought stress was not a problem in WANA's agro-climatic conditions, in which temperature rose and rainfall decreased between the heading and physiological maturity of wheat. Moreover, high flag leaf area duration positively contributes to grain filling in fewer than two irrigation conditions. Also, the genotypes with low leaf number or short plant height and low tillering capacity should receive significant irrigation, while genotypes with middling plant height should be planted during conditions of drought stress. Under both, the sets of conditions genotypes must have high flag leaf area duration (Tiryakioğlu, 2014).

The current study examined the response of yield, yield components, and other physiological parameters in durum wheat genotypes under rainfed conditions. Therefore, the objective of the study was to investigate whether or not effects on yield and other agronomical and physiological characters in durum wheat genotypes.

MATERIAL AND METHODS

The research was carried out in an experimental area at Trakia Agriculture Research Institute, Edirne, Turkey, in the 2016-2017 growing years. The experiment was composed of 30 genotypes in randomized completely blocks design (RCBD) with 4 replications. Each plot was 6 meters long and had 6 rows, spaced 0.17 meters apart. A seed rate of 500 seeds per square meter was used. Sowings of the experiment were performed by using a plot drill. In the experimental data on agronomic parameters; grain yield (GY) (kg ha^{-1}), days of heading (DH), plant height (PH) (cm), peduncle length (PL) (cm), spike length (SL) (cm), number of spikelet per spike (SNS), number of kernel per spike (KNS), and flag leaf area (FLA) (cm^2) were investigated.

Days to 50% heading: The number of days from the date of 1 October up to the date when the tips of the spike first emerged from the main shoots on 50% of the plants in a plot.

Plant height (cm): The height of ten randomly taken plants was measured at harvest maturity from the ground level to the tip of the tallest spike in centimetre and averaged.

Peduncle length (cm): The length of ten randomly taken plants was measured at harvest maturity in centimetre and averaged.

Spike length (cm): The length often randomly taken plants was measured at harvest maturity in centimetres and averaged.

Spikelet number per spike: Ten heads were randomly selected before harvest. It was determined by averaging the total number of spikelet.

Number of kernel per spike: Ten heads were randomly selected before harvest and then it was determined by averaging the total number of the kernel.

Flag leaf area (cm^2): In the research, 10 flag leaves were randomly selected in each sub-subplot and their length (FLL) and width (FLW) were measured by a ruler. Flag leaf area (FLA) was then calculated using the following formula (Fowler and Rasmussen, 1969).

FLA (cm^2) = (FLL x FLW) x 0.68

Data were analyzed statistically for analysis of variance the method described by Gomez and Gomez (1984). To evaluate significant differences among genotypes, the analysis of variance (ANOVA) was performed. The significance of differences among means was compared by using L.S.D (%5) test (Kalaycı, 2005). Correlation coefficients among all parameters were evaluated based on the means of all genotypes. Also, regression graphs are used to predict the adaptability and relationship of the characters of genotypes.

Table 1. The climatic data of the Edirne location in 2016-2017 growing season

Months	Rainfall (mm) Long year	Rainfall (mm)	Humidity (%)	Temperature (^oC)		
				Min.	Max.	Mean
September 2016	34.0	9.2	57.5	5.0	33.8	20.8
October 2016	52.9	44.4	69.5	1.3	28.8	14.3
November 2016	72.4	3.2	72.9	-9.9	15.4	0.7
December 2016	61.7	3.2	72.9	-9.9	15.4	0.7
January 2017	48.1	67.8	83.7	-17.0	8.4	-1.9
February 2017	46.9	43.4	80.0	-8.4	20.6	5.3
March 2017	52.2	51.0	73.0	-1.9	25.5	10.2
April 2017	51.0	65.6	63.1	-1.6	28.6	12.5
May 2017	56.0	85.0	65.4	4.4	30.0	17.9
June 2017	41.5	44.4	74.4	12.9	40.0	21.2
Total/Mean	516.7	417.2	71.2	-2.5	24.6	10.2

RESULTS AND DISCUSSION

This study aimed at investigating genotypic differences in the leaf area duration of durum wheat genotypes and their relation to grain yield under rainfed conditions. The research was carried out at Trakya Region, Turkey, in 2016-2017 growing years with 30 genotypes in randomized completely blocks design with 4 replications. Grain yield, flag leaf area, days of heading, plant height, peduncle length, spike length, number of spikelet per spike, number of kernel per spike, and relationship also among these characters were investigated. The analysis of variance revealed highly significant differences ($p<0.01$) among the genotypes for all parameters investigated (Table 2).

Combined analysis of variance across 2016-2017 cycles revealed highly significant variation among genotypes for grain yield under rainfed conditions (Table 3). The mean grain yield of the genotypes was 6496 kg ha^{-1}, and the highest yields were obtained in the G25 line with 7466 kg ha^{-1} and genotypes C1252, G6, and G13 had the other highest grain yield. According to days of heading, it was found that G18 and G2 were the earliest and G6 was the latest genotype. Low temperature at the end of March affects very early varieties negatively, while the high temperature in mid-May affects late varieties negatively.

Table 2. Mean square and F ratio for yield, yield component measured in durum wheat genotypes.

Parameters	Genotypes (G)	
	Mean square (MS)	F Ratio
Grain yield (GY)	276278.02	2.74**
Days of heading (DH)	38.50	10.87**
Plant height (PH)	105.90	11.09**
Peduncle length (PL)	20.99	5.79**
Spike length (SL)	1.14	3.83**
Spike number per square meter (SNM)	4593.10	4.82**
Spikelet number per spike (SNS)	7.49	6.19**
Number of kernel per spike (KNS)	178.16	8.32**
Flag leaf area (FLA)	17.83	3.43**

*, ** Significant at p<0.05 and p<0.01 respectively. ns: non-significant

The analysis of variance revealed highly significant differences (P<0.01) among the durum wheat genotypes for plant height, peduncle length, spike length. Plant height and stem solidity is an important trait in durum wheat genotypes for lodging resistance. Cultivar Kızıltan had the highest plant height with 92.8 cm, while the shortest plant height was recorded for G26 (64.0 cm). This trait was identified to be highly controlled genetically, while the environmental condition of wheat growing has an influence on this phenomenon (Pinera-Chavez et al., 2016). Peduncle length differed significantly among genotypes. In the study, cultivar Kızıltan had the highest value of 35.3 cm, while the shortest peduncle was recorded for G26 (26.1 cm). Spike length is an important yield component due to it has more grains. Spike length may vary according to genotype, environment and cultural practices. Amount and distribution of precipitation, temperature and fertilization during ear formation are important factors for spike length. There is a significant variation among the genotypes for spike length. The minimum spike length was 7.0 cm and the maximum was 9.5 cm. The longest spike was determined in genotypes G17 and Tunca79.

The number of spike per square meter of durum wheat genotypes were studied under rainfed conditions and it was found a significant difference among genotypes (Table 3). The maximum number of spikes was noted in genotype G6 (427), followed by G18, G4, and G11. Genotypes G21 and G26 produced a minimum number of spikes per square meter. Spikelet number per spike and kernel number per spike are other important yield components associated with spike length. Both parameters may vary according to genotype, environment and cultural practices such as spike length. Spikelet number per spike differed among genotypes from 16.6

to 23.4, and G26 had a higher spikelet number per spike. There was a significant difference among genotypes. Kernel number per spike varied from 30.2 to 69.5. Genotypes G28, G27 G30, G29 and Eminbey had higher kernel numbers per spike (Table 3).

Table 3. Mean grain yield and agronomic parameters of genotypes in 2016-2017

No	Genotype	GY	DH	PH	PL	SL	SNM	SNS	KNS	FLA
1	Tunca79	6553$^{b-ı}$	126.0^{b-e}	84.0^{cde}	30.5^{c-f}	9.1^{ab}	396.3^{ab}	21.4^{b-e}	39.2^{g-k}	18.6^{hı}
2	G2	6525^{b-j}	119.5^{kl}	83.0^{c-g}	27.9^{fgh}	8.5^{bcd}	381.0^{bcd}	20.1^{d-h}	43.3^{e-j}	21.4^{gh}
3	G3	6723^{a-g}	122.8$^{f-ı}$	74.8^{l}	33.8^{ab}	7.1^{hıj}	335.5^{e-h}	19.1^{f-j}	42.1^{e-j}	27.8^{ab}
4	G4	6045^{g-k}	121.0^{h-k}	78.8^{g-l}	33.6^{ab}	7.6^{d-j}	403.3^{ab}	17.8^{jkl}	36.8^{jkl}	22.2^{e-h}
5	Zenit	6813^{a-f}	120.0^{jkl}	81.0^{e-h}	28.6^{e-h}	7.0^{j}	384.0^{bc}	19.3^{f-j}	42.7^{e-j}	24.2^{b-g}
6	G6	7074^{a-d}	129.0^{a}	80.3$^{e-ı}$	27.4^{fgh}	8.1^{c-f}	427.8^{a}	22.4^{ab}	45.0$^{d-ı}$	22.6^{d-g}
7	G7	6343^{d-k}	125.0^{c-f}	78.5^{h-l}	29.4^{efg}	7.5^{f-j}	376.0^{b-e}	18.9^{g-k}	42.9^{e-j}	23.7^{c-g}
8	G8	6591^{b-h}	122.0^{g-k}	79.0^{g-l}	32.7^{a-d}	7.6^{d-j}	363.8^{b-f}	19.0^{f-k}	39.1^{g-k}	21.3^{gh}
9	G9	6786^{a-g}	121.8^{g-k}	81.3^{d-h}	26.8^{gh}	7.4^{f-j}	393.3^{ab}	20.8^{b-f}	46.4^{c-g}	23.8^{c-g}
10	Eminbey	6813^{a-f}	127.8^{ab}	85.5^{bcd}	29.4^{efg}	8.4^{b-e}	392.0^{ab}	22.2^{abc}	51.0^{bcd}	26.2^{a-d}
11	G10	6763^{a-g}	121.0^{h-k}	81.0^{e-h}	27.9^{fgh}	7.9$^{c-ı}$	401.0^{ab}	21.6^{a-d}	47.1^{c-f}	28.2^{a}
12	G11	6532$^{b-ı}$	123.3^{fgk}	75.5^{kl}	29.5^{efg}	8.0^{c-h}	402.5^{ab}	21.3^{b-e}	40.9^{f-k}	23.1^{c-g}
13	G12	7126^{abc}	120.3$^{ı-l}$	83.5^{c-f}	32.7^{a-d}	7.3^{f-j}	376.8^{b-e}	19.7$^{e-ı}$	47.5^{c-f}	21.5^{gh}
14	G13	6447^{b-k}	125.3^{b-f}	78.8^{g-l}	27.0^{gh}	7.2^{hıj}	336.8^{e-h}	20.8^{b-f}	38.2^{ıjk}	24.0^{c-g}
15	Kızıltan	6236^{e-k}	127.8^{ab}	92.8^{a}	35.3^{a}	8.5^{bcd}	338.5^{d-h}	21.2^{b-e}	40.3^{f-k}	16.4^{ı}
16	G16	6730^{a-g}	122.3^{g-j}	81.5^{d-h}	30.4^{c-f}	7.8^{c-j}	384.3^{bc}	20.3^{d-g}	48.8^{cde}	24.0^{c-g}
17	G17	6044^{g-k}	126.3^{b-e}	83.8^{c-f}	31.2^{b-e}	9.5^{a}	327.3^{fgh}	20.6^{b-g}	42.8^{e-j}	22.8^{d-g}
18	G18	6409^{c-k}	118.0^{l}	76.0$^{ı-l}$	31.2^{b-e}	7.1^{ıj}	406.0^{ab}	16.6^{l}	39.5^{g-k}	21.3^{gh}
19	G19	6141^{f-k}	120.3$^{ı-l}$	78.5^{h-l}	30.2^{def}	7.8^{c-j}	376.8^{b-e}	17.3^{kl}	33.8^{kl}	23.1^{c-g}
20	Ç1252	7164^{ab}	127.5^{abc}	88.5^{ab}	32.7^{a-d}	8.6^{bc}	316.5^{gh}	21.7^{a-d}	45.9^{c-h}	23.6^{c-g}
21	G21	5786^{jk}	123.0^{fgh}	75.5^{kl}	30.5^{c-f}	8.1^{c-g}	302.5^{h}	20.1^{d-h}	40.8^{f-k}	24.9^{a-g}
22	G22	6212^{f-k}	126.8^{a-d}	87.3^{bc}	33.4^{abc}	8.4^{b-e}	377.5^{b-e}	20.0^{d-h}	38.4^{h-k}	21.5^{fgh}
23	G23	5731^{k}	121.0^{h-k}	84.0^{cde}	34.6^{a}	7.6^{d-j}	335.3^{e-h}	18.0$^{ı-l}$	30.2^{l}	24.3^{b-g}
24	G24	6105^{f-k}	123.0^{fgh}	79.5^{f-k}	27.5^{fgh}	7.4^{f-j}	364.5^{b-f}	20.1^{d-h}	40.4^{f-k}	23.4^{c-g}

25	G25	7466^{a}	120.3^{i-l}	75.8^{jkl}	32.6^{a-d}	7.2^{g-j}	382.3^{bc}	19.1^{f-j}	46.3^{c-g}	25.2^{a-f}
26	G26	5833^{ijk}	127.5^{abc}	64.0^{m}	26.1^{h}	7.5^{e-j}	309.0^{gh}	23.4^{a}	42.4^{e-j}	22.7^{d-g}
27	G27	6363^{d-k}	124.3^{d-g}	80.0^{e-j}	26.9^{gh}	8.1^{c-g}	322.3^{fgh}	20.1^{d-h}	57.4^{b}	21.9^{e-h}
28	G28	6966^{a-e}	124.0^{efg}	78.3^{h-l}	29.1^{e-h}	7.4^{f-j}	314.8^{gh}	20.6^{c-g}	69.5^{a}	26.8^{abc}
29	G29	6631^{b-h}	118.0^{l}	83.5^{c-f}	33.2^{a-d}	7.3^{f-j}	348.5^{c-g}	18.4^{h-l}	53.0^{bc}	25.4^{a-e}
30	G30	5935^{h-k}	121.0^{h-k}	79.5^{f-k}	33.0^{a-d}	7.4^{f-j}	342.5^{c-h}	18.1^{i-l}	56.8^{b}	22.7^{d-g}
Mean		6496	123.1	80.4	30.5	7.8	363.9	19.9	44.3	23.3
CV (%)		8.1	1.5	3.8	6.2	6.9	8.48	5.5	10.4	9,7
LSD (0.05)		74.1	2.6	4.3	3.1	0.9	43.2	1.8	7.5	3.1

Significance at *: P<0.05; **: P<0.01; GY: Grain yield (kg ha^{-1}), DH: days of heading, PH: Plant height (cm), PL: Peduncle length (cm), SL: Spike length (cm), SNS: Spikelet number per spike, KNS: Kernel number per spike, FLA: Flag leaf area (cm^{2})

Under the rainfed conditions, flag leaf areas in different durum wheat genotypes during the years 2016-2017 were studied and very highly significant differences were found among the genotypes (Table 3). It was determined that there was a significant difference between the genotypes according to the flag leaf area, which contributed significantly to the yield. Flag leaf area varied from 16.4 to 28.2 cm2, and genotypes G10, G13, G28 and Eminbey had higher flag leaf areas.

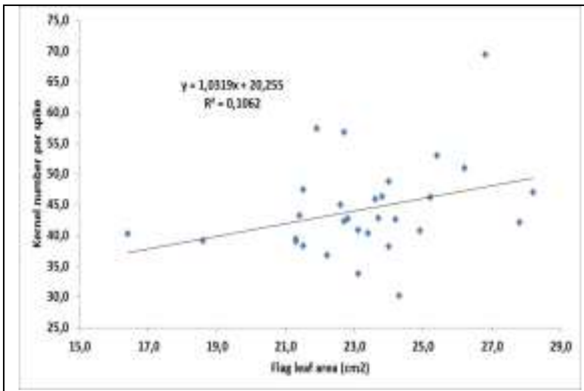

Fig 1a. Flag leaf area and kernel number per spike

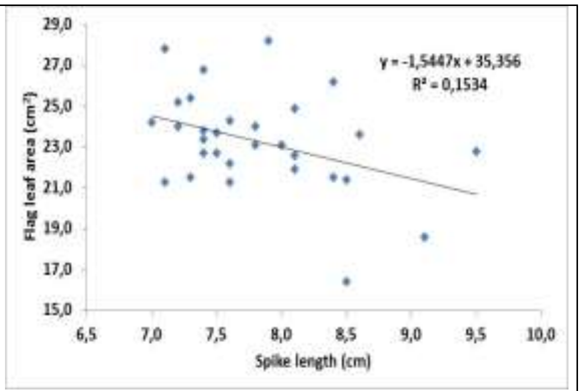

Fig 1b. Flag leaf area and spike length

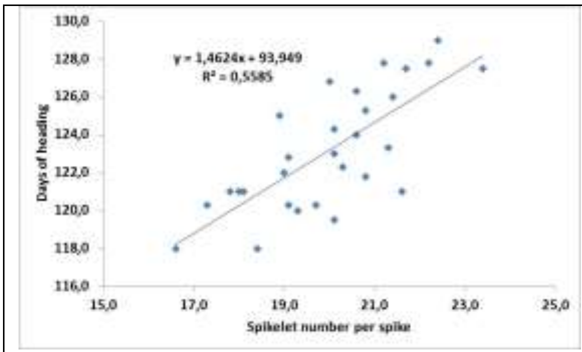

Fig 1c. Days of heading and SNS

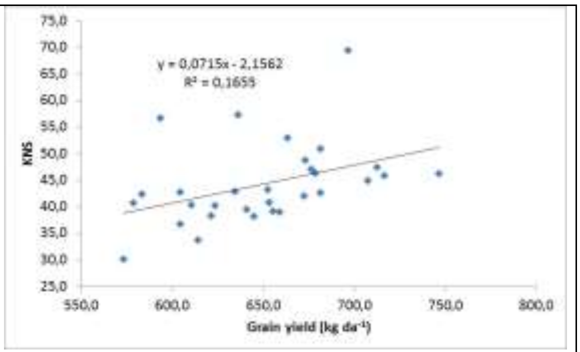

Fig 1d. Days of heading and spike length

Figure 1. Relationship between grain yield and agro-physiological parameters

Agro-physiological parameters such as days of heading with spikelet number per spike (R^2=0.558) and kernel number per spike (R^2=0.165) compared and it was found a positive association. In this research as expected, there was a positive relation between flag leaf area and kernel number per spike (R^2=0.106). Flag leaf area was also a negative associated with spike length (R^2=0.106) (Figure 1).

Table 4. Correlation coefficients among yield component and agronomic characters

Parameter	GY	SNM	SNS	KNS	PL	SL	PH	DH
SNM	0.357							
SNS	0.227	-0.092						
KNS	0.407*	-0.220	0.204					
PL	-0.073	-0.124	-0.484**	-0.210				
SL	-0.139	-0.052	0.448*	-0.099	0.047			
PH	0.174	0.102	0.035	-0.001	0.412*	0.498**		
DH	-0.026	-0.208	0.747**	0.019	-0.167	0.562**	0.182	
FLA	0.256	-0.109	0.001	0.326	-0.197	-0.392*	-0.332	-0.219

Significance at *: P<0.05; **: P<0.01; GY: Grain yield (kg ha^{-1}), SNM: Spike number per square meter, SNS: Spikelet number per spike, KNS: Kernel number per spike, PL: Peduncle length (cm), SL: Spike length (cm), PH: Plant height (cm), DH: days of heading, FLA: Flag leaf area (cm^2)

Correlation coefficients based on the investigated parameters were determined by Pearson's correlation analysis (Table 4). Days of heading was positively significant correlated with spike length (r=0.562**) and spikelet number per spike (r=0.747**). A significant positive correlation was determined between plant height with peduncle length (r=0.412*), and spike length (r=0.498**). Spikelet number per spike was negatively correlated with peduncle length (r=-0.484**), and positively correlated with spike length (r=0.747**). A negative correlation was observed between flag leaf area with plant height and spike length (r=-0.392*). Flag leaf also positively affected spike number per square meter, grain yield, kernel number per spike. There was a negative association with flag leaf area and plant height and days of heading. Grain yield was positively correlated with spike number per square meter, spikelet number per spike, flag leaf area, and significantly correlated with kernel number per spike (r=0.407*).

CONCLUSION

The analysis of variance revealed highly significant differences among the genotypes for all parameters investigated. Genotypes G25, C1252, G6 and G13 had the highest grain yield. Spike length is an important yield component due to it has more grains. Spike length may vary according to genotype so G17 and Tunca79 had the longest spike. The number of spikes per square meter of durum wheat genotypes is another yield component associated with yield. Significant differences were found between genotypes G6, G18, G4, and G11 had been higher tillering capacity. Spikelet number per spike and kernel number per spike are other important yield components associated with spike length. Both parameters may vary according to genotype, environment and cultural practices such as spike length. Spikelet number per spike differed among genotypes G26 had a higher spikelet number per spike, and G28, G27, and G30 kernel number per spike. Flag leaf in wheat makes a significant contribution to yield. There was a significant difference between the genotypes. In the current study genotypes G10, G13, G28 and Eminbey had higher flag leaf areas. Relationships between characters were investigated. Spikelet number per spike, flag leaf area, and kernel number per spike had a positive effect on grain yield. Late-heading genotypes had longer spikes and more spikelet numbers per spike. Plant height was positively correlated with peduncle length and spike length. Peduncle length and spike length had a positive effect on spikelet number per spike. Flag leaf had a positive effect on grain yield and kernel number per spike. There was also a negative association with flag leaf area and plant height and days of heading. According to the result of the study showed that the number of spikes per square meter, spikelet number per spike, kernel number per spike and flag leaf area has been yield components that contributed to grain yield of durum wheat genotypes under rainfed condition

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STUDY OF NEW DRUG CANDIDATES DERIVED FROM PLECONARILE INHIBITING COXSACKIEVIRUS B3 (CVB3) BY MOLECULAR DOCKING, ADMET, AND RETROSYNTHESIS

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ABSTRACT

In light of their serious diseases, there is an urgent and inescapable need to hunt for antiviral medications for Coxsackievirus B3 (CVB3). The current study looked at four drug candidates (P1-P4) derived from pleconaril, which has antiviral activity against CVB3. According to Lipinski's guidelines, two candidates P3 and P4 can be medications based on the results obtained after evaluating physicochemical and ADMET properties. The high antiviral activity of these two candidates (pIC_{50}=11.063 for P3 and pIC_{50}=9.580 for P4), when compared to a reference compound (MA: pIC50=8.523), was explained by the different parameters generated after optimizing their geometries employing Gaussian09 program suit with the hybrid density functional B3LYP and 6-31G(d,p) basis sets, and the molecular docking analysis (ΔG (Gibbs energy), FF(Full fitness) and bonding modes) using the SwissDock server. The principle of retrosynthesis allowed us to draw a path for the synthesis of drug candidates. This study may add more valuable and useful information to optimize further new Pleconaril derivatives.

Keywords: Molecular Docking, ADMET, Retrosynthesis, Isoxazole, Coxakievirus B3, Pleconaril, Antiviral

INTRODUCTION

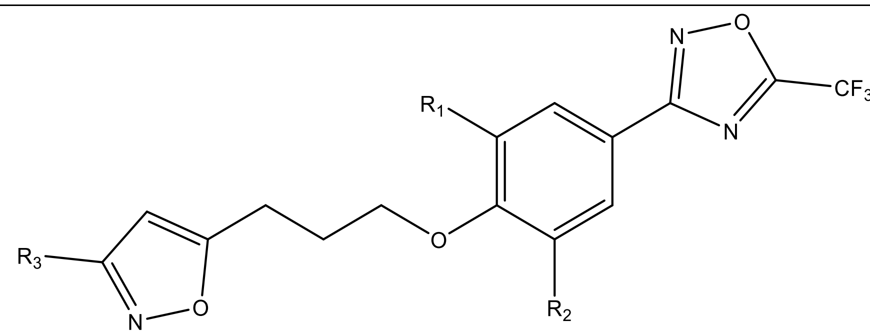
Heterocyclic compounds are among the most widely used organic chemicals, whether natural or manufactured. These substances have a broad range of biological effects (Koubi et al, 2019; El Masaoudy et al., 2020; Unni et al., 2020; Santosh et al., 2018; ElMchichi et al., 2020; HAJJI et al.,2020). The derivations of the isoxazole form a particular and interesting class of heterocyclic compounds with five links, due to their availability and their biological and pharmaceutical activities which are important and efficient such as the anti-inflammatory, analgesic, antiviral, antioxidant, anticancer and antimicrobial activities (Moukhliss et al, 2021).

Coxakievirus B3 (CVB3) is a Picornaviridae family virus, a prominent member of an ancient and widely varied family that falls under the umbrella of enteroviruses (Tapparel et al., 2013). The discovered species in these genera were discovered in both humans and animals (Tapparel et al., 2013). CVB3 can be spread by the aerosol and fecal-oral pathways, just like most viruses (Andréoletti et al.,2000). They affect everyone, especially newborns and young children under the age of 15 (Andréoletti et al.,2000; Andréoletti et al.,2009). CVB3 induce central nervous system infections, respiratory infections, mucocutaneous, muscular, and digestive diseases once they reach their intended targets (Thibaut et al, 2016; Palacios et al.,2005). Because the replication cycle begins with the entry of the virus after it attaches itself to certain receptors, these infections occur after the virus completes the process known as the replication cycle. Following this, the viral RNA is released into the cytoplasm of the cells, triggering a process. The proteins and replication, and then assembly, follow after protein expression, and new virions are produced and discharged into the bloodstream to be delivered to various destinations (American Academy of Pediatrics, 2003). These viruses, referred to as "capsid-viruses," have a diameter of approximately 30 nanometers and are nonenveloped. Their capsid possesses icosahedral symmetry. A protein-mimicking polymer (poly-protein) consists of 60 molecules of the four protein proteins VP1, VP2, VP3, and VP4. While VP4 is found on the inner surface of the capsid, VP1, VP2, and VP3 are found on the outer surface (K Muckelbauer et al, 1995). There are multiple studies that have proven that the VP1 protein has played a role in binding to the cell receptors (Makarov et al., 2015; Braun et al., 2015). Near the bottom of the canyon, below VP1, is a pocket of hydrophobic liquid containing fatty acid (Liu et al., 2016). Also, this pocket promotes the stability of the virus and is where antiviral agents find their target. These molecules occupy the hydrophobic pockets, removing lipid supplied to the hydrophobic pockets, and thus blocking the decapsidation process (Thibaut et al., 2012). In the present work, physicochemical, ADME attributes and in silico toxicity of the drug candidates to select those that can be drugs, to study by molecular docking the selected candidates to have the modes of interactions that occur between these compounds and the 3zzd receptor in order to explain the difference of the antiviral activity observed between these compounds, and finally based on the concept of retrosynthesis, synthetic routes for these drug candidates are proposed.

MATERIELS AND METHODS

Nowadays, medication research and development are being conducted at full speed. Therefore, it is essential to assess its bioavailability, its pharmacokinetics, and its toxicity before being put to experience. Four drug candidates that were proposed in our 2D-QSAR study of a series of Pleconarile derivatives recognized for their antiviral activity against Coxsackievirus B3 (CVB3)(Moukhliss et al., 2021) are the focus of our current research. In addition to their expected antiviral activity, these drug candidates' structures, and their pIC_{50}, are also included in Table 1.

Table 1 . Drug candidates studied



Drug candidate	R_1	R_2	R_3	pIC_{50}
MA*	H	NO_2	$(Me)_2NCO$	8.523
P1	NO_2	NO_2	$(Me)_2NCO$	8.644
P2	NO_2	NO_2	NO_2	10.898
P3	CN	CN	CN	11.063
P4	CHO	CHO	CHO	9.580

*MA : The most active compound in the series studied by 2D-QSAR (Moukhliss et al,2021)

Table 2 show the physicochemical properties of these drug candidates and also their pharmacokinetic properties. The physicochemical properties were evaluated using the « Swiss ADME (http://www.swissadme.ch) » database. The values of the physico-chemical properties must satisfy certain conditions known as the five rules of Lipinski (Tehseen et al., 2019). To have the suitability of these candidates to be drugs the following parameters have been evaluated (Table2): MW(Molecular Weight) $\leq$ 500g/mol , N(number of rotatable bonds) $\leq$ 10 , TPSA (topological polar surface area) $\leq 150 Å^2$, logP (Octane-water partition coefficient) $\leq$ 5, HA (hydrogen acceptor) $\leq$ 10, and HD (hydrogen donor) $\leq$ 5. The site absorption, distribution, metabolism, excretion, and toxicity (ADMET) of all drug candidates were analyzed using the pkSCM online tool (http://bleoberis.bioc.cam.ac.uk/pkcsm/) (Zafar et al., 2020). Based on the values set by the Lipinski method, two candidates P3 and P4, among the four, can be drugs. Afterwards, the selected candidates was optimized employing Gaussian09 program suit (Frisch et al., 2008) with the hybrid density functional B3LYP (Lee et al., 1988) and 6-31G(d,p) basis sets (Hariharan et al., 1973), then a molecular docking analysis to evaluate their affinities towards the target protein (PDB ID:3zzd) using the SwissDock server (http://www.swissdock.ch). Different parameters: Full fitness (FF in kcal.mol^{-1}), total number of clusters, clusters rank, Gibbs energy (ΔG in kcal.mol^{-1}), hydrogen bonds have been generated for the analysis by exploiting the output files and Discovery Studio Visualizer. Retrosynthesis is an essential basis in the modern synthesis of products. It provides a range of choices based on structure dissociation of the targeted compounds by way of a set of appropriate reaction stages from the available start compounds (McCowen et al., 2017; Robinson et al., 1991). This is validated under the term Retrosynthesis by E. J. Corey (Nobel Prize in 1990) (Corey et al., 1991). So, computer-aided formalization has been promoted over the years (Gasteiger et al., 1990; Warr et al., 2016). In our work, we took advantage of one of the most popular databases (spaya.ai) to draw a path for synthesizing the drug candidates studied.

RESULTS AND DISCUSSION

1.Lipinski's rule of five and ADME/Toxicity prediction

From the results obtained (Table 2) it can be said that drug candidates P3 and P4 can be drugs according to Lipinski's rules. These two drug candidates are likely to have a high probability of oral bioavailability and also show no violation according to the ADMET/Toxicity results. Therefore, they can be provisionally approved for preclinical testing.

Table 2 . Physicochemical property and ADME/Toxicity of the drugs candidates

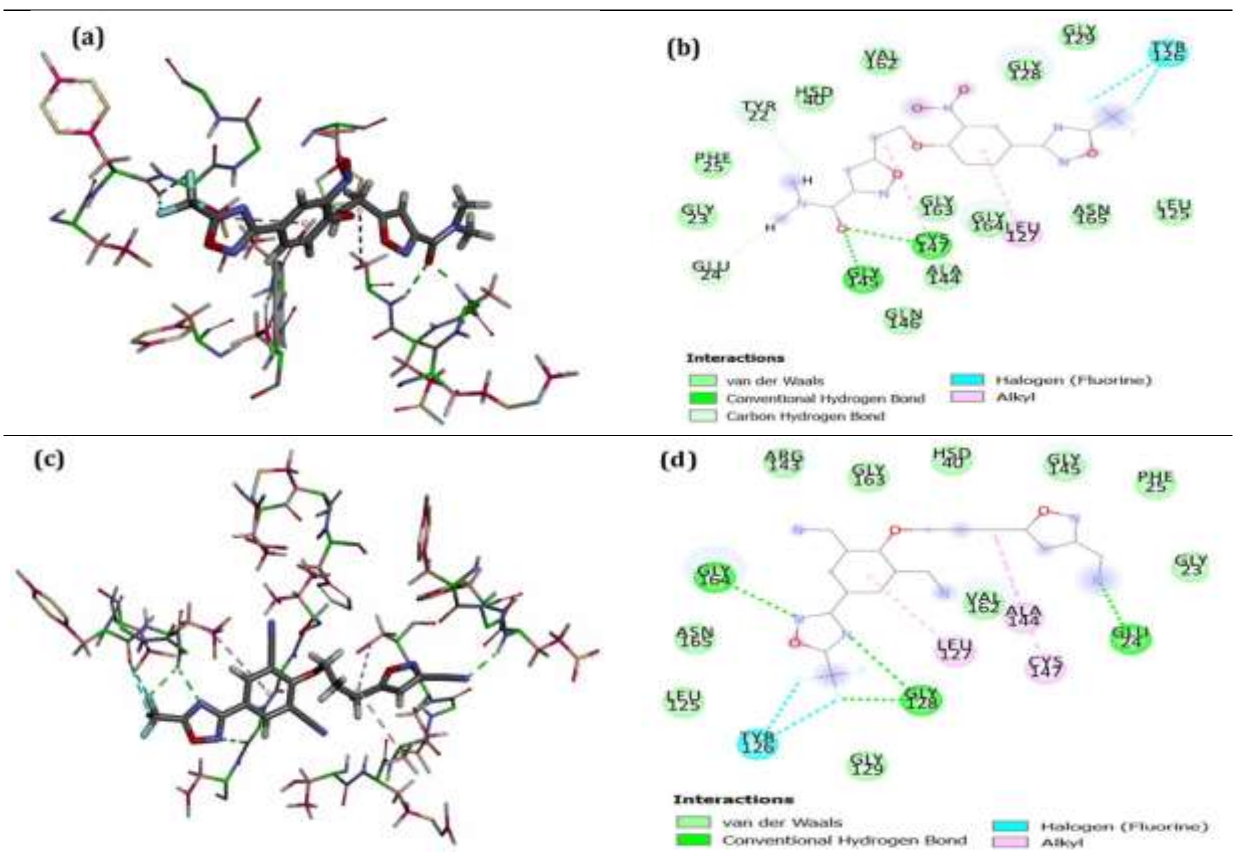
Drug candidat	Physicochemical property / ADME / Toxicity									
	MW (g.mol^{-1})	N	TPSA ($Å^2$)	LogP	HA	HD	WS	GIA	Drug-likeness Lipinski	AMES toxicity
P1	474.26	10	211.64	0.11	15	0	Moderately soluble	Low	No ; 1 violation	Yes
P2	500.34	11	186.13	0.07	14	11	Moderately soluble	Low	No ; 2 violations	Yes
P3	414.30	7	145.55	0.07	12	0	Moderately soluble	Low	Yes ; 0 violation	No
P4	423.30	10	125.39	0.07	12	0	Soluble	Low	Yes ; 0 violation	No
Optimal rang	$\leq$ 500	$\leq$ 10	$\leq$ 150	$\leq$ 5	$\leq$ 10	$\leq$ 5	–	–	–	–

2.Molecular docking analysis

Molecular docking is used to determine the potential link conformations between chemicals and receptors. In this part we will try to explain the difference in activity observed between compounds P3, P4 and MA. For this we applied the principle of molecular docking, using the SuissDock web server available on the website www.swissdock.ch. On the basis of various parameters generated at the end of the analysis (full fitness (FF), Gibbs energy (ΔG), and ligand-receptor interactions), the affinity of compounds P3, P4, and MA towards the receptor (PDB ID:3zzd) was analyzed. The results output file has multiple clusters, each of which has several rankings listed in ascending order of negative FF values. The Cluster with the best FF score is ranked 0th. The binding modes of a ligand with a higher negative FF value are more favorable (Kaviyarasi et al., 2016). We chose the cluster with the highest average FF value for the analysis. The various results are shown in Table 3 and Figure 1.

Table 3 . Results of Docking of compounds MA, P3 and P4

Ligand	Number of clusters	Selected cluster	FF (kcal.mol^{-1})	ΔG (kcal.mol^{-1})	Hydrogen bonds (Receptor-Ligand)	
MA	34 (256 rang)	3	-1042.078	-7.307	GLY145(HN)…O	1.98A°
					CYS147(HN)…O	2.57A°
P3	38 (256 rang)	0	-1056.085	-7.597	GLU24(HN)…N	2.28A°
					GLY128(HN)…O	2.38A°
					GLY128(HN)…F	2.30A°
					GLY164(HN)…N	3.05A°
P4	33 (252 rang)	2	-1059.350	-7.556	GLU25(HN)…F	2.51A°
					GLY145(HN)…N	2.65A°
					GLY145(HN)…F	2.85A°
					GLY145(HN)…O	3.00A°



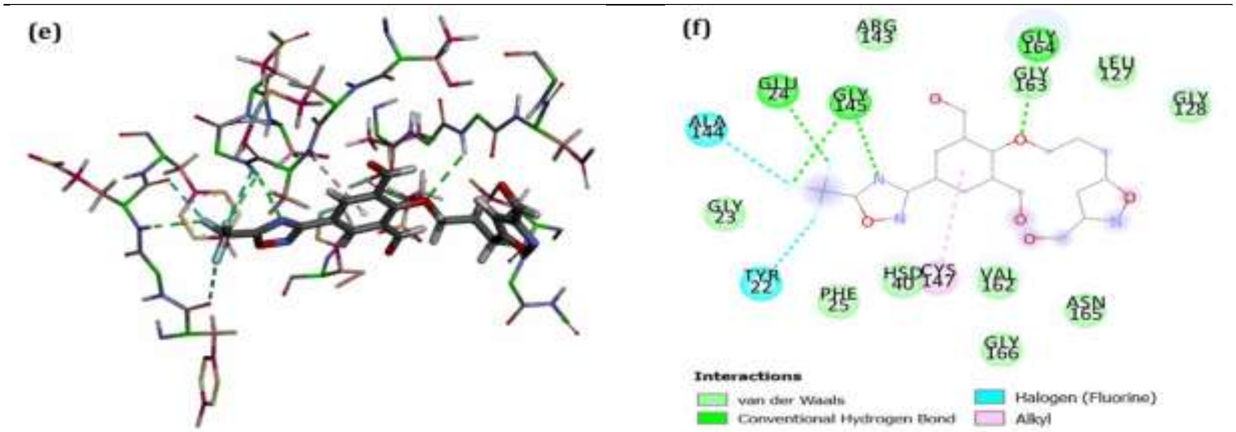


Figure 1 : the 3D and 2D graph of the binding modes between Ligand and the 3zzd receptor; (a) : 3D (MA-3zzd) ; (b) : 2D (MA-3zzd) ; (c) : 3D (P3-3zzd) ; (d) : 2D (P3-3zzd) ; (e) : 3D (P4-3zzd) ; (f) : 2D (P4-3zzd)

The FF (Full fitness) values of the two compounds P3(pIC_{50}=11.063 ; FF=-1056.085 $kcal.mol^{-1}$) and P4(pIC_{50}=9.580 ; FF=-1056.085 $kcal.mol^{-1}$) are equivalent and low when compared to MA(pIC_{50}=8.523; FF=-1056.085 $kcal.mol^{-1}$), as shown by the obtained data. P3(pIC_{50}=11.063 ; ΔG = -7.597 $kcal.mol^{-1}$) has the lowest ΔG value, followed by P4(pIC_{50}=9.580 ; ΔG =-7.556 $kcal.mol^{-1}$) and MA(pIC_{50}=8.523 ; ΔG = -7.307 $kcal.mol^{-1}$), which corresponds to the sequence in which they have antiviral activity. P3 and P4 both formed the same number of hydrogen bonds, however compound MA only formed two hydrogen bonds. P3 has a higher activity than P4 due to the strength of hydrogen bonds (short bonds) and the intervention of other types of bonding (Van Der waals, Carbon Hydrogen Bond,...).

3.Retrosynthesis analysis

To facilitate the synthesis of the drug candidates, one from target drug to reactants using the principle of retrosynthesis by exploiting the spaya platform (www.spaya.ia). We were given 300 routes by the analysis, and we chose the one with the greatest score. Each route is a collection of steps derived from similar synthesis that have been documented in the literature. (Figure 2 and Table 4).

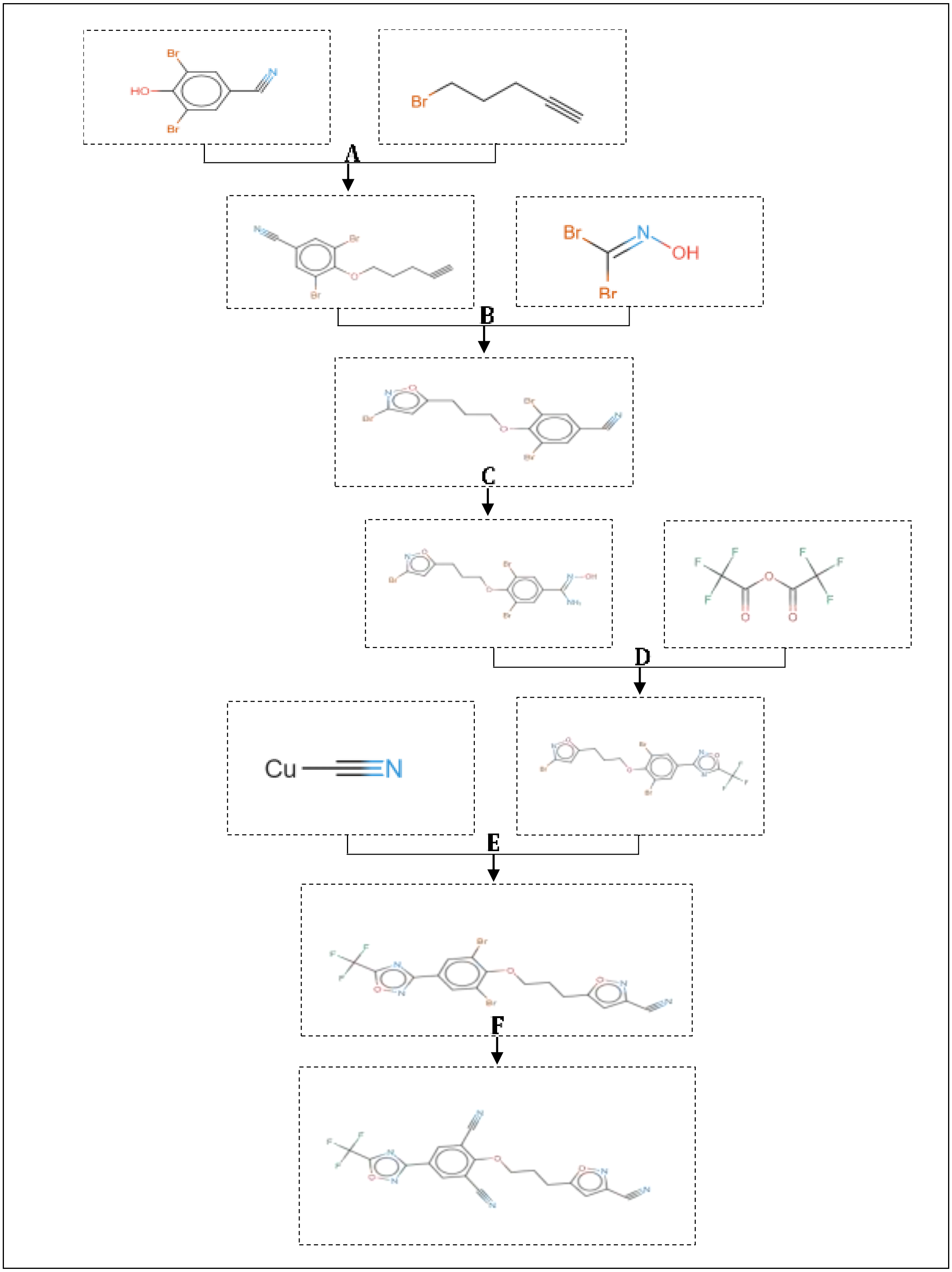
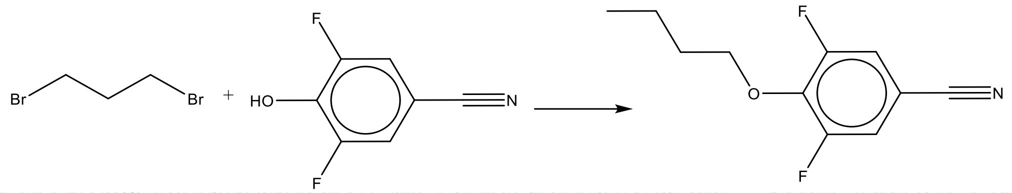
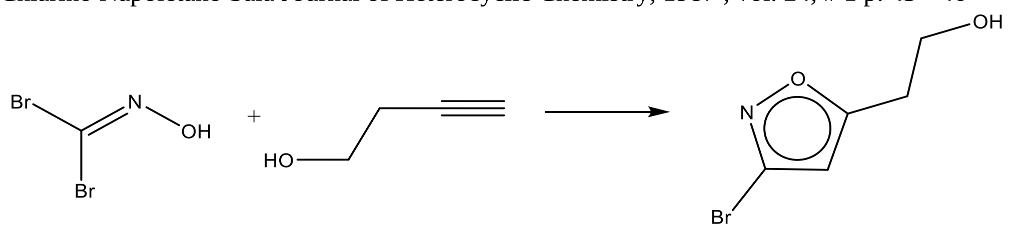
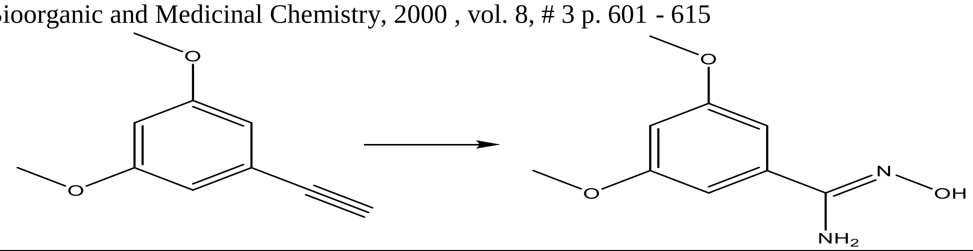
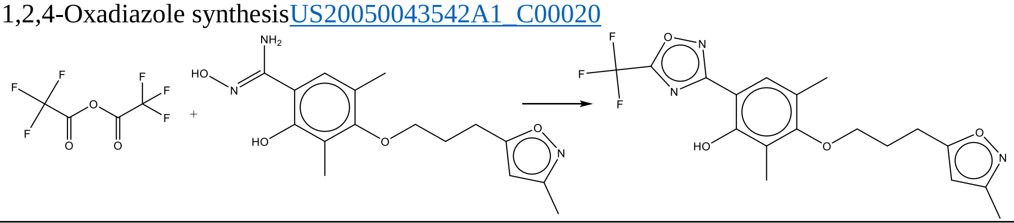
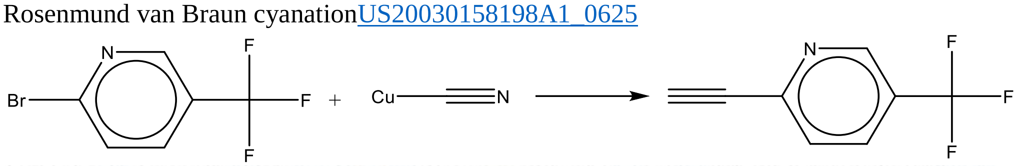
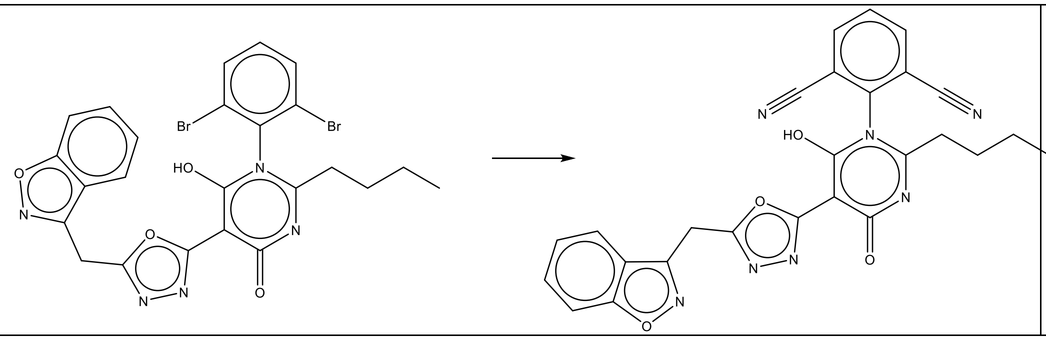


Figure 2. Different steps generated after the analysis

Table 4. Reactions as references for the synthesis of the drug candidate P3

Steps		Similarity
A	Williamson ether synthesis US20090005558A1_0501  A mixture of 2,6-difluoro-4-cyanophenol (6 g, 0.038 mol), 1,3-dibromopropane (46.9 g, 0.23 mol) and dry potassium carbonate (8 g, 0.058 mol) in dry DMF (50 ml) was stirred at RT overnight under nitrogen atmosphere. Solvent was removed under reduced pressure and the residue was purified by column chromatography over silica gel using 4% ethyl acetate in petroleum ether as eluent to yield (9.6 g) of the title compound as a liquid.	0.33
B	Chiarino Napoletano Sala Journal of Heterocyclic Chemistry, 1987 , vol. 24, # 1 p. 43 - 46 	0.35
C	Tucker, John A. Clayton, Terrance L. Chidester, Connie G. Schulz, Martin W. Harrington, Leigh E. Conrad, Steven J. Yagi, Yoshihiko Oien, Nancee L. Yurek, David Kuo, Ming-Shang Bioorganic and Medicinal Chemistry, 2000 , vol. 8, # 3 p. 601 - 615 	0.25
D	1,2,4-Oxadiazole synthesis US20050043542A1_C00020 	0.39
E	Rosenmund van Braun cyanation US20030158198A1_0625  Copper (I) cyanide (14.1 g) and 2-bromo-5-trifluoromethylpyridine (3.00 g, 13.3 mmol) in dry DMSO (70 mL) were combined and heated at 180° C. for 2 hours, cooled, and poured into NH4OH (3M). The mixture was then extracted with ethyl acetate (3×500 mL), washed with water (1×200 mL), dried (MgSO4), filtered and the filtrate concentrated under reduced pressure to provide the title compound. 1H NMR (DMSO, 300 MHz) δ 8.22 (m, 1H), 8.42 (m, 1H), 9.01 (s, 1H).	0.15
F	US20180362508A1_C00060	0.11



CONCLUSIONS

In this study, in-silico evaluation (Physicochemical properties and ADMET), molecular docking, and retrosynthesis were used to investigate a series of therapeutic candidates derived from Pleconaril, which is known for its antiviral activity against CVB3. The results of the in-silico review allowed us to choose two candidates (P3 and P4) out of the four that might potentially become medications and be provisionally approved for preclinical studies. P3 is the most active candidate (pIC_{50}=11.063), which may be explained by the metrics acquired during the molecular docking analysis (Full fitness (FF), total number of clusters, cluster rank, Gibbs energy (ΔG), hydrogen bonds) and represents P3 strong affinity for the 3zzd receptor. The results of the retrosynthesis will be used to guide the synthesis of the candidate compounds that have been proposed. Our research gives theoretical guidelines for the development of genuine pleconaril-based medications in the future.

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ACCESSION DEPENDENT MORPHOLOGICAL AND BIOCHEMICAL DIVERSITY IN APRICOT CV. TEBERZE

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ABSTRACT

In this study, some important fruit morphological and biochemical traits have been investigated in nine accessions of Teberze (Tebereze) cultivar grown in only Igdir province in Turkey. As morphological parameters, fruit mass and flesh/seed ratio were investigated. For biochemical content, specific sugars and organic acid content in fruits of nine Teberze ecotypes sampled from different parts of Igdir region were determined. Fruit mass and flesh/seed ratio were in range of 34.11 and 40.33 g and 21.15 and 26.30, respectively. HPLC results indicated the presence of four specific sugars such as sucrose, glucose, fructose and sorbitol and four organic acids such as malic, citric, tartaric and ascorbic acid in fruits of accessions. At harvest, the fruit juice content of sucrose, glucose, fructose and sorbitol ranged from 7.51 to 9.84 mg/100 g, 1.85 to 3.07 mg/100 g, 0.64 to 1.09 and 0.55 to 0.82 mg/100 g, respectively. The citric acid content varied from 756 to 933 mg/100 g, malic acid from 245 to 421 mg/100 g and tartaric acid from 30 to 48 mg/100 g, respectively. Ascorbic acid was found between 13.3 to 19.2 mg/100 g. Results indicated morphological and biochemical diversity among nine accessions of cv. Teberze supports the idea that long-term cultivation may have results natural mutation and this effects in particular sugar and organic acid metabolism in cultivars and reveal clonal variation.

Keywords: Biochemical content, clonal variation, apricot, organic acids, sugars

INTRODUCTION

Turkey is second homeland of apricot and the country shows richness of national apricot cultivars which originated in Anatolia. It is believed that those cultivars selected among wild grown apricots. In fact, each apricot growing region in Turkey has own apricot cultivars and the regions shows great effort to conserve only them. In another fact that is transition between region of these cultivars in general give negative result because there were strong environment and cultivar relationships in apricot (Ercisli, 2009).

The main apricot growing areas in Turkey including Malatya-Elazig, Aras valley (Igdir-Kagizman) and Erzincan and all these areas are located in eastern Anatolia region. In Turkey apricot cultivars classified as fresh or dried and some regions for example Malatya had only dried apricot cultivars such as Hacihaliloglu, Kabaasi etc while some regions (Mut, Mediterranean, Aegean) had only fresh apricot cultivars. However, some regions such as Igdir located in Aras valley have both make the region interesting for fresh and dried apricot production (Caliskan et al., 2012; Saridas and Agcam, 2021).

In Igdir, only a few apricot cultivars have been cultivated for a long time and ‘Salak’, as well-known fresh use apricot cultivar, dominate apricot production in Igdir region with 85% share of total production. The rest of the cultivars such as Teberze, Ordubat, Agcanabat and Agerik share 15% of total production and these cultivars are also used in orchards design for pollination. The region produces around 18.000-20.000 tons apricot annually (TUIK, 2020). The yield per tree in apricot is average around 47 kg in Turkey but in Igdir region apricot trees has exceptional high yield capacity due to used cultivars that very well adapted the region and also suitable climatic conditions of the region for apricot growing. There is only one problem for apricot growing in Igdir is the risk of spring frosts. Due to spring frost, big damage may have occur in some years because all apricot orchards in Igdir established on flat plain. In another word, topography is same for all apricot orchards in Igdir plain. The region is also one of the earliest apricot production areas in Turkey due to low altitude (around 850 m asl.). Sure, there were earlier apricot production areas in western Turkey such as Mut located in Mediterranean region but the fruit quality of early apricots in Mut is lower compared to Igdir. The apricots of Igdir are famous in Turkey due to its better fresh organoleptic traits. They have more juicy, bigger, fibrous, colorful etc.

Apricot fruit has rich nutritional and commercial importance in terms of its special functional composition and consumer demand. Currently apricot is widely and commercially cultivated in more than 50 countries, including China, USA, Iran, Pakistan, Algeria, Egypt, Ukraine, Uzbekistan, Italy, Spain, France, Greece, Morocco and Turkey. Turkey is the leading producer of apricot with 730.000 tons annual production (FAO, 2020). Consumers highly favor apricot fruit because of its mild, subacid and sweet taste, as well as an attractive flavor. Increased interest in fruits results are primarily from the need for biodiversity preservation and use as a potential source of new nutraceuticals aimed at prevention of a series of diseases (Ercisli, 2009).

Sugars and organic acids are the most common soluble constituents of fruit. They have an important influence on taste, shelf life and nutritive properties, and they are reliable indicators of acceptability by consumers (Kafkas et al., 2006). From a technological perspective, they are very important in production of juices and nectars, since they define sweetness index (Lozano, 2006). The representation of individual sugars and organic acids serves also as an indicator of authenticity of fruit products (Evans et al., 1983). In addition, sugars participate in polyphenol biosynthesis; thus, higher sugar content in fruit implies higher polyphenol concentration, which is exceptionally important from the perspective of a nutrient-enriched diet (Milivojevic et al., 2013).

It is obvious that long-term cultivation periods result some mutations on fruit trees and phenotypic deviations from the main cultivar were occurs in fruit size, fruit color, tree habitus and branching etc. Genetic variation within cultivar may be due to mutation, mechanical mixture and sexual reproduction. Somatic mutations are also known as bud mutations. The frequency of mutations is generally very low (Amanda et al., 2006; Lamo et al., 2017). A mutant allele would be homozygous only when both the alleles in the cell mutate at the same time producing the same mutant allele, or the mutant allele is already in the heterozygous condition in the original clone. Dominant bud mutations express themselves more frequently than the recessive ones, as recessive mutation get expressed only under homozygous conditions. Bud mutations often produce chimeras, i.e., individuals containing cells of two or more genotypes (El-Sharkawy et al., 2015; Zhao et al., 2019).

The aim of this study is determining fruit weight, flesh to seed ratio, individual organic acid and specific sugar contents of nine accessions of cv. Teberze grown under Igdir conditions to reveal any differences among accessions.

MATERIAL AND METHODS

Plant material

The nine accessions of genotypes were found in Igdir province in different locations. The fruits were sampled in 2020. A total of 40 healthy fruits were harvested from nine accessions of cv. Teberze in the study. Harvested fruits were quickly brought to the Laboratory and along with morphological measurements (fruit weight and flesh/seed ratio), juices of the fruits belong to nine accessions were squeezed with a blender, and the fruit juices were separated from the pulp with the help of cheesecloth. Fruit weight was determined on 30 fruits with digital balance. Same fruits were used for flesh/seed ratio determination.

Specific sugars

Sugar (fructose, sucrose, and glucose) analysis was performed with the methods described by Melgarejo et al. (2000). 1 ml of fruit extracts was centrifuged at 10 000 rev per min for 2 min at 4 °C. Supernatants were passed by SEP-PAK C18 cartridge. HPLC readings were made with μbondapak- NH_2 column using 85% acetonitrile as the liquid phase with a refractive index detector (IR). Fructose and glucose standards were used for the calculations of the sugar contents.

Organic acids

Organic acid composition of the apricot fruit was determined described by Bevilacqua and Califano (1989). Fruit extracts were obtained by crushing the fruits in cheesecloth. 0.009 N H_2SO_4 was then homogenized with shaker for 1 h. The mixture was then centrifuged at 15000 rpm for 15 min and the supernatants were filtered twice through a 0.45 μm membrane filter with a coarse filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) and passed through a SEP-PAK C18 cartridge. Organic acid readings were performed by HPLC using the Aminex column (HPX-87 H, 300 mm x 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) at 214 and 280 nm wavelengths in the Agilent package program (Agilent, USA).

Statistical analysis

All data were analyzed using SPSS software and procedures. Analysis of variance tables were constructed using the Least Significant Difference (LSD) method at $p<0.05$.

RESULTS AND DISCUSSION

Fruit weight and flesh/seed ratio

Fruit weight and flesh/seed ratio of nine accessions of cv. Teberze are given in Table 1. We found statistically significant differences among accessions in terms of both fruit weight and flesh/seed ratio at 0.05 statistical level. IT7 accession gave the highest fruit weight as 40.33 g and followed by IT5 as 39.17 g and IT4 as 38.26 g, respectively. Among the accessions the lowest fruit weight was observed in IT3 accession as 34.11 g (Table 1). As indicated before in Turkey national apricot cultivars were selected among wild populations and they have have relatively small fruits (Ercisli, 2009). Akin et al. (2008) also supported this idea and reported fruit weight between 21.16-38.24 g among the most important national apricot cultivars grown in Malatya region in Turkey. Asma and Ozturk (2005) reported that 128 Turkish apricot cultivars belong to the Iran-Caucasian ecogeographical group generally had low fruit weight. The authors reported that the fruit weight of only 7 apricot cultivars was over 50 g, and the others had lower fruit weights. Akca and Askin (1995) reported fruit weight between 40.08 and

53.73 g in 17 clones of 'Hacıhaliloğlu' apricot variety in Gurun. The fruit flesh/seed ratio were observed in descending order IT7 (26.30)>IT3 (24.41)>IT5 (23.15)>IT9 (22.78)>IT2 (22.56)>IT1 (22.18)>IT6 (21.85)>IT8 (21.55)>IT4 (21.15) (Table 1).

The flesh/seed ratio is an important fruit characteristic for apricots (Ercisli, 2009). Previously the flesh/seed ratios of the foreign apricot cultivars grown in Turkey varied between 8.9 and 21.8 (Polat and Yilmaz, 1988; Polat et al., 2004). Yilmaz et al. (2010) reported flesh/seed ratio on Levent and Özal apricot cultivars grown in Malatya between 7.90-10.38 and 10.94-13.58 according to years. Our flesh/seed ratio was higher than previous studies indicating importance of this cultivar and diverse accessions in use as genetic resources to obtain cultivars with higher flesh/seed ratio.

Table 1. Fruit weight and flesh/seed ratio of nine Teberze accessions

Accessions	**Fruit weight (g)**	**Flesh/seed ratio**
IT1	37.32b	22.18bc
IT2	38.02ab	22.56bc
IT3	34.11c	24.41b
IT4	38.26ab	21.15c
IT5	39.17ab	23.15bc
IT6	36.64bc	21.85bc
IT7	40.33a	26.30a
IT8	37.90ab	21.55bc
IT9	35.44bc	22.78bc

There were significant ($P < 0.05$) differences among the different letters in the same lines

Specific sugars

Specific sugar contents of nine accessions of cv. Teberze are given in Table 2. The accessions significantly differed each other in terms of amount of glucose and fructose concentrations ($p < 0.05$) but there were no significant differences among accessions in terms of fructose and sorbitol content. As indicated in Table 2 the predominant specific sugar in all accessions was sucrose.

Table 2. Sugars in fruits of nine cv. Teberze accessions g/100 g

Accessions	Sucrose	Glucose	Fructose	Sorbitol
IT1	8.54cd	1.85d	1.02^{NS}	0.74^{NS}
IT2	8.22d	2.34bc	0.73	0.60
IT3	9.84a	3.07a	0.84	0.70
IT4	7.97de	1.98c	1.09	0.82
IT5	7.83e	2.11bc	0.80	0.65
IT6	8.11de	2.71b	0.67	0.60
IT7	7.51f	2.60bc	0.98	0.55
IT8	9.33b	2.20bc	0.93	0.74
IT9	8.84c	2.88ab	0.64	0.76

There were significant ($P < 0.05$) differences among the different letters in the same lines

Among the genotypes IT3 and IT8 had higher level of sucrose (over 9 g/100 g). The lowest sucrose values were obtained from IT7 accession as 7.51 g/100 g and IT5 accession as 7.83 g/100 g, respectively (Table 2). Glucose content were the highest in IT3 as as 3.07 g/100 g, and followed by IT9 (2.88 g/100 g) while the lowest glucose value was observed in IT1 as 1.85 g/100 g (Table 2). Saridas and Agdam (2021) found that fruits of cv. Teberze included sucrose the highest amount (8.57 g/100 g) and followed by glucose (1.90 g/100 g), sorbitol (0.77 g/100 g) and fructose (0.60 g/100 g), respectively. Our results are good agreement with above study. Naryal et al. (2019) found that among 108 apricot genotypes sucrose was the predominant (57.8% of total amount), followed by glucose (19.4% of total amount) and fructose (14.3% of total amount) while the lowest sugar is sorbitol (8.4% of total amount). Fan et al. (2017) also reported that apricot fruits in general have 4 sugars namely fructose, sucrose, sorbitol and glucose. They also indicated that sugars, especially fructose and sucrose made the main contributions to sweetness, and further to consumer satisfaction of apricot. Imrak et al. (2017) also indicated that 'Hacıhaliloğlu' cultivar and the other selected genotypes had mostly sucrose. Su et al. (2020) found that local apricot cultivars had sucrose in the highest concentration compared to the other specific sugars. The amount and composition of sugars in apricot generally change with cultivars, maturation level, cultivation techniques, ecology and also some factors such as fruit thinning etc. affects specific sugar amount, ratio, and concentrations in apricot. Consumers are willing to pay more for fruit with high sensory quality, such as excellent taste. Fruit taste includes sweetness and acidity, which is correlated with the contents and types of soluble sugars and organic acids (Fan et al., 2017). However, among these sugar or organic acid components, which is the main contributor to consumer satisfaction is still confused.

Organic acids

Organic acid profile of nine cv. Teberze accessions are shown in Table 3. As indicated in Table 3, citric acid was predominant in apricot fruits and followed by malic acid. Citric acid and malic acid concentrations are greatly varied among genotypes ($p<0.05$). However tartaric acid concentration of the accessions was found to be non-significant (Table 3). Citric acid and malic acid varied from 756 to 933 mg/100 g; 245 to 421 mg/100 g, respectively. Among the tested accessions, IT2 had the highest citric acid content whereas IT6 had the lowest (Table 3).

Table 3. Sugars in fruits of nine cv. Teberze accessions g/100 g

Accessions	Citric acid	Malic acid	Tartaric acid	Ascorbic acid
IT1	884ab	281f	35^{NS}	14.1ab
IT2	933a	245g	44	13.3b
IT3	790c	309e	30	16.8ab
IT4	907ab	330d	37	15.7ab
IT5	848bc	421a	40	14.6ab
IT6	756d	404b	48	19.2a
IT7	911ab	378c	35	15.4ab
IT8	863b	336d	30	19.0ab
IT9	815bc	298ef	48	13.9ab

There were significant ($P < 0.05$) differences among the different letters in the same lines

Organic acids are known to particularly affect the fruits' taste formation and many physiological processes. Previous studies are also indicated that citric acid is the predominant organic acid in apricot fruits (Saridas and Agdam, 2021, and it was followed by malic acid and tartaric acid. Saridas and Agdam (2021) found that organic acid compositions of the apricot cultivars changed significantly and citric and malic acid content were between 92 and 1098 mg/100 g indicating a great variability. Besides the genotype, differences in malic acid concentrations could be resulted from ecology, cultivation techniques, harvest date etc. Fan et al. (2017) showed that malic acid was mainly responsible for sourness of apricots, although malic acid was not the prominent organic acid in all apricot cultivars. It has also been reported that malic acid has an apparent acidic taste than citric acid or other organic acids in fruit (Colaric et al., 2005).

CONCLUSIONS

In literature, very limited studies have assessed on organic acid and sugar content of clones of apricot. Accessions of cv. Teberze presented considerable variation with reference to fruit weight, flesh/seed ratio, major sugars and organic acids. A systematic understanding of the variability of accessions of cv. Teberze by using biochemical traits may enable the development of a reference classification system for commercial purposes.

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HORMONAL AND BIOCHEMICAL PARAMETERS ANALYSIS OF THE YELD COWS BLOOD

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ABSTRACT

The authors of this article selected animals with various pathologies of the reproductive system in order to study the hormonal and biochemical status of the yeld (infertile) cows. Further, groups were formed in accordance with the disease type. The experimental groups included clinically healthy fertilized animals and animals with a physiological ovary cycle. The authors of this article measured the levels of sex steroids, adrenal and thyroid hormones, and also determined the indices of the main metabolic processes, the levels of macro- and micronutrients, and vitamin A in the blood serum. Excluding the group of cows with luteal cysts, the infertile animals demonstrated a noticeable decrease in progesterone by 2.4-14.5 times. The yeld (infertile) cows without clinical changes in the genitals, cows with uterine subinvolution and ovarian hypofunction showed a markedly reduced (by 1.6-4.1 times) testosterone level in the blood. A decrease in the estradiol concentration was noted in all the studied groups, some animals demonstrated the deviations in the cortisol and triiodothyronine levels from the indices in the healthy cow groups. The hormonal status of the infertile animals indicated probable violations in the regulation systems of the endocrine glands, the initial links of which are the hypothalamus and pituitary gland. The biochemical blood characteristics, in turn, indicate that yeld (infertile) cows suffer from changes in metabolic processes, a macro-and micronutrients deficiency, especially the selenium deficiency. To restore the reproductive function, the use of hormone therapy in combination with additional sources of macro- and micronutrients is highly recommended.

Keywords: hormones, reproductive system, cows**.**

INTRODUCTION

The main place in the regulation of vital processes belongs to the endocrine system. The endocrine system glands produce hormones, together with which and the nervous, immune and tissue systems, metabolic processes in the animal body are controlled (Sundrum, 2015). Hormones also play a particularly important role in ensuring the reproductive animals function (Safonov, 2008).

During one ovary cycle, cows have three increases and decreases in the estrogenic and androgenic hormones levels. The concentration of progesterone in the blood plasma is the highest in the luteal phase of the cycle and the lowest in the follicular phase. While the follicle growth, the production of 17β-estradiol also increases. The ovary cycle, characterized by fluctuations in the sex steroids levels, is accompanied by the changes in the corticosteroid and thyroid hormones synthesis, for example, the follicles' growth is accompanied by an increase in the concentration of these hormones, mostly evident in the case of cortisol.

Metabolic hormones, which include cortisol and triiodothyronine, in turn, have the ability to influence the development of dysfunctional ovarian conditions. Thyroid hormones affect the gonadotropins' synthesis, while creating conditions for the follicles' maturation and ovulation. In addition to a sufficient iodine intake into the body for the production of thyroid hormones, it is important to ensure an adequate selenium intake from feed. According to the number of studies, it has been found that pathological conditions of the reproductive system develop against the background of an increased level of oxidative stress, aggravated by a lack of micronutrients included in the structure of antioxidant enzymes (Awadeh et al., 1998; Safonov et al., 2008, Safonov et al., 2014, Kireev et al., 2018).

An increase in cortisol concentration characterizes the cattle in the early lactation period, which is associated with a high-stress load; it allows to increase the activity of compensatory mechanisms aimed at catabolism of adipose tissue and to obtain fatty acids from it. Also, it helps to obtain amino acids from muscle tissue proteins in order to provide the gluconeogenesis process with the necessary metabolism components (Plemyashov, Moiseenko, 2010; Simonov et al., 2017). It is also worth noting and taking into account during the determination of the hormonal animals status that the hormone level is characterized by the high phenotypic variability and susceptibility to daily fluctuations. The unfavorable external factors can have a very strong influence on the hormone level (Mazurina, 2017, Bova et al., 2014).

The physiological changes in the steroid and thyroid hormones levels are accompanied by a change in the biochemical blood composition. This can be clearly noted for such indices as: total protein and its fractions, glucose, total lipids, cholesterol, calcium, phosphorus, vitamins. For example, during the follicular phase of the ovary cycle, the indices of total protein, glucose, calcium and phosphorus, vitamins A and E increase in the blood of cows against the background of a decrease in cholesterol and vitamin C. In the luteal phase, the lowest level of total protein, glucose and phosphorus is noted as well, at a time when the level of cholesterol and vitamin C becomes maximum. There is also a direct dependence of the animals' ability to fertilize on the albumins, sugar, phosphorus, vitamins A, E, and trace elements levels (Bisinotto et al., 2012; Michael et al., 2019).

This research was organized in order to establish the hormonal and biochemical status of dairy cows that were repeatedly unsuccessfully inseminated or suffer from various pathologies of the reproductive system.

MATHERIALS AND METHODS

To conduct the experiment, the authors of this article selected Black-and-White Dutch cows at the age of no earlier than 2 lactations, of a similar milk productivity level, belonging to one of the breeding farms of the Voronezh region. Therefore, for the purpose of the research, the following groups of animals were formed: yeld (infertile) cows without clinical changes in the reproductive organs – 7 heads, yeld (infertile) cows with uterine subinvolution – 7 heads, successfully fertilized cows – 10 heads, yeld (infertile) cows with ovarian pathologies: with follicular cysts – 8 heads, with luteal cysts – 8 heads, with ovarian hypofunction – 9 heads, healthy non-pregnant cows – 9 heads.

The maintenance of animals is carried out according to a loose housing, feeding – from the feed table. The cows' diet is compiled according to generally accepted zootechnical methods for dairy cows, while taking their productivity into account.

The assessment of the clinical animals condition was carried out while taking into account the anamnesis during visual examination and the use of the transrectal palpation method and ultrasound scanner. The hormonal animals status was determined by means of the laboratory blood plasma analysis, the ELISA test systems and the Uniplan spark analyzer. The level of total protein was determined by the refractometric method, total lipids, glucose, calcium, inorganic phosphorus

– by the “Hitachi-902” biochemical analyzer, trace elements – by the “Perkin Elmer-703” atomic absorption spectrometer, vitamin A-by the spectrophotometric method. Animals’ blood was taken from the jugular vein in the morning hours before feeding in compliance with aseptic and antiseptic measures. The manipulations with animals were carried out according to the norms of humane treatment set out in the European Community Directive (86/609/EEC) and the Helsinki Declaration. The obtained data were processed by mathematical analysis methods adopted in veterinary and biological studies, by the use of MS Excel and Statistica 6.0 Statsoft Inc. programs. The results in the tables are given as a mathematical expectation ± standard deviation.

RESULTS AND DISCUSSION

The research results of the hormone level in the blood of fertilized and yeld (infertile) cows are presented below (Table 1). The obtained data show that yeld (infertile) cows, characterized by repeated unsuccessful attempts to inseminate them, demonstrate reduced functional activity of the thyroid gland and ovaries.

Table 1. Hormones concentration in the blood of successfully fertilized and yeld (infertile) cows, nmol/l

Indices	Yeld cows		Fertile cows (N = 10)
	No clinical changes (N = 7)	Uterus subinvolution (N = 7)	
Progesterone	8.5 ± 3.64 *	7.3 ± 2.96 *	20.5 ± 3.85
Testosterone	8.7 ± 0.95 **	5.4 ± 1.48 **	14.1 ± 2.89
17β-Estradiol	0.76 ± 0.04 *	0.71 ± 0.03 *	0.85 ± 0.02
Cortisol	30.9 ± 7.35	27.0 ± 7.52	22.3 ± 3.25
Triiodothyronine (T3)	2.08 ± 0.72 *	2.08 ± 0.78 *	3.38 ± 1.34

* – $P < 0.05$, ** – $P < 0.01$, in comparison with fertilized cows

The progesterone level in the yeld (infertile) cows without any visible clinical pathologies is significantly reduced – by 2.4 times compared to pregnant animals, the testosterone level is 1.6 times lower, 17β-Estradiol – by 1.1 times, the triiodothyronine level – by 1.6 times. At the same time, animals have an increase in the adrenal hormone production – cortisol – by 38.6%. A higher cortisol level indicates the presence of stress factors affecting the cows’ bodies, and at the same time it leads to the mobilization of compensatory mechanisms in it.

Only 3 animals among the yeld (infertile) cows with undetected clinical changes in the reproductive organs demonstrate the presence of an ovary cycle with the formation of a functioning yellow body. The progesterone level in these animals was 15.9±8.84 nmol/l, at a time when the progesterone level remained at a low level in animals with anovulatory cycles. The absence of ovulation and a low concentration of progesterone may indicate atresia in the follicles ovaries at an early stage of their maturation, which is probably due to a violation of regulation at the level of the hypothalamic-pituitary system (Shkuratova, Ryaposova, 2011). The anovulatory cycles in such animals may be corrected with the help of special stimulating or hormone replacement therapy.

The changes in the hormonal profile of animals similar to those previously considered are noted in the case of the chronic uterus subinvolution. The progesterone concentration in the blood of cows with pathology is 2.8 times lower than it may be in healthy pregnant animals, the testosterone is 2.6 times lower, 17β-Estradiol is 1.2 times lower, triiodothyronine is 1.6 times lower. There is a tendency to an increasing of the cortisol level by 1.2 times compared to the one in fertilized cows. The cyclic activity of the sex glands was preserved only in 2 animals from the experimental group, in the rest the progesterone level in the blood during the observation period was 4.17±1.8 nmol/l, i.e. there was a disorder of the functional ovaries activity. Thus, chronic uterus subinvolution is accompanied by a disorder of the morphological structure functions of the sex glands involved in the hormonal regulation of the hypothalamic-hypophysial-gonad axis, as well as the absence of the ovulation of the mature follicle with the subsequent formation of the corpus luteum. Treatment of such animals should be aimed not only at restoring the uterus morphological structure and functions, but also at correcting the functional ovaries activity.

Table 2. Biochemical status of fertilized and yeld (infertile) cows, blood serum

Indices	Yeld cows		Fertile cows (N = 10)
	No clinical changes (N = 7)	No clinical changes (N = 7)	
Total protein, g/l	81.0 ± 1.55	84.9 ± 2.39 **	79.4 ± 1.24
Glucose, mmol/l	1.84 ± 0.14	1.54 ± 0.17	1.69 ± 0.26
Total lipids, g/l	4.03 ± 0.17 **	4.31 ± 0.28	4.28 ± 0.14
Cholesterol mmol/l	5.90 ± 0.42	6.02 ± 0.85	5.78 ± 0.26
Calcium, mmol/l	2.59 ± 0.03 **	2.52 ± 0.07 **	2.65 ± 0.04
Phosphorus, mmol/l	1.99 ± 0.07 **	1.96 ± 0.14 **	2.16 ± 0.11
Iodine (associated with protein), μg%	2.07 ± 0.18 **	2.62 ± 0.21 *	2.35 ± 0.15
Manganese, μg%	15.5 ± 0.69 **	16.8 ± 1.21 **	19.0 ± 0.63
Copper, μg%	78.1 ± 1.75 **	77.9 ± 2.35 **	85.3 ± 1.44
Selenium, mcg %%	15.4 ± 0.83	13.4 ± 1.20 **	16.2 ± 0.87
Vitamin A, μg%	71.5 ± 4.85 **	43.9 ± 11.2	53.6 ± 5.44

* – P < 0.05, ** – P < 0.01, in comparison with fertilized cows

A comparative assessment of repeatedly inseminated infertile cows without pronounced clinical changes in the reproductive system shows a reduced level of lipids in the blood serum by 5.8% in comparison with springing animals (Table 2). They also demonstrate a slight decrease in the calcium and phosphorus levels, a decrease in the manganese level by 18.4%, copper level – by 8.4%, protein-bound iodine — by 13.5% and a higher concentration of vitamin A – by 34.4% higher than in fertilized cows. The incomplete satisfaction of the need for macro-and micronutrients acts as an additional etiological factor of reproductive disorders in yeld (infertile) animals (Nikolaev, Konopeltsev, 2019). While chronic uterus subinvolution, cows also have a decrease in the calcium and phosphorus level in the blood serum – by 4.9 and 9.3%, respectively, and a lack of trace elements: the concentration of manganese is reduced by 11.6% compared in comparison with springing animals, copper – by 8.7%, selenium – by 17.3%. There is a tendency to a reduced vitamin A level. The iodine level in such cows was 11.5% higher than in springing animals. The state of uterine subinvolution is accompanied by a high protein level in the blood serum, which was 6.9% higher than in the group of fertilized cows.

In the second research series, the hormonal status of infertile cows with various ovarian pathologies was analyzed in comparison with the indices of clinically healthy animals with cycles (Table 3).

Table 3. Hormones concentration in the blood of the yeld (infertile) cows with ovarian disease and healthy cows with cycle, nmol/l

Indices	Yeld (infertile) cows with ovarian disease			Healthy cows with cycle
	Follicular cysts (N = 8)	Luteal cysts (N = 8)	Hypofunction (N = 9)	(N = 9)
Progesterone	1.3 ± 0.46 **	17.4 ± 4.30	1.04 ± 0.25 **	15.1 ± 3.85
Testosterone	12.8 ± 0.67 *	10.1 ± 0.49	2.44 ± 0.40 **	9.9 ± 3.37
17β-Estradiol	0.71 ± 0.02 **	0.79 ± 0.05 *	0.67 ± 0.10 **	0.90 ± 0.12
Cortisol	64.4 ± 13.40 **	12.6 ± 2.78 **	29.0 ± 7.97 *	40.2 ± 11.20
Triiodothyronine (T3)	3.77 ± 1.19 **	2.13 ± 1.15	1.86 ± 0.70	2.25 ± 0.86

* – $P < 0.05$, ** – $P < 0.01$, in comparison with fertilized cows

Depending on the form of the ovarian disorder, the hormonal animal status was characterized by significant differences. While detecting follicular cysts in cows, a low progesterone level in the blood was found, therefore its level was 11.6 times lower than in healthy animals, and the level of 17β-Estradiol was also lowered by 25.3%. At the same time there was an increase in the testosterone concentration by 29.3%. Such a situation characterizes a violation of the enzymatic processes of estrogens and progesterone synthesis.

The highest concentration of triiodothyronine and cortisol in the blood is observed while the presence of follicular cysts. An increase in the functional activity of the adrenal glands leads to a 1.5 times higher hormone level than in healthy animals. It is assumed that this causes a blockade of the production and incretion of gonadotropin hormones of the thyroid gland and further leads to a violation of ovulation and cyst formation.

Animals with identified luteal cysts show a high progesterone level in comparison with other groups of cows with pathology, in addition, the hormone level is 15.3% higher than in cyclical cows. The amount of testosterone corresponds to the indices of healthy animals, the concentration of 17β-Estradiol is reduced by 14%, the concentration of cortisol is significantly reduced – by 3.2 times. The authors of the article suppose that luteal cysts in this case are associated with the cyclic release asynchrony of luteinizing hormone while the regulatory function of the hypothalamic-pituitary system changes. The hypofunctional state of the ovaries in cows is manifested by prolonged anaphrodisia, inability to fertilize and low production of sex hormones. In the group of cows with hypofunction, the progesterone level was reduced by 14.5 times, testosterone level – by 4.1 times, and 17β-estradiol level – by 34.3% in comparison with the animals having a healthy cycle. There was also a decrease in the triiodothyronine concentration. The results of measuring the cortisol level in the blood of cows of this group were uneven. In 5 animals, the reduced hormone secretion was detected with a blood concentration of 13.2±1.63 nmol/l and in 3 animals it was an increased one, with a concentration of 60.7±10.63 nmol/l. In the first case, cows may be under the influence of chronic distress, and in the second – in a state of the acute one.

Table 4. Biochemical status of the yeld (infertile) cows with ovarian disease and healthy cows with cycle

Indices	Yeld (infertile) cows with ovarian disease		Healthy cows with cycle (N = 9)
	Ovarian cysts (N = 16)	Hypofunction (N = 9)	
Total protein, g/l	81.0 ± 1.65	84.5 ± 2.02 **	80.2 ± 2.08
Glucose, mmol/l	1.70 ± 0.14	1.86 ± 0.17 *	1.62 ± 0.16
Total lipids, g/l	4.52 ± 0.27 **	3.08 ± 0.25 **	3.58 ± 0.13
Cholesterol mmol/l	6.03 ± 0.48 **	4.93 ± 0.40	5.09 ± 0.33
Calcium, mmol/l	2.65 ± 0.04	2.57 ± 0.02 *	2.61 ± 0.05
Phosphorus, mmol/l	2.23 ± 0.12	2.02 ± 0.21 **	2.19 ± 0.06
Iodine (associated with protein), µg%	2.03 ± 0.16 **	2.56 ± 0.19	2.69 ± 0.19
Manganese, µg%	17.1 ± 0.91 **	18.1 ± 0.85 **	15.6 ± 0.65
Copper, µg%	82.8 ± 3.39 *	81.4 ± 1.77 **	80.0 ± 1.87
Selenium, mcg %%	14.6 ± 0.68 **	12.7 ± 0.32 **	16.0 ± 0.73
Vitamin A, µg%	53.2 ± 4.07	49.9 ± 6.85	50.2 ± 5.21

* – $P < 0.05$, ** – $P < 0.01$, in comparison with fertilized cows

The biochemical status of animals with cystic ovarian changes is characterized by high concentrations of lipids and cholesterol: 37.8 and 18.5% higher, respectively, than in the group of animals with a healthy cycle, which may be explained by an imbalance of metabolic body processes. The macronutrient status did not differ from that of healthy cows, while there was a reduced selenium level by 8.8% and iodine level by 24.5% against the background of high functional activity of the thyroid gland in follicular cysts. The availability of manganese exceeded the healthy animals level by 9.6%, the availability of copper – by 3.5%. Therefore, the hypofunctional ovarian disorder in cows is accompanied by a sufficiently high level of total protein and glucose, and at the same time a decrease in the supply of macronutrients. The most noticeable was a decrease in the selenium level– by 20.6%, at a time when the manganese level was 16.0% higher than in animals with a healthy cycle. Thus, pathological processes in the ovaries of cows occur against the background of changes in the biochemical profile of blood and the lack of individual macro- and micronutrients.

CONCLUSIONS

The failure of the steroidogenesis regulation in yeld (infertile) animals in most cases occurs due to the changes in the levels of progesterone, estrogen and testosterone, which, through feedback, affect the hypothalamic-pituitary system responsible for controlling the hormone synthesis by the ovaries.

The study of the hormonal status of yeld (infertile) cows with various ovarian pathologies shows that the pathophysiological pathways of the formation of luteal cysts are associated (to a greater extent) with the hypothalamic-hypophysial-adrenal axis. The changes in the indices of sex hormones in this cows group were the smallest ones. In addition to the elements of this system, changes in the activity of the thyroid gland and the ovaries themselves are involved in the follicular cyst formation.

Changes in the biochemical profile of blood are associated with the development of pathologies of the reproductive organs and infertility in dairy cows. Shifts in the balance of lipid, protein and carbohydrate metabolism are usual, there was an insufficient supply of mineral elements in all the groups (in comparison with healthy animals). A particularly strong deficiency was observed in providing the yeld (infertile) cows bodies with selenium – its deficiency is inherent in all the considered groups and amounted to 4.9-20.6%.

In order to correct the cows' infertility, in all the cases, the specialized hormone therapy may be used in combination with providing them with a full-fledged diet and the addition of additive mineral elements sources.

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BREEDING OF SAFFLOWER SUITABLE FOR TEA PRODUCTION

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ABSTRACT

As a result of ecological variety testing of safflower at Aktobe Agricultural Experimental Station, new varieties and variety samples were developed that are highly productive in petals intended for obtaining therapeutic safflower tea. Methods and results of research. Safflower is a new oilseed crop for the Aktobe region. A significant proportion of safflower samples obtained from the international center CIMMYT (Mexico), and in recent years from VIR (St. Petersburg, Russia), the State Scientific Institution "Nizhne-Volzhsky Research Institute of Agriculture" (Volgograd, Russia), the Caspian Research Institute of Arid Agriculture, Kazakh Research Institute of Agriculture and Plant growing". The safflower gene pool at the Agricultural Experimental Station of Aktobe comprises more than 100 samples. According to the results of the EVT, the Akhram variety of safflower was created and transferred to the State variety testing. It exhibits high ecological plasticity and seed and petal yield. Comparative studies on the yield and quantitative content of the petals (rims) of flower in the context of safflower varieties and variety samples were conducted in the breeding experiment, along with the study of productivity. The collection of flower petals by varieties was made in the phase of the beginning of seed ripening, with an aim to study the yield of flower petals of safflower in the nursery of competitive variety testing. Regarding the late-ripening varieties "Akhram" and "Aqbayan" the largest number of petals was contained at one plant - 1.79-1.51 g, and at 100 plants - 33.6-32.5 g. The actual timing of harvesting the flower mass was 5-6 kg / ha. The highest quantitative indicator of the yield of safflower by-products (flower beaters) was noted for late-ripening varieties, in which each plant has the highest number of baskets. The creation of late ripening varieties with high yields of the flower mass has a prospect.

Keywords: safflower, safflower tea, petals, plant, oil-seed crop, variety, breeding

INTRODUCTION

Safflower is a drought-resistant oilseed crop, which can give a stable crop of oil seeds with an oil content of 27-38% in the conditions of arid Aktobe region. In arid conditions safflower complements the "basket" of oilseeds. Safflower is not a competitor to sunflower, since it spreads to the south of the cultivation zone due to its drought tolerance. This crop is capable of forming crops in extremely dry years, as it is adapted to the conditions of a sharply continental climate. Due to the high concentration of cell sap, it uses soil moisture sparingly. The entire above-ground part of the safflower plant at different periods of the growing season may be used in animal husbandry: green mass for silage, substandard seeds for feed to poultry. Waste obtained after squeezing the oil - cake, is given for animal feed.

Cultivation of safflower is known since antiquity. It was cultivated initially as a dyeing plant and only later it began to be used as an oil plant. It was known to the ancient farmers, who grew it in arid areas. Dried safflower flowers are found by archaeologists in the Egyptian pyramids: the hieroglyphs denoting this plant are preserved on the walls of the temples. The ancient Egyptians wrapped mummies In the fabric, dyed with safflower. Dried flowers of safflower were found in the tombs of the Egyptian Pharaoh, who ruled the country in the sixteenth century. BC er.

Flowers contain nutrients and are used in the treatment of many diseases. In addition, safflower prevents proliferation (cell death), which can make an extract from it suitable for the treatment of psoriasis and mutagenic diseases. Oil is a good cosmetic product for problematic skin and a basis for hair care products, especially weakened, brittle and dry. In creams, oil is used to care for dry and aging skin. Due to the presence of vitamin K, this oil is a valuable component of remedies for rosacea. Derivatives of serotonin and vitamin E have antioxidant and regenerating properties. Safflower flower petals have two different coloring pigments - yellow and red. The yellow pigment is considered less valuable and is removed by washing the petal mass with water. The red substance of safflower - cartamine – is hardly dissolving in water, but easily soluble in alcohol and alkali, which caused the use of safflower flowers as a dye for fabrics. It has been used for a long time as a raw material for the production of safflower tea. The taste of this tea is pleasant and unusual, does not contain caffeine, it has been recommended for centuries as a sedative and as a cure for intestinal diseases. Tea is recommended not only for psoriasis, but also for lacerations, impaired digestion and bowel movements, toxemia and ulcers. It has a positive effect on the stomach and intestines and helps to facilitate the nutrition of the skin caused by disorders in the digestive tract. The greatest benefit of this tea is cleansing the liver and kidneys, increasing sweating and healing damage to the intestinal walls, and is also an intestinal antiseptic. Currently, antioxidant, analgesic, anti-inflammatory and antidiabetic properties of safflower tea have been discovered. With a long reception safflower tea gives a diuretic effect, the urge to urinate can be even with an empty bladder. This is due to its cleansing effect on the kidneys, creating a constant flow of urine and, as a consequence, more frequent emptying of the bladder. One of the varieties of tea is safflower water. It is useful in severe cases of psoriasis. The idea is to have a certain amount of safflower in all drinking water, not in the same concentration as in a tea, but the cleaning effect will be similar. Safflower steam is useful for psoriatic skin lesions. Such rashes cause considerable concern to the patient, as they are visible. With the help of safflower vapor, you can clear the skin of psoriatic lesions.

METHODS AND RESULTS

Safflower is a new oilseed crop for the Aktobe region. The collection of safflower has been studied and enlarged at Agricultural experimental station of Aktobe for 11-15 years. A significant proportion of samples obtained from the international center CIMMYT (Mexico), and in recent years from VIR (St. Petersburg, Russia), the State Scientific Institution "Nizhne-Volzhsky Research Institute of Agriculture" (Volgograd, Russia), the Caspian Research Institute of Arid Agriculture, Kazakh Research Institute of Agriculture and Plant growing”.

The safflower gene pool at the Agricultural Experimental Station of Aktobe comprises more than 100 samples. According to the results of the Ecological variety testing, the Akhram variety of safflower was created and transferred to the State variety testing. It exhibits high ecological plasticity and seed and petal yield (Fig. 1).

Fig.1. Petals of safflower flowers of "Akhram" variety

Comparative studies on the yield and quantitative content of the petals (rims) of the flower in the context of safflower varieties and variety samples were conducted in the breeding experiment, along with the study of productivity. The collection of flower petals by varieties was made in the phase of the beginning of seed ripening, with an aim to study the yield of flower petals of safflower in the nursery of competitive variety testing. Regarding the late-ripening varieties “Akhram” and “Aqbayan” the largest number of petales was contained on one plant - 1.79-1.51 g, and on 100 plants - 33.6-32.5 g (table 1).

Table 1 - The yield of safflower flower petals, 2015-2018.

Variety name	Weight of collected flower petals		
	from 1 plant, g	from 10 plants, g	from 100 plants, g
Akhram	1,79	4,08	33,6
Aqbayan (SI-CE 1230/PC-162)	1,51	3,92	32,5
AK-VO-11	1,43	3,88	31,5
S H A R - 1 1	1,60	3,98	32,4
Aqmay(St)	1,36	3,33	17,3

The actual timing of harvesting the flower mass was 5-6 kg / ha. The highest quantitative indicator of the yield of safflower by-products (flower beaters) was noted for late-ripening varieties, in which each plant has the highest number of baskets. The creation of late ripening varieties with high yields of the flower mass has a prospect. In august 2017 a proposal for long-term cooperation as regards the use of safflower petals as raw material for natural dyes, have been received from Sensient Colors LLC (founded in 1905, St. Louis, Missouri, USA, http://sensientfoodcolors.com/en-us/) via Representation of the German Economy in Central Asia, and in 2018 an another proposal for cooperation from the Moscow Institute of Applied Biotechnology.

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CEREAL Β-GLUCANS IN NUTRITION

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ABSTRACT

β-glucans ((1/3,1/4)- β-D-glucans) are the major components of cell wall structure of cereal grains such as barley and oats. The β-glucans in the endosperm cell walls may be covalently bonded to protein, forming large molecules. Levels of β-glucan can vary dramatically between varieties, but usually range from 2 to 9% dry weight. Genetic and environmental factors impact on β-glucan content of the cereals. Despite their relatively small contribution to the total weight of the grain, it is clear that β-glucans have a disproportionate impact on the nutritional value of the grain and the technology of cereal utilisation as well. Cereal β-glucans possess a number of functionalities and roles that make it unique as a plant cell wall component and as a dietary fibre. It is classified generally as a soluble dietary fibre which attenuate postprandial blood glucose and insulin levels and lower serum cholesterol levels. In this presentation it was aimed to review the potential effects of cereal β-glucans in nutrition.

Key words: β-Glucans, cereals, nutrition, dietary fiber

Introduction

β-Glucans are major components of starchy endosperm and aleurone cell walls of cereals, such as, barley, oat, rye and wheat. β-glucan, content of cereals ranges from 1% in wheat, to 3–7% in oats, and 5–10% in barley (Table 1) (Ahmad and et al., 2012). Thus, barley grains are a rich source of β-glucans. In oat and barley, β-glucans are located throughout the starchy endosperm whereas in wheat the highest concentration is in the subaleurone layer with little in the rest of the starchy endosperm (Figure 1) (**Brennan and Cleary, 2005; Lazaridou and Biliaderis, 2007; Cui and Wand, 2009**)

Table 1. β-glucan content in different sources (Ahmad and et al., 2012)

Source	β-Glucan content (%)
Barley	5-10
Hulless barley	3.7-9
Oat	3-7
Rye	0.7-2.4
Winter wheat	0.4-1.4
Spring wheat	0.6-1.1

Corn/maize	0.1-1.3
Spelt	0.6-1.2
Millet	0.5-1.0
Rice	0.4-0.9
Beans	2.4-3.5
Lentils	0.4-1.1
Peas	0.3-0.7
Flax	0.3-0.7
Canary seed	1.1-2.3

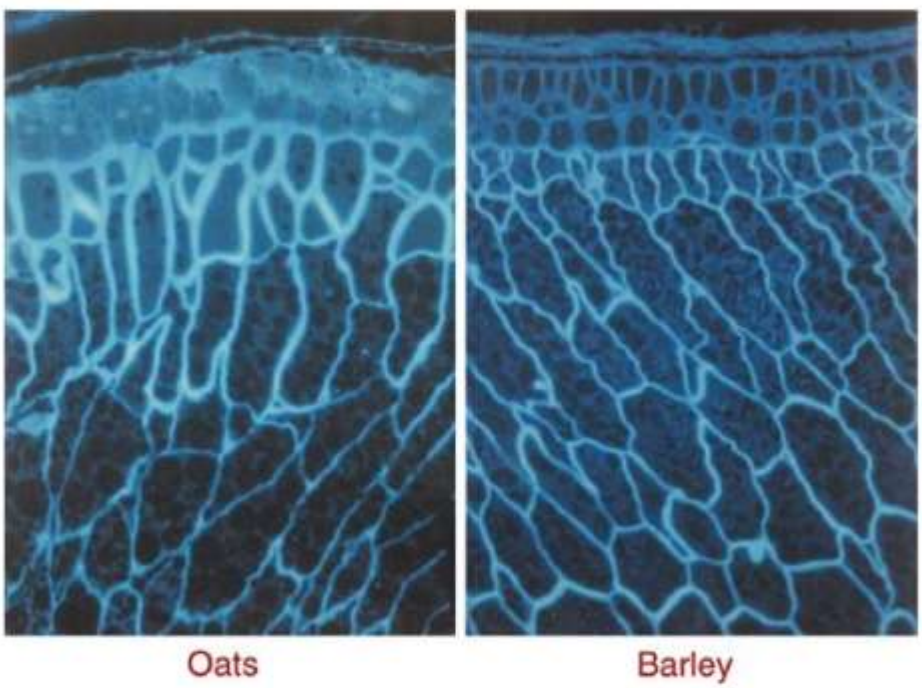

Figure 1. Fluorescence micrographs of sections of oat (left) and barley (right), showing the fluorescence (white) of calcofluor-stained β-glucan in the endosperm cell walls. Oat, which has thicker cell walls, is enriched in b-glucan (**Cui and Wand, 2009)**.

β-Glucans from cereals are glucose units linked via a mixture of β-(1→3) and β-(1→4) linkages (**Figure 2**). They exhibit considerable diversity in their structures, including the ratio of tri- to tetramers, the amount of longer cellulosic oligomers and the ratio of b-(1-4):b-(1-3) linkages (**Zhu and et al., 2016**).

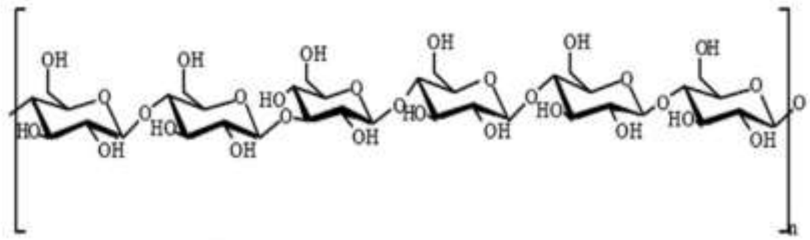

Figure 2. The structures of (1→3) β-glucans with ramifications β-(1→4) (**Zhu and et al., 2016**).

These structural features appear to be important determinants of their physical properties, such as water solubility, viscosity, and gelation properties as well as of their physiological action in the gastro-intestinal tract.

Over the last two decades the acceptance of β-glucans as functional, bioactive ingredients has increased the popularity and consumption of cereal-based foods (**Lazaridou and Biliaderis, 2007; Izydorczyk and Dexter, 2008; Lovegrove and et al., 2017**).

B-glucans in Food Industry

Due to the specific physical properties of β-glucan, such as water solubility, viscosity, and gelation, it has been increasingly used by the food and other industries such as medicine, chemistry and cosmetic (**Zhu and et al., 2016**). β-glucans can be utilised as thickening agents to modify the texture and appearance of food formulations or may be used as fat mimetics in the development of calorie-reduced foods. β-glucan-rich fractions from cereals or purified β-glucans have in fact been successfully incorporated into products such as breakfast cereals, pasta, noodles and baked goods (bread, muffins), as well as dairy and meat products. Recently, non-dairy ready-to-use, lactose-free, milk substitutes (oat milk beverage, yogurt, ice cream etc.) have been introduced into the market. These products contain cereal β-glucans as bioactive ingredients as well as acting as stabilisers and texturisers **(Lazaridou and Biliaderis, 2007; Izydorczyk and Dexter, 2008; Ahmad and et al., 2012).**

β-glucans in Nutrition

The physiological functions of β-glucans as a dietary fibre component are attributed to their physico-chemical properties such as water holding capacity, swelling, viscosity enhancement and gel-formation, binding properties, and susceptibility or resistance to bacterial degradation and fermentation. They are not digested and are fermented by the colon microbiota to produce short chain fatty acids. It has been suggested that cereal β-glucans decrease the absorption and reabsorption of cholesterol, bile acids, and their metabolites by increasing the viscosity of the gastro-intestinal tract contents as well as delaying gastric emptying and the intestinal absorption of nutrients, such as digestible carbohydrates, and thereby reducing post-prandial hyperglycemia and insulin secretion. The latter have health benefits for those with type-2 diabetes, and are also associated with reduced risk of developing the disease and insulin insensitivity **(**Brennan and

Cleary, 2005; Lazaridou and Biliaderis, 2007; Wood, 2007¸ Jayachandran and et al., 2018; Din and et al., 2018).

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COMPARATIVE ANALYSIS OF RICE PERFORMANCE AND PROFITABILITY WITH THE SYSTEM OF RICE INTENSIFICATION (SRI) AND TRADITIONAL PRACTICES (TP) IN ZIGUINCHOR DISTRICT, SENEGAL

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ABSTRACT

The "System of Rice Intensification"(SRI) is a combination of three elements(soil, water, plant and light) that allow the plant to express its production potential hidden by inappropriate farming practices. It represents a sustainable alternative to improve household yields and incomes. The aim of this study was to evaluate the yields and the profitability of the SRI and the Traditional Practices (TP) in Ziguinchor district, Senegal. A directed sampling based on criteria for selecting the system used and the cultivated varieties common to both systems was applied to collect the yield parameters and yields of paddy rice. Thus, 18 producers in the Badiate, Essyl and Fanda sites were selected, nine per system and four 1 m2 yield squares were installed in each selected producer plot. A total of 72 yield squares, 36 per system, were installed, and an individual questionnaire was randomly administered to 55 producers using at least one of the systems to collect data on rice production and costs. The yield parameters including the number of fertile tillers per m2, the number of spikes, the weight of the 1000 grains are significantly higher ($p<0.05$) in the SRI including yield compared to the traditional system. Transplanting density and plant duration are higher in TP (26±5.6 plants/m2 and 26 days) than in SRI (16±0.4 plants/m2 and 16 days). The lower the transplanting density, the higher the yield parameters and the yield. The economic profitability, determined on the basis of the benefit/cost ratio, is higher in SRI (1.5) than in TP (1.2). The SRI required a lot of technicality in its implementation and generated more cost of production. However, SRI was more productive and economically more profitable than the traditional system.

Keywords:, SRI, Traditional practices, Rice, performance, Productivity, profitability

Introduction

Rice is a cereal that plays an important role in food security. It is the staple food of more than half of the world's population and remains the most widely grown cereal for human consumption (Pascualand Wang, 2017). Thus, in regions such as West Africa, which only covers 60% of its rice needs, a large part of the rice consumed comes from Asia (Boutsen and Aertsen, 2013). In Senegal, despite the efforts noted in terms of improving rice yields, production remains insufficient in relation to the need of a population that is constantly growing and is only asking to produce more (Fall and Dieye, 2008). These deficiencies in production are linked to a lack of material resources, poor technology transfer and dissemination and non-compliance with good rice production practices (Poussin and Boivin, 2002; Zingore et al., 2014). While facing their growing needs, traditional subsistence rice farming communities are increasingly faced with the need to increase their production to meet their monetary needs and provide cities with food (Radanielina, 2010).

However, as the expansion of arable land is less and less possible, the increase in production would require the adoption of new agricultural practices and technologies, which would not only maximize yields but also adapt to climate variability and particularly to rainfall irregularities. Thus, various agro-ecological practices, particularly the intensive rice farming system, have been popularized by State structures, but also by some NGOs among small producers. This system of rice intensification(SRI) is a combination of the elements of the soil-water-plant-light relationship in a harmonious way that allows the plant to express its production potential hidden by inappropriate farming practices. It is based on some essential practices: transplanting young plants (1 seedlings/pot) from 08 to 15 days, intermittent water management, organic amendment, reduction of competition between plants and weeding (Gathorne-Hardy et al., 2016), which could therefore be an effective alternative to increasing rice yields in Lower Casamance (Ziguinchor Province) where small rice farms have difficulty in reaching the two tonnes per hectare (VECO, 2015; Sène, 2018).
Despite the enormous potential that exists in these areas, rice farming, dominated by smallholders, continues to face a lack of equipment and to suffer the adverse effects of climate variability and its corollaries (reduced rainfall duration, land salinization, soil poverty, etc.).It is in this perspective that this work, aims to study the yields and evaluate the profitability of the SRI compared to the Traditional Practices (TP) in Lower Casamance.

Material and methods

Study area

This study was carried out in three villages which are Badiate and Essyl located in the municipality of Enampore and Fanda in the municipality of Niaguis (Fig 1). These municipalities belong to the province of Ziguinchor, located in southern of Senegal. The Province of Ziguinchor is geographically located at 12°33' latitude north and 16°16' longitude west and covers a total area of 7339 km^2. Ziguinchor is characterized by a mean annual rainfall varying between the 1400 and 1600 mm . The Sudano-Guinean climate is dominated by two seasons: a long dry season from November to mid-June and a short rainy season from mid-June to October. The economic activities are dominated by mainly agriculture, livestock and fisheries.

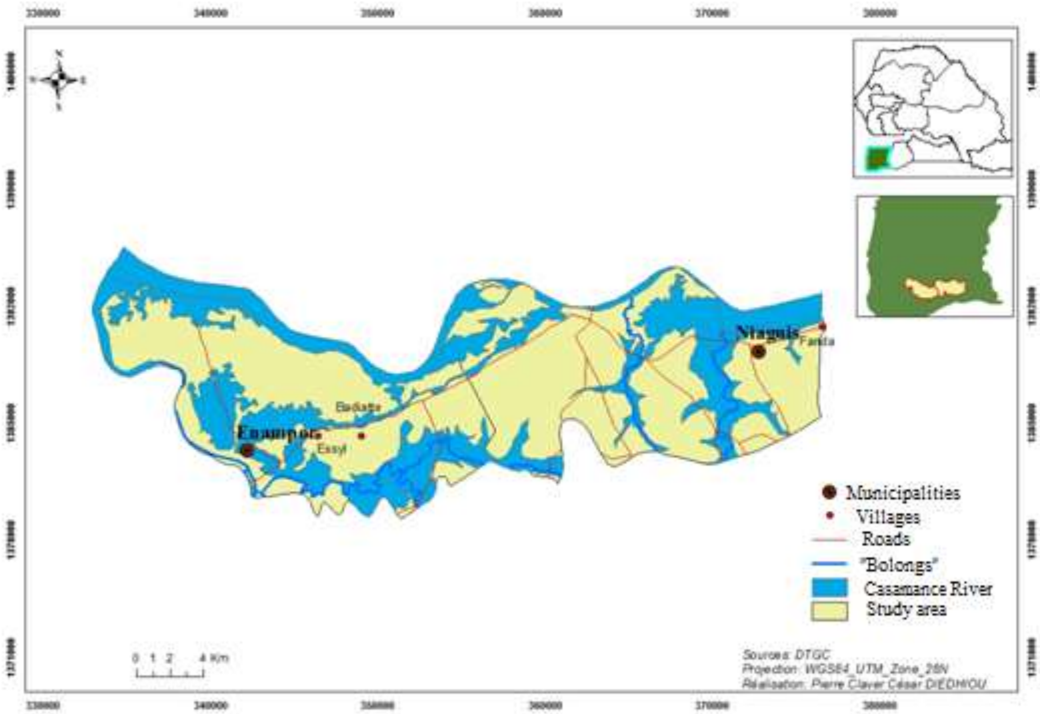

Figure 1:Study area location

- **Vegetal material**

The vegetal material consists of four rice varieties including Sahel 108, Nerica 4, Nerica 14 and Niamlissou which is a local variety. They are grown in the SRI and TP by producers.

- **Experimental design**

Yield squares (1 m^2) were placed in the producers' plots and made it possible to collect yield parameters and yields. In each plot, four yield squares of one meter side were randomly placed along the diagonals while avoiding the edge effect (Figure 2). A total of 12 yield squares per system for each site with a total of 72 yield squares for the three sites. Stakes were used to materialize the yield squares and its geographical coordinates were recorded using a GPS. In Each yield square, the agronomic parameters were recorded (Sanou et al., 2016).

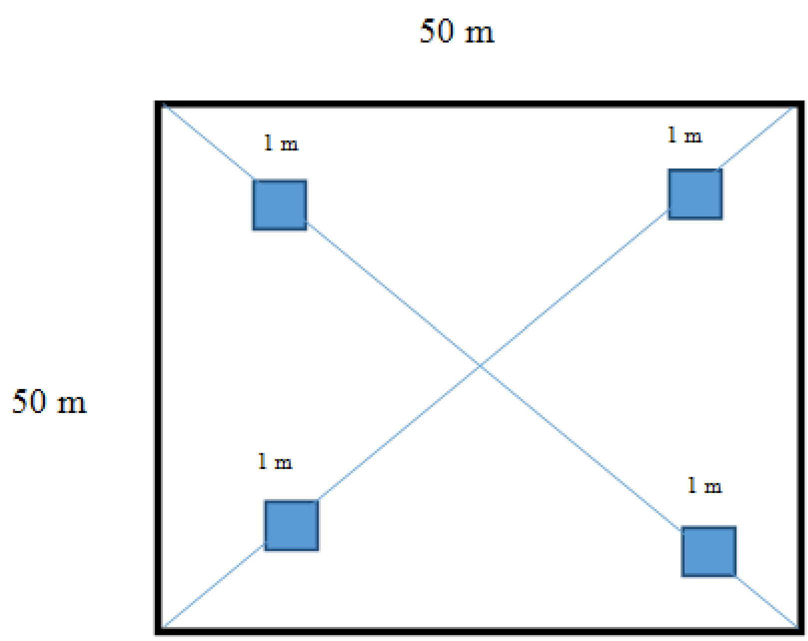

Figure 2: Experimental design of the yield squares on a 50m side plot

- **Data collection**

✓ ***Agronomic parameters***

A sampling based on criteria for selecting the practiced system and the cultivated varieties common to both systems was applied to assess the agronomic performance of each system as well as cropping practices. And for this purpose, 18 producers, three of whom were chosen in each site and per system. Agronomic parameters such as plants, fertile tillers and spikelets density, weight of 1000 grains and yields were measured in each system for four rice varieties grown by producers to assess rice productivity in each system.

✓ **Profitability indicators**

The sample size was calculated based on the following Fisher equation:

$$nf = \frac{n}{(1+\frac{n}{N})} \qquad (1)$$

with nf = The desired sample size;
N =the size of the population;
n = 1 / d², desired accuracy levels (d=10%)

The rice farmers' population was 77, 48 and 32 respectively in Badiate, Essyl and Fanda. Thus, 55 producers, 20 in Badiate and Essyl and 15 in Fanda, were randomly selected for survey. A questionnaire was administered to producers who have adopted at least one of the rice systems at the different sites to assess the economic performance of the systems. Individual interviews were used to collect data on production costs and production (Paraïso et al., 2011). The economic profitability of each system has been calculated on the basis of costs using two indicators

(Agbohessi et al., 2011; Yabi, 2010; Yabi et al., 2012): The Net Margin (NM) and the Benefit/Cost Ration (BCR).

- **The Net Margin (NM)** is expressed in African Financial Community Franc (AFCF)/ha, the net production margin is obtained by deducting from the gross product in value (GPV) per hectare the total costs (TC) per hectare. It is calculated by the following equation:

$$NM = \mathbf{GPV} - TC$$

The price of paddy rice was estimated at 125 AFCF/kg based on the market cost in Senegal (VECO, 2015).

- **Benefit/cost ratio (BCR) was determined by the following equation** :

$$BCR = \frac{GPV}{TC}$$

where GPV= gross product in value, TC= total costs

The economic profitability analysis was interpreted on the basis of the net margin and the BCR. If the BCR were greater than one, the investment would be positive that one invested franc generates more than one AFCF as a profit, and the activity is said to be economically profitable. Of course, if the BCR were less than 1, the value of the benefits do not cover the value of the costs, therefore the net value of the investment would be negative, then one invested franc generates less than one AFCF as a profit, and the activity is considered economically unprofitable, because the producer earns less than he invests. It is correlated to the net margin.

- **Statistical analysis**

The yield parameters and yields were subjected to a comparison of averages and an Analysis of Variance (ANOVA) to determine the components and cultivation practices that influence yields. The Fisher test at the 0.05 threshold was used to determine the degree of significance of the parameters measured between the systems. When the differences were significant, Tukey's test was used for multiple mean comparisons to detect the significant differences of the yield parameters and yields between the systems, the village and the varieties. Statistical significance was fixed at 0.05. A constrained ordination using Principal Component Analysis (PCA) as implemented in R was performed for the relationship between the yield parameters, the yields and the cultivation practices.

Results

- **Cultivation and management methods**

Rice cultivation was practiced differently in different areas. In Badiate and Essyl, the men were in charge of preparing the nursery and labour while the women were transplanting. In Fanda, on the other hand, women were fully involved in rice cultivation. The labour equipment was essentially the tractor, the "kadiandou" and the hoe for the SRI. However, the "Kadiandou" was used in Badiate and Essyl and the hoe in Fanda for the TP. The average age of seedlings was 16 days for SRI and 26 days for TP. The transplanting was done in line on a flat surface for the SRI and in a scattered way on ridges (Badiate and Essyl), and on a flat surface (Fanda) for the TP. The number of seedlings transplanted per hill varied according to the production system in Badiate and Essyl: one seedling per hill for SRI and two to three seedlings for TP. However, the number of seedlings per hill is one in both systems in Fanda. Weeding and water management were practiced in both systems, but frequencies ranging from 25 to 100% are more important in SRI (Table 1). The organic fertilization was the most used in both systems with frequencies varying from 40 to 85%.

Table1: Standard paddy cultivation practices and management for SRI and TP

Villages	Systems	Age of seedlings (d)	No. of seedlings per hill	Water management (%)	Weeding (%)	Organic fertilization (%)	Inorganic fertilization (%)
Badiate	SRI	14	1	70.59	100	82.35	29.41
	TP	28	2	68.75	25	68.75	25
Essyl	SRI	16	1	61.54	100	84.62	69.23
	TP	24	2	45	50	50	40
Fanda	SRI	17	1	80	80	40	6.67
	TP	27	1	40	60	40	0

Yield parameters

The analysis of variance (ANOVA) showed that the yield parameters according to the systems Were significantly different (p<0.05). The number of fertile tillers, the number of filled spikelets and the weight of the 1000 grains were higher in the SRI. On the other hand, the transplanting density was higher in the traditional system (Table 2).Yield parameters were higher in SRI compared to the traditional system in all villages and were lower in Fanda with the exception of transplanting density (29 plants/m2) (Table 3). There was a significant difference (p<0.05) in transplanting density, number of tillers, number of spikelets and weight of 1000 grains for Sahel 108 variety between systems. And for the Nerica 14, the difference was only significant for the transplanting density and weight of the 1000 grains parameters. The number of spikelets and the weight of the 1000 grains of the variety Nerica 4 were significantly different between systems (p<0.05) while a significant difference is not observed for the transplanting density parameters (p=0.124) and number of tillers. The local variety, Niamlissou, had a significant difference (p<0.05) in transplanting density, number of spikelets and weight of 1000 grains. The yield parameters were all higher in the SRI compared to the TP (Table 4).

Table 2: Average yield parameters by production system

Yield parameters	SRI	TP	Pr<0,05
seedlings (no./m^2)	16 (±0,4) ^{a}	26± (5,6) ^{b}	5,84$^{e-16***}$
Tillers (no./m^2)	201 (±69,4) ^{a}	159 (±49,6) ^{b}	0,00501**
Spikelets (no./m^2)	1165 (±315,9) ^{a}	790 (±159,5) ^{b}	1,95$^{e-08***}$
1000 grains weight (g)	24,9 (±2,4) ^{a}	22,7 (±2,0) ^{b}	6,68e^{-05***}

Table 3: Average yield parameters by production system and by site

Village	Systems	seedlings (no. /m²)	Tillers(no./ m$^{2)}$	Spikelets(no./m^{2})	1000 grains weight (g)
Badiate	SRI	16 (±0,3) ^{b}	188 (±53,8) ^{a}	1271(±293,7) ^{a}	26,2(±2,2$^{)}$ ^{a}
	TP	24(±5,3) ^{a}	163(±48,1) ^{b}	739(±89,8) ^{b}	23,5(±1,6) ^{b}
Essyl	SRI	16(±0,3) ^{b}	230 (±54,6) ^{a}	1226 (±260,9) ^{a}	23,3 (±0,0) ^{a}
	TP	25 (±4,4) ^{a}	176 (±41,0) ^{b}	911 (±165,7) ^{b}	21,3 (±0,0) ^{b}
Fanda	SRI	16(±0,6) ^{b}	184(±89,9) ^{a}	997(±340,5) ^{a}	25,4(±3,1) ^{a}
	TP	29(±6,0) ^{a}	140(±54,0) ^{a}	722(±141,8) ^{b}	23,2(±2,7) ^{b}

Table 4: Average yield parameters by production system and by variety

Systems	Varieties	Plants(no. /m²)	Tillers(no./ m$^{2)}$	Spikelets(no./m^{2})	1000 grains weight (g)
SRI	Sahel 108	16,12 (±0,5)^{b}	224 (±58,5)^{a}	1251 (±286,6)^{a}	23,33 (±0,0)^{a}
	Nerica 14	16, 25 (±0,5)^{b}	144,25 (±119,0)^{a}	7507 (±383,0)^{a}	29,67 (±0,0)^{a}
	Nerica 4	16,25 (±0,5)^{a}	176,8 (±29,3)^{a}	1244 (±205,5)^{a}	28,67 (±0,0)^{a}
	Niamlissou	16 (±0,0)^{b}	139,5 (±25,7)^{a}	971,8 (±77,2)^{a}	26,67 (±0,0)^{a}
TP	Sahel 108	27,96 (±5,3)^{a}	179,5 (±44,1)^{b}	802,8 (±183,5)^{b}	21,33 (±0,0)^{b}
	Nerica 14	22,5 (±3,1)^{a}	90,5 (±38,5)^{a}	823,5 (±105,4)^{a}	27 (±0,0)^{b}
	Nerica 4	18,75 (±2,7)^{a}	140,2 (±8,3)^{a}	701(±71,5)^{b}	25 (±0,0)^{b}
	Niamlissou	24,5 (±2,8)^{a}	127 (±12,1)^{a}	773(±92,6)^{b}	24,33 (±0,0)^{b}

Yield

The analysis of variance showed a significant difference (p= 5.61e-07) between the yields of the production systems. SRI was more productive than TP. Indeed, the average yields were 394.47 g/m2 for SRI and 227.47 g/m2 for TP. Average yields were highly variable within and between systems. They were more variable in SRI with a large difference of 600 g/m2 between producers (minimum = 67 g/m2 and maximum = 786 g/m2) (Figure 3). However, there were no significant difference in yields between villages for the same system. Yields were higher in the SRI. Badiate had the highest yields (459.6 g/m2) followed by Essyl (392 g/m2) and Fanda (330 g/m2) in the SRI. On the other hand, yields are higher in Essyl than in Badiate for TP. The lowest yields were recorded in Fanda with average values of 330g/m2 for SRI and 203 g/m2 for TP (Figure 4). Comparing the yield between the varieties, the analysis of yield variance showed a significant difference between yields (p=0.01). Sahel 108 (333.8g/m^2) was the most productive followed by Nerica 4 (323.2 g/m2), Nerica 14 (237.5 g/m2) and Niamlissou (235.4g/m2). Yields were highly variable within the same variety characterized by larges standard deviations . However, the greatest variation in yields was also observed in the Sahel 108 with a minimum of 106 g/m2 and a maximum of 786 g/m2. A significant difference was noted between yields of Sahel 108 , Niamlissou and Nérica 4 . However, there was no significant difference between the yields of Nerica 14. The yield of varieties increased for Sahel 108 by 2/3, Nerica4 by twice (200 g/m2) and Niamlissou by 100 g/m2 from PT to SRI (Figure 5). The Principal Component Analysis (PCA) showed that there was a strong and positive correlation between the number of fertile tillers and yield (r=0.65) and the number of spikelets and yield (r=0.90). On the other hand, the transplanting density has a negative effect on the number of spikelets, the weight of the 1000 grains and the rice yield. This decrease in yields is correlated with high density (Table 5 and Figure 6).Based on the local people perception, the analysis of variance showed a significant difference (p=0.0207) between yields. The average SRI yields were higher than those of the traditional system with values of 2093.02 kg/ha and 1523.02kg respectively (Figure 7). The yields of Badiate and Essyl were significantly different (p<0.05). However, no significant difference (p=0.846) was observed in Fanda. Rice yields were higher in the SRI system except in Fanda where they were lower. For SRI, the higher yields were recorded in Badiate (2694 kg/ha) followed by Essyl (2589 kg/ha) and Fanda (982.5kg/ha). While in ST, the best yields were obtained in Essyl (1890 kg/ha) followed by Badiate (1206kg/ha) and Fanda (1072kg/ha) (Figure 8).

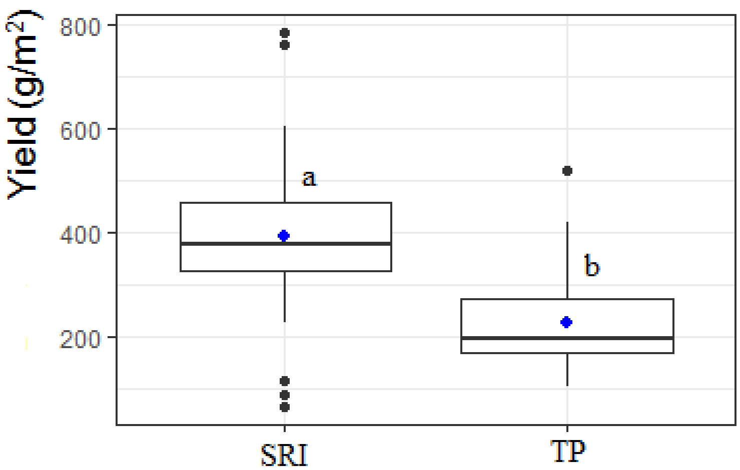

Figure 3: Variation of yields by production system

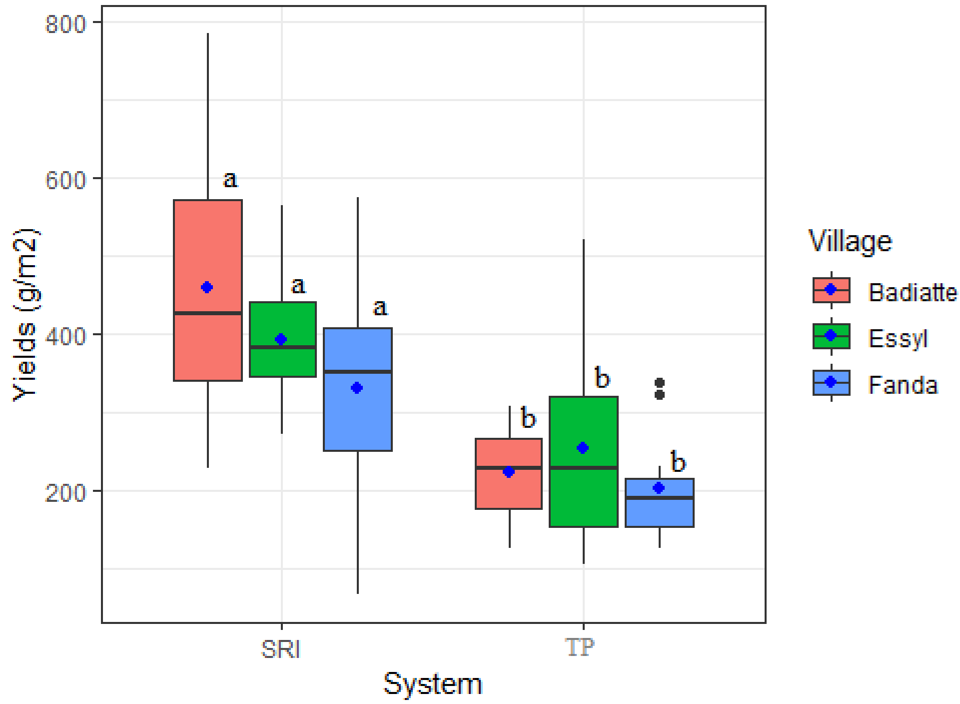

Figure 4 : Variation of yields by production system and by site

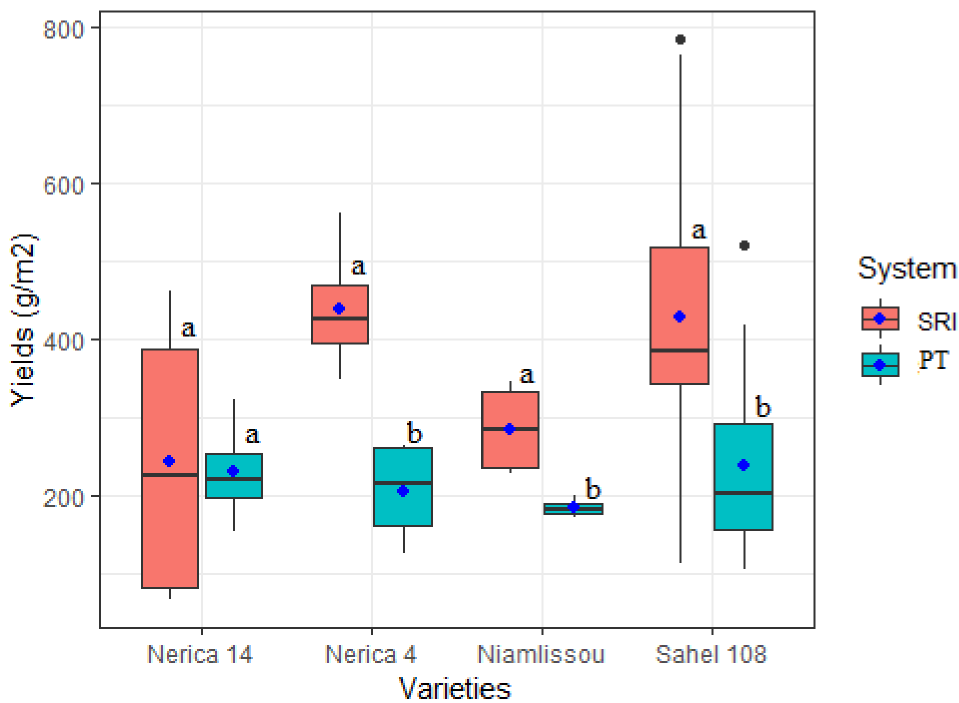

Figure 5. Variation of yields by production system and by variety

Table 5: Correlation matrix (Pearson) of yield parameters and yields

Variables	Plants density	Number of fertile tillers	Number of Spikelets	1000 grains weight (g)	Yield (g/m^2)
Plants density	**1**				
Number of fertile tillers	-0,2647	**1**			
Number of Spikelets	**-0,6303**	**0,6663**	**1**		
1000 grains weight (g)	**-0,5679**	-0,3928	0,0708	**1**	
Yield (g/m^2)	**-0,5979**	**0,6568**	**0,9072**	0,0869	**1**

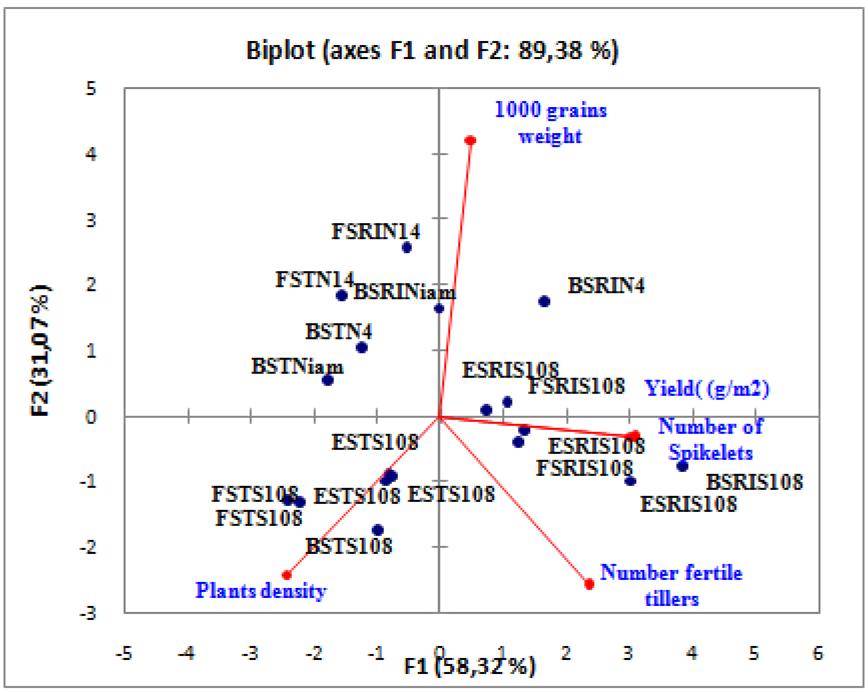

Figure 6:Relationships between yield parameters and yields

Where B=Badiate; E=Essyl; F=Fanda; SRI=System of Rice Intensification; PT=Traditional Practices; S108=Sahel 108; N4=Nerica 4; Niam=Niamlissou; N14=Nerica 14**.**

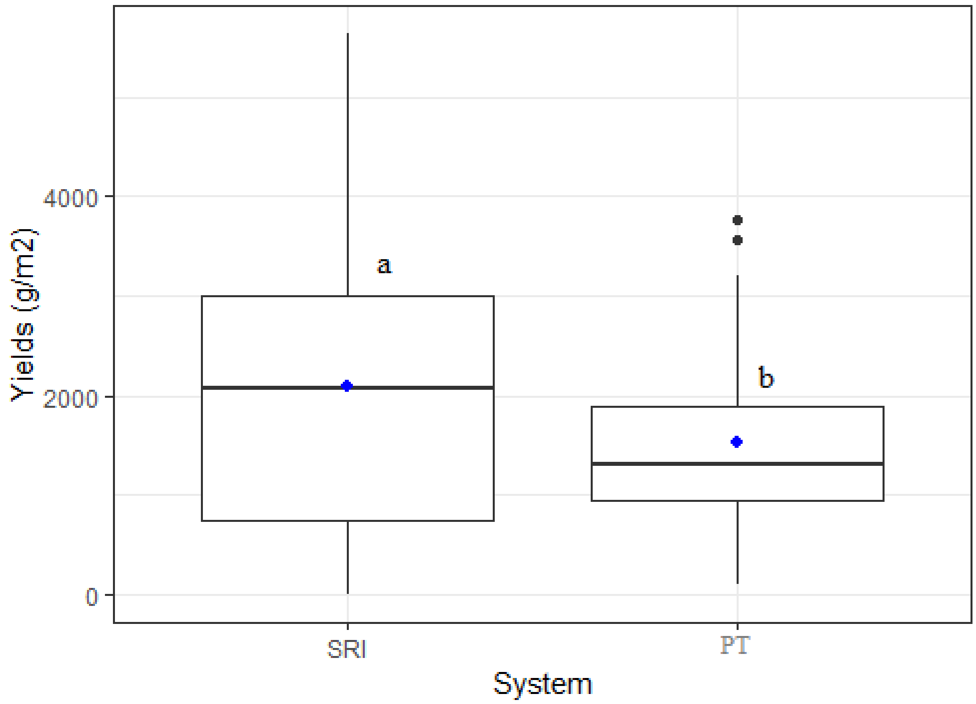

Figure7:perception on yields by production system

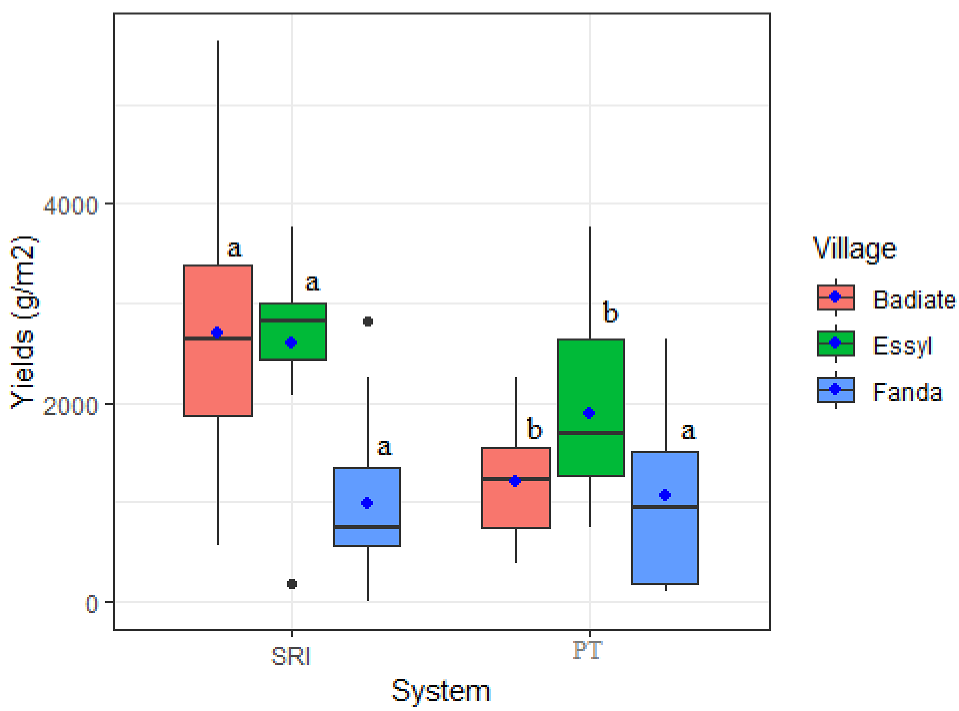

Figure 8:Perception on yields by system and by site

Economic Profitability

- **Production costs**

The total production costs of rice mainly concern labour, transplanting, harvesting, inputs (fertilizers) and other expenses such as and whatever the producers' consumption needs. The total production costs of the SRI system were higher than those of the traditional practices with total average values of 176644 and 145741 AFCF for SRI and TP respectively. The largest loads were encountered in Badiate in the SRI system. Labour and transplanting operations were those that required more work in any village except Fanda. Labour loads were higher in the SRI system in all villages (Table 6).

Table 6: Total rice production costs for SRI and TP (AFCF)

Villages	System	labour	transplanting	harvesting	Other expenses	Organic fertilizers	inorganic fertilizers	Total costs
Badiate	SRI	89176	49882	41412	17647	5965	3313	207395
	ST	50375	34500	27500	17688	4000	5498	139560
Essyl	SRI	88462	40308	23692	15077	4938	9255	181732
	ST	65800	41000	29400	11800	2990	2752	153742
Fanda	SRI	71600	52000	15200	0	980	1024	140804
	ST	52000	52000	37600	400	1920	0	143920
Mean cost	SRI	83079	47397	26768	10908	3961	4531	176644
	ST	56058	42500	31500	9963	2970	2750	145741

Economic profitability analysis

The total production costs of the SRI system are the highest. The higher the production costs, the higher the yields increased and the profits increased also except in Fanda. Rice production is more profitable in Essyl (141893 AFCF) compared to Badiate (129355 AFCF). It was in Essyl where rice cultivation was most beneficial to the population regardless of the production system, although a producer in SRI earns more than 59385 AFCF more than the one who practices TP. Economic analysis between the systems showed that SRI was eleven times more beneficial than TP in Badiate. In Fanda, rice cultivation is not profitable because of the negative Net Margin. Because the production costs were higher than the GPV and the BCR was less than one (Table 7).

Table 7: Profitability by production system

Villages	System	TC (AFCF /ha)	Yield (kg/ha)	GPV (AFCF /ha)	N M (AFCF /ha)	BCR
Badiate	SRI	207395	2694	336750	129355	1.6
	ST	139560	1206	150750	11190	1.1
Essyl	SRI	181732	2589	323625	141893	1.8
	ST	153742	1890	236250	82508	1.5
Fanda	SRI	140804	982.5	122812.5	-17991.5	0.9
	ST	143920	1072	134000	-9920	0.9
Mean	SRI	176644	2088.5	261062.5	84418.5	1.5
	ST	145741	1389.3	173662.5	27921.5	1.2

DISCUSSION

Yield parameters and Yields

The transplanting density depended on the production system. It was higher in TP than in SRI. In Burkina Faso, Sanou et al. (2016) showed that the number of plants per hill was 20 to 26% lower in SRI than the Rice Growers' Habitual Practices plots. The number of tillers was higher in the SRI at all sites and was strongly influenced by the transplanting density. Various comparative studies between SRI and conventional practices have shown similar cases in the difference in tillers production (Gani et al., 2002; Krishna et al., 2008; Bagayoko et al., 2017). Indeed, the higher the transplanting density, the more the tillering capacity of the plants increases. This would be related to the competition of plants for light and nutrients due to the high densities observed in TP. This hypothesis was confirmed by Thakur et al. (2010) who stipulated that transplanting a plant per inch as well as spacing between plants contributed to reducing competition. In addition, the growth rate of a group of tillers is itself affected by the level of competition to which the stand is subjected (Pigeaire, 1980).Rice plants in the SRI plots are much more productive in spikelets by more than 19%. Indeed, in the SRI, young plants were transplanted rather and reach the productive phase, especially at the maturation stage, contrary to what was observed in TP. And therefore almost all spikelets may be more likely to be filled before the recurrence of the rainfall deficits noted at the end of the season. Sanou et al. (2016) found a difference in the average number of grains filled per panicle in the plots of producers who have adopted the SRI and Usual practices of rice farmers in Burkina Faso. In Indonesia, Hidayati et al. (2016) also showed that all yield parameters with a higher number of grains per inch in SRIs than in conventional system plots. The results of the study showed a distinction between the yield parameters of the varieties according to the system. The Sahel 108 variety has a higher tillering capacity and a higher number of spikes followed by the Nerica 4, Niamlissou, a local variety, recorded the lowest parameters except for the weight of the 1000 grains, which is higher than that of the Sahel 108. This could explain why the low production observed in rural areas is linked to the use of low-yielding varieties. Nerica 14 has the lowest average tillering, however its grain weight is the highest. In addition, Sahel 108 has a large tillering and number of spikes. And despite its low grain weight, it has the highest average yield (333.4 g/m²). The number of tillers was one of the parameters used to distinguish rice varieties (Kouakou, 2017). The results of the study showed a distinction between system performance. There was an increase of yield (22%) in SRI compared to traditional practice (Zhao et al., 2009).

Factors influencing rice yield

Concerning cultural practices, it is noted that the level of adoption of the SRI principles has not been 100% effective. Rice practices varied from one system to another and strongly influenced yields (Bagayoko et al., 2017). Indeed, a negative correlation was noted between the duration of nursery plants and yield, and a positive correlation between organic fertilizers and yield. The duration of the plants in the nursery reduced their production capacity and had a negative influence on yield. The intermittent water management in plots allowed weeds to grow quickly because of the low water level, unlike in the traditional system where plots were practically permanently flooded. This would inhibit the development of weeds. Analysis of these factors has enabled to identify that differences between potential and actual yield were related to biophysical factors, cultural practices and socio-economic conditions, or technology transfer and diffusion (FAO, 2004). The rice practices (fertility, water, weed management, etc.) interacted to determine yield (Husson et al., 2004). The low yields in PT could be linked to late planting by farmers (Deschènes and Dubuc, 1981) but also to poor soils because there was little organic and mineral amendment. The lack of yields in the TP system was caused by the late planting (Dobelmann, 1976). The low yields were related to non-compliance with the technical itineraries and crop schedules recommended by the extension (Poussin and Boivin, 2002). The average yields of SRI in Fanda according to survey data were very low (982.5kg/ha), which clearly showed that the system did not meet the expectations of some producers. According to producers, the valley was not cultivated from 1992 to 2017 due to the conflict in the area. This could be explained by the presence of iron toxicity in producers' plots, leading to yield losses (Gnago et al., 2017). One of the main factors explaining the difference in yields would be related to transplanting and spacing between young plants because competition for nutrients reduces plant performance and therefore leads to lower yields. A case study on the physiology of SRI plants compared to conventional system showed that transplanting young plants into SRI minimizes the effect of transplant shock and competition for nutrients, water and light (Hidayati et al., 2016). Final yield results from the interaction between the characteristics of the varieties, environmental factors and conditions and cultural practices (IRRI, 1984; Hassani and Persoons, 1995).

Production cost and Profitability

Rice production was labour-intensive and also required a lot of transplanting. The village of Badiate used more labour than the other villages. However, there was not a large gap in the average labour difference between the systems. This decrease in the SRI workforce would be due to the experience of producers in transplanting young plants. Indeed, many producers in the village of Essyl have confirmed that the longer the SRI is practiced, the shorter the time required for transplanting. In Madagascar, Uphoff (2007) has shown in this dynamic that as farmers gain experience, they reduce the need for labour. The profitability of rice cultivation is determined by total production costs and yields obtained at the end of the harvest. Lower production costs and higher yields provide producers with a return on investment. Among rice production operations, labour was the most labour-intensive in all areas and therefore generated the highest costs. With regard to total production costs and yields, the survey results showed that the average cost per producer was higher in SRI than in TP. Although the SRI generated an excess of expenses, it nevertheless generated a greater benefit than the TP (Gathorne-Hardy et al., 2016).

Conclusion

Rice cultivation played an important role in local people's lives. Despite its role in food security, local production remained low due to low yields, linked on the one hand to rainfall variability and on the other hand to a lack of material resources and especially to inappropriate agricultural practices. The duration of the plants in the nursery, transplanting density, organic amendment and weeding were the main factors influencing yield. Thus, despite the high costs of implementing the SRI, it was more productive and economically profitable than TP. SRI was therefore a sustainable alternative to improving yields and increasing household incomes in a context of climate change. In the future, it would be important to repeat the study in the station in order to properly study the requirements of the SRI in terms of inputs and working time, to adapt the system to local conditions and to study the limiting factors (biotic and abiotic) related to low yields in the Fanda Valley.

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EFFECTS OF ULTRASONICATION ON ANTHOCYNANIN CONTENTS OF FRUIT JUICES

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ABSTRACT

Fruit juices are processed against microbial contamination, most commonly via thermal preservation techniques, of which reduce nutritional composition and organoleptic properties. Rising public interest in safer and more nutritional foods have increased the popularity of non-thermal methods, including ultrasonication. Ultrasound, when applied to liquid mediums like fruit juices, creates acoustic cavitation that generates bubble formation, which then will be collapsed causing thermal, mechanical and chemical impacts. This impacts of ultrasonication, make it useful in the fruit juice processing. Anthocyanins are colored pigments that are found in plants including fruits, especially berries, cherries and grapes. As a functional ingredient, anthocyanins show anti-diabetic, anti-inflammatory and antimicrobial effects, also helps preventing cancer and cardiovascular diseases. This review summarizes the effects of ultrasonication on anthocyanin contents of fruit juices.

Keywords: Ultrasound, Ultrasonication, Anthocyanin

INTRODUCTION

Unprocessed fruit juices are vulnerable to microbial contamination which adversely effects sensory and nutritional properties [9], hence preservation methods are applied. Besides being the most common and the most effective preservation techniques, thermal methods reduce organoleptic properties and freshness of juices [9]. It has been reported that anthocyanins degrade during thermal processing, because of their instability [16]. Rising public interest in safer and more nutritious foods on the other hand, have increased the popularity of non-thermal preservation techniques [2]. It has been documented that nonthermal methods have much less effect on anthocyanin degradation when compared to the thermal techniques [16]. Moreover, considering the reduction in water consumption, wastewater production, energy requirement and chemical consumption [3]; non-thermal preservation methods including ultrasound, have gained acceptance as green and innovative technologies. Ultrasound also meets the US. Food and Drug Administration's (FDA) requirement of 5-log reduction in microorganisms in fruit juices [17].

Ultrasound is the term given to the sound waves which have higher frequencies than human hearing, ranges from 20 kHz to some gigahertz level and ultrasonication [4] is the name given to the process that utilizes ultrasound. Ultrasound is categorized into two [4] as low power and high power. While low power ultrasound has intensities below 1 W/cm2 and frequencies above 100 kHz, high power ultrasound has intensities higher than 1 W/cm2 and has frequencies in between 20 kHz and 500 kHz [4]. Although high power ultrasound is used to have insight on physicochemical properties of foods, low power ultrasound is used to affect these properties instead [4]. When ultrasound is applied to a liquid medium like fruit juices, acoustic cavitation occurs, creating bubbles [4] which will be collapsed ending up thermal, mechanical and chemical impacts in the environment. Such impacts of ultrasound are convenient to be used in the field of food preservation.

Anthocyanins are colored pigments, a member of flavonoid [11] class, found in plants, being the source of red, orange [11], purple and blue colors [10]. Cyanidin-3-glucoside (Cy-3-glu) is the most common anthocyanin type that found in fruits [14].

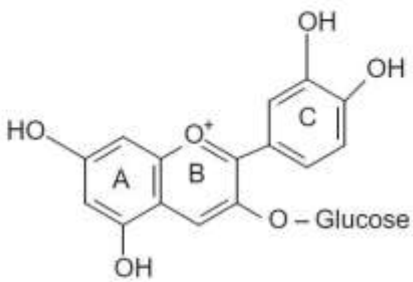

Figure-1: Molecular structure of cyanidin-3-glucoside.

As a functional ingredients [9] they show anti-diabetic, anti-inflammatory and antimicrobial effects, help preventing cancer and cardiovascular diseases, all mainly because of their anti-oxidative properties [10]. Most common anthocyanin containing fruits are berries, cherries, strawberries and grapes [11].

In this paper, the effects of ultrasonication on fruit juices in terms of their anthocyanin content was examined.

BACKGROUND

There are vast amount of studies in the literature on ultrasonication of fruit juices, such as on bayberry juice (Xiamin et. al., 2019) pomegranate juice (Alighourchi et. al., 2013), orange juice (Valtramidis et. al., 2010), strawberry juice (Tiwari et. al., 2008), blackberry juice (Tiwari et. al., 2009) and the like.

Besides generally being at a lesser degree than thermal methods [13], studies proved that anthocyanin content of fruit juices treated with ultrasound, decreases [6][8][9][13][14]. These factors affecting anthocyanin degradation are reported as; duration of ultrasonication time [13], temperature [13] and amplitude level [13]. Most studies was run at a stable temperature of 25 °C [9][16], whereas different temperatures such as 40°C [13] and 55°C [13] was also examined. Installations like heat exchangers using water, preferred to stabilize the temperatures of the samples [9][13][16]. Amplitudes tested in the studies varies between %20 [8] and %100, concentrated mostly between %40 and %100 [9][13][16][17]. Sonication durations studied in most works were between 0 and 10 minutes [9][13][16][17].

Dubrovic et. al. (2011) reported an inverse proportion between the anthocyanin content and duration of ultrasonication or temperature [13]. An example of the effects of amplitude and treatment time on total anthocyanin content are shown in Figure-2.

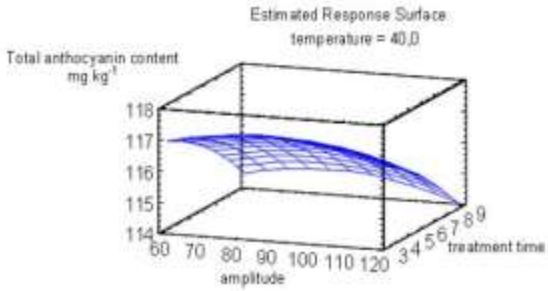

Figure-2: Response surface plots for total anthocyanin content in sonicated strawberry juice [15].

Xiamin et. al. reported no considerable differences in anthocyanin concentration at low amplitude values (90, 181, 271 W/cm^2) with treatment times under 8 minutes and also high amplitude values (362, 452 W/cm^2) with treatment times under 6 minutes [8]. Similarly, Tiwari et. al reported a high level of stability of anthocyanins against ultrasound treatment [16]. Nevertheless, while Xiamin et. al. observed increased durations cause anthocyanin degradation, up to %9.95 as shown in Figure-3 [8], Alighourchi et. al. recorded %8.41 degradation level as maximum in their research with pomegranate juice [9] and Tiwari et. al. reported a maximum decrease of %5 in blackberry juice [16].

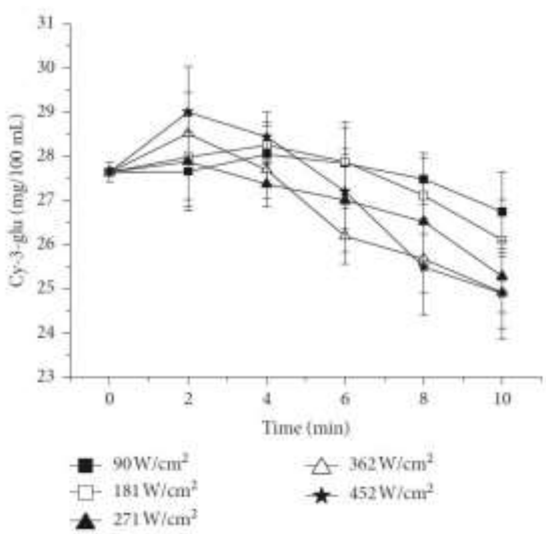

Figure-3: Effects of application time of ultrasound treatment on Cy-3-glu [8].

Anthocyanin decomposition that occurs during ultrasound treatment is caused mainly by 3 factors; (a)high temperature and pressure caused by cavitation, (b)forming of free OH^- radicals and (c)mechanical shear forces [8]. With a more precise suggestion, Yao et. al. (2016) reported that the main factor affecting anthocyanin degradation is cavitation, which makes water molecules create OH^- free radicals that deteriorate anthocyanins [14]. Kamal et. al. (2014) reported that free radicals produced by high intensity ultrasound may have undesired effects on fruit based foods [2] and similarly Jianxia et. al. (2016) observed formation of OH^- free radicals during high intensity ultrasonication of berry juices, causes degradation of anthocyanin, up to a ratio of %3.2 and lower their antioxidant activities up to %74.77 [6]. Yao et. al. reported a direct relationship between OH^- radicals and the degradation rate of anthocyanin [14]. Figure-4 shows the reduction in the clearance rate of OH^- free radicals by Cy-3-glu, depending on treatment time and ultrasonic power [14].

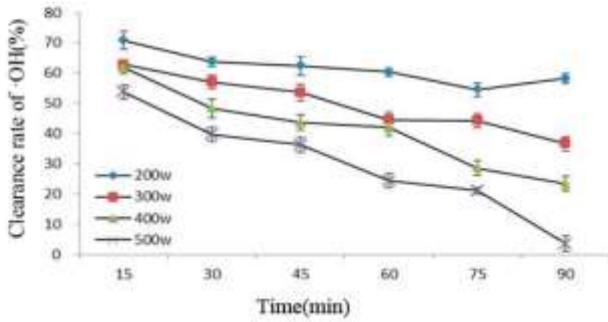

Figure-4: Correlation between clearance rate of OH and ultrasonic power and treatment time (n=3) [14].

Xiamin et. al [8], Alighourchi et. al. [9] and Tiwari et. al. separately recorded increases in anthocyanin contents, up to a level of %6.84, at especially low amplitudes and durations of ultrasonication process, and both researchers explained this happening with extraction of bound anthocyanins in fruit pulp [8]. However, Dubrovic et. al. [13] studied ultrasound on strawberry juice and did not report such an increase in anthocyanin level [13].

Tiwari et. at. (2009), Dubrovic et. al. (2011) and Espinosa et. al. (2019) used response surface methodology to design their experiments and optimize the conditions of ultrasonication. All researches noted that RSM is applicable and useful for the stated purpose.

CONCLUSION

However ultrasound causes degradation of anthocyanins in fruit juices, depending on treatment time, temperature and amplitude, recent studies showed that the degradation ratio varies between %5 and %10, a lesser degree than thermal techniques. Hence ultrasound is considered an effective preservation technique to be utilized in fruit juices.

Many studies make use of response surface methodology (RSM) to determine optimum conditions of the laboratory-scale tests to specify minimal anthocyanin degradation level. This method can also be applied in full-scale designing of ultrasonication processes to be used for fruit juice pasteurization.

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REMOVAL OF CONGO RED (CR) FROM AQUEOUS SOLUTIONS BY ADSORPTION ONTO APRICOT STONE ACTIVATED CARBON (ASAC)- THERMODYNAMIC AND KINETIC MODELING

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ABSTRACT

The preparation of activated carbon from apricot stone (ASAC) with H_3PO_4 and its ability to remove the Congo Red (CR) used in textile industry from aqueous solutions are reported in this study. The FTIR spectroscopy is used to get information on interactions between the adsorbent and CR. A series of contact time experiments were undertaken in stirred batch adsorber to assess the effect of the system variables. The results were discussed and showed that ASAC can be used in the wastewater treatment. A comparison of two models on the overall adsorption rate showed that the kinetic of adsorption was better described by the pseudo-second order model. The adsorption isotherms of CR onto ASAC are determined and correlated with common isotherms equations. The smaller RMSE values obtained for the Langmuir and Dubinin-R models indicate the better curves fitting, the monolayer adsorption capacity of CR is found to be 32.85 mg.g^{-1} at temperature 25 ^{o}C and 23.42 mg.g^{-1} at temperature 65 ^{o}C at pH 13. The thermodynamic parameters indicate the spontaneous and endothermic nature of the adsorption process. The positive value of the entropy ΔS clearly that the randomness indecreased at the solid-solution interface during the CR adsorption onto ASAC, indicating that some structural exchange may occur among the active sites of the adsorbent and the ions.

Keywords: Apricot stone, Congo Red, Kinetic, Isotherm, Adsorption, Thermodynamic

1. Introduction

The dye production plants and many other industries which utilize dyes are increasing globally by the day with advancement in technology **[1-3].** The effluents from textile, leather, food processing, dyeing, cosmetics, paper, and dye manufacturing industries are important sources of dye pollution **[4].** Many dyes and their break down products may be toxic for living organisms particularly Congo Red (CR) **[5].** Therefore, decolorizations of dyes are important aspects of wastewater treatment before discharge. It is difficult to remove the dyes from the effluent, because dyes are not easily degradable and are generally not removed from wastewater by conventional wastewater systems **[6].** Presence of colors and dyes in water gives rise to chemical oxygen demand, biochemical oxygen demand and high-suspended solids. Considering volume discharged and effluent combustion, the wastewater from the textile industry is rated as the most polluting among all industrial sectors. Their presence in water, even at very low concentrations, is highly visible and undesirable and may significantly affect photosynthetic activity in aquatic life due to reduced light penetration. As dyes are toxic to micro-organisms, stable to light irradiation and heat, they cannot be easily removed by conventional wastewater treatment processes due to their complex structure and synthetic origins. The adsorption process provides an attractive alternative, especially if the adsorbent is inexpensive and readily available. Activated carbon is the versatile adsorbent and has been used regularly for adsorption process, however, it is expensive**.** Hence, most researchers worldwide have focused on the search of new low-cost precursors especially issued from agricultural wastes such as rubber seed coat **[9]**, pecan shells **[10]**, jute fiber **[11]**, Indian rosewood sawdust **[12]**, olive stones **[13]**, pinewood **[14]**, sawdust **[15]**, coir pith **[16]**, rice husk **[17]**, bamboo **[18]**, rattan sawdust **[19],** oil palm fiber **[20]** and apricot stone **[21]**. The remarkable adsorption capacity of activated carbons is due to their well-developed porous structure and pore size distribution, as well as the surface functional groups, and it depends on the polarity, solubility, and molecular size of adsorbate, the solution pH, and the presence of other ions in solution. The apricot stone used in the present study was prepared by chemical and physical activation this study was carried out with the aim to optimize conditions such as initial dye concentration, pH, particle size, contact time, adsorbent dosage, agitation speed and temperature. Besides this, the equilibrium adsorption data were fitted to various equations to obtain constants related to the adsorption phenomena. Equilibrium and kinetic analysis were conducted to determine the factors controlling the rate of adsorption, the optimization of various parameters in dye recovery and to find out the possibility of using this material as low-cost adsorbent for dye removal.

2. Experimental

2.1. Materials and methods

Analytical grade reagents are used in all experiments. Basic dye, CR (99 %) is purchased from Merck Company. In this work, required activated carbon was prepared by a conventional method: carbonization and chemical activation with phosphoric acid as follows: Apricot stones obtained from Boumerdes region in Algeria, are air-dried, crushed and screened to obtain two fractions with geometrical mean sizes ranging from 63 to 2.5 mm. 100 g of the selected fraction are impregnated with concentrated H_3PO_4 (85 %) and dried in air. Then, it is activated in a hot air oven at 250 °C (4 h). The carbonized material is washed with distilled water to remove the free acid until the pH reaches 6.8 and dried at 105 °C. The clean biomass is mechanically ground and sifted to get a powder of different particle sizes.

2.2. Batch mode adsorption studies

The effects of the experimental parameters such as the initial CR concentration (40-100 mg.L^{-1}), pH (2-14), adsorbent dosage (1-10 g.L^{-1}), Agitation speed (100-1200 rpm) and temperature (298-338 K) on the adsorptive removal of CR ions is studied in a batch mode of operation for a specific period of contact time (0-60 min). The CR solutions are prepared by dissolving the accurate amount CR (99 %) in distilled water, used as a stock solution and diluted to the required initial concentration. pH is adjusted with HCl (0.1 mol.L^{-1}) or NaOH (0.1 mol.L^{-1}). For the kinetic studies, desired quantity of ASAC is contacted with 10 mL of CR solutions in Erlenmeyer flasks. Then, the flasks are placed on a rotary shaker at 300 rpm and the samples are taken at regular time intervals and centrifuged at 3000 rpm for 10 min. The CR content in the supernatant was measured spectrophotometrically on a Perkin Elmer UV-visible spectrophotometer model 550S at wavelength of 494 nm. The amount of CR ions adsorbed by activated carbon q_t ($mg.g^{-1}$) is calculated by using the following equation (A1):

$$q_t = \frac{(C_0 - C_e) \cdot V}{m} \qquad \textbf{(A1)}$$

Where C_o is the initial CR concentration and C_e the CR concentrations ($mg.L^{-1}$) at equilibre, V the volume of solution (L) and m the mass of the activated carbon (g). Due to the inherent bias resulting from linearization of the isotherm models, the non-linear regression Root Mean Square Error (RMSE) equation (A2), the Sun of Error Squares (SSE) equation (A3) and Chi-Squares (X^2) equation (A4) test are employed as criterion for the quality of fitting .

$$\mathbf{RMSE} = \sqrt{\frac{1}{N-2} \cdot \sum_1^N (q_{e,exp} - q_{e,cal})^2} \qquad \textbf{(A2)}$$

$$\mathbf{SSE} = \frac{1}{N} \sum_{n=1}^{\infty} (qe_{cal} - qe_{exp})^2$$

(A3)

$$\mathbf{X^2} = \sum_1^N \frac{(q_{e,exp} - q_{e,cal})^2}{q_{e,cal}}$$

(A4)

Where, $q_{e(exp)}$ ($mg.g^{-1}$) is the experimental value of uptake, $q_{e(cal)}$ the calculated value of uptake using a model ($mg.g^{-1}$), and N the number of observations in the experiment (the number of data points). The small the RMSE values, the better the curve fitting **[22]**.

3. Results and Discussion

The FTIR spectra of the adsorbent display a number of absorption peaks, indicating that many functional groups are present in the adsorbent. Peak positions are observed at 3436, 2929, 1732, 1599 and 1508 cm^{-1}. The band in the region between 3122-3680 cm^{-1} is related to the hydroxyl (-OH) groups (libber and intermolecular hydrogen band). The band at 2929 and 1508 cm^{-1} suggest the presence of ($-CH_2$) groups (symmetric and antisymetric) while the band in the region 1600-1665 cm^{-1} suggest the presence of groups (C-H, -C=C- and C=C). The peak at 1732 cm^{-1} is assigned to C=O in the carboxylic groups. These results clearly indicate that functional groups including.

3.1 Effect of analytical parameters

In the first stage of batch adsorption experiments on ASAC, the effect of particle sizes on the acid dye adsorption by ASAC is examined. Significant variations in the uptake capacity and removal efficiency were observed at different particles sizes, indicating that the best performance is obtained with lower particle sizes (315-800 μm). In general, smaller particles provide large surface area, resulting in high acid dye uptake capacity and removal efficiency. The range (315-800 μm) is subsequently used in all adsorption experiments.

Dye removal increases consistently with decreasing pH. The effect of pH on the adsorption by ASAC can be explained on the basis of the point of zero charge pH_{zpc}, for which the adsorbent surface is neutral. The surface charge of the adsorbent is positive when the medium pH is under the pH_{zpc} value and negative for pH ions is favored. The pH(zpc) of ASAC is 7.05 and the surface charge of ASAC is negative at higher pH. As the pH decreases, the number of positively charged sites increases and favours the adsorption of CR ions by electrostatic attractions. Similar experimental details have been reported by Demirbas.

The maximum uptake was obtained for a stirring speed of 300 rpm. Such moderate speed gives a good homogeneity for the mixture suspension.

The adsorption capacity of CR increases with time and attains a maximum value after 40 min and thereafter, it reaches a constant value indicating that no more CR ions are further removed from the solution. The equilibrium time works out to be 40 min. Thus changing the initial concentration of acid dye from 50 to 100 $mg.L^{-1}$, the adsorbed amount increases from 10.08 to 34.51 $mg.g^{-1}$. This may be attributed to an increase in the driving force of the concentrations gradient with increasing the initial basic dye concentration in order to overcome the mass transfer resistance of CR ions between the aqueous and solid phases.

For the first stage of batch adsorption experiments on ASAC, the effect of adsorbent dosage on the acid dye adsorption by ASAC is examined. Significant variations in the uptake capacity and removal efficiency are observed at different adsorbent dosages (1 to 10 $g.L^{-1}$) indicate that the best performance is obtained with an adsorbent dosage of 1 $g.L^{-1}$, this result was expected because the removal efficiency is generally increased by the fact that more mass available, more the contact surface offered to the adsorption. Moreover, the higher dose of adsorbent in the solution, the greater availability of exchangeable sites for the ions, i.e. more active sites are available for binding of CR ions. Our results are qualitatively in a good agreement with those found in the literatures**.** This result is subsequently used in all isotherms adsorption experiments.

3.2. Adsorption Isotherms

The shape of the isotherms is the first experimental tool to diagnose the nature of a specific adsorption phenomenon. The isotherms have been classified according to Giles et al. **[23]** in four main groups: L, S, H, and C. According to the above classification, the isotherms of ASAC at different temperatures (25 ^{o}C and 65 ^{o}C) displays L and S type curve **(Figs.1a and 1b).** The results for the different modelization are reported in **Table. 1.**

3.3. Adsorption kinetics

The kinetic study is important for the adsorption process, it describes the uptake rate of adsorbate and controls the residual time of the whole adsorption process. Two kinetic models namely the pseudo first order and pseudo second-order are selected in this study to describe the adsorption. The pseudo first order equation **[24]** is given in equation (C1):

$$\log(q_e - q_t) = \log q_e - \frac{K_1}{2.303} \cdot t$$

(C1)

The pseudo second order model **[25]** is expressed by the equation (C2):

$$\frac{t}{q_t} = \frac{1}{K_2 \cdot q_e^2} + \frac{1}{q_e} \cdot t$$

(C2)

Where q_t (mg.g^{-1}) is the amount of metal adsorbed on the adsorbent at various times t (min), K_1 the rate constant of the pseudo-first order kinetic (min^{-1}), K_2 the rate constant of the pseudo-second order kinetic (g.mg^{-1} min^{-1}).

For the pseudo-first order kinetic, the experimental data deviate greatly from linearity. This was evidenced by the low values of q_e and determination coefficients. Therefore, the pseudo-first order model is inapplicable to this system. The determination coefficient and $q_{e,cal}$ of the pseudo-second order kinetic model are in good agreement with the experimental results **(Table.2)**.

3.4. Intraparticle diffusion study

An empirically found functional relationship common to most adsorption process is that varies almost proportionally with $t^{1/2}$, the Weber-Morris plot (q_t versus $t^{1/2}$), rather than with the constant time t **[26]** equation **(D1).**

$$.q_t = K_{in}\, t^{1/2} + C$$

(D1)

Where K_{in} is the intraparticle diffusion rate constant. Values of intercept C gives an idea about the thickness of boundary layer, that is, larger, the intercept the greater is the boundary layer effect **[37].**This is attributed to the instantaneous utilization of the most readily available adsorbing sites on the adsorbent surface. The values of K_{in} and C obtained from the slope and intercept of linear plots and the constant of the Freundlich modified model and Elovich model are listed in **Table. 3.** The adsorption mechanisms and the kinetics can be described according to several kinds of models, and all of them can predict the breakthrough curves at different levels of times and accuracies.

3.5. Effect of temperature

Figure.2 clearly shows that the adsorption capacity of ASAC decrease (21.64 to 7.33 mg.g^{-1}) with increasing temperature (295 to 323 K), indicating that the adsorption is disfavored at high temperature. Thermodynamic parameters, the free energy ΔG, enthalpy ΔH and entropy ΔS are determined from the following equations **(E1)** and **(E2) [27]**.

$$\Delta G = - RT \ln K \quad \text{(E1)}$$

$$\Delta G = \Delta H - T\Delta S \quad \text{(E2)}$$

The thermodynamic equilibrium constant K for the sorption was determined by Khan and Singh **[28]** by plotting q_e/C_e versus C_e and extrapolating to zero q_e, T is the absolute temperature in Kelvin and R is the universal gas constant (8.314 J.K^{-1}mol^{-1}). The ΔH and ΔS values obtained from the slope and intercept of Von't Hoff plots of LnK versus 1/T and the ΔG values at various temperatures are summarized in **Table 4.**

4. Conclusions

This study has shown that activated carbon prepared from apricot stone can be employed as effective adsorbent for the removal of CR from aqueous solution. The Dubinin-R and Langmuir isotherms model provided a better fit of the equilibrium adsorption data one. It gave a maximum adsorption capacity of 34.51 mg.g^{-1} at temperature 25 ^{o}C which decreased up to 23.08 mg.g^{-1} at 65 ^{o}C at 13. The pseudo-second order model proved the best description of the kinetic data. The negative value of ΔG and positive value of ΔH indicate that the adsorption of CR onto ASAC is spontaneous and endothermic over the studied range of temperatures. The positive value of ΔS state clearly that the randomness increased at the solid-solution interface during the CR adsorption onto ASAC, indicating that some structural exchange may occur among the active sites of the adsorbent and the ions.

The adsorption of CR ions by ASAC follows a pseudo-second order kinetic model, which relies on the assumption that chemisorptions may be the rate-limiting step. In chemisorption, the CR ions are attached to the adsorbent surface by forming a chemical bond and tend to find sites that maximize their coordination number with the surface.

This study in tiny batch gave rise to encouraging result, and we wish to achieve the adsorption tests in column mode under the conditions applicable to the treatment of industrial effluents and the present investigation showed that ASAC is a potentially useful adsorbent for the metals, acid and basic dyes.

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BANANA BUNCHY TOP DISEASE (BBTD) SITUATION IN CAGAYAN, PHILIPPINES

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ABSTRACT

Banana is one of the most important fruit crops of the Philippines (Damasco,*et.al*.,2019). In Cagayan Valley, banana is the prime commodity under fruit crops. The production in 2019 was pulled down due to the occurrence of super typhoons in 2nd Semester of 2018 (PSA, 2019). Aside from the typhoon, the occurrence of pest and diseases especially the banana bunchy top disease (BBTD) is one of the major constraints in the production. In Cagayan province, a study was conducted to determine the occurrence and distribution of BBTD in the banana farms using the Geographic Information System (GIS). A survey and mapping were used to the major banana growing municipalities namely: Allacapan, Lasam, Gattaran and Baggao. The NAMRIA form was used to gather data to the banana farmers thru an individual interview from October to December 2020. The gathered data from the respondents were analyzed using the frequency counts, weighted means, and percentages. The geo-referenced locations of the banana farms were analyzed using the QGIS software. Results revealed that the banana growers had no management to the BBTD or commonly called "tungro". In the actual farm visits, GPS recordings and collection of BBTD samples were done from March to May 2021. The highest BBTD incidence was obtained in Gattaran with 24.9% followed by Allacapan, and Baggao with 20.77% and 6.97% respectively. With this situation, as part of the BBTD management of the project a series of trainings was immediately conducted to the selected banana farmers due to IATF protocols. The site selection and validation for the BBTD rehabilitation and processing of collected banana samples are the on-going activities of the project.

Keyword: BBTD, occurrence, BBTV, incidence, GIS, survey

Introduction

Banana (*Musa spp*) is one of the most important fruit crops of the Philippines, both as a source of local and international revenues for farmers and an important component of the daily diet of all diet of all Filipinos (Damasco,*et.al*.,2019).

In Cagayan Valley, banana is the prime commodity under fruit crops. During the first quarter of 2019, the production was pulled down by 45.48 or a shortage of 15,680 metric tons from its 2018 level of 34,481 metric tons. This is due to the occurrence of super typhoons Ompong and Rosita in 2nd Semester of 2018 (PSA, 2019).

Aside from the typhoon, other factors such as changing weather patterns, pest and diseases affect the production of banana. And one of the major constraint diseases is the banana bunchy top (BBTD).

The BBTD caused by the banana bunchy top virus (BBTV) (*Babuvirus*, *Nanoviridae*) is one of the most economically important diseases of bananas in many production regions of the world including the Philippines (Dale 1987). The virus is characterized by isometric virions of 18-

20nm with an ssDNA genome consisting of at least six components (Burns et al., 1995). Three species of aphid, *Aphis gossypii, Rhopalosiphum maidis* and *Pentalonia nigronervosa* are known to transmit the virus in a non- persistent manner through plant to plant transmission. BBTD was first recorded in Fiji in 1879 and has spread to number of countries in the south Pacific, Asia and Africa (Magee, 1927, Thomas, and Iskra-Caruana, 2000; Geering 2009a).

Symptoms of bunchy top include a narrow, upright appearance of the plant apex, and dark green streaks on the petioles, midribs and leaf veins. Infected plant produce no fruit, or reduced bunch with no market value. Bunchy top is caused by Plant to plant transmission is by the black aphid, (Magee, 1927) in a persistent manner, although the virus does not replicate in the vector (Hafner, et al 1995) Vegetative transmission occurs through rhizomes, suckers, and tissue cultured plants and is the major factor for long distance movement of the virus (Thomas et al 1994).

So far, very limited related study has been conducted regarding the occurrence, distribution and symptomatology of BBTD in banana production in Region 2, hence this study.

Objectives

1. To determine the occurrence and distribution of banana bunchy top disease (BBTD) in banana plantations in Cagayan using Geographic Information System (GIS);

2. To examine, describe, document the BBTD symptoms and detect the virus from BBTD banana plantations; and

3. To establish BBTD management.

Methodology

Site Selection- Banana areas with historical BBTD infection in the major banana growing areas in Cagayan was considered in the survey. The roving survey was conducted to ascertain per cent disease incidence in banana plantations. The specific locations for the survey were selected after consulting the Department of Agriculture and the Municipal Agriculture Office.

Baseline Data Gathering for Banana Plantation. The data was collected based on records of all reported banana growers and farmers in the municipality. The banana plantations were validated by reconnaissance survey with the aid of Global Positioning System (GPS) and a survey form developed by National Mapping and Resource Information Authority (NAMRIA). Land use (banana) systems data to be gathered were capital intensity, market orientation, labor intensity, mechanization and power usage in relation to mechanization, farm size, infrastructure requirements, cropping characteristics and cultural management practices. The occurrence of BBTD was the prime focus of the survey. The survey was dependent on the top banana growers and top banana plantations determined in baseline data gathering. The number of respondents was pre-determined based on the initial data generated.

Interview with Farmers/ Baseline Data Gathering for Banana Plantation. Information on the disease previously observed and production/cultivation techniques such as: planting materials sources, fertilization cultivation, pest management and other practices will be gathered through interview with the farmers in the municipalities.

Spatial Analysis. All data to be gathered including the geo-referenced banana locations and the qualitative data was spatially analyzed using Arc Geographic Information System software. The software was used to generate the banana map of the province.

Disease distribution and incidence of BBTD. Disease surveys and mapping was conducted on four (4) major banana farm locations in Cagayan.

For disease incidence, from each municipality, three plantations were located to cover the total banana plantations for this survey. Samples were collected from individual field to confirm the disease and numbers of infected plants were counted to calculate the percentage disease incidence. The per cent disease incidence was recorded at random in different locations by counting number of plants infected out of total number of plants observed using the formula given below:

$$\text{Per cent (PDI)} = \frac{\text{No of plants infected}}{\text{Total no. of plants observed}} \times 100$$

Results and Discussion

As regards to the socio-demographic profile of banana growers in four municipalities at Cagayan Philippines, the findings revealed that the average age of farmers were 37, 44, 47 and 49 of which farmers from Allacapan (49 years old) were the oldest followed by Gattaran (47 y/o), and the youngest were farmers from Lasam (37 years old). The farmers from Allacapan were into farming for 22 years, 20 years, 16 years and 13 years for Lasam, Gattaran and Baggao, respectively. Likewise, majority of these farmers were elementary level 32%, 36% and 39% in Allacapan, Lasam and Gattaran while in Baggao farmers were elementary graduates (30%).

Socio-demographic profile of respondents in four municipalities at Cagayan, Philippines

Table 1. Socio-demographic profile of respondents in four municipalities at Cagayan, Philippines

Municipality	Av Age	Av. years in banana farming	Educational Attainment (%)					
			Elementary Level	Elementary Graduate	High School Level	High School Graduate	College Level	College Graduate
Allacapan	49	22	32	9	18	19	12	10
Lasam	37	20	36	14	23	12	10	5
Gattaran	47	16	39	14	11	22	6	6
Baggao	44	13	12	30	24	24	3	6

Farm size (%) of Banana Growers in Four Municipalities at Cagayan Province

The farm size (%) of banana growers was shown in Figure 1. The findings showed that majority of the banana farmers in the selected four municipalities have a farm size of one hectare below. In Lasam, Cagayan, the farm size of banana growers were 80 %, followed by Baggao, Cagayan with 74 and 59.3 for Gattaran. Majority of farmers from Allacapan, Cagayan have a wider farm size (74%) ranging from 2-5 hectares. Only few farmers 8.10%, 4% and 3.70 % have 5-10 has from Allacapan, Lasam and Gattaran, respectively while 2.70% and 2% for more than 10 has at Allacapan and Baggao.

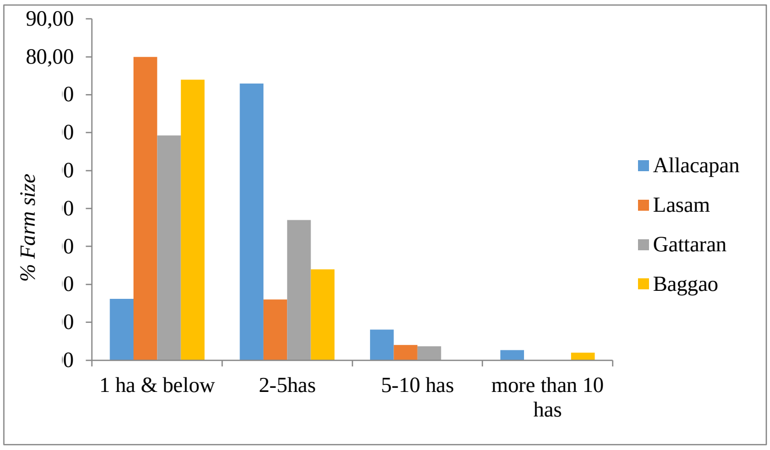

Figure 1. Farm size (%) of Banana Growers in Four Municipalities at Cagayan, Philippines

Market orientation, Capital and Labor Intensity, and Power Usage of Banana Growers.

Market Orientation of Banana Growers in Four Municipalities at Cagayan, Philippines

The market orientation of banana growers is commercial with subsistence (figure 2) wherein in Baggao obtained the highest percentage with 98% followed by Lasam with 96, Allacapan with 95 and the least is from Gattaran with 80%. This means that the banana growers sell all their harvest for income and the fruits considered reject by the traders was left and served as their subsistence and food for animals. For their subsistence, some of them processed it into vinegar, banana que, and used for animal food.

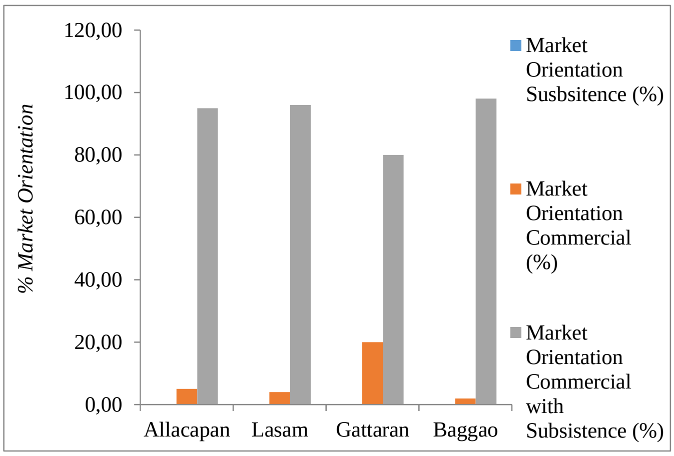

Figure 2. Market Orientation (%) of Banana Growers in Four Municipalities at Cagayan,

Table 2. Capital, Labor Intensity and Power Usage of Banana Growers in Four Municipalities at Cagayan,

Philippines

Municipality	Capital Intensity	Labor Intensity	Power Usage	Mechanization	Material Inputs
Allacapan	traditional	high	Human Labor	Non-mechanized	Low-no purchased
Lasam	traditional	medium	Human Labor	Non-mechanized	Low-no purchased
Gattaran	traditional	high	Human Labor	Non-mechanized	Low-no purchased
Baggao	traditional	high	Human Labor	Non-mechanized	Low-no purchased

The capital intensity is traditional wherein the farmers do not require high initial investment to establish their farm. The cultivation practices were done manually. Using machines or any equipment is not the practice but rather human labor. Some farmers were still practicing the " Bayanihan or ammuyo system. The material inputs used in banana farming is low-no purchased since majority of the farmers were not using fertilizers, insecticides, and any other pesticides. This is because farmers were not aware to such input needed in the banana production, only the use of weedicide.

C.Cultivation Practices of Banana Growers in Four Municipalities at Cagayan, Philippines

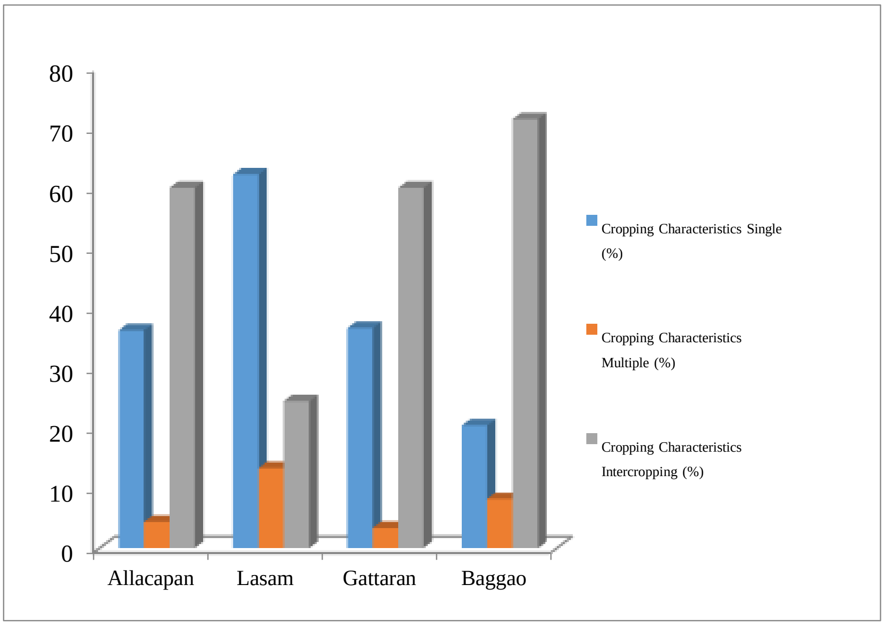

Figure 3. Cropping characteristics of Banana Growers in Four Municipalities at Cagayan, Philippines

The cropping system of the farmers is intercropping of which 71.4 % practiced in Baggao and 60 % in Gattaran and Allacapan. Majority of the farmers intercropped corn, upland rice, fruit trees and vegetables such as eggplant and sitaw. In Lasam, 62.22% practiced single cropping (solely banana).

c.2 Land Preparation

The area is prepared through the slash and burn practice of the farmers. Some farmers practiced the land clearing manually or with the application of weedicides. The preparation period usually starts from March-June of the year. Digging of holes is the common practiced of the farmers wherein its depth ranges from 1-2 feet.

c.3 Planting Practices

Majority were 95-100% farmers got their planting materials from their own field. Within the locality, only few bought or exchanged suckers from the co-farmers. For the variety planted, the mixed planting of the two varieties namely: saba or commonly known as *dippig* or *tagalog*, latundan or locally known as *murusa* or *manila* obtained the highest percentage of 72%, and 56.60% at Lasam, and Allacapan, Cagayan. It was followed by the mixed planting of saba, lakatan and latundan which obtained 43% at Baggao, Cagayan. According to the farmers, this practiced gave them the highest income compare to planting of one variety alone. Other varieties planted were bungulan and bullilising.

Table 3. Planting Practices of Banana Growers in Four Municipalities at Cagayan, Philippines

Municipality	***Planting Practices***						
	Source of Planting Materials (%)		**Variety Planted (%)**				
	Own suckers	Bought/Ex-change from co-farmer	Pure (Saba)	Pure Lakatan	Mixed (Saba & Lakatan	Mixed (Saba & Latundan	Mixed (Saba, Lakatan & Latundan)
Allacapan	98.00	2.00	15.10	3.80	9.43	56.60	15.10
Lasam	99.00	1.00	24.00	0.00	4.00	**72.00**	0.00
Gattaran	95.00	5.00	**31.90**	2.90	**40.60**	7.24	17.40
Baggao	100.00	0.00	30.00	2.27	20.45	6.82	43.00

The 4 x 4 m planting distance of banana growers in Lasam, Cagayan had the highest % of 40 followed by 30 % both in 3 x 3 and 2 x 2 m. In Allacapan, Cagayan, 7 x 7 m obtained the highest percentage of 27.27 followed by 6 x 6 m with 21.21 %, and 4 x 4 and 2 x 2 m with 15.15 %. This means that the banana growers practiced in planting distance is wider. The 5 x 5 m is the planting distance of banana growers in Gattaran, Cagayan with 24.24 % followed by 3 x 3 m with 21. 62 % and 10 x 10 m with 16.21 %. For Baggao, Cagayan, the planting distance is 3 x 3 m and 5 x 5 m with both 24%. It was followed by 10 x 10 m with 19.04% and the least is the 4 x 4 and 6 x 6 m with both 10% .

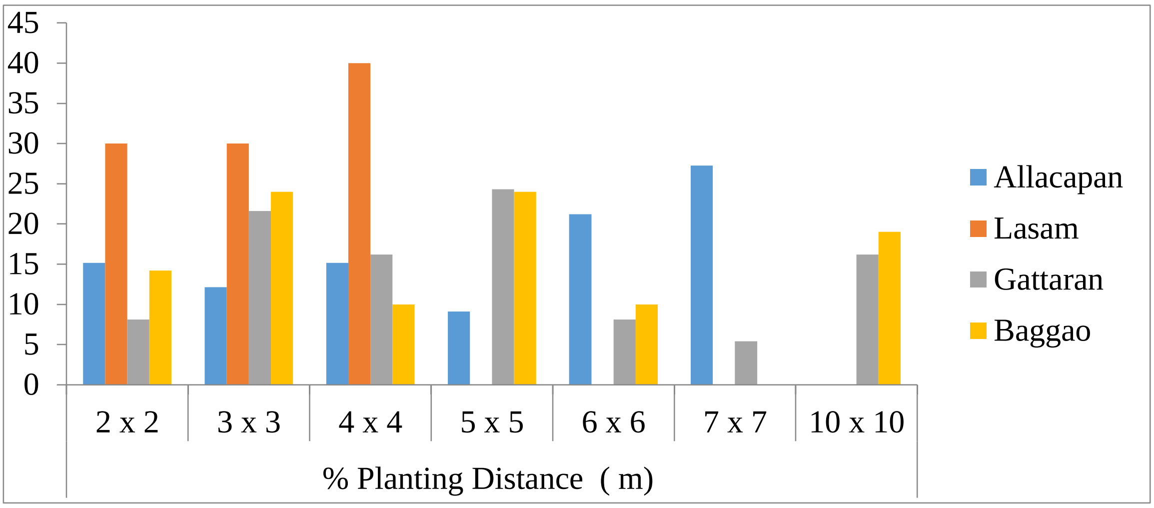

Figure 4. Planting Distance (m) of banana growers in Four Municipalities at Cagayan, Philippines.

c.4 Fertilizer Application

Farmers were not using fertilizers or no application at all. Only few farmers were using fertilizers at Gattaran and Baggao. This is done when they applied fertilizers in the intercropped corn.

c.5 Pest Management

Farmers do not have management for the insect pest. For the BBTD disease or commonly called " tungro", majority of the farmers cut the banana plants and leave it until it will decompose. The most susceptible variety of banana with this disease is the lakatan.

c.6 Harvesting and Hauling

The harvesting is usually done manually by the members of the family or if not hired laborer. The hauling is usually done by using the animal (carabao) to pull the harvested banana bunch.

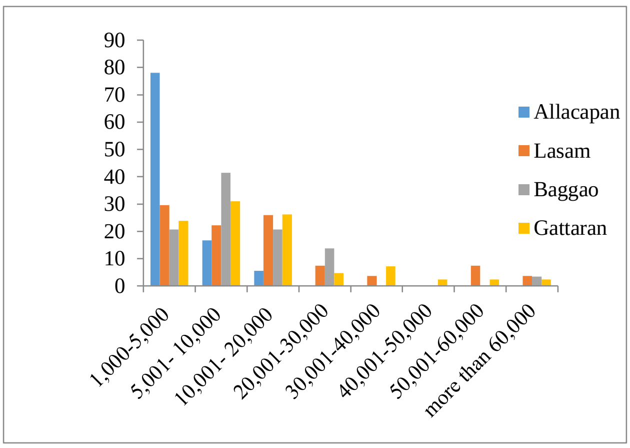

Figure 5. Yield (kg) per hectare per year of banana growers in four municipalities at

The lowest banana yield was obtained by the banana growers from Allacapan, Cagayan with 1,000-5,000 kg per hectare/year. It was observed that low yield is due to the wider planting distance (7 x 7 m) and intercropping practices by the growers. For the banana growers in Baggao, Cagayan, the highest yield was 5001- 10,000 kilograms per hectare per year. This might be due to the slightly closer plant spacing.

D. BBTD Disease Incidence

The highest incidence was observed in Gattaran with 24.9 % followed in Allacapan and Baggao with 20.77% and 6.97 %. This might be due to the planting of susceptible variety (lakatan mixed with saba) and to the none application of chemicals by the growers.

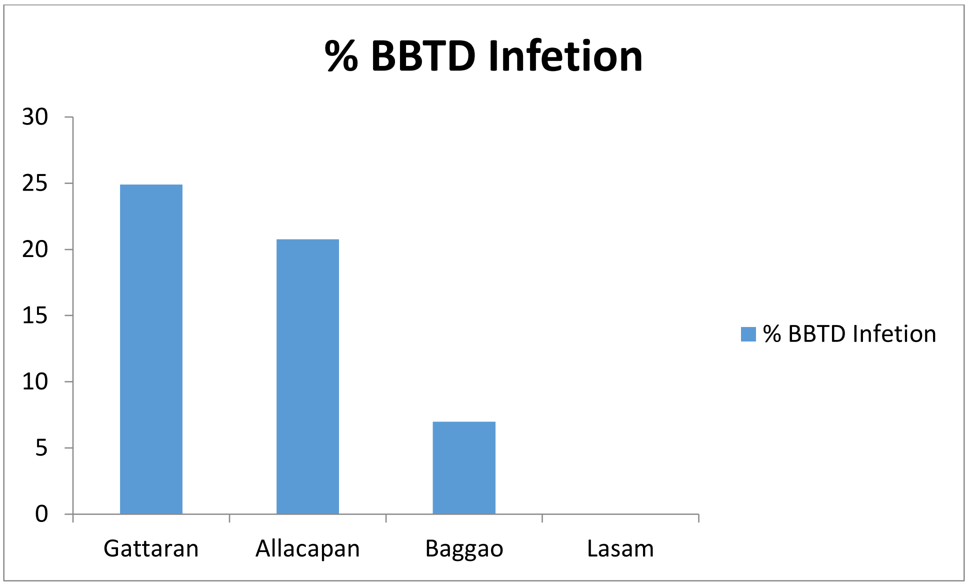

Figure 6. BBTD Incidence (%) in Four Municipalities at Cagayan, Philippines

E. Mapped Banana Farms

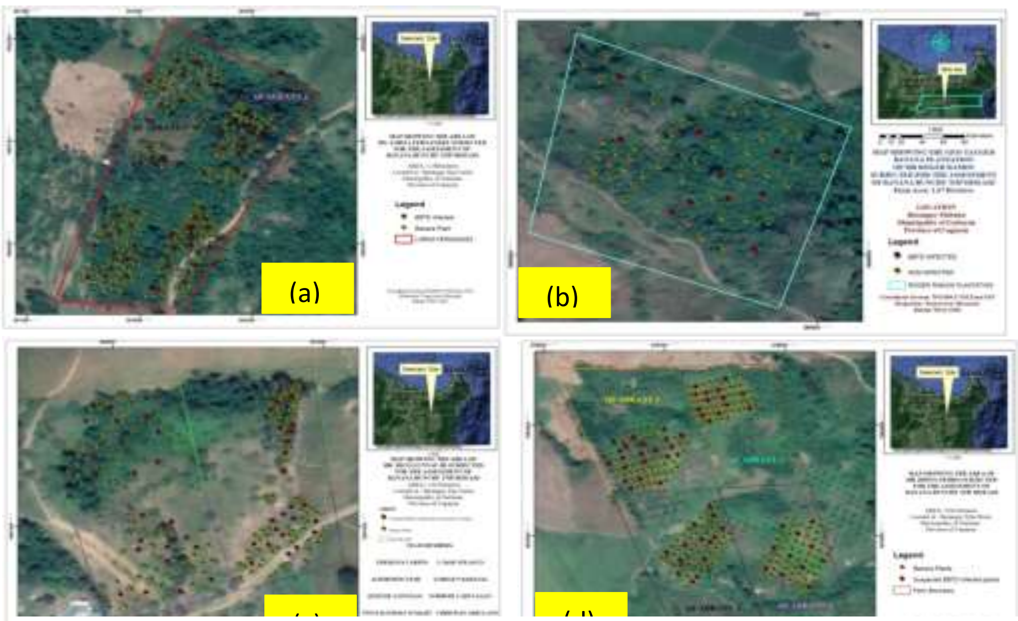

Figure 7. Map indicating the surveyed areas at Gattaran, Cagayan, Philippines (a & c) San Carlos(b) Mabuno (d) Pina Weste

The GPS recordings of banana farms in the three barangays were collected on third week of March to May 2021 using the GPS and geo-tracker app installed in the android phones. 25% of the total population of the plants in an area was the basis in tracking the sample plants. A mat that contained at least one plant with visible BBTD symptoms was considered as infected. The BBTD case of Gattaran was very visible during the conduct of mapping compare to other municipalities.

Conclusions

Based on the initial results of the study, the actual field visits, tracking of the banana plants using the GPS and geo tracker application installed in android phones and collection of banana sample plants were reliable techniques in assessing the visual symptoms of the banana bunchy top virus diseases (BBTVD). It is concluded that the areas with banana bunchy top disease (BBTD) were in the covered barangays of the project in Gattaran, Allacapan and Baggao, Cagayan, Philippines.

Recommendations

It is recommended that to control the widespread of the BBTD, all banana farmers within the covered barangays of the project should undergo in the BBTVD training its identification and management. Also, a massive application on the control of this disease should be done immediately since the banana areas were contiguous. Moreover, a follow up study (laboratory trial) would be conducted to determine the symptomatology and the type of virus presence on the banana plants infected from selected municipalities. Likewise, another study would be recommended to continue the surveillance of this disease not only to the covered municipalities of this study but to all banana farms in the province.

Acknowledgement

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EFFECTS OF GENOTYPE BY ENVIRONMENT INTERACTION ON THE YIELD AND QUALITY TRAITS OF POTATO BREEDING LINES IN THE CENTRAL ANATOLIA REGION OF TURKEY

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ABSTRACT

The current research was conducted to assess the effect of GEI on physiological, yield and quality traits of potato breeding lines in the Central Anatolia Region of Turkey. The research was laid in randomized complete block design at three locations (environments) with four replications and the data collected was analysed using AMMI software. Genotypes, location and GEI showed highly significant ($P \leq 0.01$) differences for all the physiological, yield and quality traits except stem establishment, which depict non-significant ($P>0.05$) results among the environments. The maximum plant yield, tuber yield, and marketable tuber yield was 967.03 t/ha, 41.77 t/ha and 41.60 t/ha, respectively, while the minimum was found to be 400.77 t/ha, 17.04 t/ha and 16.66 t/ha, respectively. Highly significant ($P \leq 0.01$) effects were also observed for variables such as marketable tuber weight, dry matter content and specific gravity, with ranges of 159.83 kg to 81.65 kg, 25.62% to 19.15%, and 1.106 g/cm^3 to 1.076 g/cm^3 respectively. The study classified the breeding lines into high, medium and low based on yield dry matter content and specific gravity. Among the three locations, the highest tuber yield and quality were obtained from Sivas's location. Genotype by environment interaction greatly affects the physiological, yield and quality traits of the potato breeding lines and is considered eminent in plant breeding in this era of climate change. Therefore, it is suggested that such studies should be conducted at multiple locations and years to achieve stable high yielding cultivars adapted to a wide range of environmental conditions. Moreover, some stable breeding lines identified in our study had a broad future prospective in climate change scenarios.

Keywords : AMMI, potato breeding lines, genotype by environment interaction, physiological traits, yield traits, quality traits.

INTRODUCTION

Food security is a global issue and no country has escaped the zone of food insecurity despite its level of development. It is defined as the physical, social, and economic access of sufficient, safe, and nutritious food always to meet the dietary needs and food preferences for active and healthy living (Gibson, 2012). The glaring effects of climate change behoves on plant breeders and agronomists to work assiduously to identify high yielding, and stable crop varieties to meet the food security and nutritional needs of human life. The current global population growth along with industrialization and urbanization are drastically reducing arable lands, degrading the fertile agricultural fields, diversification of agro-zones, and establishment of intra-climate modification. This make it difficult for cultivated crops to adapt and give high and stable yield (Islam and Karim, 2020). Plant breeders and agronomists need to keep pace with this trend of rise in population and climate change at all costs to sustain life through the breeding of high yielding and stable crop genotypes.

Potato (*Solanum tuberosum* L) is a vital annual tuber crop of the *Solanaceae* family which is ranked the 1st and 3rd most important tuber and food crop respectively on a global scale per human consumption (Devaux *et al.* 2014), and is cultivated in over 160 countries for its nutritional and life sustaining value. Potato is the most important non-grain crop whose global significance has caused its production to increase from 267 million metric tonnes to about 374.5 million metric tonnes since 1983, with approximately 19.25 million hectares of cultivated land area. Potato is rich in carotenoids, flavonoids, caffeic acid, Vitamin A, B6, and C, carbohydrates (Ezekiel *et al*., 2013; Premkumar, 2014) and antioxidant properties which help in digestion, heart health, blood pressure maintenance, lower risks of stroke, brain function, and nervous system coordination (Szalay, 2017). It is used in various ways such as French fries, chips, dehydrated potatoes, freshly used products, and alcohol production. The diverse golden benefits of potato, its high yield per unit area than cereals and other major crops (Miheretu *et al*., 2014; FAO, 2014) including its diverse agronomic and climatic features led to its diversified distribution in the temperate, subtropical and the Mediterranean zones from Peru (centre of origin) in the South American continent. This triggered global interest and the recommendation of the International Potato Centre (CIP), the Food and Agriculture Organization of the United Nations (FAO), and food processing industries to take a keen interest and acting as the major driving force behind the growth of the potato cultivation and market (Floros *et al*., 2010), as food security, poverty alleviation, and global health improvement crop (FAO, 2017). This has projected the crop average production growth rate (CAPGR) at 1.06% during the 2019-2024 forecast period (USDA 2015; FAO 2017) with competing production efforts among nations including Turkey in recent times which increase potato demand and consumption.

The rise in population along with climate change has diversified agro-ecological zones in the world which affect the biological and physiological yield performance of crops (Raza *et al*., 2019) making adaptation very difficult for crops (Adavi *et al*., 2018; Onyango, 2019) due to differences in traits, resistance and /or susceptibility (Dube *et al*., 2016; Di Vittorio *et al*., 2016; Singh and Singh, 2017). Upon climate change, previously cultivated fields behave and present themselves as different agro-zones (FAO, 2017) and so affect crop adaptation to the different agro-ecological environments (Nyahunda and Tirivangasi, 2019). Anthropogenic activities including other biotic and abiotic stresses induce soil nutrient depletion hinder the progress of potato breeders, as the energy and efforts of potato breeders or farmers do not reflect the yield output and so mitigating against the full realization of potato yield and production to meet market demand (Kang *et al*., 2004; Voss-Fels *et al*., 2019).

It is worthwhile for plant breeders to keep pace with these effects through the sustainability of agriculture (Lammerts van Bueren *et al*., 2018) to identify strategies to breach the production gap and the realization of the potentials of potato to feed the ever-rising global population. Agronomist and potato breeders in their role to feed the world has employed genotype by environment interaction and stability analysis in their breeding programs as a mechanism to produce new potato cultivars suitable for the diverse agro-ecological conditions, adaptation, and yield stability levels created by climate change and to meet global consumer demand and preference (Kivuva *et al*., 2014; Aliche *et al*., 2018).

Genotype by environment interaction (GEI) is a multifactorial phenomenon that leads to the differential phenotypic expression of genotypes qualitatively and quantitatively because of different environmental parameters and nutrient accessibilities (Kivuva *et al*., 2014). The extent of response of a genotype to environmental fluctuation defines the genotype as wide or specific adaptation, and the resilience of the genotype against environmental fluctuation defines its stability. This phenomenon is a fundamental principle in all fields of agriculture in the identification of desired, suitable, and stable genotypes by reducing the association between phenotypic and genotypic values and causing a natural selection of living organisms from one environment to another. GEI has been employed in several crop breeding programmes to facilitate selection and cultivar certification which brings about suitable crop production, adaptation (Raymundo *et al*., 2018; Ngailo *et al*., 2019), release and provision of the right cultivar and thus the study of GEI is never out of breeding programmes. Though this delay certification processes (Dwivedi *et al*., 2019; Rono *et al*., 2016), breeders can identify superior cultivars, and the best environments for the crop cultivation (Kang and Gauch, 1996). This has necessitated this research on potato in Turkey over diverse environmental locations to identify potato breeding lines with broad (general) and specific adaptability before registration as a new cultivar.

The analysis of GEI and stability parameters have been made feasible by the development of several statistical tools and models which have been employed globally to analyse several crops including potato either singly, jointly or in comparison with other models and tools. These evaluate and estimate the interaction and relationship of crop genotype and environment (Hongyu *et al*., 2014) through regression coefficient bi (Finlay and Wilkinson, 1963), the sum of squared deviations from regression S^2di (Eberhart and Russell, 1966), stability variance σ^2 (Shukla, 1972), coefficient of determination and coefficient of variability (Francis and Kannenberg, 1978) and stability parameters of α' and λ (Tai, 1971). These models include; general linear model (GLM) procedure of SAS software, bilinear models (AMMI and GGE), GENSTAT software among others to perform principal component analysis, ANOVA, regression on the mean, and factorial regression models for the establishment of adaptability and stability analysis of GEI.

This study was designed to investigate the effect of genotype by environment interaction on the yield and quality traits of potato breeding lines in the central Anatolia Region of Turkey using AMMI model. In this research, a combined general ANOVA and AMMI ANOVA was performed for all the traits and their relationships through principal component analysis eigenvalue method and spearman's correlation analysis. While Additive Main Effects and Multiplicative Interaction (AMMI) Analysis ANOVA and biplot was carried out for tuber yield (total tuber yield, marketable tuber yield) and quality traits (marketable tuber weight, dry matter concentration and specific gravity).

2 Material and method

2.1. Plants materials

Fifteen (15) potato genotypes consisting of twelve (12) breeding lines developed at the Agricultural Genetic Engineering Department, Niğde Ömer Halisdemir University, and three (3) standard check cultivars (Table 1), were used in the study. These were tested for yield and quality performances in three different environmental locations in the Central Anatolia Region; Niğde, Konya, and Sivas, of Turkey in 2019.

2.2. Site selection and location

The research was conducted in three different provinces (Niğde, Konya and Sivas) in 2019 in the Central Anatolia Region of Turkey. These provinces were selected based on their similar crop growing seasons from April to October. The monthly temperature and rainfall of the study site during the research period are presented in Table 2.

2.3. Experimental design and Agronomic Management

The field experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications in all three locations. Each plot was consisted of two rows of 8.1 m in length and 0.75 m apart. The potato seed tubers were planted on 05/05/2019, 16/05/2019, and 20/05/2019 respectively in Konya, Nigde, and Sivas using a two-row planter with 30cm in-row spacing and planting distance and planting density of 54 tuber seeds per plot (27 tubers per row). In Sivas and Konya locations the plots were fertilized with 150 kg/ha of 15–15–15 N-P-K fertilizer before planting and an additional 250 kg/ha N (as urea) was side-dressed during tuber initiation and bulking stages while in Niğde, 100 kg/ha of 15–15–15 N-P-K fertilizer and 200 kg/ha urea was applied before planting and during tuber initiation and bulking stages. The seed potato was treated with a fungicide (active ingredient: 100 g/l Penflufen+18 g/l Prothioconazole) at 20 23 ml/100 kg seed rate and an insecticide (active ingredient: 350g Thiamethoxam) at 20 ml/100 kg seed rate before planting. The pre-sowing herbicide (active ingredient: 70% Metribuzin) was applied to soil at the rate of 750 g/ha, and the plots were maintained as weed-free by hand weeding during growing period. The plots were irrigated with overhead sprinklers when needed in all locations.

The harvesting was done on 09/10/2019 in Nigde, on 11/10/2019 in Konya and on 15/10/2019 in Sivas location using a two-row harvest machine. The harvested potato tubers were collected in bags per plot and labelled with the block and plot number for yield and quality analysis in the Faculty of Agricultural Sciences and Technologies Laboratory.

2.4. Data Collection

Stand establishment (%): The number of plants in each plot was counted at the flowering stage and the emergence rate was calculated as by dividing the plants germinated over the number of seed tuber sowed multiplied by hundred. Plant height (cm): The heights of ten randomly selected plants for each plot were measured at the flowering stage using measuring tape at ±1 cm accuracy and their mean calculated. Number of stems per plant: The number of main stems per plant was counted on ten randomly selected plants in each plot at the flowering stage and their mean number computed. Total tuber yield (t/ha): Total yield of each genotype was calculated by counting and weighing of all tubers in each plot. Marketable tuber yields (t/ha): The marketable sizes of the tubers (>30 mm) were calculated using total yield and percentage of marketable tubers. Number of tubers per plant: All tubers in each plot were counted and the number of tubers per plant was calculated by dividing the total tuber number with the number of plants per plot (stand establishment). Mean tuber weight (g): Mean tuber weight was calculated by dividing the total tuber weight of each plot by the number of tubers of each plot. Dry matter content (%) and specific gravity analysis. The dry matter content and the specific gravity of the potato tubers was measured by digital hydrometer PW2050 (Martin Lishman). Two kilograms of potato tubers were weighed in air and then in water to obtained displaced dry weight and the specific gravity of the tubers using Martin Lishman protocols.

2.5. **Data Analysis**

2.5.1. Analysis of Variance

The data for all the traits were analysed using the General Linear Model (GLM) procedure of SAS software (SAS Institute, Cary, N.C., United States) to examine genotype, environment, and G x E interaction effects by analysis of variance. Statistix statistical software (version 8.1) was used to perform the mean comparison test of Duncan's Multiple Range Tests at a 5% level of significance where the genotype effect was considered as fixed and the environmental effects as random. Correlation analysis was also performed to assess the interrelationships among the various traits.

2.5.2 AMMI model

The genotype and environment interaction on the yield, dry matter concentration and specific gravity was accessed using AMMISOFT statistical software following the protocol of Gauch and Moran, 2019. This model operates on two analytical principles: the variance and singular value decomposition principles on one component (principal component analysis), and the additive component for the genotype main effects (gi), environmental effects (ej), and multiplicative for the interaction effect (ge)ij as elaborated by Gauch et al. (2011), and Gauch (2013) based on (Gauch, 1992) model:

Yijr = μ + gi + ej + br(ej) + Xn k=1 λkαikγjk + ρij + εij (1)

Where; Yijr is the phenotypic trait of genotype i in environment j for replicate r, μ is the grand mean, gi is the genotype main effects as deviations from μ, ej is the environment main effects as deviations from μ, λk is the singular value for the Interaction Principal Component (IPC) axis k, αik, and γjk are the genotypes and environment IPC scores (i.e. the left and right singular vectors) for axis k, bi(ej) is the effect of the replication r within the environment j, r is the number of replications, ρij is the residual containing all multiplicative terms not included in the model (1); n is the number of axes or principal components (PC) retained by the model, and εijr is the experimental error.

3. Results

3.1. Analysis of Variance of the studied traits

The results of the combined ANOVA revealed very high significant ($P \leq 0.001$) effects of genotype, environment, and genotype by environment interaction on the PH, NSP, TPY, TTY, MTY, MTW, DMC, and SG with a CV of 5.4, 9.4, 11.3, 3.3, 3.87, 3.06, 1.89 and 0.19 respectively (Table 3). Also, there were very high significant differences For the stem establishment, there was significant ($P \leq 0.01$) and ($P \leq 0.05$) differences among the genotypes, and G x E respectively, however, there was no significant differences among the three environments. The combine AMMI ANOVA also revealed highly significant ($P \leq 0.001$) effects of the treatments, genotypes, environments and the GEI on the PH, NSP, TPY, TTY, MTY, NTP, DMC, and SG (Table 4). For the SE, there was a significant ($P \leq 0.05$) effect of the treatment, genotype and G x E but no-significant effect of the environment. In addition, the AMMI ANOVA also revealed high significant ($P \leq 0.001$) effects of the IPC1 on the PH, NSP, TPY, TTY, MTY, NTP, DMC, and SG, and a significant ($P<0.05$) effect on the SE. Table 4 showed that the residual have no-significant ($P>0.05$) effect on the SE, but it had significant ($P<0.05$) effect on the TPY, and a high significant ($P \leq 0.001$) effect on the PH, NSP, TTY, MTY, NTP, DMC and SG. Furthermore, for the Block/environment interaction there was significant ($P<0.05$) effect on the PH, NSP, TTY, and MTY but the there was no-significant ($P>0.05$) difference on the SE, TPY, MTW, DMC and SG (Table 4).

3.2. AMMI Analysis of Tuber Yield and Tuber Quality

The results of the AMMI ANOVA on the tuber yield; total tuber yield (TTY) and Marketable tuber yield (MTY) revealed that there were highly significant ($p < 0.001$) effects of the treatment, Genotypes, Environments, Genotype by Environment Interaction, IPC1 and Residual on the potato tuber yield (Table 5 and Table 6). There was also significant ($P<0.05$) effect of the Block/Env on the potato yield (Table 5 and Table 6). The CV among the genotypes and the environments of was found to be 3.3 and 3.8 respectively for TTY and MTY. The treatment explained 99.08% and 94.75% of the total sum of squares (TSS) respectively for TTY and MTY. For the TTY, genotype accounted for 31.6 %, environment accounted for 52.9 and GEI accounted for 15.5% of the treatment sums of squares (TRT) explained (Table 5), while for the MTY, genotypes accounted for 28.58%, environment accounted for 52.49%, and GEI accounted for 15.5% of the treatment sums of squares explained (Table 6). Of the GEI explained IPC1 accounted 77.44% while residual accounted for 22.56% for the TTY (Table 5); whereas for the MTY, IPC1 accounted for 77.62% while residual accounting for 22.38% (Table 6).

For the tuber quality; marketable tuber weight (MTW), dry matter content (DMC) and specific gravity (SG), the AMMI ANOVA revealed highly significant ($p < 0.001$) effects of the treatment, genotypes, environments, GEI, IPC1, and residual on the MTW, DMC and SG (Table 7, Table 8 and Table 9) while Block/env had no-significant ($P>0.05$) effects on the MTW, DMC and SG. Of the total sum of squares, the treatments explained 94.75%, 93.96%, and 93.16% respectively for MTW, DMC and SG. Of the treatment sums of squares explained, genotypes accounted for 50.53%, 51.95%, 51.95% for MTW, DMC and SG respectively, whiles environment accounted for 25.46%, 39.41%, and 38.83% respectively for MTW, DMC and SG respectively. More so, GEI accounted for 24.02%, 8.65% and 9.71% respectively for MTW, DMC and SG of the treatment sum of squares explained. In addition, the IPC1 accounted for 70.8%, 75.53%, 50% respectively for the MTW, DMC and SG of the GEI explained while the residual accounted for 29.2%, 24.47% and 50% respectively for the MTW, DMC and SG.

3.3. Principal component analysis and AMMI Biplots: Genotype and Environment Classification of variables

The number of PCA to retain of the AMMI biplot was determined by using the scree plot based on the eigenvalues. The PCA with an eigenvalue greater than or equal to 1 had a significant effect on the GEI and so was retained whilst those with eigenvalue less than 1 was discarded. The first four PCA (F1, F2, F3, and F4) had their eigenvalues greater than 1 which had a cumulative variability of 85.63% (Figure 1). However, to avoid disturbance and complication in the analysis, the first two interaction principal component axes (IPCA1 and IPCA2) were used to construct the biplot for the PCA analysis to evaluate the relationships between the traits and their environmental adaptations (Figure 2). The IPCA1 and IPCA2 explained 64.72% of total GEI, were used. The degree of GEI was proportional to the length of the vector of an environment from the biplot origin. Thus, the environments with longer vectors indicated strong forces of interaction whilst the environments with shorter vectors indicated weak forces of interaction. The PCA results of the genotypes by environment interaction effects on the traits show that number of tubers per plant (NTP), marketable tuber yield (MTY), total plant yield (TPY), dry matter concentration (DMC), and specific gravity (SG) moved in the same direction and so exhibited positive correlations. They also had longer vectored away from the PCA origin which depicted that they had very high GEI. There was almost a 100% positive correlation between DMC and SG, and between MTY and TTY. N11, S1, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, and S14 are observed to cluster around the same quadrant of the PCA axis which indicated that they had the same response in similar environmental conditions for NTP, MTY, TPY, TTY, DMC, and SG. This suggested that Sivas' location was the best environment for TTY, TPY. MTY, NTP, SG, and DMC. This confirmed the Spearman’s correlation analysis (Table 10).

Genotype K1, K2, K5, K9, K10, K11, K12, K14, N12, and S2 were also clustered in the same quadrant of PCA axis which also indicated that they correlate positively in the same environmental conditions for MTW, PH, and BTN. The genotypes in this quadrant had yield above average for MTW, PH, and BTN and thus were moderately tolerant. This depicted that Konya environment was the best for PH, BTN, and MTW. On the contrarily, genotypes; N14, N15, N13, N4, N8, N10, N7, N1, S15, and K8 had similar field behaviour as they were clustered in the quadrant and were positively and strongly correlated with SE, STN, and MTN. Nigde environment was depicted to found in this quadrant which was moderately susceptible to unfavourable environmental conditions. It also had high GEI. Among all the variables, only NSP had less GEI though it was very susceptible to unfavourable environmental conditions. Genotype N3, N2, N5, N6, N9, K3, K6, K7, K3, K4, and K13 were segregated to be susceptible to unfavourable environmental fluctuations.

The AMMI IPC1 biplot against mean TTY group the genotypes into general stability or specific stability, and high or low yielding genotypes. The degree of adaptability of a genotype to the environment is determined by the distance of the genotype IPC1 scores to the IPC1 axis. In, Figure 3, AGRIA, MEÇ1402.09, MEÇ1402.07, MEÇ1411.06 are stable genotypes and broadly adapted to a wide range of environments with only MEÇ1411.06 high yielding while MACAR1406.04 and MACAR1406.07 are low yielding and very much interactive with favourable environmental conditions. On the other hand, MACAR1409.09, MEÇ1405.06, MEÇ1407.08, and MEÇ1407.05 are high yielding genotypes and highly interactive with favourable environmental conditions. Furthermore, MACAR1402.11 and Russet Burbank are low yielding genotypes and highly sensitive to unfavourable environmental conditions while MACAR1402.10, MEÇ1407.17, and MADELEINE are high yielding and highly interactive to adverse environmental fluctuations. The environments are widely interactive more than the genotypes with Sivas being the most interactive and best environment while Nigde and Konya being very much sensitive (Figure 3)

The AMMI biplot analysis of the genotype for MTY (Figure 4) showed that AGRIA, MEÇ1402.09, MEÇ1402.07, and MEÇ1411.06 had general adaptation regardless of the environmental influences which are like the genotype characterization in Figure 4.11. Genotype partitioned closed to each other had a positive correlation, thus the genotypes in this biplot were generally segregated into three groups; Russet Burbank and MACAR1402.11 forming one group, AGRIA, MEÇ1402.09, MEÇ1402.07, MACAR1406.07, and MACAR1406.04 forming one group, and MACAR1402.10, MADELEINE, MEÇ1411.06, MEÇ1407.17, MEÇ1407.08, MEÇ1407.05 MACAR1409.09, and MEÇ1405.06 also forming one group. The environments, on the other hand, were scattered and relatively forming two groups: Nigde (NGDE) and Konya (KNYA) on one site and Sivas (SVAS) on the other site. This similarity in the grouping of genotypes may be associated with genetic similarities between cultivars and the similarity in the environment is predictively due to environmental inputs. The Nigde (NGDE) environment was relatively stable while the Konya (KNYA) and Sivas (SVAS) environments were highly unstable for marketable tuber yields for the genotype.

The AMMI biplot analysis of the potato tuber weight (Figure 5) showed that MACAR1406.07 had a large positive IPC1 value and low weight tubers below the grand weight of the genotypes and so was moderately susceptible to environmental conditions. Russet Burbank, AGRIA, MACAR1402.10, MEÇ1407.08, MEÇ1402.09, and MEÇ1405.06 produced low weighted tubers and had large negative IPC1 values, making them susceptible and highly interactive to environmental condition and thus unstable. MACAR1406.04, MACAR1402.11, MADELEINE, and MACAR1409.09 produce above-average weighted tubers, with high IPC1 scores, making them unstable due to their high interaction with the environments. On the other hand, MEÇ1402.07, MEÇ1411.06, MEÇ1407.05, and MEÇ1407.17 had high IPC1 scores which depicted high GEI and unstable in tuber weight in unfavourable environmental conditions.

The AMMI biplot analysis (Figure 6) also revealed that MACAR1406.07, MACAR1409.09, MEÇ1402.09, MEÇ1405.06, and MEÇ1411.06 had high DMC and were less influenced by environmental conditions due to their small IPC1 score making them generally stable whilst MACAR1402.11, MACAR1406.04, MEÇ1402.07, MEÇ1407.05, and MADELEINE had low DMC below the grand mean but generally stable due to their small IPC1 scores. Contrarily, MACAR1402.10, MEÇ1407.08, and MEÇ1407.17 on the other hand had very high DMC and high IPC1 scores which is an indication of high GEI making them unstable whereas AGRIA and RUSSET BURBANK were very susceptible to environmental condition and so highly unstable for DMC and so have specific adaptability. The environments were grouped in to two mega environments which are highly heterogeneous and unstable with large IPC1 values for DMC. Konya and Sivas were positively correlated and produced high DMC whilst they were negatively correlated with Nigde environment. Konya environment was ideal for majority of the genotypes in terms of DMC.

The AMMI biplot of SG (Figure 7) revealed that MACAR1406.07, MACAR1409.09, MEÇ1402.09, MEÇ1405.06, and MEÇ1411.06 had high SG with less interaction with environmental influences making them generally stable whilst MACAR1402.11, MACAR1406.04, MEÇ1402.07, MEÇ1407.05, and MADELEINE had low SG and generally stable. Contrarily, MACAR1402.10, MEÇ1407.08, and MEÇ1407.17 on the other hand, had very high values of SG and high IPC1 scores indicating a high GEI making them unstable whereas AGRIA and Russet Burbank were very susceptible to environmental condition and so highly unstable for SG and so had specific adaptability.

Table 1.

SN	Code	GENOTYPE	Pedigrees (♀ x ♂)
1	MCR1	MACAR1402.10	White Lady x W870
2	MCR2	MACAR1402.11	White Lady x W870
3	MCR3	MACAR1406.04	01.509 x Latona
4	MCR4	MACAR1406.07	01.509 x Latona
5	MCR5	MACAR1409.09	99.463 x Kolibri
6	MEÇ1	MEÇ1402.07	Alegria x Lindita
7	MEC2	MEÇ1402.09	Alegria x Lindita
8	MEC3	MEÇ1405.06	(Alegria x Challenger) x Borwina
9	MEC4	MEÇ1407.05	(GalaxChallenger) x (Allegria)
10	MEC5	MEÇ1407.08	(GalaxChallenger) x (Allegria)
11	MEC6	MEÇ1407.17	(GalaxChallenger) x (Allegria)
12	MEC7	MEÇ1411.06	Ke-11 x Jelly
13	AGRA	AGRIA	Quarta x Semlo
14	MADA	MADELEINE	Leyla x KO 85-1002
15	RUBB	RUSSET BURBANK	Mutated Burbank (no specific parent)

Environment								
SN	Code	Environment	SN	Code	Environment	SN	Code	Environment
1	NGDE	Nigde	2	SVAS	Sivas	3	KNYA	Konya

Table 2.

	Locations	May	June	July	August	September	October
Mean Temperature (ºC) (1981-2010)	Konya	15.7	20.4	23.6	23.4	18.9	12.7
	Niğde	15.3	19.7	22.9	22.7	18.2	12.4
	Sivas	13.6	17.4	20.4	20.5	16.5	11
Mean Temperature (ºC) -2019	Konya	19.7	23	24.3	24.8	21	17.3
	Niğde	17.6	21.3	22.2	22.6	18.2	15.1
	Sivas	16	20.6	19.8	20.8	?	?
Rainfall (mm) (1981-2010)	Konya	38.6	20.5	7.8	5.6	11.3	29.7
	Niğde	46.4	24.5	5.7	5.3	7.6	30.4
	Sivas	58.8	34.8	10.3	5.9	16.7	41
Rainfall (mm) -2019	Konya	5.4	31.8	8.2	2.0	10.2	6.4
	Niğde	26.2	43.4	8.3	6.0	6.0	13.7
	Sivas	18.8	48.4	18.6	6.0	?	?

Location	Coordinates	Elevation above sea level
Konya	37.8746° N, 32.4932° E	1029m
Niğde	37.9698° N, 34.6766° E	1244m
Sivas	39°45′N 37°01′E	1,278 m

Source: Turkish State Meteorological Service

Table 3: Combined ANOVA for studied traits of 15 potato genotypes evaluated in three different environments.

Source of variation	DF	Total Sum of Squares								
		SE	PH	NSP	TPY	TTY	MTY	MTW	DMC	SG
G	14	1287.56 **	5786.9 ***	80.746 ***	3970464 ***	7986 ***	8053.9 ***	81636 ***	483.443 ***	0.0105 ***
E	2	145.6 ns	36638.1 ***	37.595 ***	8750810 ***	13368.2 ***	13215.3 ***	38730 ***	366.738 ***	0.0083 ***
G x E	28	1880.54 *	5171.2 ***	95.583 ***	1476318 ***	3915.9 ***	3907.1 ***	32452 ***	80.457 ***	0.0018 ***
Error	135	5132.19	1680.9	27.575	959436	192.4	197.1	1895	23.413	0.0006
Total	179	8445.89	49277.2	241.5	1.52E+07	25462.5	25373	154713	954.052	0.0212
CV		6.6	5.4	9.4	11.3	3.3	3.87	3.06	1.89	0.19

DF, degree of freedom; SE, stem establishment; PH, plant height; NSP, number of stem per plant; TPY, total plant yield; TTY, total tuber yield; MTY, marketable tuber yield; MTW, marketable tuber weight; DMC, dry matter concentration; SG, specific gravity; G, genotype; E, environment; G x E, genotype by environment interaction; ns not significant, *Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$; ***Significant at $P \leq 0.001$.

Table 4: Combined AMMI ANOVA for studied traits of 15 potato genotypes evaluated in three different environments

Source	DF	Mean squares									
		SE	PH	NSP	TPY	TTY	MTY	NTP	MTW	DMC	SG
Total	179	47.2	275.3	1.345	84651.6	142.2	141.751	4.06	895	5.3299	0.0001
Treatment	44	75.33 **	1081.6 ***	4.85 ***	304587 ***	574.321 ***	572.189 ***	15.828 ***	3431.34 ***	21.1511 ***	0.0005 ***
G	14	91.95 **	413.3 ***	5.75 ***	270973 ***	570.43 ***	575.276 ***	19.665 ***	5449.176 ***	34.5317 ***	0.0008 ***
E	2	72.41 ns	18317 ***	18.73 ***	4067506 ***	6684.09 ***	6607.66 ***	144.391 ***	19216.11 ***	183.369 ***	0.0042 ***
G x E	28	67.22 *	184.69 ***	3.41 ***	52613.7 ***	139.855 ***	139.541 ***	4.726 ***	1294.938 ***	2.87347 ***	0.0001 ***
IPC1	15	95.86 *	227.96 ***	4.65 ***	73220.1 ***	202.169 ***	202.184 ***	6.528 ***	1711.479 ***	4.05114 ***	0.0001 ***
Residual	13	34.18 ns	134.77 ***	1.98 ***	28837.1 *	67.954 ***	67.26 ***	2.646 ***	814.314 ***	1.51463 ***	0.0000 ***
Error	135	38.03	12.44	0.2	12969	1.425	1.46	0.23	67.9	0.17343	0
Blocks/Env	9	39.41 ns	9.744 *	0.43 *	16328.6 ns	3.281*	3.242 *	0.223 ns	72.303 ns	0.20365 ns	0.0000 ns
Pure Error	126	37.93	12.64	0.19	12729	1.293	1.333	0.23	67.6	0.17127	0

DF, degree of freedom; SE, stem establishment; PH, plant height; NSP, number of stem per plant; TPY, total plant yield; TTY, total tuber yield; MTY, marketable tuber yield; MTW, marketable tuber weight; DMC, dry matter concentration; SG, specific gravity; G, genotype; E, environment; G x E, genotype by environment interaction; ns not significant, *Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$; ***Significant at $P \leq 0.001$; IPC1, interaction principal component one

Table 5: AMMI analysis of variance for total tuber yield (TTY) of 15 potato genotypes grown in three environments

Source	DF	SS	MS	% TSS explained	% TRT SS explained	%GEI SS explained
Total	179	25462.5	142.249			
Treatment	44	25270.1	574.321***	99.08		
Genotype	14	7986.02	570.43***		31.6	
Environment	2	13368.2	6684.09***		52.9	
G x E	28	3915.94	139.855***		15.5	
IPC1	15	3032.54	202.169***		(12)	77.44
Residual	13	883.401	67.954***		(3.5)	22.56
Error	135	192.39	1.425	0.92		
Blocks/Env	9	29.529	3.281*			
Pure Error	126	162.861	1.293			

DF= degree of freedom, SS= sum of square, MS= mean square, TSS = total sum of squares, TRT SS = treatment sum of squares. GEI SS = genotype by environment interaction sum of squares, *** $p \leq 0.001$

Table 6: AMMI analysis of variance for marketable tuber yield (MTY) of 15 potato genotypes grown in three environments

Source	DF	SS	MS	% TSS explained	% TRT SS explained	%GEI SS explained
Total	179	25373.4	141.751			
Treatment	44	25176.3	572.189***	99.04		
Genotype	14	8053.86	575.276***		28.58	
Environment	2	13215.3	6607.66 ***		52.49	
G x E	28	3907.15	139.541 ***		15.52	
IPC1	15	3032.77	202.184 ***		(12.05)	77.62
Residual	13	874.383	67.26 ***		(3.47)	22.38
Error	135	197.096	1.46	0.96		
Blck/Env	9	29.181	3.242*			
Pure Error	126	167.915	1.333			

DF= degree of freedom, SS= sum of square, MS= mean square, TSS = total sum of squares, TRT SS = treatment sum of squares. GEI SS = genotype by environment interaction sum of squares, *p≤0.05, *** p≤0.001 ns= not significant

Table 7: AMMI analysis of variance of MTW for 15 potato genotypes grown in three environments

Source	DF	SS	MS	% TSS explained	% TRT SS explained	%GEI SS explained
Total	179	160149.3	894.689			
Treatment	44	150978.9	3431.34 ***	94.75		
Genotype	14	76288.46	5449.176***		50.53	
Environment	2	38432.21	19216.11***		25.46	
G x E	28	36258.27	1294.938***		24.02	
IPC1	15	25672.19	1711.479***		(17)	70.8
Residual	13	10586.08	814.314***		(7.01)	29.2
Error	135	9170.322	67.928	5.25		
Blocks/Env	9	650.73	72.303 ns			
Pure Error	126	8519.592	67.616			

DF= degree of freedom, SS= sum of square, MS= mean square, TSS = total sum of squares, TRT SS = treatment sum of squares. GEI SS = genotype by environment interaction sum of squares, *** p≤0.001 ns= not significant

Table 8: AMMI Analysis of variance of dry matter content (DMC) for 15 potato genotypes grown in three environments

Source	DF	SS	MS	% TSS explained	% TRT SS explained	%GEI SS explained
Total	179	954.052	5.3299			
Treatment	44	930.639	21.1511***	93.96		
Genotype	14	483.443	34.5317***		51.95	
Environment	2	366.738	183.369***		39.41	
G x E	28	80.4573	2.87347***		8.65	
IPC1	15	60.767	4.05114***		(6.53)	75.53
Residual	13	19.6902	1.51463***		(2.12)	24.47
Error	135	23.4131	0.17343	6.04		
Blocks/Env	9	1.83286	0.20365 ns			
Pure Error	126	21.5803	0.17127			

Table 9: AMMI Analysis of variance of specific gravity (SG) for 15 potato genotypes grown in three environments

Source	DF	SS	MS	% TSS explained	% TRT SS explained	%GEI SS explained
Total	179	0.0212	0.0001			
Treatment	44	0.0206	0.0005 ***	93.16		
Genotype	14	0.011	0.0008 ***		53.4	
Environment	2	0.008	0.0042 ***		38.83	
G x E	28	0.002	0.0001 ***		9.71	
IPC1	15	0.001	0.0001 ***		(4.85)	50
Residual	13	0	0.0000 ***			
Error	135	0.001	0.00	6.84		
Blocks/Env	9	0	0.0000 ns			
Pure Error	126	0.001	0			

DF= degree of freedom, SS= sum of square, MS= mean square, TSS = total sum of squares, TRT SS = treatment sum of squares. GEI SS = genotype by environment interaction sum of squares, *** p≤ 0.001 ns= not significant

Table 10 Spearman Correlation analysis between parameters

Trait	SE	NSP	PH	MTW	TPY	TTY	MY	DM	SG
SE	**1**	0.15	-0.09	-0.37	0.05	0.23	0.23	-0.13	-0.13
NSP		**1**	0.24	-0.16	-0.11	-0.08	-0.08	0.06	0.06
PH			**1**	0.26	0.11	0.06	0.07	0.41	0.42
MTW				**1**	0.57	0.47	0.48	0.41	0.41
TPY					**1**	0.98	0.98	0.62	0.62
TTY						**1**	1.00	0.57	0.56
MTY							**1**	0.57	0.56
DMC								**1**	1.00
SG									**1**

SE= stem establishment, PH= plant height, MTW = marketable tuber weight, NSP =stem per plant, TPY = total plant yield, TTY = total tuber yield, MTY = marketable tuber yield, DMC = dry matter, SG = specific gravity

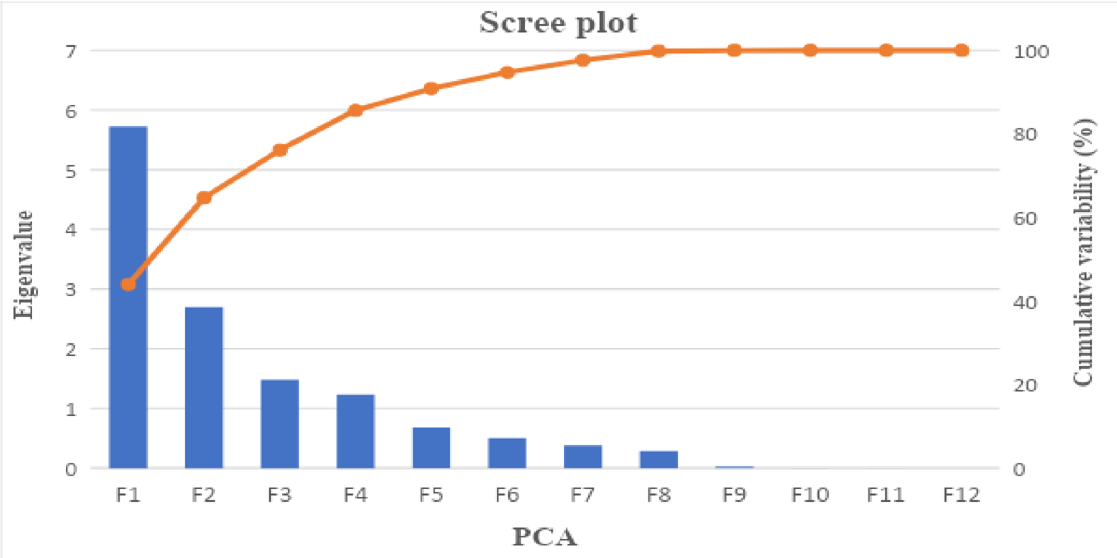

Figure 1: Scree plot of Eigenvalues against PCA with cumulative variability (%) for GEI of the studied traits, where F1 to F12 indicates IPCA1 to IPCA12.

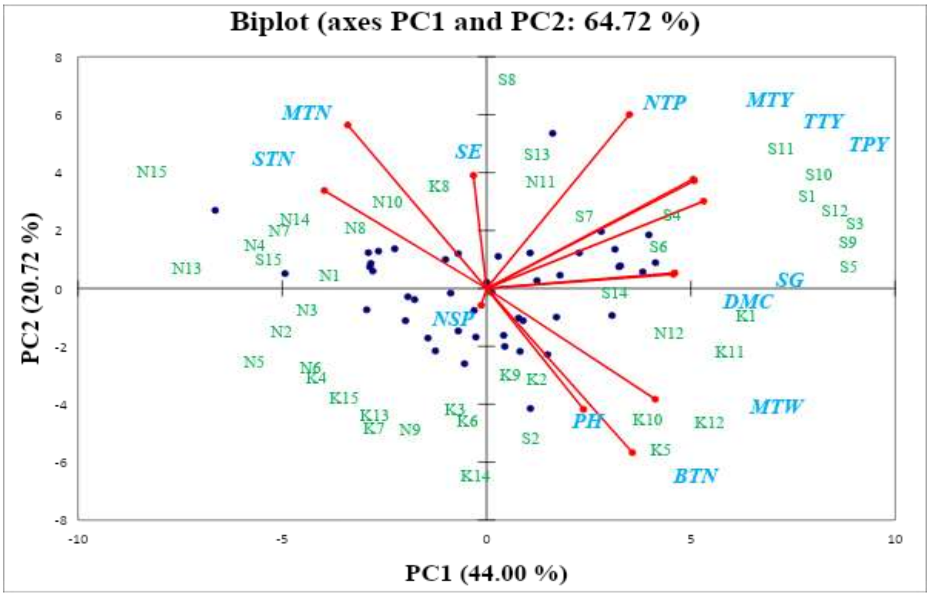

Figure 2. PCA1 and PCA2 biplot of genotype by environment interaction (GEI) relationship of the variables in the three different environments. Note: N1, N2, N3, …, N15, S1, S2, S3, …, S15 and K1, K2, K3, …,K15 respectively refers to genotype 1, 2, 3, …, and 15 interactions respectively with Nigde environment, Sivas environment, and Konya environment; N = Nigde, S = Sivas, K = Konya, 1 =MACAR1402.10, 2 = MACAR1402.11, 3 = MACAR1406.04, 4 = MACAR1406.07, 5 = MACAR1409.09, 6 = MEÇ1402.07, 7 = MEÇ1402.09, 8= MEÇ1405.06, 9= MEÇ1407.05, 10 = MEÇ1407.08, 11 = MEÇ1407.17, 12 = MEÇ1411.06, 13 = AGRIA, 14 = MADELEINE, 15 = RUSSET BURBANK; MTN= Medium tuber number, STN= small tuber number, SE=stand establishment, NTP= number of tuber per plant, MTY = marketable tuber yield, TTY = total tuber yield, TPY =total plant yield, SG=specific gravity, DMC=dry matter concentration, NSP=number of stem per plant

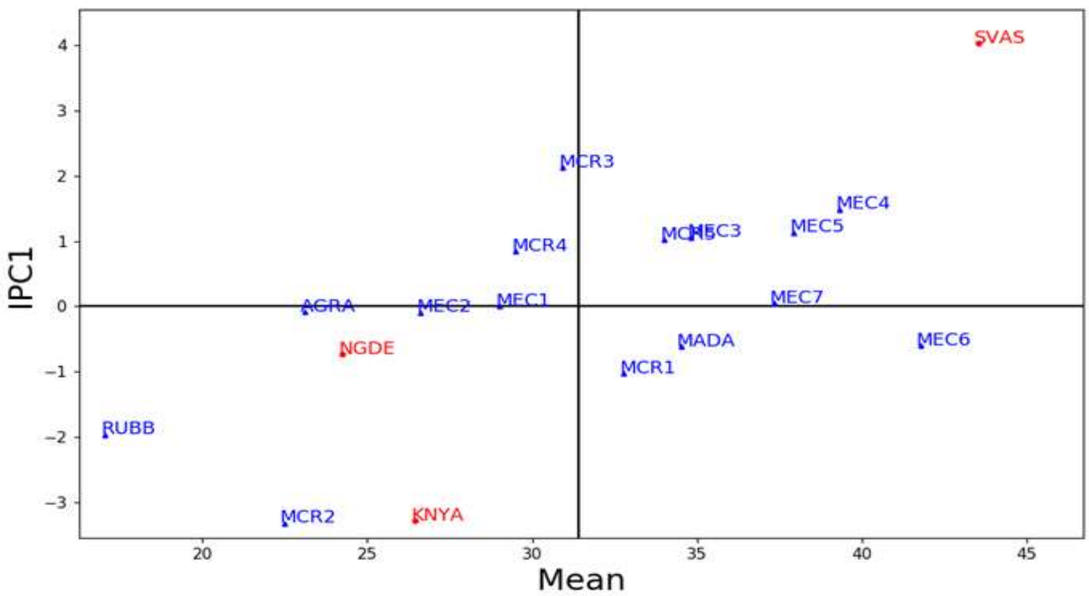

Figure 3: AMMI biplot analysis of interaction principal component axis (IPCA-1) with mean of total tuber yield (TTY) of potato genotype evaluated across three different environments. Note: MCR1 = MACAR1402.10, MCR2 = MACAR1402.11, MCR3 = MACAR1406.04, MCR4 = MACAR1406.07, MCR5 = MACAR1409.09, MEÇ1 = MEÇ1402.07, MEC2 = MEÇ1402.09, MEC3 = MEÇ1405.06, MEC4 = MEÇ1407.05, MEC5 = MEÇ1407.08, MEC6 = MEÇ1407.17, MEC7 = MEÇ1411.06, AGRA = AGRIA, MADA = MADELEINE, RUBB = RUSSET BURBANK; KYNA = Konya, NGDE = Nigde, SVAS = Sivas

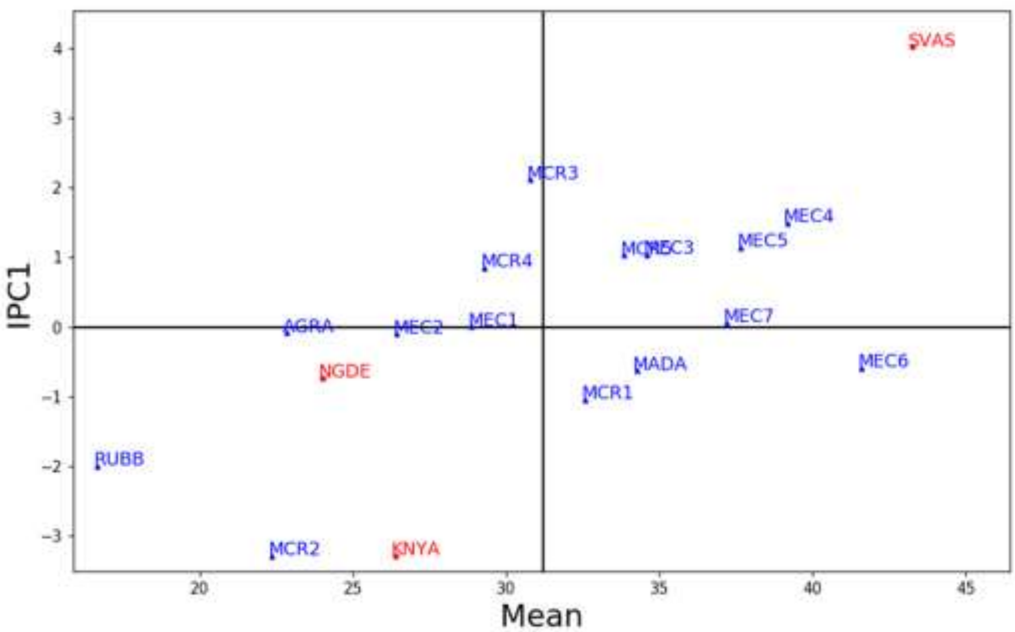

Figure 4. AMMI biplot analysis of interaction principal component axis (IPCA-1) with mean of marketable tuber yield (MTY) of potato genotype evaluated across three different environments. Note: MCR1 =MACAR1402.10, MCR2 = MACAR1402.11, MCR3 = MACAR1406.04, MCR4 = MACAR1406.07, MCR5 = MACAR1409.09, MEÇ1 = MEÇ1402.07, MEC2 = MEÇ1402.09, MEC3 = MEÇ1405.06, MEC4 = MEÇ1407.05, MEC5 = MEÇ1407.08, MEC6 = MEÇ1407.17, MEC7 = MEÇ1411.06, AGRA = AGRIA, MADA = MADELEINE, RUBB = RUSSET BURBANK; KYNA = Konya, NGDE = Nigde, SVAS = Sivas

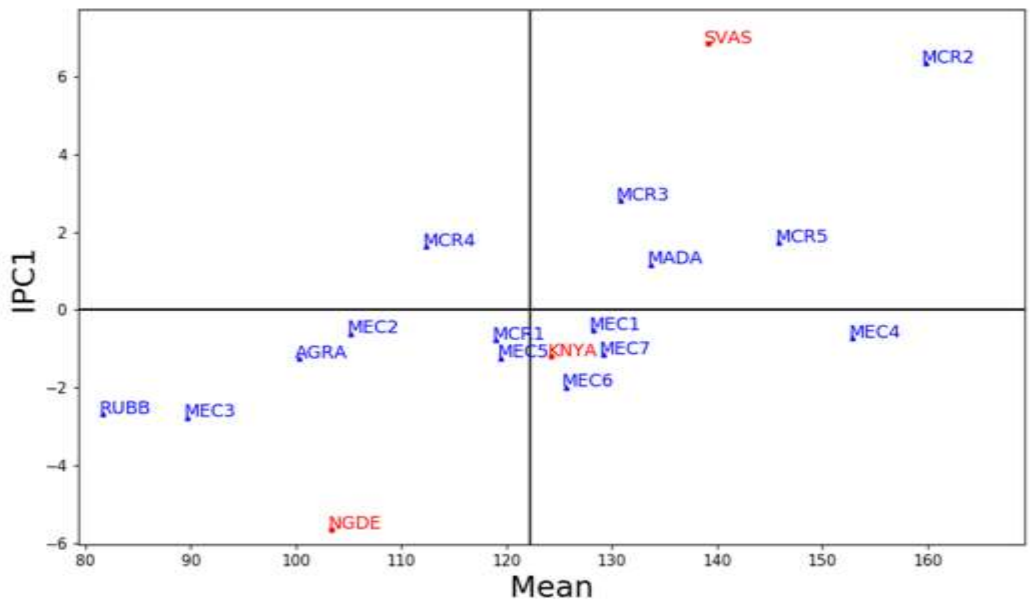

Figure 5. AMMI biplot analysis of interaction principal component axis (IPCA-1) with mean marketable tuber weight (MTW) of potato genotype evaluated across three different environments. Note: MCR1 =MACAR1402.10, MCR2 = MACAR1402.11, MCR3 = MACAR1406.04, MCR4 = MACAR1406.07, MCR5 = MACAR1409.09, MEC = MEÇ1402.07, MEC2 = MEÇ1402.09, MEC3 = MEÇ1405.06, MEC4 = MEÇ1407.05, MEC5 = MEÇ1407.08, MEC6 = MEÇ1407.17, MEC7 = MEÇ1411.06, AGRA = AGRIA, MADA = MADELEINE, RUBB = RUSSET BURBANK; KYNA = Konya, NGDE = Nigde, SVAS = Sivas

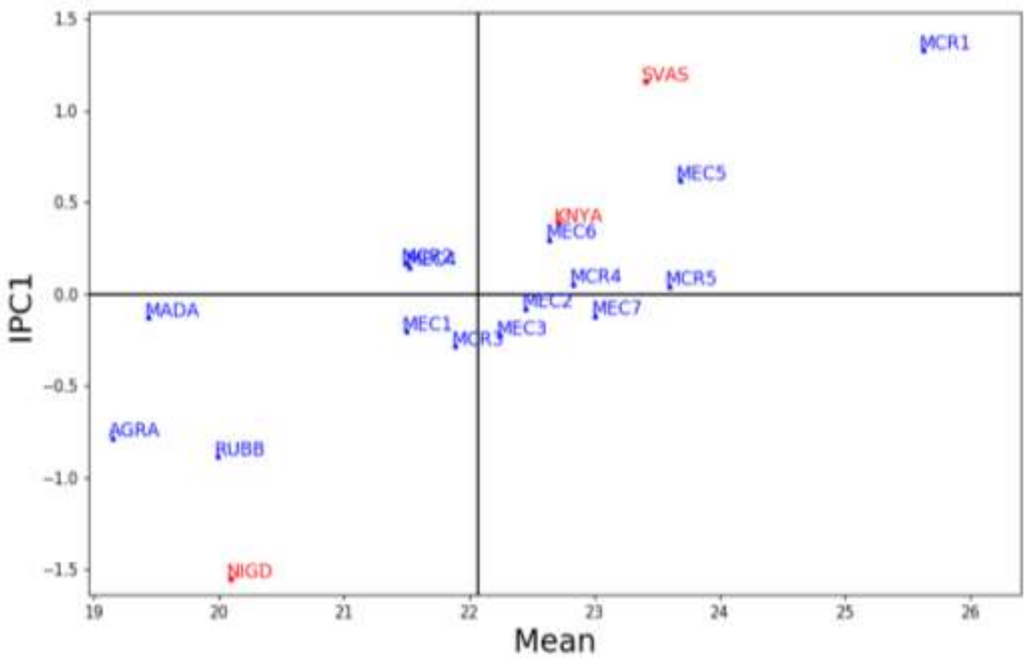

Figure 6: AMMI biplot analysis of interaction principal component axis (IPCA-1) with mean of dry matter concentration (DMC) of potato genotype evaluated across three different environments. Note: MCR1 =MACAR1402.10, MCR2 = MACAR1402.11, MCR3 = MACAR1406.04, MCR4 = MACAR1406.07, MCR5 = MACAR1409.09, MEÇ1 = MEÇ1402.07, MEC2 = MEÇ1402.09, MEC3 = MEÇ1405.06, MEC4 = MEÇ1407.05, MEC5 = MEÇ1407.08, MEC6 = MEÇ1407.17, MEC7 = MEÇ1411.06, AGRA = AGRIA, MADA = MADELEINE, RUBB = RUSSET BURBANK; KYNA = Konya, NGDE = Nigde, SVAS = Sivas

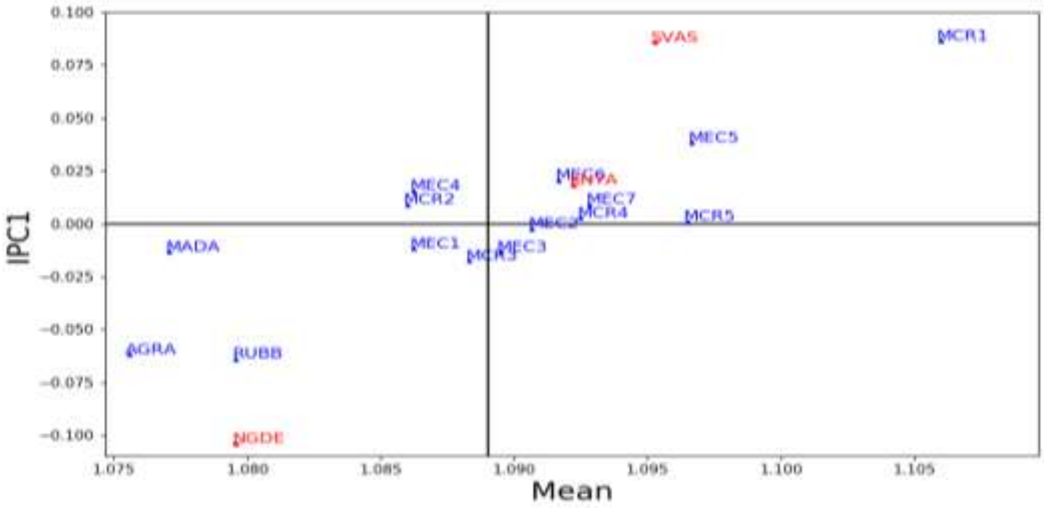

Figure 7: AMMI biplot analysis of interaction principal component axis (IPCA-1) with mean of specific gravity (SG) of potato genotype evaluated across three different environments. Note: MCR1 =MACAR1402.10, MCR2 = MACAR1402.11, MCR3 = MACAR1406.04, MCR4 = MACAR1406.07, MCR5 = MACAR1409.09, MEÇ1 = MEÇ1402.07, MEC2 = MEÇ1402.09, MEC3 = MEÇ1405.06, MEC4 = MEÇ1407.05, MEC5 = MEÇ1407.08, MEC6 = MEÇ1407.17, MEC7 = MEÇ1411.06, AGRA = AGRIA, MADA = MADELEINE, RUBB = RUSSET BURBANK; KYNA = Konya, NGDE = Nigde, SVAS = Sivas

4.0 Discussion

4.1 Significance of Genotype by Environment Interaction (GEI) in plant breeding

This study was focused to elucidate the effect of genotypes by environment interaction on the tuber yield and quality of potato breeding lines in three different environments in the Central Anatolia Region of Turkey. The application of GEI in breeding programmes have severally helped to compare and identify the contributing elements for better yield or performance of a breeding line and their responses to specific environment. This can be implored to identify the ideal conditions and formulate crop management recommendations (Mateus-Rodriguez et al., 2014). GEI is stated to be important in accounting for the phenotypic differences in the quantitative traits of crops and is accommodated in statistical models designed for multi-environmental trials (Xavier et al., 2018)

It can be deduced (Table 5 and Table 6) that a negligible error was observed on the tuber yield of the potato and so is not responsible for the yield differences among the genotypes. The greater differences in the yield can be stated to be environmentally induced more than genetically induced and the genotypic differences is attributed to the differences in the crossing parents of the cultivar which confers different responses with the environments and led to the significant difference in the yield of the genotypes. This differences in the pedigree of the breeding lines and their responses to environmental parameters led to a crossover GEI on the TTY and MTY across the three locations of the study. The TTY of the genotypes ranges from Russet Burbank(17.033t/ha) to MEÇ1407.17 (41.77t/ha) whiles among environment; Nigde (24.24t/ha), Sivas (44.56t/ha), and Konya (26.45t/ha) with statistically significant yield differences between the highest and lowest yield of the genotypes and with the rest of the genotypes (data not presented). The mean MTY of the genotypes ranges from 16.66 t/ha to 41.60 t/ha which was found in Russet Burbank and MEÇ1407.17 respectively. Among the environments; Nigde (24.00 t/ha), Sivas (43.25 t/ha), and Konya (26.38 t/ha). The highest TTY across the environments were found in MEÇ1407.17 (41.01 t/ha) in Nigde, MEÇ1407.05 (56.69 t/ha) in Sivas, and MACAR1402.10 (35.52 t/ha) in Konya.

On the other hand, the lowest TTY and MTY in Sivas was found in MACAR1402.11 (21.06 t/ha), while Russet Burbank was recorded the lowest TTY of 10.47 t/ha and 19.09 t/ha respectively in Nigde and Konya. Across the environments, different genotypes occupy different ranks with MEÇ1407.17 (40.70 t/ha), MEÇ1407.05 (56.52 t/ha), and MACAR1402.10 (35.5 t/ha) recording the highest MTY respectively in Nigde, Sivas, and Konya whereas Russet Burbank recorded the lowest MTY for all the three environments with 10.14t/ha, 20.92 t/ha and 18.91 t/ha respectively in Nigde, Sivas and Konya. This give the genotypes differential ranking across the environments.

These findings confirm several researches that genotypes, and environment have great effect on the yield of crops (Wassu, 2017; Shah et al., 2020). Biru (2017) reported a highly significant effect of genotype, environments, and G×E on the yield and yield components, implying the genetic variability among genotypes and the segregating possibility for selecting stable. Raja et al. (2018), Rymuza et al. (2015), and Wassu, (2017) reported that the genetic difference among potato genotypes account for the difference in marketable tuber yield, total tuber yield. The higher contribution of the treatment sum of squares by genotypes than the environment and the genotype by environment interaction depicts that the variation in the MTW, DMC and SG of the potato genotypes is largely due to genotypic differences followed by the environments than the GEI component. These differences in the quality traits of the potato tubers can be attributed to the difference sources of the crossing parents and the pedigree of the breeding lines and the check cultivars. It may also be due to the agro-ecological disparities. Differences in evaporative demand due to high global solar radiation flux density, low relative humidity, and/or high wind speed cause closure of stomata leading to reduction in photosynthesis accompanied with restriction of CO_2 uptake, thus resulting in light intensities outside the required optimum for maximum dry matter production (Stark and Love, 2003). The differences in altitude of the study areas might also have a contributory factor in the disparities in the dry matter content as a result of air and soil temperature differences as shown in Table 2 which have been ascribed to have varietal effect on specific gravity of the potato tubers causing differences in the dry matter concentration of the potato tubers (Pereira et al 2008).

4.2. Implication from quality traits in food security and industrial food processing

Food security is important in this era to provide enough and quality food in this era of rise in human population. Thus, it is paramount to beefed up food safety needs to ensure the food consumed is of good quality.

In potato, the suitability of a cultivar industrial food processing is dependent on its quality traits especially dry matter concentration and specific gravity. Potato dry matter concentration is important in the food processing industry, especially for French fries or chips. Dry matter content of >20% gives good chips quality (Kabira and Berg, 2003; CIP 2007). Again, the Acquisition and Distribution Unit of CIP (2009) distinctively categorized that, DMC of >23% is termed as high, DMC from 20 to 23% as medium and DMC <20% as low according to the CIP (CIP, 2009). Wassu (2017) stated that, the industrial use of potato is dependent on tuber dry matter concentration and starch and that tubers with 20 to 24% dry matter concentration are ideal for French fries and crisps processing. In the study, MACAR1402.10, MACAR1409.09, MEÇ1407.08, and MEÇ1411.06 had high DMC (>23%); MACAR1402.11, MACAR1406.04, MACAR1406.07, MEÇ1402.07, MEÇ1402.09, MEÇ1405.06, MEÇ1407.05, and MEÇ1407.17 had medium DMC (20 to 23%) whilst Agria, Madeleine and Russet Burbank had low DMC (<20%) (data not presented here). Potato SG is a key parameter in the food processing industry especially for French fries or chips (Kabira and Berg, 2003; CIP, 2007; Wassu, 2017).

For French fry and potato chips, CIP (2007) set the minimum acceptable levels of potato tubers with a specific gravity of ≥1.080. Potato varieties with ≥1.080 reduces the amount of oil uptake by potato chips and French fries during frying and enhances texture and yield quality of the finished products.
All potato breeding lines in this study had DMC >21% and SG >1.081 gcm^{-3} as compared to the check cultivars (AGRIA, Madeleine, and Russet Burbank). Thus, making them ideal for the French fries and chips industry. The environments were highly heterogeneous with large IPC1 values and were unstable for SG. Averagely, Konya environment was ideal for majority of the genotypes in terms of SG. Overall, 8 (53%) of the 15 genotypes had SG above grand mean and 7 (47 %) genotypes had SG below the grand mean of 1.089. Also, 53% of the genotypes have positive IPCA1 whilst 47% of the genotypes had negative IPCA1 values. Larger IPC1 scores values of the genotypes indicate higher instability and specific adaptability of the genotypes to the environment due to higher interaction (Temesgen *et al*. 2015).

5. Conclusion

The study of genotype by environment interactions in plant breeding has once again been proven to be essential for agronomists and plant breeders. The Additive Main effect and Multiplicative Interactions (AMMI) revealed the significant effect of genotype by environment interaction on the 15 potato breeding lines in the Central Anatolia Region of Turkey. There were significant genotypic, environmental and GEI effects on all traits, which shows the existence of high variabilities among the potato breeding lines due to the different parental sources and productivity of each environment. Crossover type of GEI was present which resulted in genotypes ranking differently in each environment. MEÇ1407.17, MEÇ1407.05, MEÇ1407.08 and MEÇ1411.06 were outstanding genotypes in respect to marketable tuber yield while the standard cultivars Agria, Russet Burbank and MACAR1402.11 were found as low yielding genotypes as average of three locations. Genotypes MEÇ1407.17, MEÇ1411.06, MADELEINE, MACAR1406.07, MEÇ1402.07, MEÇ1402.09, and Agria were more stable genotypes with broad adaptability to diverse environments. MEÇ1405.06, MEÇ1407.05, MEÇ1407.08, MACAR1409.09, MACAR1406.04, MACAR1402.10, MACAR1402.11 and Russet Burbank were unstable with specific adaptation. All the 12 breeding lines have DMC >20% and SG > 1.080, which are threshold levels for potato processing industries and are ideal for chips and French fries processing. Sivas location was found as the most productive environment for potato yield while the Niğde and Konya have similar performance. The breeding lines MEÇ1407.17, MEÇ1407.05, MEÇ1407.08 and MEÇ1411.06 were identified as promising cultivar candidates due to their high tuber yield and stable performances across different environments.

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DETERMINATION OF SOME AMPELOGRAPHIC CHARACTERS OF AMERICAN GRAPE CULTIVARS HAVE Vitis labrusca TRAITS INTRODUCED FROM NORTH AMERICA TO TURKEY

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ABSTRACT

The study was conducted in application area of Agriculture Faculty of Ondokuz Mayıs University between 2011 and 2012. The study carried out for the determination of ampelographical characteristics of grape cultivars of *Vitis labrusca* have foxy grape traits introduced from North America to Turkey in 2006. Totally fourteen cultivars (August Giant, Bluebell, Buffalo, Champbell Early, Delicatessan, Delaware, Munson, Mars, Niagara, Neptune, Steuben, Venus, Valiant ve Concord) were evaluated considering a several ampelographical traits (shoot, mature leaf, one-year-old canes, flower bunch, grape bunch, berry and seed) described by the methods of IBPGR's "Descriptors of the Grapes". It has been determined that all varieties have hermaphrodite flower structure, leaf areas vary between 146.50 (Neptune) and 228.70 cm^2 (Mars), Bluebell has the largest inflorescences and the number of berry per bunch varies between 36.0 (Neptune) and 57.6 (Bluebell). It was also revealed that the maturity period lasted throughout August and three of the studied varieties were seedless (Bluebell, Mars and Venus).

Key Words: American grapes, hybride, foxy grape, Black Sea Region, ampelography

INTRODUCTION

Viticulture is quite old in Turkey, one of the most important reasons for this situation is that our country is located in the most suitable climatic zone of the world in terms of viticulture. In addition to its location and climate advantage, Turkey is motherland to many plants with its fertile soil structure. Thanks to the civilizations that lived in Anatolia throughout the history, different tastes and consumption habits, as well as the difference in ecological conditions, increased the variety of grapes (Ağaoğlu, 1999). It served as a bridge in the spread of Anatolian Viticulture to Europe, America, Asia, Africa and the Far East. Grape cultivation can be done in almost all regions of Turkey, which has a rich culture in viticulture, and the product obtained after harvest is used by turning it into different products for table grapes, raisins, wines or must Çelik et al., 1998). For the restructuring of viticulture, which is important for the future of Turkey, it is necessary to collect, protect and develop grapevine gene resources (Çelik et al., 1998). In addition to collecting genetic resources and taking them under protection, it is important for the development of viticulture to determine the regional adaptations and phenological morphological characteristics of new grape varieties developed around the world. The further development of viticulture in Turkey and the further increase in its contribution to the country's economy will be realized with the importance given to the studies carried out for the determination, protection and evaluation of our existing grapevine gene resources (Soylemezoğlu, 1996). In these studies, ampelographic characteristics of grape varieties grown according to regions are determined, yield and quality characteristics are determined in detail. Despite the ampelographic studies to reveal our rich genetic assets in viticulture and the collection vineyards created from these varieties, not all grape varieties in Turkey have been examined and some varieties have disappeared (Çelik and Karanis, 1998).

The word ampelography means the science of describing the vine, which consists of the words "ampelos" vine and "graphe". Various methods have been used by different scientists for the identification of grapes. In order to eliminate these confusions and to identify varieties in a true way, "Grape Descriptors" were determined as a result of the joint studies of "International Center for Plant Genetic Resources" (IBPGR), "International Viticulture and Winemaking Office" (OIV) and "International Union for the Protection of New Plant Varieties" (UPOV). ” (Descriptor for Grape) has been published and a method that can be valid all over the world has been put forward (Anon., 1983).

Identification of grape varieties is extremely important for viticulture, wine producers, authorities that regulate grape products, sellers and consumers. It is estimated that there are between 5000-6000 varieties of *Vitis vinifera* in the world. However, very few of these cultivars are grown commercially (Tessi, 2010). Phyllis Burger et al. (2009) states that there is a great genetic diversity in vines that most of them are commercially grown, which can adapt to different soil and climatic conditions, belong to the *Vitis vinifera* species. In the USA, some other *Vitis* species such as *Vitis labrusca* are grown commercially and have local economic importance.

Many morphological parts of the vine are used to distinguish grape varieties from each other in ampelography, and these organs are examined in certain periods. Ampelographically revealed characteristics may have changed by region due to genetic, environmental and cultural processes (Tessi, 2010). Tessi (2009) stated that DNA techniques have been used in addition to ampelographic, ampelometric, isoenzyme and phytopathological features in the identification of varieties in recent years. The same author also revealed that the identification of grape varieties by revealing their DNA profiles is more advantageous than the subjective nature of ampelography, and it is an adequate and verified method by eliminating the variability that may arise morphologically and unknown grape varieties in the database can be easily identified.

Ampelographic studies date back to ancient times. As in the world (Levadoux et al., 1971; Morton, 1979; Nosul’chak, 1986; Calo et al., 1990; Martinez and Perez, 2000; Ortiz et al., 2004; Santiago et al, 2005; Gonzalez et al., 2007), many ampelographic studies have been carried out on many grape varieties, types and varieties grown in Turkey (Odabaş, 1984; Altın, 1991; Çelik and Odabaş, 1991; Cangi, 1999; Ünal, 2000; Çelik et al., 2003; Köse et al., 2004; Köse and

Çelik, 2007; Çelik et al., 2008; Uyak, 2010; Keskin et al., 2013; Doğan et al., 2017; Arslan et al., 2018; Binay, 2013; Çelik et al., 2018).

Although the climate in the coastal parts of the Black Sea Region is abundant rainy and temperate, a transitional or continental climate prevails in the interior. Rize, which is one of the provinces with the highest rainfall in the coastal regions, has an annual precipitation of 2537 mm (Anon., 2000). In many parts of the Black Sea region, especially in the coastal part, it can be difficult or even impossible to grow commercial viticulture with grape varieties of the *Vitis vinifera* type due to the increase in fungal diseases arised from excessive rainfall in spring and autumn and the fewer sunny days compared to other regions (Çelik, 2004). However, it is stated that grape varieties or types, which are the foxy grapes of the region and fall into the *Vitis labrusca* type, can grow even under these conditions and yield quality grapes (Cangi, 1999; Çelik, 2004).

By conducting extensive surveys in the Black Sea Region, grape varieties or types that are known to be resistant to fungal diseases, have economic value and are known to be resistant to fungal diseases have been revealed (Çelik et al., 2003; Melek and Çelik, 2005; Cangi et al., 2006a and 2006b; Çelik et al., 2008; Çelik et al., 2018). In fact, as a result of these studies, 5 fragrant grape varieties with superior characteristics ('Rizessi', 'Rizpem', 'Çeliksu', 'Rizellim' and ‘Ulkemiz’) were registered and added to the National Variety List (Çelik et al., 2017).

In this study, the phenological, morphological and ampelographic characteristics of the newly bred cultivars (*Vitis labrusca* L.) grown in the Northern Regions of America were determined under Samsun conditions. Thus, it is aimed to add new varieties to the variety richness of the Black Sea Region. In the study, broad ampelographic characteristics of grape varieties with strawberry scent and "foxy" flavor, which are classified as *Vitis labrusca* L., which are brought from America and grown in Atakum, Samsun, resistant to diseases and even pests and can grow in high humidity conditions, were determined. Thus, the first step has been taken to introduce new varieties of foxy grapes, which have existed in the region for centuries and show a wide variety of species, as well as new cultivars that grow in the world and show foxy, by revealing their phenological, biological and morphological characteristics. This situation is important for the inclusion of new varieties in the variety preferences for the vineyards being established in the region.

MATERIAL AND METHODS

This research was carried out in the vineyard on the campus area of Ondokuz Mayıs University, Faculty of Agriculture, in Atakum district of Samsun province, from the bud burst period to the harvest period. In the study, 14 grape cultivars (August Giant, Bluebell, Buffalo, Champbell Early, Delicatessan, Delaware, Munson, Mars, Niagara, Neptune, Steuben, Venus, Valiant and Concord) were brought to Turkey by introduction from North America in 2006 and have 10-year-old vines (Table 1) Their ampelographic characteristics were determined according to the grape descriptor named “Descriptors for Grape” (Anon, 1983) published by IBPGR, which provides international unity in this field. The characteristics of shoots, mature leaves, sticks, flowers and grape clusters, and berry and seed characteristics were determined in cultivars.

RESULTS AND DISCUSSION

Shoot, mature leaf and shoot characteristics

In terms of shoot tip shape in the examined varieties, Blubell, Champbell Early, Mars, Valiant and Concord varieties have "closed" shoot tips, while shoot tips of other varieties are "open"; has been observed. According to Kara (1990) and Çelik (1998), the shoot tip is "open" in *Vitis vinifera* varieties and "closed" in *Vitis labrusca*. These results were generally found in Cangi et al. (2006a) is consistent with the findings of Melek and Çelik (2005) and Şanlı and Odabaş (2005). Anthocyanin distribution was not observed in the shoots of Buffalo, Neptune and Venus cultivars. In August Giant and Concord cultivars, anthocyanin was found “all over” the shoot tip, but in other cultivars the anthocyanin distribution was “partially” (Table 2 and 3). The color of a healthy shoot tip is an important character in determining the difference between cultivars (Morton, 1979). The better the anthocyanin distribution at the shoot tip, the better the shoot development. This result has been demonstrated in many studies (Çelik et al., 2008).

Niagara, Steuben, Valiant, Concord, Blubell, Buffalo cultivars examined in the experiment had "semi-upright" shoot habit, while August Giant, Champbell Early, Delicatessan, Delaware, Munson, Mars, Neptune and Venus cultivars were determined to have "upright" shoot habit. Cangi et al. (2006b) made an ampelography studies on *Vitis labrusca* types that grow naturally in Trabzon and they determined that 3 of the types have semi-upright, 4 horizontal and the other 3 drooping exile habits among the findings of 10 types.

In Champbell Early, Munson, Mars, Steuben and Concord cultivars, tendrils are "continuous" on the shoot, and "dashed" in other cultivars (Table 2 and 3). Çelik (1998), Cangi et al. (2006a), Çelik and Odabaş (191) stating that the tendrils of the cultivars of *Vitis labrusca* L. type are continuous, and they also stated that this situation may be different in hybrids. The length of the tendrils is less than 11 cm (very short) in August Giant, Delaware and Steuben cultivars, and between 11-17.4 cm (short) in other cultivars. As a matter of fact, Çelik et al. [20] reported that the tendril lengths of the types included in the *Vitis labrusca* type vary between 10-15 cm in their studies in Artvin and Rize.

While the leaf size of the examined cultivars was less than 75 cm^2 in the Neptune cultivar and classified as "very small"; It is between 225-300 cm^2 in Mars and Venus varieties and is classified as "large". In other cultivars, the leaf size was found to be “medium” and between 150-224 cm^2 (Table 4). Melek and Çelik (2005) determined that 17 of the 26 grape types grown in Sinop and included in the group of foxy grapes had a "medium" leaf size, while the leaf size of the remaining types was "small". The examined cultivars similarly have “medium” sized leaves. Keller (2015) reported that leaf size in the vine depends on the water uptake of the plant. On the other hand, Morton (1979) reported that leaf size may differ from variety to variety, as well as climatic conditions, applied training system, soil fertility and vine growth strength are also effective in leaf size. Leaf length were “short” (8-14.4 cm) in Neptune, Valiant, Concord and Munson cultivars; in other cultivars, it was found to be at “medium” level (14.5-19.4 cm) (Table 4).

Table 1. American foxy grape varieties used as a plant material for this ampelographic study

Variety	*Parents*	*Breeding year*	*Referance*
August Giant	*V. labrusca* x *V. vinifera*	1861	Hedrick, 1992
Bluebell	Beta x *V. labrusca*		Anon., 2018
Buffalo	*V. labrusca* x *V. vinifera*	1928	Smiley and Cochran, 2016
Champbell Early	*V. labrusca* hyb.	1880	Rambough, 2002
Delicatessan	*V. labrusca* x *V. vinifera*	1902	Rambough, 2002
Delaware	*V. vinifera* x *V. labrusca* x ‘bourquiana’ (*V. aestivalis*)	1850	Weaver, 1976
Munson	*V.vinifera* x *V. rupestris*	1897	Rambough, 2002
Mars	*V.vinifera* x *V. labrusca*	1975	Smiley and Cochran, 2016
Niagara	*V. labrusca* (Concord x Cassady)	1868	Smiley and Cochran, 2016
Neptune	*V. labrusca* x *V. vinifera*	1998	Smiley and Cochran, 2016
Steuben	*V. labrusca* x *V. vinifera*	1931	Smiley and Cochran, 2016
Venus	*V. labrusca*	1850	Smiley and Cochran, 2016
Valiant	*V. labrusca* x *V. riparia*	1972	Smiley and Cochran, 2016
Concord	*V. labrusca*	1843	Weaver, 1976

Table 2. Ampelographic characters of some foxy American grape varieties.

TRAITS	OIV	August Giant	Bluebell	Buffalo	Champbell Early	Delicatessan	Delaware	Munson
YOUNG SHOOTS								
Form of tip	001	Half-open	Closed	Half-open	Closed	Half-open	Half-open	Half-open
Density anchocyanin colouraion of tip	002	Allaround	Partially	No	Partially	Partially	Partially	Partially
Shoot habit	006	Erect	Half erect	Half erect	Erect	Erect	Erect	Erect
Erect hairs on nodes	011	Very sparse	Very sparse	Very sparse	Medium	Very sparse	Very sparse	Very sparse
Erect hairs between nodes	012	Sparse	Very sparse	Very sparse	Medium	Very sparse	Very sparse	Very sparse
Very sparse Prostrate hairs on nodes	013	Medium	Sparse	Very sparse	Medium	Very sparse	No	Very sparse
Prostrate hairs between nodes	014	Medium	Very sparse	Very sparse	Medium	Very sparse	No	Very sparse
Number of consecutive tendrils	016	Discontinuous	Discontinuous	Discontinuous	Continuous	Discontinuous	Discontinuous	Continuous
Length of tendril	017	Very short	Short	Short	Short	Short	Very short	Short
MATURE LEAF								
Size of blade	065	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Leaf length	066	Medium	Medium	Medium	Medium	Medium	Medium	Short
Shape of blade	067	Wedge	Wedge	Pentagonal	Pentagonal	Pentagonal	Wedge	Wedge
Number of lobes	068	Lobeless	Three	Three	Three	Three	Three	Five
Colour of upper blade	069	Green	Green	Green	Dark green	Green	Dark green	Green
Length of teeth	077-1	Short	Short	Very short	Very short	Medium	Short	Short
General shape of petiole sinus	080	V shape	U shape	V shape	V shape	U shape	U shape	V shape
Shape of petiole sinus	081	No	No	No	No	No	No	No
Length of petiole	092	Very short	Very short	Short	Very short	Very short	Very short	Very short
ONE YEAR OLD SHOOT								
Transverse section	101	Round	Elliptic	Elliptic	Round	Elliptic	Round	Flat elliptical
Upper side	102	Veined	Flat	Lined	Lined	Lined	Lined	Lined
Yellowish brown	103	Yellowish brown	Dark brown	Yellowish brown	Yellowish brown	Yellowish brown	Red-brown	Red brown
Lenticel	104	Present	No	No	Present	No	No	No
FLOWER								
Sex of flower	151	Hermaphrodite	Hermaphrodite	Hermaphrodite	Hermaphrodite	Hermaphrodite	Hermaphrodite	Hermaphrodite
First flower bunch appeared node	152	3.-4 nodes	3.-4 node	3.-4 node	3.-4 node	3.-4 node	3.-4 node	3.-4 node
Flower bunch per shoot	153	1-2 bunch	2-3 bunch	1-2 bunch	2-3 bunch	2-3 bunch	1-2 bunch	2-3 bunch
Length of first flower bunch	154	Short	Medium	Short	Medium	Short	Short	Medium
BUNCH								
Berry bunch per shoot	201	1-2 Bunch	2-3 Bunch	1-2 bunch	2-3 bunch	2-3 bunch	1-2 bunch	3'den fazla bunch
Bunch length	203	Short	Medium	Very short	Short	Very short	Very short	Short
Compactness	204	Sparse	Medium	Very sparse	Sparse	Sparse	Medium	Sık
Berry per bunch	205	Very little	Little	Very little	Very little	Very little	Very little	Very little
Length of bunch stalck	206	Short	Short	Short	Short	Very short	Very short	Short
BERRY								
Berry length	221-1	Short	Short	Short	Short	Short	Short	Very short
Berry shape	223	Short oval	Long oval	Short oval	Short oval	Round	Round	Short oval
Skin colour	225	Blue-black	Blue-black	Blue-black	Reddish black	Blue-black	Blue-black	Blue black
Skin thickness	228	Thin	Medium	Thin	Thin	Medium	Thin	Thick
Flesh colour	230-31	Lightly colored	Too Lightly colored	Too Lightly colored	Colorless	Very Lightly colored	Very Lightly colored	Colorless
Juiceness of flesh	232	Juicy	Juicy	Juicy	Juicy	Juicy	Juicy	Juicy
Taste	236	Foxy	Foxy	Foxy	Foxy	Foxy	Foxy	Foxy
Berry stalck length	238	Short	Short	Short	Short	Very short	Very short	Short
SEEDS								
Seed weight	243	Low	Seedless	Low	Low	Medium	Medium	Medium
Transverse grooves on the dorsal side	244	No	Seedless	No	No	No	No	No

In terms of leaf shape, the leaf blades of August Giant, Bluebell, Delaware, Munson, Steuben, Valiant and Concord varieties are wedge-shaped, while Buffalo, Champbell Early, Delicatessan, Mars, Niagara and Venus varieties have pentagonal leaf blades; It was determined that the Neptune cultivar had "heart" shaped leaf blades. As a general characteristic of *Vitis labrusca* L., mature leaves are “lobeless” (Çelik, 1998). However, leaves can be lobed in American vines obtained at the end of hybridization (Çelik, 1998). The leaves of the Munson variety, which is one of the varieties examined in the study, are “five” lobed; In the cultivar August Giant, Venus, Valiant and Concord the leaves are “lobeless”; In other cultivars, leaves were identified as “three” lobed (Table 2 and 3). As reported by Aktepe (1994) and Diri (1996), it is emphasized that leaf shape and slicing are a definite feature used in cultivar differentiation. They also states that this feature is related to the growth strength and soil structure of the vine and the number of lobes seems to be one of the characters least affected by environmental conditions for the same variety and clone.

Table 3. Ampelographic characters of some foxy American grape varieties

TRAITS	OIV	CULTIVAR						
		Mars	Niagara	Neptune	Steuben	Venus	Valiant	Concord
YOUNG SHOOT								
Form of tip	001	Closed	Half open	Half open	Half open	Half open	Closed	Closed
Density anchocyanin colouraion of tip	002	Partially	Partially	No	Partially	No	Partially	All around
Shoot habit	004	Dense	Dense	Dense	Dense	Dense	Medium	Dense
Erect hairs on nodes	005	Medium	Medium	Medium	Sparse	Very sparse	Very sparse	Very sparse
Erect hairs between nodes	006	Erect	Half erect	Erect	Half erect	Erect	Half erect	Semi erect
Prostrate hairs on nodes	011	Medium	Very sparse	Very sparse	Sparse	Medium	Very sparse	Very sparse
Prostrate hairs between nodes	012	Medium	Sparse	Sparse	Sparse	Sparse	Very sparse	Very sparse
Number of consecutive tendrils	013	Medium	Very sparse	Very sparse	Sparse	Medium	No	Sparse
Length of tendril	014	Medium	Sparse	Very sparse	Sparse	Sparse	Very sparse	Sparse
Form of tip	016	Continuous	Discontinuous	Discontinuous	Continuous	Discontinuous	Discontinuous	Continuous
Density anchocyanin colouraion of tip	017	Short	Short	Short	Very short	Short	Short	Short
MATURE LEAF								
Size of blade	065	Big	Medium	Small	Medium	Big	Medium	Medium
Leaf length	066	Medium	Medium	Short	Medium	Medium	Short	Short
Shape of blade	067	Pentagonal	Pentagonal	Heart shape	Vedge	Pentagonal	Vedge	Kama
Number of lobes	068	Three	Three	Three	Three	Lobeless	Lobeless	Lobeless
Colour of upper blade	069	Green	Dark green	Green	Green	Dark green	Dark green	Green
Length of teeth	077-1	Very short	Long	Long	Short	Short	Short	Very short
General shape of petiole sinus	080	V shape	V shape	U shape	V shape	V shape	U shape	U shape
Shape of petiole sinus	092	Very short	Very short	Very short	Very short	Very short	Very short	Very short
ONE YEAR OLD SHOOT								
Transverse section	101	Round	Round	Elliptic	Elliptic	Round	Elliptic	Round
Upper side	102	Lined	Lined	Lined	Veined	Veined	Veined	Veined
Original colour	103	Dark brown	Yerllow	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown
Lenticel	104	Absent	Absent	Absent	Present	Present	Present	Var
FLOWER								
Sex of flower	151	Hermaphrodite	Hermaphrodite	Hermaphrodite	Hermaphrodite	Hermaphrodite	Hermaphrodite	Hermaphrodite
First flower bunch appeared node	152	3-4 nodes	3-4 nodes	5 node	3-4 nodes	3-4 nodes	3-4 nodes	3-4 nodes
Flower bunch per shoot	153	2-3 bunch	2-3 bunch	1-2 bunch	1-2 bunch	2-3 bunch	2-3 bunch	More than 3 bunch
Length of first flower bunch	154	Short	Medium	Medium	Short	Short	Short	Short
BUNCH								
Berry bunch per shoot	201	2-3 bunch	2-3 bunch	1-2 bunch	1-2 bunch	2-3 bunch	2-3 bunch	2-3 bunch
Bunch length	203	Medium	Short	Short	Short	Short	Very short	Short
Compactness	204	Sparse	Medium	Medium	Sparse	Sparse	Medium	Medium
Berry per bunch	205	Very little	Very little	Very little	Very little	Very little	Very little	Very little
Length of bunch stalck	206	Short	Short	Very short	Short	Short	Very short	Short
BERRY								
Berry length	221-1	Short	Short	Short	Short	Very short	Very short	Short
Berry shape	223	Long oval	Long oval	Long oval	Long oval	Long oval	Round	Short oval
Skin colour	225	Blue black	Green-yellow	Green-yellow	Red-black	Blue black	Blue black	Mavi siyah
Skin thickness	227	Strong	Strong	Strong	Strong	Medium	Strong	Medium
Flesh colour	228	Medium	Thick	Very Thick	Thick	Thick	Thick	Medium
Juiceness of flesh	230-231	Colorless	Very light colored	Colorless	Very Lightly colored	Lightly colored	Lightly colored	Lightly colored
Taste	236	Foxy	Foxy	Foxy	Foxy	Foxy	Foxy	Foxy
Berry stalck length	238	Short	Very short	Short	Short	Very short	Very short	Short
SEED								
Seed weight	243	Seedless	Medium	Medium	Medium	Seedless	Medium	High
Transverse grooves on the dorsal side	244	Seedless	Absent	Absent	No	Seedless	Absent	No

Stem opening grade is “too open” in Concord variety; It was found to be "closed" in August Giant and "open" in other varieties examined. The main shape of the leaf stem pocket is classified as “U” and “V”. Accordingly, among the examined cultivars, Delicatessan, Delaware, Neptune, Valiant and Concord, the leaf stem pocket is "U" shaped; In other varieties, it is "V" shaped. The leaf stem pocket shape is defined as “closed U”, “closed V”, “open U”, “open V” shapes. One of the features of the leaf stem pocket is that the leaf stem pocket is limited by the lateral veins or has teeth on the edge. It has been determined that the leaf stem pockets of the examined cultivars do not have these features. The shape of the upper side pockets; It is “closed” in August Giant and Champbell Early cultivars and “open” in other cultivars. In terms of the main shape of the side pockets, only Concord cultivar has “U” shaped side pockets, while other cultivars have “V” shaped side pockets (Table 2 and 3). The degree of stem opening is leaf size, it varies according to varieties such as tooth shape and tooth length. In addition, it has been found that stem spacing is inversely related to the time or rate of cell division during leaf formation, so that the depth of the stem pocket is less in leaves with more cell division (Keller, 2015).

Table 4. Mature leaf characteristics of American foxy grapes

Variety	*Leaf length* (cm)	*Petiole length* (cm)	*Leaf area* (cm^2)
August Giand	15.20±0.64	5.60±0.47	174.54 ± 6.94
Bluebell	17.90±0.40	5.86±0.31	217.90±12.00
Buffalo	18.90±0.28	6.76±0.42	207.20±5.84
Champbell Early	17.50±0.80	5.06±0.24	214.60±8.30
Delicatessan	17.90±0.51	4.98±0.47	215.30±9.67
Delaware	18.50±0.42	6.48±0.36	215.80±2.79
Munson	13.80±0.34	4.58±0.38	190.10±9.90
Mars	16.40±0.73	6.12±0.28	228.70±11.70
Niagara	16.30±0.55	5.96±0.36	193.30±10.70
Neptune	14.10±0.18	6.20±0.45	146.50±9.47
Steuben	16.20±0.62	6.34±0.28	182.10±3.94
Venus	16.10±0.57	6.80±0.36	205.10±13.20
Valiant	14.30±0.44	6.30±0.42	170.90±8.54
Concord	12.60±0.44	6.50±0.34	178.20±5.26

In the experiment, the length of the petiole was generally less than 7 cm and it was found to be in the "very short" group. In the Buffalo variety, the petiole length is between 7-11.4 cm and is classified as "short". The ratio of petiole length to midrib length was found to be less than 0.50 in Munson, Niagara and Valiant cultivars and "much shorter", while this ratio was between 0.50-0.80 and "shorter" in other cultivars (Table 4). Mature leaf characteristics are considered as one of the most important parameters examined in ampelography (Çelik et al., 2018). The mature leaf characteristics of the cultivars examined in the experiment differed according to the cultivars. These differences are mostly due to the characteristics of the variety. On the other hand, leaf characteristics are closely related to the fertility of the soil where the vine grows, its growth strength and climatic conditions.

The main color of the shoots of American grape varieties examined in the experiment; It is "dark brown" in Blubell and Mars cultivars, "reddish brown" in Delaware and Munson cultivars, and "yellowish brown" in other cultivars. It was determined that Steuben, Venus, Valiant and Concord cultivars had lenticels on the rods, and other cultivars had no lenticels. Bluebell, Delicatessan, Delaware, Mars and Concord cultivars do not have vertical hairs at the nodes and between the nodes; in other cultivars, there are “very sparse” vertical hairs on the nodes; It has been determined that there are no vertical hairs between the nodes (Table 2 and 3).

Flower, bunch, berry and seed characteristics

It was determined that all the examined cultivars had a " hermaphrodite" flower structure. The first inflorescence is generally emerging from the 3rd or 4th nodes, only in the Neptune variety the first inflorescence is on 5th node. Although the number of inflorescences per shoot varies according to the varieties; 1 or 2 bunches were found in August Giant, Buffalo, Delaware, Neptune and Steuben varieties; While 2 or 3 clusters" were found in Blubell, Champbell Early, Munson, Mars, Niagara, Venus and Valiant varieties, the number of flower clusters per shoot was found to be more than 3 in Concord variety (Table 2 and 3). Çelik et al. (2008) examining the labrusca types in the Northeast Anatolian region and they reported that the number of inflorescences arised from the shoot may be 1– 2, 2– 3 or more than 3. Although the length of the first inflorescence varies according to the cultivars, it was determined that it was between 6.00-17.40 cm. In the cultivars August Giant, Buffalo, Delicatessan, Delaware, Mars, Steuben, Venus, Valiant and Concord, the length of the first inflorescence is between 6.00-12.40 cm, the length of the panicle is “short”; It was determined that the length of the first inflorescence was between 12.5-17.4 cm and the other varieties were in the “middle” class (Table 2, 3).

It was determined that the berry length of the foxy grape varieties examined in the experiment and brought from the USA by introduction was "short" or "very short". In Venus, Valiant and Munson varieties, the berry length is "very short" and less than 11 mm; In other cultivars, the berry length was found to be "short". In terms of berry length, the longest grain was determined in Munson variety with 13.68 mm, and the shortest one was determined in Valiant variety with 8.6 mm. Berry width “too narrow” (<11.5 mm) in Neptune; “narrow” (11.5-14 mm) in August Giant, Blubell, Buffalo, Champbell Early, Munson, Venus and Concord varieties; While it was determined to be “medium” (14.1-17.5 mm) in Delicatessan, Delaware, Mars, Niagara and Steuben varieties, the highest value in terms of berry width was observed in Mars variety of 16.4 mm, the lowest value was determined in Valiant variety with 11.42 mm (Table 4).

Table 5. Bunch characteristics of American foxy grapes

Variety	*Bunch length* (cm)	*Bunch area* (cm^2)	*Bunch stem length* (cm)
August Giand	12.0±0.53	136.12±9.05	3.96±0.57
Bluebell	21.0±0.51	235.80±10.30	5.42±0.27
Buffalo	10.2±0.79	145.50±13.10	3.31±0.37
Champbell Early	11.1±0.42	103.40±6.69	3.68±0.11
Delicatessan	9.8±0.50	114.40±4.87	2.50±0.22
Delaware	10.9±0.39	113.10±13.90	2.72±0.16
Munson	14.5±0.79	154.30±6.70	3.90±0.36
Mars	18.4±0.40	206.20±5.60	4.18±0.57
Niagara	16.1±0.57	142.60±12.40	3.84±0.28
Neptune	14.1±0.50	142.50±9.08	2.88±0.39
Steuben	17.1±0.30	160.10±7.74	3.70±0.41
Venus	17.0±0.28	136.20±4.42	3.76±0.25
Valiant	10.7±0.56	97.00±9.89	2.38±0.24
Concord	16.0±0.20	146.50±6.97	4.22±0.18

There are also differences among the varieties in terms of uniformity in berry size. It was determined that the berry shape was "wide oval" in Mars, Niagara, Neptune, Steuben, Venus, Bluebell cultivars, "short oval" in August Giant, Buffalo, Champbell Early, Munson and Concord cultivars, and "round" in Delicatessan, Delaware and Valinate cultivars. The cross-section of the berry was examined in two categories as "not round" and "round" and it was determined that the cross section of the berry was "round" in all of the examined varieties. Although the color of the bark varies according to the cultivars, it has been determined that "blue black", "red black" and "green yellow".

It has been determined that the number of grape clusters per shoot varies according to the varieties. August Giant, Buffalo, Delaware, Steuben varieties have less cluster number (1-2 clusters) per shoot than other varieties; The number of clusters per shoot is 2-3 in Blubell, Champbell Early, Delicatessan, Mars, Niagara, Venus, Valiant and Concord; It was determined that more than 3 clusters were formed in Munson variety. As a matter of fact, Çelik et al. (2008) determined the same situation in the labrusca types in the Northeast Anatolian region,

Table 6. Berry characteristics of American foxy grapes

Variety	*Berry stem length* (mm)	*Berry length* (mm)	*Berry diameter* (mm)
August Giand	7.64±0.54	11.72±0.63	13.10±0.44
Bluebell	6.98±0.42	12.10±0.33	13.40±0.27
Buffalo	8.62±0.48	13.50±0.82	12.90±0.62
Champbell Early	5.52±0.29	13.90±0.41	12.60±0.46
Delicatessan	4.10±0.28	12.20±0.70	15.60±0.52
Delaware	4.76±0.36	12.10±0.85	15.80±0.62
Munson	7.04±0.48	13.68±0.47	12.10±0.41
Mars	7.16±0.48	15.10±1.03	16.40±0.98
Niagara	5.10±0.46	13.20±0.78	14.30±0.24
Neptune	7.04±0.37	12.60±0.48	13.90±0.76
Steuben	7.72±0.35	15.10±0.54	16.70±0.36
Venus	5.38±0.42	9.82±0.69	11.90±0.15
Valiant	5.06±0.23	8.60±0.36	11.42±0.36
Concord	7.38±0.39	16.10±0.30	13.50±0.11

It has been determined that the size of the cluster in the cultivars is generally “small”. Blubell and Mars cultivars have larger clusters than other cultivars and are in the "medium" class; August Giant, Munson and Steuben are in the “small” class; other cultivars were classified as “very small” in terms of cluster size. Çelik et al. (2008) detected “small”, “medium” and “large” clusters in labrusca types. Cluster length is “medium” in Blubell and Mars varieties; Buffalo, Delicatessan, Delaware and Valiant cultivars were determined to be "very short", while other cultivars were determined to be "short". Among the cultivars, the highest cluster length was found in the Bluebell variety with 21 cm, and the lowest in the Delicatessan variety with 9.8 cm (Table 3). The frequency of grape clusters; “frequent” in the Munson variety; “medium” in Blubell, Delaware, Niagara, Neptune, Valiant and Concord cultivars; In the other varieties examined, it was determined that they had “sparse” berry. It has been determined that the number of berry is generally "very few" in the varieties, and it has been determined that the varieties generally contain less than 51 berries. It has been determined that only Blubell has 51-124 berry number among the examined cultivars and unlike the others, it is classified as "low". While the Bluebell variety had the "highest" number of berry (57.6) in the cluster, the Neptune variety had the "least" number of berry (36.0). Bunch stem length; “Very short” in Neptune, Valiant, Delicatessan and Delaware varieties; It was determined that it was “short” in other cultivars and the length of the bunch stem was the “shortest” (2.38 cm) in the Valinat cultivar, and the 'longest' (5.42 cm) in the Blubell cultivar (Tables 3, 4, 5) . The lignification of the bunch stem is “medium” in Champbell Early, Dellicatessan, Delaware, Neptune and Valiant; other varieties were found to be "weak". The cluster characteristics are under the influence of various factors such as soil type, irrigation, training system, pruning, environmental conditions at the time of flowering and chemical applications (Keller, 2015).

In Niagara and Neptune varieties, the rind color of the grain is “green yellow”; “red black” in Steuben and Champbell Early cultivars; In other cultivars, it was observed to be "blue black". While the bark color was not uniform in Champbell Early, Neptune, Steuben, Venus and Concord cultivars, the bark color showed a homogeneous distribution in other cultivars. Cangi et al. (2006a) reported that the berry color was similarly blue-black, dark red-violet and red-gray, and the coloration was not uniform in most of the examined types of *V. labrusca* examined by the researchers. According to Çelik (2004), in contrast to the labruscas, which are known as grapes that come out of the skin because of their thick skin, the skin thickness in the trial varieties is "very thin" in Neptune; “Slim” in August Giant, Buffalo, Champbell Early, Delaware, Munson, Niagara, Steuben, Venus and Valiant cultivars; In Bluebell, Delicatessan, Mars and Concord varieties, it was determined to be "medium" thickness. While the flesh was "colorless" in Champbell Early, Munson, Mars and Neptun cultivars, it was also found to be "very lightly colored" in other cultivars. As a matter of fact, Çelik et al. (2008) state that the flesh of some labrusca berry is slightly colored. It was determined that the fruit flesh was juicy in all of the examined cultivars. Varieties in terms of taste; It was evaluated as “tasteless” (none), “musket”, “foxy” and “other” and it was determined that the taste characteristics of all varieties were “foxy”, that is, fragrant. In the classification of taste, it has been determined that all varieties are in the "aromatic" group. Taste classification results are consistent with previous studies and foxy aroma is characteristic of *V. labrusca* cultivars (Cangi et al., 2006a and 2006b). In Delicatessan, Delaware, Niagara, Venus and Valiant cultivars, which are evaluated as "very short" in terms of berry stem length, the berry stem is smaller than 6 mm; In other cultivars evaluated as "short", it was determined that the grain stalk was between 6-10.4 mm (Table 4). As a matter of fact, Melek and Çelik (2005) state that some labrusca types have very short or short berry stalk length. As mentioned before, many factors affect cluster characteristics. Indirectly, the same environmental factors and cultural practices also affect grain characteristics (Morton, 1979).

Table 7. Inlorescence, tendril, berry and seed characteristics of American foxy grapes

Variety	First inflorescence length (cm)	Tendril length (cm)	Berry number (numb/bunch)	Seed weight (g/100 seeds)
August Giand	11.50±0.45	9.90±0.60	42.8±3.70	26.00±3.73
Bluebell	14.50±0.89	12.60±0.57	57.6±2.72	Seedless
Buffalo	11.80±0.38	16.20±0.60	45.1±2.96	20.70±0.83
Champbell Early	13.70±0.85	16.20±0.52	37.6±3.44	18.50±1.33
Delicatessan	12.60±0.21	15.50±0.62	39.2±5.23	39.20±2.62
Delaware	11.60±0.76	10.20±0.50	45.2±2.99	37.80±3.37
Munson	13.60±0.70	15.60±0.71	45.4±1.85	38.00±2.20
Mars	11.20±0.52	15.50±1.09	42.0±2.60	Seedless
Niagara	13.70±0.66	15.90±0.87	39.2±2.31	39.10±3.02
Neptune	14.20±0.64	12.50±0.56	36.0±1.78	37.50±2.86
Steuben	11.30±0.57	10.10±0.38	46.8±3.18	39.10±2.22
Venus	11.50±0.60	16.20±0.31	38.8±3.31	Seedless
Valiant	11.90±0.36	15.90±0.38	48.2±1.32	41.20±1.94
Concord	12.20±0.59	16.50±0.46	44.0±2.82	51.30±1.78

While Bluebell, Mars and Venus cultivars examined within the scope of the study do not have seeds, other cultivars have seeds (Table 5). Seed weight “low” in August Giant, Buffalo and Champbell Early; “medium” in Bluebell, Delicatessan, Delaware, Munson, Niagara, Neptune, Steuben and Valiant cultivars; The seed weight of the Concord variety was found to be "high" (Table 5).

CONCLUSIONS

As a result, in order to bring viticulture to a higher level in Turkey, it is necessary to determine the characteristics of existing genetic resources in international norms. For this purpose, it is important to determine the ampelographic characteristics and to fully reveal the characteristics of the cultivars. The *V. labrusca* species or types or varieties included in this species have been used for cold resistance breeding in Korea, Japan, China and Thailand. In these areas, winter temperatures can drop down to -20, -35 °C. On the other hand, labrusca grapes attract the attention of breeders with their special aroma. Grape varieties of *V. labrusca* type are used extensively in breeding in Asia and America.

Determination of ampelographic characteristics is important in terms of defining and revealing the characteristics of the varieties, as well as in determining the varieties that are adapted to the region where the cultivation is made. In this study, extensive ampelography of grape varieties that are disease resistant, can be grown in high humidity conditions, brought to Turkey by introduction from North America and have the characteristics of foxy grapes, grown in Atakum district of Samsun province were carried out. The varieties covered in the study differed from each other in terms of the ampelographic characteristics examined. It was determined that 3 of the cultivars were seedless (Blubell and Mars, Venus). On the other hand, by determining these differences of the varieties, the varieties can be evaluated as breeding materials for future breeding studies, and the superior characters they have can be transferred to varieties adapted to our region in terms of yield and quality, and breeding of new varieties can be achieved. In recent years, foxy grape varieties and types, which have been determined to be resistant to some fungal diseases, have been started to be used in breeding studies.

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HISTORICAL DEVELOPMENT OF HORSE BREEDS

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ABSTRACT

This study was conducted to examine the historical development process of modern horse breeds. Horses are among the few species that have managed to become domesticated on earth. The domestication of horses took place after dogs, goats, sheep, pigs, reindeer and poultry. It is accepted that the first ancestor of the horse species existed in the Eocene geological period (55 million years ago). In the fossil record, 13 genera have been identified in the equine family. Of these, Equus is the only surviving genus today. Different ecological regions, climates and cultural conditions have led to the development of well-adapted horse breeds. In addition, the changing needs of human beings have had important effects on the formation and development of these breeds. The changing needs of people have been effective in the differentiation of horses in areas such as hunting and food source, battlefields, agricultural activities, cargo transportation, transportation, show, entertainment, hobby, sports, horse racing and finally therapy and support. As mechanization increases as a result of technological developments in the world, there is a decrease in horse population and genetic diversity. In order to prevent these, measures should be taken such as doing more studies on horse breeds, drawing attention to the relationship between human and horse in the historical process, and protecting genetically endangered breeds.

Keywords: Horse breed, historical development, horse coat color

1. INTRODUCTION

In the domestication of the horse, human beings have made important contributions, and the development and formation of the breeds has been directed in line with the desired characteristics. Features such as hunting and food source, battlefields, agricultural activities, cargo transportation, transportation, show, entertainment, hobby, sports, horse racing and finally therapy and support have been effective in the domestication of the horse and the formation of breeds.

The horse is among the few domesticated species on earth (Rothe et al., 2005). It is generally accepted that the first ancestor of the horse species existed 55 million years ago in the *Eocene* geological period (Sakınç, 2007).

In the fossil record, 13 genera have been identified in the *equine* family. Of these, *Equus* is the only surviving genus and it has been found to contain about 30 species (*Equus ferus caballus*) (Waran et al., 2007). Today, 7 members of the *Equus* genus remain, apart from horses. These; Wild Donkey (*Equus ferus*), African Wild Donkey (*Equus africanus*), Onager 'Asian'

Donkey (*Equus hemionus*), Kiang (*Equus kiang*), Strike Zebra (*Equus scratchi*), Burçel 'Plain' Zebra (*Equus quagga*) and Mountain Zebra (*Equus zebra*) species (Yılmaz, O., 2012). The domestication of horses dates back 6,000 years. This process began in Dereivka, Ukraine, with their preservation as a food source in their natural habitat (Waran et al., 2007). In the process from *Eohippu*s to the modern horse; loss of the soles of the feet, elongation of the legs, the formation of the ankle bones by fusing the independent bones under the legs with the knee, an increase in the size and complexity of the brain, and developments in the teeth suitable for grazing (Anonymous, 2021a). In addition, while they were the size of a fox and their feet were five-toed at the beginning; it was later determined that they grew in size and size, the number of fingers decreased, and the toe bones extended over time and their middle fingers developed (Anonymous, 2021b).

In the research of this subject, an overview of the adventure of the horse breed, which has always had an important place in the history of mankind, is aimed to be examined, although its usage area changes according to production and technological developments.

2. HISTORICAL DEVELOPMENT OF HORSES

Interventions shaped by human experience and needs, rather than natural ways, had an impact on the domestication process of the horse (Rothe et al., 2005). domestication of horses; after dogs, goats, sheep, pigs, reindeer and poultry (Koçkar, 2012).

We can summarize the development of the modern horse in the historical process chronologically as follows (Sakınç, 2007; Koçkar, 2012):

-Little horses (55 million years ago)

- ✓ *Hyracotherium (Eohippus)*
- ✓ *Orahippus*
- ✓ *Epihippus*

-Leaf-fed, low-crowned teeth, medium-sized horses (45-24 million years ago)

- ✓ *Mesohippus*
- ✓ *Miohippus*
    - -*Anchitherium*
    - -*Kalobatippus*
    - -*Archaeohippus*
    - -*Hypohippus*
    - -*Megahippus*

-The arrival of horses on the plains (18 million years ago) and horses with slender legs and high-crowned teeth

- ✓ *Parahippus*
- ✓ *Merychippus*

- One-toed horses (10-1 million years ago)

- ✓ *Pliohippus*
- ✓ *Dinohippus*
- ✓ *Astrohippus*
- ✓ *Neohipparion*
- ✓ *Equus*

-Modern horses (4 million years ago)

3. MODERN HOURSE BREDS

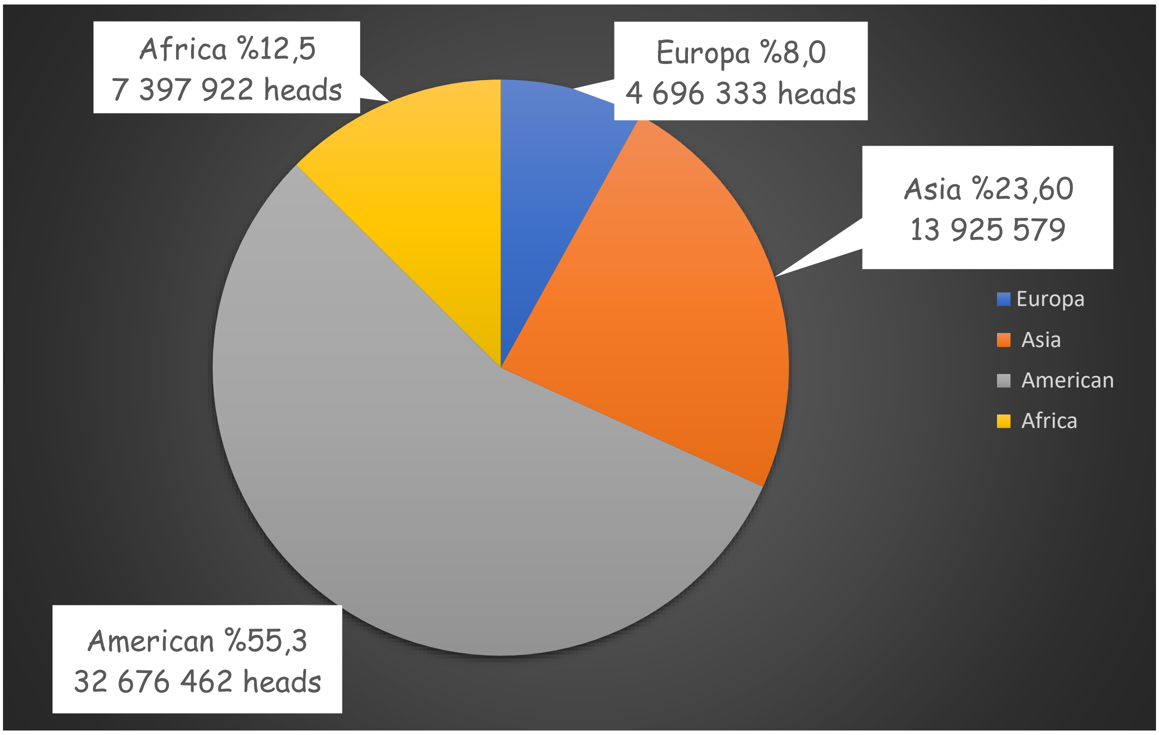

Figure 1. Distribution of Horse Population by Continent in 2019 (FAO, 2021).

In world horse breeding, the Americas constitute 55.3% of the total population with 32 676 462 heads (Figure 1). Other continents in horse breeding are ranked as Asia (23.60%), Africa (12.5%) and Europe (8.0%), respectively. As a matter of fact, as given in Figure 2, located in the Americas; USA (10 702 799 heads), Mexico (6 382 699 heads) and Brazil (5 850 154) are the most important countries in the world in horse breeding.

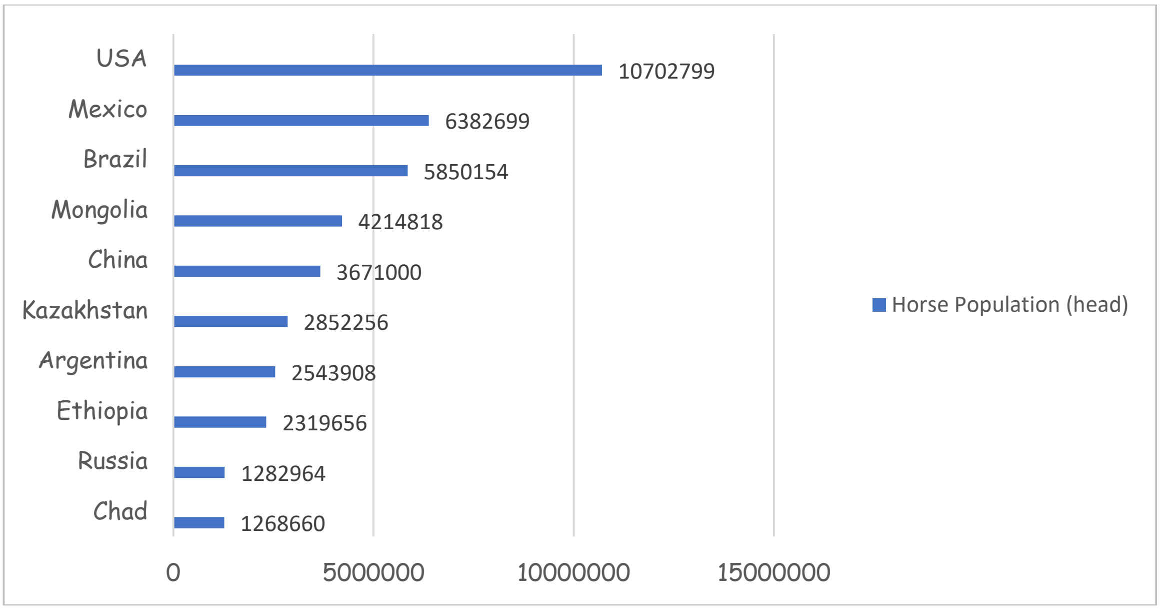

Figure 2. Top 10 Horse Breeders Country in the World in 2019 (FAO, 2021).

There are more than 300 breeds of horses and ponies in the world and they can be classified in several different ways. For example, horses can be classified as hot-blooded, cold-blooded, or pony. In addition, it can be grouped as light (light), heavy (draft) or pony according to size, weight and structure. Horses in these groups; It can also be further subdivided according to its uses, such as riding, racing, driving, jumping, or use. Horse classifications are also significantly dependent on the horse's withers height and weight (Parker, R., 2007).

All modern horses can be divided into three main categories: hot-blooded, cold-blooded and warm-blooded:

1. Hot-blooded Horses: Hot-blooded Horses: It has been reported that warm-blooded horse breeds originated from E. Tarpan (Köseman and Şeker, 2016). Fast and well-tempered, warm-blooded breeds are best suited for experienced riders. The most popular hot-blooded horses are the Arabian horse, originating from the Middle East and dating back thousands of years. Arabian horses are known for their noble appearance, intelligence and endurance over long distances. These horses are used for show purposes or as race horses (McIntosh, 2014). Another famous hot-blooded horse is the Thoroughbred, the classic racehorse. It is a special breed developed to reach speeds of 40 miles per hour in thoroughbred middle-distance running. They are trained to start racing at age two and often retire at age five. Thoroughbreds are suitable for equestrian sports such as horse shows and polo, although they are prone to a nervous temperament and health problems (McIntosh, 2014). Thoroughbreds have been bred in the UK for over 300 years and 5,000 thoroughbred foals are produced annually. There is also an important part of the breeding industry devoted to producing sport horses (Anonymous, 2005).

2. Cold-blooded Horses: It has been reported that the cold-blooded horse breeds originated from E. Preçevalski (Köseman and Şeker, 2016). Cold-blooded horses are heavy, solid and strong load horses with a calm temperament (Parker, 2007; McIntosh, 2014). As a result of the development of technology, machines have largely taken their place. The most famous coldbloods in the United States are the Clydesdales, popular at parades and shows. Derived from Scottish farm horses, these gentle giants weigh a ton, stand over six meters high, and have large feet (horseshoes the size of a dinner plate) with distinctive hairs hanging over their hooves. (McIntosh, 2014).

3. Warm-blooded breeds: The term warm-blooded is not related to horses with a certain blood temperature. It refers to the general temperament of light medium horse breeds. Warm-blooded horses are thin-boned and suitable for riding. In some countries, it is used for horses that have the gene for the warm-blooded Arabian horse breed. According to another opinion, all light horses are expressed as warm-blooded, while another opinion suggests that all breeds that are not purebred, heavy or pony can be classified as warm-blooded (Parker, R., 2007). Warm-blooded breeds combine the agility of the warm-blooded and the moderate temperament of the cold-blooded. There are many warm-blooded breeds in the United States. Good-natured and easy to handle, these horses are excellent pets and show horses. Originally herding cowboys in the western United States, these breeds are now considered ideal for riders of all levels and even for introducing children to the joys of horseback riding (McIntosh, 2014).

Grouping of horses by size, weight and structure:

A) Heavy Horse Breeds (Draft Horse):

Heavy horses have a height of 144-175 cm and a weight of 635 kg and above. They are mainly used for heavy work or towing loads (Parker, R., 2007).

1. Belgian Horse: The main area of use of this breed, which has been found to spread to 12 countries in the world, is towing works that require power (Khadka, 2010). Mainly used for heavy draft. The height of these Belgian-origin horses is 155.5-172.8 cm. They are massive and powerful animals. They have short and sloping shoulders and short, thick necks. It has a short back and wide legs (Anonymous, 2021a).

2. Clydesdale Horse: Although the Clydesdale is a heavy draft horse, it has the attitude and grace of a much lighter horse. In addition to being one of the most athletic breeds for their size, they have beautiful proportions. The withers height of these horses originating from Scotland is 163-183 cm. It is characterized by a well-curved neck with large eyes and a graceful head, high shoulders and a short, strong back, muscular rump, long, silky hair on long legs with sturdy legs. Available in true, black, grey, navy blue or red. White spots appear on the face and legs. It is suitable to be used as a heavy tow, farm work, equestrian, show and cavalry horse (Anonymous, 2021c).

3. Percheron Horse: It is a breed used in draft and sports fields spread over 15 countries worldwide (Khadka, 2010). It is of French origin. The height is approximately 163 cm. It is typically characterized by a gray or black color, high chest width and hairless legs (Anonymous, 2021a).

4. Shire Horse: Originated from England. Although it is usually around 173 cm, it has been reported to reach withers heights of up to 193 cm. It has a convex profile, relatively long neck, long, sloping shoulders, short back and heavily feathered ankles below the knee (Anonymous, 2021a).

B) Light Horse Breeds:

The height of light horses varies between 122-175 cm and their weight varies between 408-635 kg. They are primarily used for boarding, driving, show or racing purposes but are also suitable for use in farm and livestock work. Light horses are more active, agile and fast compared to heavy horses (Parker, R., 2007).

1. Akhal-Teke Horse: Akhal-Teke is one of the oldest, most remarkable and extraordinary horses in the world. These horses were first bred 3000 years ago around the oases of the Turkmenistan Desert in northern Iran (Ustyantseva et al., 2019). The height of Akhal-Teke is 150-170 cm. It holds its head upright, has a straight profile and a long, narrow, rather high neck. It also has a medium length, sometimes straight shoulder and a long, straight back (Anonymous, 2011d). It has long and slender legs (Anonymous, 2021a). It has small, stiff, hairless hooves and a rather sparse tail and silky-looking mane. Ears are longer and wider than other breeds and are slightly sickle-shaped. The eyes are usually almond-shaped (Anonymous, 2011d). These horses; The fact that it is fast, durable and easily trainable has enabled it to be used in a versatile way (Leisson et al., 2010). Akhal-Teke; The racehorse is a suitable horse for long-distance travel and equestrian sports riding (Ustyantseva et al., 2019).

2. American Quarter Horse: These horses are adept at short-distance (quarter-mile) racing. Its origin is based on horses brought to America by Spanish immigrants in the 17th and 18th centuries (Koçkar, 2012). Reported to have spread to 28 countries, this horse breed is the most popular breed in the USA (Khadka, 2010). They were used in cattle herding activities due to their athletic and courageous features, and while running these pursuits, they gained the ability to turn in the opposite direction and run quickly back. With the mechanization, its

importance in agricultural activities decreased and it started to be used as a hobby and sports horse. Withers height is 143-160cm. Different colors are available. It has a short and broad head structure, a small nose, large eyes, upturned ears, long and elastic neck structure, and rounded shoulders. The chest and abdomen are broad and the back is short. The rump is large, deep and muscular. Its legs are strong. Their nails are rectangular in shape, have a deep and wide nail structure (Koçkar, 2012).

3. American Saddlebred Horse: American Saddlebred horses were crossbred and selection with Morgan and Narragansett Pacer horses (Koçkar, 2012; Anonymous 2021e) of Pony (Galloway and Hobby horses) brought by immigrants from England to North America in the late 1600s. developed as a result of their breeding. Their average height is 145 – 170 cm. American Saddlebred horses have a broad forehead, straight profile and large black eyes. It has a thin neck and smooth mane. It has a deep and sloping shoulder structure. Their legs are smooth, muscular and strong (Koçkar, 2012). Their average lifespan is between 25-35 years (Anonymous 2021e).

4. Andalusian Horse: It originates from Spain. Its height is 155 – 160cm. It has large almond eyes. The nose is straight or slightly protruding. Their necks are strong, broad, beautifully curved and long. The neck is wide, the back is strong and the muscles are visible. Chest width is high. They have long tails, bushy and slightly wavy hair. It has long manes. It has short and strong legs. It has high and solid hooves. Although there is no standard color of Andalusian horses, 80% of them are gray. Other important colors are Doru, Dark Doru and Yağız (Koçkar, 2012).

5. Appaloosa Horse: It originates from the states of Idaho, Oregon and Washington in the United States of America. Claimed to be a cross between horses brought by Spanish explorers and native wild Mustangs, they were first bred in North America by the Nez Perce Indians. Some Appaloosas have a solid coloration interspersed with small, round spots of the same color as the body, except for a white patch on the hips. Others are covered with white spots in a solid solid color. Its height is 145-163 cm. Used as a riding horse (Anonymous 2021a).

6. Arabian Horse: These horses originate from the oases along the Tigris river in Syria, Iran and Iraq and from other parts of the Arabian peninsula (Koçkar, 2012). Today, this breed is cultivated in approximately 59 countries. They are widely used in different branches of equestrian sport (Khadka, 2010). Big eyes, upturned tail, short and straight back, muscular and broad chest, muscular legs, small nails enlarging on the heel are some of its characteristic features. Colors; it can be nutshell, iron grit, rain and light frost. Whiteness on the face and legs is common. It has a thin and silky skin, bushy mane and tails (Koçkar, 2012).

7. Criollo (South America) Horse: Criollo is a horse breed originating from Spain. These horses, which were released to nature in the USA, where they were brought, tried to adapt to the environment for 4 centuries and were subjected to natural selection (Koçkar, 2012).They have a broad forehead and sometimes convex profile, watchful eyes, small and pointed ears, neck muscular and slightly crested, broad and strong hindquarters, short, strong and strong legs (Anonymous 2021f), short and deep trunks and a muscular structure (Anonymous, 2021a). Its height is 140- 150 cm. It is used as farm work, draft, show and riding horse (Koçkar, 2012; Anonymous 2021f).

8. Hanoverian Horses: It is a noble warm-blooded horse breed originating from Germany and distributed in 12 countries of the world (Khadka, 2010). They are calm and dignified horses (Koçkar, 2012). It is one of the leading sport horse breeds in the world. This warm-blooded breed, which is the leader in horse breeding worldwide, is considered one of the main breeds of the modern sport horse (Anonymous 2021g). Show jumping, activity and recreational riding are other uses (Khadka, 2010). Its height is 155-172 cm. They have long and muscular necks, high chest depth and strong hindquarters structure (Anonymous, 2021a).

9. Lipizzan Horses: This breed dates back to the 16th century, when this breed was developed by the Habsburg family to meet their need for a strong but agile horse for use in the army and riding schools. In 1562, the Emperor brought Spanish horses to the palace and established a stud farm. Likewise, he established a similar breeder in Lipizza in Slovenia. These two horse farms produced the basic stallions of the modern Lipizzan breed. The offspring of native Karst horses, originally crossed with Spanish, Barb, and Arabian stallions, were crossed with the now-extinct Neapolitan horse, Kladruber, Frederiksborg, and other Baroque horses of Spanish origin. Between 1765 and 1819, the parents of the Lipizzan horses known today were formed (Anonymous, 2021h). It has been found to spread to 18 countries, mostly in Europe (Khadka, 2010). Its height is between 145-155 cm. Their heads are long and large, their eyes are expressive, their ears are small and their nostrils are wide. They have a short, curved neck, broad and deep chest, solid shoulders and very rounded rumps. Their tails are high and very thick and long like a mane. They have short and strong legs and small and hard nails. The dominant color on most Lipizzan horses is gray (Anonymous, 2021h).

10. Missouri fox trotter horse: It is a horse breed that settlers developed in the Ozark Mountains of Missouri in the early 19th century by crossing the horses they brought with them with strong breeds. Its maneuverability and smooth walking on rocky terrains has earned it acceptance by the locals. It was used for plowing fields, logging and cattle work. Missouri fox-trotting horses; It has a flat facial profile, a muscular body and a short back. It is a strong horse breed with sloping and strong shoulders, and solid legs. Their heads and tails are almost always high and their ears are pointed. This breed has many color variations. Available in chestnut, gray, champagne, black and many more. Some horses have white spots on their legs and faces, while others have spots (Murphy, 2019).

11. Morgan Horse: This horse of American origin was born as a colt in 1789 and began to be named after its owner. Although it is not known for certain, the ancestors of this stallion were a mix of English purebred, Arabian, Welsh Cob and Dutch breeds. Its height is 141-152 cm. Its color is usually true, chestnut, oily. The head is wide forward. It has large eyes, short and erect ears, a slightly angled neck, and sloping shoulders. Its hindquarter is muscular and its tail is up. Their straight and strong legs have short and thin bone structure. It has a soft and bushy tail and mane (Koçkar, 2012).

12. Paso Fino: These horses are descended from Spanish horses brought by immigrants in 1493. Today it has spread from the Dominican Republic to the entire Caribbean and many countries of South America. In the 1960s, it was imported for the first time by the USA and used and cultivated in this country. They are often considered recreational horses as they are easy to ride, tireless and reliably carry their riders across trails with a comfortable, consistent gait at varying speeds. In addition to being a means of entertainment, they are also used as tracking horses, work animals and show animals. The height of Paso Fino horses varies between 132 and 152 cm. It carries its tails high like a flag. It can be any color except the leopard color (Anonymous 2021). Paso Poodle horses are well profiled. Head and body are proportional. The nostrils are large and wide. The neck, which extends harmoniously from the body, takes a sharp curve when it comes to the head and is arched. It has a muscular, round and strong hindquarter structure. It has muscular and strong legs, a long and hairy mane and tail (Koçkar, 2012).

13. Standardbred Horse: The Standardbred is a horse breed developed in the United States in the 19th century and used primarily for running races. This breed was developed using the English Thoroughbred Messenger (1780-1808), which was imported from England to the United States in 1788. With considerable strength and stamina, Standardbreds are very similar to Thoroughbreds, but are generally smaller and have straighter ribs and heavier bones (Anonymous, 2021a). The height of this horse is 142-163 cm. The color of these horses can be any color, mostly brown, bay, black and chestnut (Parker, 2007).

14. Tennessee Walking Horse: The Morgan horse has a significant genetic contribution to the formation of this breed (Anonymous, 2021a). Their weight is between 410-540 kg. It has a long neck, small ears that are placed in proportion to the head. It has a short back, muscular and moderately deep legs (Anonymous 2021k). Its height is between 145-173 cm. There are sorrel, chestnut, black, white, gold, gray, bay and brown colors (Anonymous, 2021L). It was originally developed for use on farms and fields in South America. Later, it started to be used as a popular riding horse due to its calm stance, soft gait and solid feet (Anonymous, 2021k). It is used for entertainment, touring and show purposes (Anonymous, 2021L).

15. Thoroughbreds: Thoroughbreds are outstanding racehorses developed in the 18th century. Thoroughbreds have been racing since they were first bred. They got their speed from horses from Spain, Italy, Africa and Turkey. While there are many important stallions in thoroughbred history, the Byerly Turk, a Turkmen horse, Darley Arabian, a thoroughbred Arabian racehorse named after its owner, and the Moroccan horse Godolphin Barb are just a few basic stud stallions used. Thoroughbreds have very muscular shoulders. Normally, their necks are long and almost straight. Thoroughbreds have small but graceful heads with a neat profile. They have movable ears that are proportional to the head. Purebreds have large and lively eyes. The skin of these horses is thin and silky. They can be laurel, chestnut, black or gray in color. White spots are usually seen on their skin (Anonymous, 2021m).

C) Ponies

1. Connemare Horse: The Connemara pony is a world famous Irish horse with its athletic, intelligent and docile temperament. There are different rumors about the origins of this race. According to one view, the origin of the Connemara pony goes back to the Vikings, while another view traces its roots to the Irish Hoby horses, which became extinct in the 13th century. According to another rumor, it was formed by crossing Andalusian horses brought by Spanish ship in 1588 with Irish native breeds. Whatever its origin, the Connemara pony has become a hardy species that can thrive on the region's scanty vegetation (Hughes and O'Callaghan, 2015). Their height is between 132-144 cm. It is widely used as a riding and light draw horse. It has a tail that it carries above, a long neck with a full mane, and well-muscled legs with a bushy mane (Anonymous, 2021a).

2. Pony of the Americas: A good riding horse for children, the American pony was developed in 1954 by crossing Shetland ponies with Appaloosa mares. The little colt born from this union had a white body that looked like patches of black paint all over his body (Anonymous, 2021n). Its height is between 114-134 cm. It has erect ears and large eyes (Anonymous, 2021a).

3. Shetland Horse: The origin of Shetland is from the Scottish archipelago in the middle of the North Sea, off the coast of Scotland and Norway, from which it derives its name. In the past, the Shetland pony was used mainly by the inhabitants of the Shetland Islands to work in the fields and as a means of transportation. It was used as a horse carriage in coal mines with the industrial revolution in the middle of the 19th century. It has been used for entertainment purposes since the beginning of the 20th century. Harsh climatic conditions, nutritional instability and isolated conditions on the island have kept the Shetland pony small. Their height is 102-107 cm. Intelligent eyes, broad forehead; It has small ears and wide open nostrils. The mane and tail are covered with abundant and hard hairs. The neck is muscular. The chest is wide and deep, the back is short, the waist is strong and wide. It has strong legs, round, hard and durable nails (Anonymous, 2021o).

4. Welsh Pony: Welsh Pony horses are a pony horse bred in the Wales region of England. This horse was first domesticated by the Celtic people of ancient England. The harsh climatic conditions and food shortages in the region have increased the endurance of this small mountain

horse. Thanks to the Arabian horses that the Crusaders brought on their return, Arab blood was also mixed. In addition to the Arabian horse, the blood of Hackney horses and Thoroughbred horses after the 1950s were added over time and 4 different types developed:

- ✓ - Welsh Mountain Pony: Known as the Welsh Mountain Pony. Its height is maximum 122cm. It is one of the most used horses in equestrian sports and horse-drawn carriage. With its large eyes, it has a round head that has not been influenced by Arabian horses, a short back with a large bone structure, and straight legs.
- ✓ Welsh Pony: The height of the Welsh Pony is 137cm. is It is used a lot, especially in car driving races. He has a very harmonious body structure.
- ✓ Welsh Cob Pony: Its height is up to 137cm. It is a resistant, strong, calm and harmonious horse that can compete at a high level in equestrian sports. One of its most distinctive features is its silky skin. It has a long hindquarters, dark eyes, hard nails, deep chest and strong legs.
- ✓ Welsh Cob: No definitive figure for its height has been determined. According to some experts, they are the best horses for riding. It is a strong, durable and agile animal. He has a good temperament. Their head is smooth, their ears are small and erect, their hindquarter is round, their back is short, their legs are strong and their nails are hard (Koçkar, 2012).

4. COAT COLORS IN HORSES

The three basic colors formed by two pigments in horses are derived from the interaction of two genes (melanocortin-1 receptor (MC1R) and agouti-signaling protein (ASIP)). These colors are; straight, black and chestnut. Other genes (for example, dilution genes) determine variations of each of the three primary colors.

MC1R gene: Dominant allele genes (EE) determine eumelanin (brown-black) production; recessive (recessive) genes (Ee) determine the production of pheomelanin (yellow-red). According to the forms of this gene, it becomes a black color when there is at least one dominant allele ((E^EE^E or E^EE^e)) or a red color (reddish color) when the recessive allele genes are homozygous.

ASIP gene: Dominant allele (AA) encodes the production of agouti signaling protein, which has the ability to block the melanocortin receptor found in present melanocytes. When the receptor is blocked, eumelanin is not produced and only pheomelanin is produced.

Interaction results of MC1R and ASIP genes:

In the case of a dominant allele (A^AA^-) at the agouti locus, the body color of an eumelanic (E^EE^-) horse will have a bay color, which can be expressed as a reddish to brownish body colour.

A horse with the genotype $E^EE^-A^aA^a$ will be black. This color is quite common in the Percheron breed. Here, the superscript hyphen characters (-) are not used to mean "negative". This sign means that the allele at that locus is unimportant for the phenotype.

If the genotypes are located at locus as EeEe AAA- or EeEe AaAa, the horse is chestnut.

4.1. Basic colors in horses:

4.1.1. Bay: It is believed to be found in primitive herds and to be an effective camouflage against predators (Neves et al., 2017). The horse's body color can range from brownish red to almost yellow, including shades of mustard or light reddish. Mane, tail and lower leg appendages are black or sometimes dark brown. The Bay is one of the most common colors and is usually seen in American Quarter horses. It is also frequently encountered in Viatka and Bashkir horses and other domestic horses. This color is rarely seen in some races originating from the Iberian peninsula, such as Andalusia and Lusitano (Kurskaya, V., 2017).

4.1.2. Black: The skin, mane, tail and body hair are all black. Whiteness can only be found on the face and legs (Anonymous, 2021p). It is a rare color in horse populations. Black color in horses; dark glossy black, dull black and dirty black tones are available (Yılmaz and Ertuğrul, 2011).

4.1.3. Chestnut: It is characterized by the horse's appendages (mane, tail and legs) and all the hair on its body being red or different shades of red. Chestnut color in horses; It has normal (ordinary) red, light red, cherry red, whitewing, dark red and burnt red tones (Yılmaz and Ertuğrul, 2011).

4.2. Other Colors: Occur when proteins determined by specific genes change the amount of pigment in receptive structures (keratinocytes and hairs). The effect creates a dilution in the intensity of the original color due to its pigment lightening feature (Neves et al., 2017).

4.2.1. Gray: Involves the gradual whitening of all hairs on the horse's body, but the skin may remain dark for many years. Sometimes, the skin may remain unpigmented gradually (for example; Lipizzan). In such cases, at each shedding time, the horse's true color will be replaced by white hairs. When animals are born, their feathers have distinctive colors. Graying first begins around the eyes. Whitening can take years from horse to horse individually. This mutation causes excessive melanin production. This is why horses that turn gray are born with more vibrant dark colors. This feature is clearly observed in foals with jet black fur, unlike the mutation-free black foals that are born with a grayish color. It is common among various breeds such as Thoroughbred, Arabian, Percheron, Andalusian and is the only color seen in Lipizzan (Neves et al., 2017).

4.2.2. Cream: In these, the hairs covering the body are in different shades of yellow, while the hairs on the extensions are observed in light colors close to white. The "C" (Cream) gene, which has a color dilution feature, is active in the formation of this color (Yılmaz and Ertuğrul, 2011).

4.2.3. Champagne: The champagne gene sometimes gives the skin color a metallic sheen; it also causes the skin to change to a light brown color and amber eyes (Neves et al., 2017).

4.2.4. Dun: It is observed in horses with red coats. It is the covering of the whole body with white hairs and the formation of a red-white mixture. If the white hairs are more than the red hairs, they are called light gray, if they are equal, they are called ordinary gray, and if they are less, they are called dark gray (Yılmaz and Ertuğrul, 2011).

5. CONCLUSION

The relationship between horse and human, which started as a food source before domestication, has developed as a service tool that facilitates the work with domestication. This union, which has an important place in the historical adventure and existence of human beings, has been interrupted due to technological developments and there has been a significant decline in the horse population in the world. It is inevitable that these decreases will continue as the technological development in the world increases and becomes widespread. However, as a result of the demand for horse breeds suitable for new needs, there is also the danger of genetic extinction of a significant number of breeds. In order to prevent these, measures should be taken such as doing more studies on horse breeds, drawing attention to the relationship between human and horse in the historical process, and protecting genetically endangered breeds.

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EFFECT OF DIFERENT FATTENING SISTEMS ON FATTENING PERFORMANCE, AND CLINICAL INDICATIONS IN SHARRI LAMBS

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ABSTRACT

Determining the impact of fattening systems on fattening performance and clinical characteristics in sharri lambs is the aim of this research. Two different groups of lambs were formed: the control group (n = 12) cultivated and fed in the traditional form that is also practiced on most farms in Kosovo, and the experimental group (n = 12) cultivated and fed with concentrated mixtures dedicated to lamb fat and quality hay. The studies were completed in a period of 100 days, for which time the weight of the lambs in the control group was 24,903 kg, while in the experimental group 27,704 kg (P <0.01), and the daily live weight gain (DLWG) in the control group was 0.207 kg, while in the experimental group 0.240 kg (P <0.01), the yield of hot bodies (53,017% and 53,241%), the yield of cold bodies (52,006% and 50,406%).

Keywords: Fattening systems, Fattening performance, clinical characteristics and Sharri lambs

INTRODUCTION

Sheep farming is of particular importance in terms of its contribution to the economy and to human nutrition. This is attributed to the ability of sheep to utilize a large number of plants from nature (bulky food), especially from meadows and pastures that other animals have difficulty using or can not use at all, and their transformation into valuable products. high nutritional value such as milk and meat.

Despite having an important place in the economy, sheep farming in Kosovo is within the framework of traditional and semi-intensive farming. Breeds which are cultivated 95.01% are autochthonous and only 4.99% are mixed and other breeds (Mehmeti H.2000) where the sharri sheep is represented by 48.80% of the total number. The average weight of carcasses taken from slaughtered sheep is 12.423 kg and 13.455 kg. According to the Statistical Yearbook (Kosovo Agency of Statistics), 189,102 sheep were cultivated in Kosovo during 2019. The amount of meat obtained from slaughtered sheep and lambs is quite small, about 2520 tons, due to low yield and lack of intensive fattening.

Profit during lamb breeding depends on the quantity of production as well as the quality of the meat obtained, within the short period and the cheap production. Fattening systems, the fattening period as well as the final weight determine the quality and quantity of lamb meat. Breastfeeding of lamb is based on the principle of achieving the weight of slaughter for a short period by applying quality voluminous and concentrated food (ad libitum) as well as the absorption of milk from their mothers. In this system of fattening, lambs aged about 3.5 months are slaughtered, when they are separated from their mothers, reaching a live weight of up to 35 kg. While the traditional cultivation system is based on simple nutrition that is also practiced in most farms in Kosovo, which in addition to breastfeeding have available hay and ground corn. In this cultivation system up to the age of 3.5 months the live weight up to 30 kg is reached.

This study was conducted with the aim of comparing sharri sheep lambs cultivated in different fattening systems, ie lambs fed under different feeding conditions, in terms of fattening performance, slaughter and clinical indicators.

MATERIAL AND METHOD

The study was conducted on the farm where sharri sheep are cultivated in the village of Rakaj-Kosovo, starting from January 27 to May 10, 2018. The sharri sheep is an autochthonous sheep breed which as a breeding-cultivation area are sharri mountains.

Treatment of lambs and feeding

The lambs for the study (24 heads - 12 males and 12 females) were selected from the day of birth when the birth weight was measured, matriculated and divided into two groups of 12 lambs each (6 males and 6 females), from which one is the control group, while the other group is the researcher. The lambs until the 10th day stayed with their mothers and were fed with their mothers milk. The control group of lambs from the 10th day in addition to breast milk available have hay and ground corn until the 40th day, and after this day until the age of 100 days, those days are separated from their mothers while at night are together, ie during the night they can suck milk. The sheep and lambs of the control group are together at night, and during the day they are separated.

The experimental group of lambs after reaching the age of 10 days, in addition to breast milk have at their disposal concentrates and hay meadows (ad libitum) until the 40th day when they are separated from their mothers. They were fed lamb fat concentrates with 15.10% protein, and quality hay (ad libitum) until slaughter. The composition of concentrated feed is given in Table 1. The lambs received the water through special containers, where the water was filled twice a day.

Table 1. Nutrient kontents of concentate feed

Composition	%
Water	13,55
Dry matter	86,45
Crude protein	15,10
Crude fat	1,27
Crude cellulose	3,43
Mineral matter	4,35
Calcium	2,34
Phosphorus	0,48

Performance of growth and food spending

The monitoring of the live weight of the lambs was done every 10 days, from birth to slaughter with electronic scales with a capacity of 100 kg and a sensitivity of 5 gr. Also the consumption of food (ground corn and concentrate) in the whole experimental period is measured daily. In the morning before feeding the lambs, the unspent food left in the feed is first weighed, and then the new food is placed. Based on the food consumed, the total daily food consumption for the whole group and per head is calculated, as well as the food conversion, ie the expenditure of food per kilogram of growth. The tracking of the body weight of the lambs was done every 10 days in order to track the increase in body mass. Weighing of lambs is done in the morning before they are fed.

Based on the tracking of the body weight of lambs from birth to slaughter, ie up to the age of 100 days, the tracking of live weight of lambs is done in order to calculate the total daily increase for the period of fattening, overall daily lambs growth in the fattening period, as well as total and daily growth for 100 days. Based on the data obtained on body weight and total food expenditure of the groups, the cost of lamb feed for the respective time period was calculated, as well as the average feed conversion per kilogram of growth.

The slaughter process and clinical indicators

The slaughter of the lambs was carried in a private slaughterhouse (Dajti) without stunning, and the bleeding of the lambs was carried out after hanging on a hook, by cutting the blood vessels in the neck: *vena jugularis externa and arteria carotis communis*. After slaughter, the skin and lower parts of the legs (below the carpal and tarsal joints) were removed, then the internal organs of the abdominal cavity (stomach, intestine, spleen and liver) and the thoracic cavity (trachea, lungs and heart), which are weighed individually. After removing the internal organs at the time of slaughter, each carcass was weighed, recording the measured weight in a warm-headed body, and then sent to the cooling chamber. Without staying in the cooling chamber for 24 hours, carcasses were measured to determine the losses during cooling. The 30 kg electronic scale with a sensitivity of 5 gr was used for weighing the internal organs after slaughtering the lambs.

The results obtained from research done on the effect of different fattening system on fattening performance and clinical indicators of sharri lambs, are processed according to the usual statistical methods used for scientific purposes, using computer technology from Microsoft Excel by Microsoft Office software package. The processed statistical results are presented in tables for easier presentation of the differences displayed.

RESULTS AND DISCUSSION

The results obtained from the researches carried out for the breeding of lambs of the sharri breed sheep by the control group and the experimental group of lambs for the fattening period from the 1st day to the 100th day, are given in Table 2. The average birth weight of lambs in the control group is 4,156 ± 0.134 kg, while in the experimental group 4.307 ± 0.157 kg. At the end of the 40-day period, the average weight of lambs in the control group was 11,116 ± 0.343 kg, while in the experimental group 13,057 ± 0.603 kg. Weight the final fattening of the lambs of the control group is 24,903 ± 0.813 kg, while in the experimental group it is 27,704 ± 0.991 kg.

The fattening performance of lambs in both groups of lambs is presented in Table 3, where it is seen that the increase in DLWG in the control group was 207 gr, while in the experimental group was 240 gr, while the CCF in the control group was 764 gr and in the experimental group 889 gr. Food conversion per kilogram of body weight gain was 3,423 kg in the control group, while in the experimental group was 3,663 kg.

Table 4 presents the clinical characteristics of slaughtered lambs of the control group and the experimental group. There were statistically significant differences between the two groups in terms of live weight before slaughter, hot carcass weight, carcass percentage, head, legs, skin, liver, lungs, complete and empty digestive organs and heart.

The average birth weight of lamb breeds of sharri sheep in the control group is 4,156 kg with variation from 3.53 to 4.84 kg, while in the experimental group of lambs is 4.307 kg with variation from 3.20 kg to 5.32 kg. The difference in birth weight of lambs from the control and experimental group is 0.157 kg, to the benefit of the experimental group. The birth weight difference is statistically insignificant $p > 0.05$.

The difference in the total average growth for lambs of the control group (6,960 kg) and the experimental group (8,750 kg) from birth to the 40th day is 1,790 kg in favor of the experimental group, while the difference in the average daily growth is 0.045 kg in benefit of the experimental group. The differences in the average total growth per lamb and the average daily growth per lamb up to day 40 are not statistically significant p> 0.05. The lambs of the control group on the 100th day had an average weight of 24,903 kg, while the lambs of the experimental group weighed 27,704 kg. The difference in live weight of lambs on the 100th day between the two groups is 2,801 kg, which is statistically very significant p <0,001, which means that the method of cultivation, ie feeding the lambs during the fattening period had better results in growth on the experimental group, compared to the control group

The lambs of the control group from birth to the age of 100 days had an average increase for lambs of 20,474 kg, respectively the daily average for this period is 0.207 kg, respectively from day 40 to day 100 of the total average is 13,787 kg, or the daily average for a lamb is 0.230 kg. The average growth of lambs in the experimental group from birth to the 100th day when they were slaughtered, for lambs is 23,397 kg, ie the average daily growth is 0.240 kg. The average growth for a lamb from the 40th day when fattening started until the 100th day is 14,647 kg, and the average daily increase for the 60-day period is 0.244 kg. The results of the average daily growth of lamb breeds of sharri sheep obtained in our research are higher than those stated by: Ramljak et al. (2005), Mioć et al. (2012), Ana Kajić et al. (2012), while the lowest stated by: Cmiljnić et al. (2003), Antunović et al. (2017) etc. Our results are somewhere in between, when compared to those findings, which is normal. The daily growths of lambs are breeds characteristics (Krajinović et al. 2009, Obućina et al. 2014, Ghita 2011) which are greatly influenced by the quality of food used to feed the lambs during the fattening period. Differences in the daily growth of lambs of different breeds, species and half-breeds, as well as in different cultivation and feeding conditions are due to the influence of genetic and paragenetic factors and it is very difficult to distinguish which one is the most important.

Table 2. Dynamics of lamb body weight growth

Live weight	Fattening Systems										P
	Control group (n=12)					Experimental group (n=12)					
	$X \pm s_x$	s	Cv	min	max	$X \pm s_x$	s	Cv	min	max	
Birth weight	4,156 ± 0,134	0.463	11.149	3,51	4,84	4.307 ± 0,157	0.544	12.634	3,20	5,32	NS
10th Day	6,648 ± 0,242	0.804	12.095	5,61	7,92	7.336 ± 0,291	1.009	13.726	4,95	8,40	NS
20th Day	8,381 ± 0,348	1.207	14.397	6,85	10,01	9.480 ± 0,297	1.031	10.871	7,92	11,01	NS
30th Day	9,972 ± 0,407	1.412	14.06	8,22	12,61	11.239 ± 0,455	1.577	14.029	8,19	13,62	NS
40th Day	11,116 ± 0,343	1.189	10.693	9,35	14,19	13.057 ± 0,603	2.088	15.991	9,83	16,73	*
50th Day	13,714 ± 0,470	1,626	12,325	10,40	16,58	15,391 ± 0,557	1,930	12,541	11,91	18,75	*
60th Day	15,325 ± 0,570	1,975	12,865	12,25	19,82	17,516 ± 0,665	2,302	13,144	13,03	20,87	**
70th Day	18,377 ± 0,662	2,294	12,474	14,02	22,62	20,51 ± 0,792	2,743	13,377	15,93	24,56	**
80th Day	20,629 ± 0,717	2,485	12,049	16,31	24,87	23,011 ± 0,909	3,151	13,691	17,66	26,85	**
90th Day	22,975 ± 0,856	2,967	12,914	18,30	28,31	25,496 ± 0,946	3,276	12,850	20,36	30,15	**
100th Day	24,903 ± 0,813	2,818	11,315	19,85	30,45	27,704 ± 0,991	3,433	12,399	22,90	32,75	***

NS-not signifikant; *P<0,05; **P<0,01; ***P<0,001

Table 3. The fattening performance of lambs in different fattening groups

Characteristics	Fattening Systems		P
	Control group (n=12) (n=12)	Experimental group (n=12)	
	X	X	
Birth weight (kg)	4,156 ± 0,134	4.307 ± 0,157	NS
Final weight (kg)	24,903 ± 0,813	27,704 ± 0,991	***
Daily live weight gain (g)	207	240	**
Daily concentrated feed consumption (g)	764	889	**
Feed conversion ratio (kg)	3.423	3.663	*

NS-not signifikant, *P<0,05; **P<0,01; ***P<0,001

Dressing percentage (Table 5) is a measure of meat quantity and carcass quality, which is the difference between the carcass weight of a lamb slaughtered hot, or cold, with or without a head, kidney with wrapper, liver, lungs, heart and omentum compared to the live weight of the lamb. The differences in dressing percentage in the warm carcass between the two groups (53.017%, and 53.241%) are 0.224% and in the cold carcass (52.006%, and 50.406%) it is 1.60%, which are not statistically significant p> 0,05. The difference in cold carcasses without head and internal organs (48.263% and 46.773%) is 1.53% which is not statistically significant p>0,05.

Table 4. Descriptive statistics of clinical indicators

Organs (kg)	Control group (n=12)				Experimental group (n=12)			
	X	s	CV	%	X	s	CV	%
Live weight	23,432	2,781	11,870	100,00	25,727	3,580	13,916	100,00
Hot carcass weight	12,423	1,634	13,155	53,017	13,455	1,726	12,840	53,241
Blood	0,854	0,142	16,64	3,644	0,959	0,162	16,944	3,728
First legs	0,334	0,037	11,216	1,425	0,365	0,046	12,585	1,419
Hind legs	0,322	0,035	10,815	1,374	0,342	0,038	11,298	1,329
Skin	2,554	0,426	4,263	10,899	2,895	0,269	9,314	11,253
Omental weigt	0,085	0,049	57,296	0,363	0,114	0,055	49,991	0,443
Liver	0,478	0,072	15,062	2,040	0,509	0,109	21,447	1,978
Spleen	0,084	0,027	32,224	0,358	0,084	0,033	39,245	0,326
Lung	0,484	0,076	15,781	2,065	0,456	0,072	15,936	1,772
Heart	0,107	0,018	17,162	0,456	0,128	0,031	24,667	0,497
Stomach with intestines	2,028	0,312	15,398	8,655	2,068	0,427	20,689	8,038
Content of food, secretions and other confiscates	3,607			13,025	4,286			16,659
Weight of portions to be eaten	1,238			5,283	1,291			5,018
Weight of inedible parts	4,136			17,651	4,618			17,950

Table 5, Dressing percentage of lambs (Randman)

Indicators	Sharri sheep				P
	Control group		Experimental group		
	kg	%	kg	%	
Slaughter weight (kg)	23,432	100,00	25,727	100,00	**
Hot carcass weight with head and carcass percentage	12,423	53,017	13,455	53,241	NS
Cold carcass weight with head and carcass percentage	12,186	52,006	12,968	50,406	NS
Headless cold carcass weight and carcass percentage	11,309	48,263	12,023	46,733	NS

NS-not signifikant; *P<0,05; **P<0,01; ***P<0,001

The results obtained from the research conducted on slaughter indicators (dressing percentage on hot and cold carcass with head and parts to be eaten and cold carcass without head and parts to be eaten) are in line with the results obtained by the other researchers: Živković et al. (1981), Kozarovski et al. (1998), Djabirski et al. (2000), Žgur et al. (2003) etc. The lowest dressing percentage ascertains Djabirski et al. (2000) in cold headless carcass and internal organs of lambs obtained by crossing local merinized sheep with rams of Sardinian breed, which is 38.51%. Similar results shows Cividini et al. (2007) on Jezerosolvaçkaka breed which is 43.36%, while Žujović et al. (2004) ascertains the dressing percentage of the pirot breed sheep from 45.18% to 47.18%. The highest dressing percentage ascertained Smiljanoivić et al. (1983) on lambs obtained by crossing sharri sheep with rams of the virtemberg breed which is 60.06%, then Kozarovski (1988) of 58.80%, Djabirski et al. (2000) finds a dressing percentage on hot carcass of 57.83%, etc.

In the dressing percentage of lambs, the parts that are eaten and the parts and organs that are not eaten and which are acquired during the slaughter of animals have a great influence. The amount of parts eaten by the control group 1.238 kg, respectively in the live weight of the lambs participate with 5.283%, while in the experimental group their quantity is 1.291 kg or 5.018%. The empty stomach and complete intestines in the control group is 2,028 kg, respectively in the live weight participate with 8.655%, and in the experimental group in the empty stomach with complete intestines is 2,068 kg which in the live weight of the animals share with 8.038%.

The results obtained from our research on the weight of the parts that are eaten and those that are not eaten are in line with the results obtained by other authors, although there are certain deviations that we consider to be the result of breed characteristics and age of lambs during slaughter. Results similar to ours for the weight of the head, skin, lungs, liver and heart are also emphasized by Djabirski et al. (2000), Žgur et al. (2003), Mioć et al. (2013), Drzaić et al. (2015) and many other authors.

CONCLUSIONS

The analysis identifies differences between fattening systems in the traditional form and the use of concentrated foods based on developmental performance and clinical indicators. With the introduction of concentrated foods in food rations, the qualities of lamb carcasses are improved for the benefit of the portions that are eaten.

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A SAMPLE OBSERVATION FOR GASTRONOMIC APPLICATIONS: INTERACTION OF LEMON OR APPLE VINEGAR ADDED OLIVE OIL WITH SEA BASS FILLET AND BEEFSTEAK

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ABSTRACT

Sensory parameters for the consumers come to the fore to estimate the shelf life of the meat products. Microbial growth may lead to the sensory deterioration which affects the consumers liking and may also create some defects in terms of gastronomic point of view. Therefore, role of 5% apple vinegar and 5% lemon juice added olive oil marination liquid on raw beefsteak (BV and BL) and seabass (SV and SL) samples presented in this study. pH values of apple vinegar-olive oil and lemon juice-olive oil marination liquids were measured as 3.13 and 3.01, respectively. Sensory evaluation of these products is examined by odor, color, texture scores from the initial day, at refrigeration temperature in every 24 hours, until they reached unacceptable score of <5. According to overall acceptability scores, beefsteak samples were defined as unacceptable at 144th hour (BV:4.34, BL:3.86) which are supported by visual photographs. Seabass samples had shorter shelf life depending on marination liquid contents.Significant differences observed in terms of overall scores between SL and SV group on 72nd, 96th, and 120th hours ($p<0.05$). The higher sensory evaluation scores were determined in beefsteak and seabass samples marinated with olive oil-apple vinegar in final stages of storage period. Marination by vinegar resulted in significant differences compared to lemon juice group regarding overall scores at 120th hour for beefsteak samples, and 72nd, 96th, 120th hour for seabass samples. Results of this study showed that marination of samples by olive oil-lemon juice or apple vinegar contributes to the product quality and may extend the shelf life during refrigerated storage. These additives in marination liquids may have positive effect on sensory quality of meat products and can be used at different ratios for gastronomic applications.

Keywords: sensory analysis, marination, sous vide, lemon juice, apple vinegar

INTRODUCTION

Food safety must be ensured from slaughter or harvest to the fork for meat product. Various packaging technologies, processing technologies, and different preservation methods are generally applied to ensure food safety for consumers. Marination, one of the traditional food preservation techniques, is used to improve sensory properties and to prolong the shelf life of meat products. Marination aims to soften the texture of meat products treated with various sauces, spices, and oils while offering a different flavor to the consumer (Ceylan et al., 2021). Marination of meat by soaking in acidic solution decreases the pH meat. In this respect, microbiological growth inhibited, and sensory characteristics improved. Other beneficial effects of marination on meat texture are a juicier texture and reduction of water loss during cooking (Zochowska-Kujawska et al., 2012). Meat properties can be differently influenced from acidic marination depending on used additives and concentration. It has been stated that acidic solutions up to 1% concentrations were acceptable and positively effected taste and aroma (Sengun et al., 2021). Acidic marination can be used to improve meat tenderness and enrich it with different flavors. Tenderization and flavoring of treated meat are influenced through the marinade. pH causes the swelling of muscle fibers and connective tissue and increases the extractability of myofibrillar proteins. This process weakens muscle structure and increases the solubilization of collagens during cooking. Furthermore, soaking meat in an acidic solution affects various meat quality-related traits, such as its water holding capacity, juiciness, and color (Tänavots et al., 2018). It is also important to present “sous vide” meat products with different spices such as lemon and basil. It can be briefly defined as cooking under a vacuum at a certain temperature and time relation for the consumer as ready to eat (RTE). (Baldwin, 2012; Ceylan et al., 2021). The meat product cooked with this method can better preserve its nutritional composition (Ceylan Zafer & Ünal Şengör, 2019). In terms of gastronomy, various spices such as garlic, onions, lemon, and oils can be added to produce different aromas and flavors on the meat. For instance, as an important component of the diet, olive oil has been widely used for marination. Some of its constituents, mainly phenolic antioxidants, inhibit or modulate oxygen-related reactions and have a substantial favorable effect against oxidative injury. Olive oil contains lipid soluble bioactive compounds (monounsaturated oleic acid, squalene, carotenoids, and lutein (Lopez et al., 2014; Topuz et al., 2016). Lemon fruit (*Citrus limon*) is a rich source of nutrients—including bioactive compounds such as flavonoids, citric acid, vitamin C, and minerals such as potassium, which provide many health-promoting properties. It has been shown that vitamin C and flavonoids may also play a role in relation to antioxidant properties (González-Molina et al., 2009; Topuz et al., 2016). Vinegar is a special kind of condiment produced from fermentable carbohydrates through the activity of yeasts and acetic acid bacteria. During vinegar fermentation, acetic acid bacteria mainly produce acetic acid. It has long been used as a preserving and flavoring agent. Research demonstrated that vinegar effectively inhibit microorganisms, and pathogens were successfully eliminated from vegetables by vinegar rinsing or soaking (Karabiyikli & Sengun, 2017; Sengun et al., 2020). Homogenization of olive oil with lemon juice or vinegar could enhance solubility and dispersibility of bioactive compounds in marinated food (Topuz et al., 2016). The effects of different sauces on quality of meat have been investigated in few studies. For instance, the effects of vinegar, sunflower oil, on the meat quality of marinated herring were investigated by (Szymczak et al., 2013a). In another study, it has been reported that the combined addition of vinegar and sake is a possible alternative with minimal loss of organoleptic quality when longer storage is required (Karam et al., 2020).

As can be seen in literature, marination and sous-vide methods provide a gastronomic presentation for meat products and can be considered healthy and safe for consumers. In this respect, the objective of the present study is to evaluate the effect of lemon juice or vinegar added olive oil-based marination liquids on the sensorial quality of raw fish fillet and beefsteak during refrigerated storage.

MATERIAL AND METHOD

Material

Beefsteak and seabass samples, olive oil and apple cider vinegar were bought from an international supermarket. Fresh lemon fruits (*Citrus limon*) were purchased from a local market. Extraction of lemon juice was done by manual squeezing. The apple cider vinegar was a product from Efor Co. (Eskisehir, Turkey). Apple vinegar had a concentration of 4-5% acidity, and also contains sodium metabisulfite as an antioxidant, according to the manufacturer. HANNA HI 221 (Hanna Instruments Inc., Woonsocket-RI-USA, Romania) pH meter was used for pH analysis. The pH values of lemon juice and apple vinegar were measured as 2.76, and 2.99 respectively.

Method

Olive oil-based marination liquids were prepared using apple vinegar and lemon at 5% (v/v). Lemon juice and apple vinegar added to olive oil then beefsteak and seabass fillets were immersed into the marinades. The composition of marinated olive oil was (per 100 mL): 5 mL apple vinegar and 95 mL olive oil (pH:3.13), and 5 mL lemon juice and 95 mL olive oil (pH:3.01). Beefsteak samples raw marinated with marination liquid prepared with vinegar and lemon juice were stated as BV and BL respectively, while seabass samples raw marinated with marination liquids prepared with apple vinegar and lemon juice were stated as SV and SL respectively. Samples were stored at refrigerated conditions (4 ± 2 °C) and were analyzed per 24 hours until the sensory scores reached low values. To reveal consumer awareness at home conditions, overall acceptance, color, odor, and texture properties of samples were evaluated by using numerical scoring test (10 = extremely good 1 = extremely bad) and 5 points were accepted as the limit value for the rejection. Visual changes in course of time were supported by using photographic images.

Statistical Analysis

Collected data were subjected to analysis of variance (ANOVA) to determine the sensory quality changes. GraphPad Prism software (California Corporation, USA) was applied to reveal significant differences between groups by ANOVA. Once a significant ($p<0.05$) main effect was obtained, the mean values of the samples were further analyzed using Tukey's multiple range comparison tests.

RESULTS AND DISCUSSION

Beefsteak

Color, odor, and texture are the most important attributes of the sensory quality of a raw meat product. The consumers initially can assess these sensory attributes when they purchase the product or before cooking the product. Therefore, the sensory properties of BV and BL samples are evaluated on the initial hour and after marination up to the 144^{th} hour. In Figure 1, the overall acceptability scores of the BL and BV groups every 24 hours can be seen from statistical results. On the initial day of the experimental period odor, color, texture scores for beef steak were found to be 9.84, 9.84, and 9.86, respectively. These high scores confirmed the freshness of the raw product. After the 24th hour, sensory scores slightly decreased. The overall score values were 9.34 and 8.82 on the 24th hour for BL and BV groups, respectively ($p>0.05$). BV group had lower odor, color, texture scores than the BL group in the 24th hour. Odor scores were 8.96 and 9.36, for BV and BL groups respectively. Lower odor scores for BV could be attributed to the sharp fermented flavor of apple vinegar. Usage of apple vinegar in meat marination caused also lower texture scores than the BL group at 24th, 48th, and 72nd, hour probably softening effect of vinegar on texture. (Sengun et al., 2021) stated that fruit vinegar contains organic acids that could affect the texture of meat. As can be seen from the results, significant differences were observed in terms of texture scores between storage hours for both groups ($p<0.05$). However, between treatment groups, a significant texture difference (6.65%) was only observed in the 48th hour (BV:7.82; BL:8.34) (CHANG et al., 2010) revealed that after marination with organic acids, meat fiber diameter and thickness were decreased, and collagen fiber arrangements were disordered. Meat textural characteristics were affected by structural changes of intramuscular connective tissue collagen (Zochowska-Kujawska et al., 2012) marinated meat samples in lemon juice, wine, or kefir, and detected textural changes. Therefore, a significant difference in texture scores between storage hours is not surprising.

On hours 48th, 72nd, 96th, and 120th, overall scores for the BL group were 8.22, 7.58, 6.06, 5.02 respectively. BV group scored 7.86, 7.48, 6.4, 5.74 in these hours. The soaking of meat in marinates solution affects tenderness through pH-induced swelling of connective tissue and accelerated additional proteolytic weakening of muscle structure (Zochowska-Kujawska et al., 2012). This may also affect overall scores since very soft or very hard structures are not desirable for consumers. At the 72nd hour, the scores were close and similar, and no significant difference was observed between treatment groups. ($p>0.05$). At 96th hour, the vinegar group had higher overall scores. After 96 hours odor, color, texture scores were also compatible with overall acceptability. These results concluded that vinegar may have higher long-term antimicrobial activity and may prolong shelf life better than lemon juice. Higher BV group scores could also be attributed to the presence of sodium metabisulfite as a preservative agent inside apple vinegar liquid.

Table 1: Sensory scores of BL and BV samples every 24 hours.

	Group	Initial	24th	48th	72nd	96th	120th	144th
Color	BV		8.86±0.26^{aA}	8.02±0.48^{aB}	7.14±0.25^{aC}	6.46±0.45^{aCD}	5.76±0.54^{aD}	4.4±0.23^{aE}
	BL	9.84±0.17	9.22±0.39^{aA}	7.84±0.23^{aB}	6.92±0.73^{aC}	6.16±0.23^{aC}	4.98±0.59^{bD}	3.94±0.31^{bE}
Texture	BV		8.98±0.40^{aA}	7.82±0.25^{aB}	6.98±0.04^{aC}	6.3±0.45^{aD}	5.16±0.21^{aE}	3.96±0.18^{aF}
	BL	9.86±0.17	9.42±0.33^{aA}	8.34±0.11^{bB}	7.16±0.64^{aC}	5.9±0.22^{aD}	5.08±0.11^{aE}	3.82±0.04^{aF}
Odor	BV		8.96±0.29^{aA}	7.90±0.24^{aB}	7.58±0.40^{aB}	6.50±0.47^{aC}	5.76±0.48^{aD}	4.92±0.11^{aE}
	BL	9.88±0.11	9.36±0.42^{aA}	8.48±0.16^{bB}	7.84±0.45^{aAB}	6.02±0.32^{aC}	5.08±0.11^{bD}	4.78±0.47^{aD}
Overall	BV		8.82±0.41^{aA}	7.86±0.30^{aB}	7.48±0.48^{aB}	6.40±0.44^{aC}	5.74±0.53^{aC}	4.34±0.21^{aD}
	BL	9.88±0.13	9.34±0.43^{aA}	8.22±0.41^{aB}	7.58±0.44^{aAB}	6.06±0.33^{aC}	5.02±0.36^{bD}	3.86±0.31^{aE}

$^{a\text{-}b}$ Within each column, different superscript lowercase letters show differences between treatment groups for same storage hour ($p < 0.05$).
$^{A\text{–}D}$ Within each row, different superscript uppercase letters show differences between the storage hours within same analysis group ($p < 0.05$).
BV: Samples treated with apple vinegar-olive oil marination liquid.; BL: Samples treated with lemon juice-olive oil marination liquid.

Previous studies have reported that meat products prepared with acidic antimicrobials such as vinegar and lemon juice, increase the microbiological, chemical quality and improve sensory properties (Sengun et al., 2021). Overall scores confirm this statement as groups were still acceptable until the 120th hour. These scores can be used as a guide for the evaluation of the shelf life of steak samples for consumers.

All Pairs Tukey-Kramer test results and visual photographs of BV and BL group with initial, and 144th-hour photographs are presented in Figure 1 and Figure 2. As could be seen from the Figures, beefsteak had bright, shining pinky color with perfect structure in the initial photo.

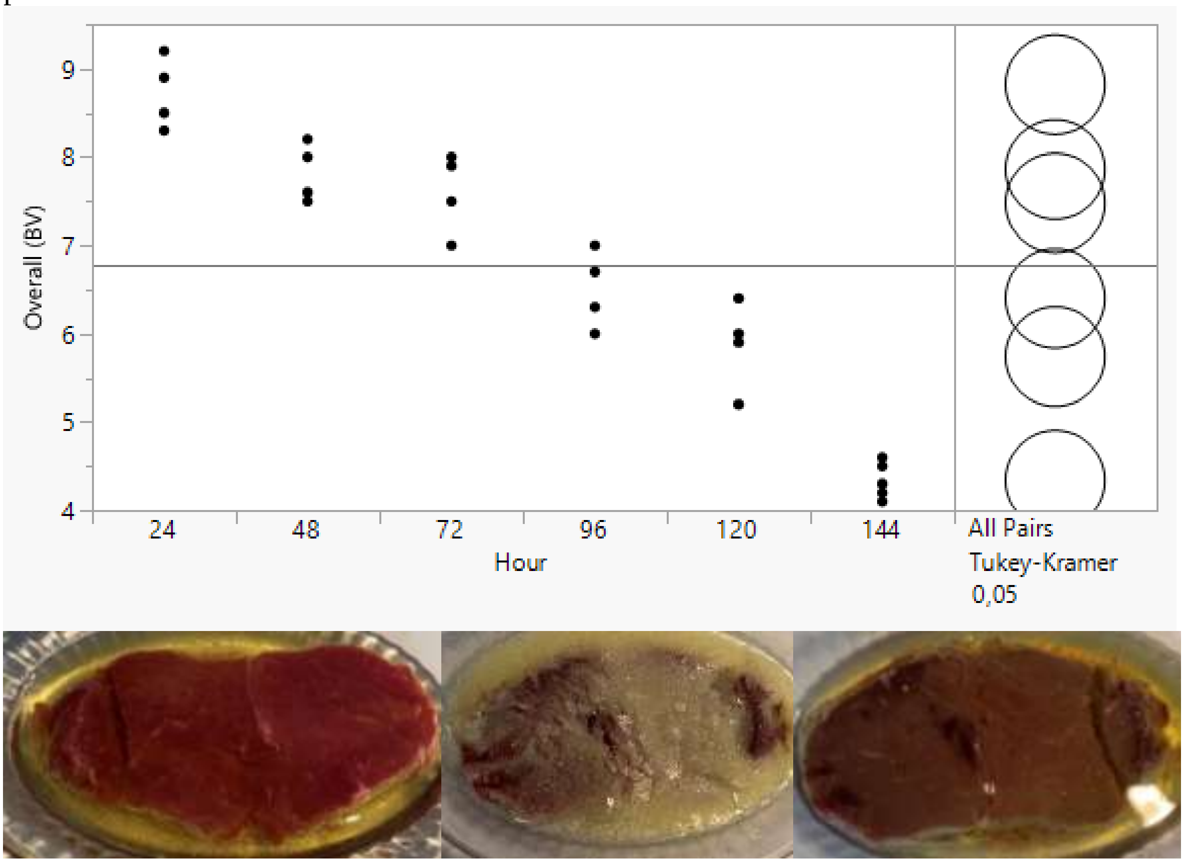


Figure 1: All Pairs Tukey-Kramer test results of BV and visual photographs on initial (left), 144th hour before thawing (middle), 144th hour after thawing (right)

As can be noticed from photos at the right side, after thawing the meat at room temperature, color defects such as darkening and black spot on some parts of meat observed on 144th hour. Ceylan et al. (2021) mentioned that darkening and browning can be seen in meat products with spoilage. As bacterial growth increases, characteristics such as rapid breakdown of connective tissue can be expected (Zagorec and Champomier-Vergès, 2017; Ceylan 2021). After 78 hours, structure deformation was observed. Acidic marination increases the water-binding of muscle proteins, which lead to removing less amount water from the structure, which results in softening (Zochowska-Kujawska et al., 2012) stated that due to the 7 days of aging, the addition of lemon juice 30–36% reduced hardness, respectively relative to the control. So, a softer structure is expected. However, this could affect consumer preferences and texture scores since consumers do not prefer very soft or very hard structures.

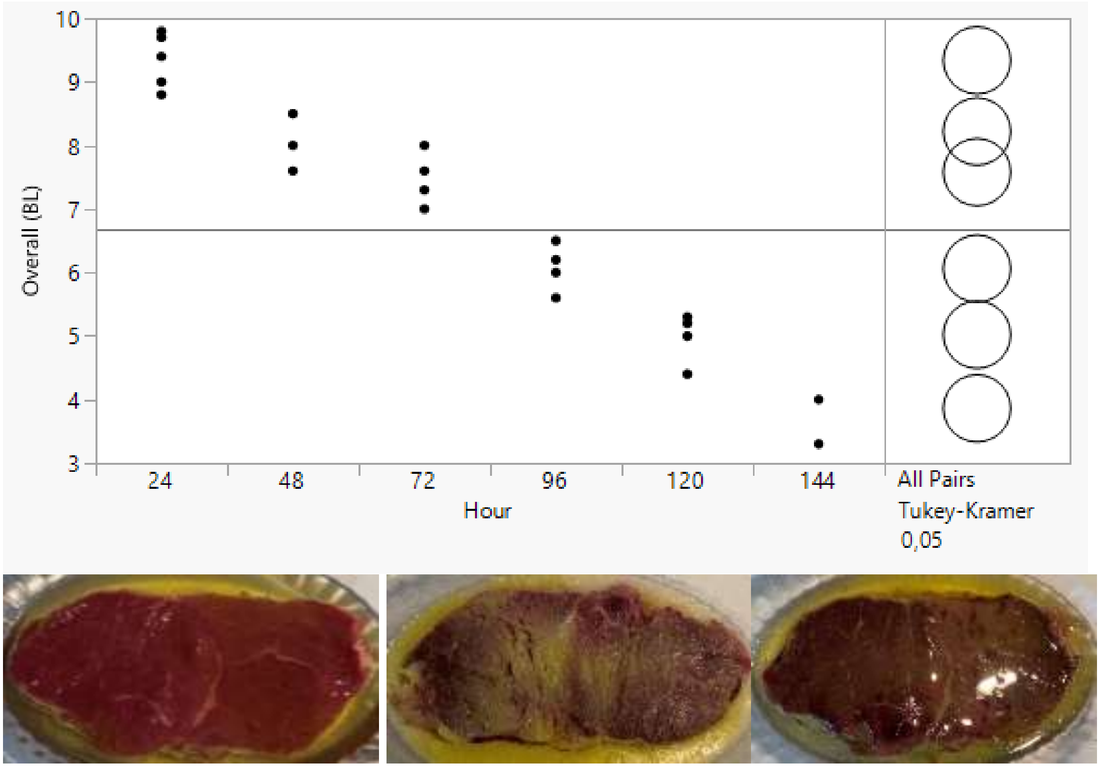


Figure 2: All Pairs Tukey-Kramer test results of BL and visual photographs on initial (left), 144th hour before thawing (middle), 144th hour after thawing (right)

At 144th hour, vinegar and juice lemon containing olive oil marinades did not have any positive effect on samples since scores were <5. Although both groups were observed as unacceptable at the 144th-hour BV group had higher scores. Differences can be seen from thawed products visual photographs on the right side, BL group had more dark spots and color fluctuations. Results showed that after 144-hour samples are visually unsuitable from a gastronomic point of view.

Seabass

Sensory evaluation is the most popular way of assessing the freshness of fish and other seafood. It is fast, simple, and provides immediate quality information. The sensory characteristics of fish and other seafood are clearly visible to the consumer and are essential for consumer satisfaction. Olive oil containing lemon juice and apple vinegar on the sensory properties of raw marinated seabass samples are presented in Table 2. Penetration of oil and vinegar into meat during marinating was confirmed by (Szymczak et al., 2013). It was evident that the color and bioactive ingredients such as phenolic compounds even of lemon juice and olive oil penetrated and distributed efficiently throughout meat. However, (Topuz et al., 2016) stated that using a concentration of lemon juice higher than 35% may result in undesirable sensory attributes. As mentioned in method section, 5% lemon juice and apple vinegar added to olive oil marinade in the current study. On the initial day of the experimental period odor, color, texture scores were found to be 9.8, 9.82, and 9.84, respectively. As for the effect of lemon juice and vinegar in marination liquids, sensory attributes were found to be affected during storage periods, with the vinegar producing higher scores than lemon juice group. For instance, hardness scores for BV group were higher in all storage periods. Recent studies indicated that texture socores may depend on the type of additives. (Tänavots et al., 2018) treated the pork samples with white wine, apple vinegar, mustard-honey, and kefir marinades and found that vinegar samples turned tougher than compared to the mustard-honey, kefir marinades. Significant difference between treatment groups occurred on 72nd, 96th, and 120th hour in terms of overall scores, indicating apple vinegar has more antimicrobial effect than lemon juice in long term. Organic acids and phenolic compounds in fruits and metabolites produced by acetic acid bacteria are responsible for the antimicrobial activity of fruit vinegars. In this respect, the inhibition effect of apple vinegar can be associated with its organic acid contents, which is predominantly acetic acid (Sengun et al., 2021).

Table 2: Sensory scores of SL and SV samples in every 24 hours.

	Group	Initial	24th	48th	72nd	96th	120th
Color	SV	9.82±0.2	8.68±0.65^{aA}	7.5±0.56^{aB}	6.72±0.59^{aBC}	6.16±0.23^{aC}	4.92±0.18^{aD}
	SL		8.54±0.54^{aA}	7.04±0.8^{aA}	6.36±0.35^{aAB}	5.4±0.55^{bB}	4.4±0.55^{aB}
Texture	SV	9.84±0.18	8.84±0.25^{aA}	7.4±0.55^{aB}	6.7±0.4^{aBC}	5.98±0.71^{aC}	4.94±0.22^{aD}
	SL		8.78±0.29^{aA}	6.96±0.74^{aA}	6.1±0.55^{aA}	5.16±0.48^{aA}	3.46±0.46^{bB}
Odor	SV	9.8±0.23	8.86±0.33^{aA}	7.8±0.45^{aB}	7.48±0.19^{aB}	6.24±0.34^{aC}	4.92±0.23^{aD}
	SL		8.64±0.45^{aA}	7.36±0.48^{bA}	6.82±0.52^{bA}	4.56±0.52^{bA}	3.46±0.45^{bB}
Overall	SV	9.78±0.22	8.8±0.27^{aA}	7.76±0.34^{aB}	7.08±0.23^{aB}	6.18±0.25^{aC}	4.96±0.22^{aD}
	SL		8.86±0.33^{aA}	7.12±0.99^{aB}	6.34±0.24^{bB}	4.92±0.11^{bC}	3.6±0.55^{bD}

$^{a-b}$ Within each column, different superscript lowercase letters show differences between treatment groups for same storage hour (p <0.05).
$^{A-D}$ Within each row, different superscript uppercase letters show differences between the storage hours within same analysis group (p <0.05).
SV: Samples treated with apple vinegar-olive oil marination liquid.; SL: Samples treated with lemon juice-olive oil marination liquid.

On 96th hour, although SL group was evaluated as unconsumable in terms of overall and odor scores (overall 4.92; odor 4.56), SV group had still higher scores (overall 6.18; odor 4.56, which was significantly different (p<0.05). On 120th hour, both groups had <5 scores, proved that products are not fresh and chemical, microbiological spoilage is occurred.

All Pairs Tukey-Kramer test results and visual photographs of SV and SL group with initial, and 120th-hour photographs are presented in Figure 3 and Figure 4. On the initial period of storage fresh seabass had typical odor and color with shiny appearance. However, after 120 hours, shiny appearance changed to mat appearance, and darker, reddish color was confirmed in photos at the right side. The visual results were compatible with other studies. In a study by (Szymczak et al., 2013) the sensory analysis of herring fish showed numerous brown discolorations on marinades surface.

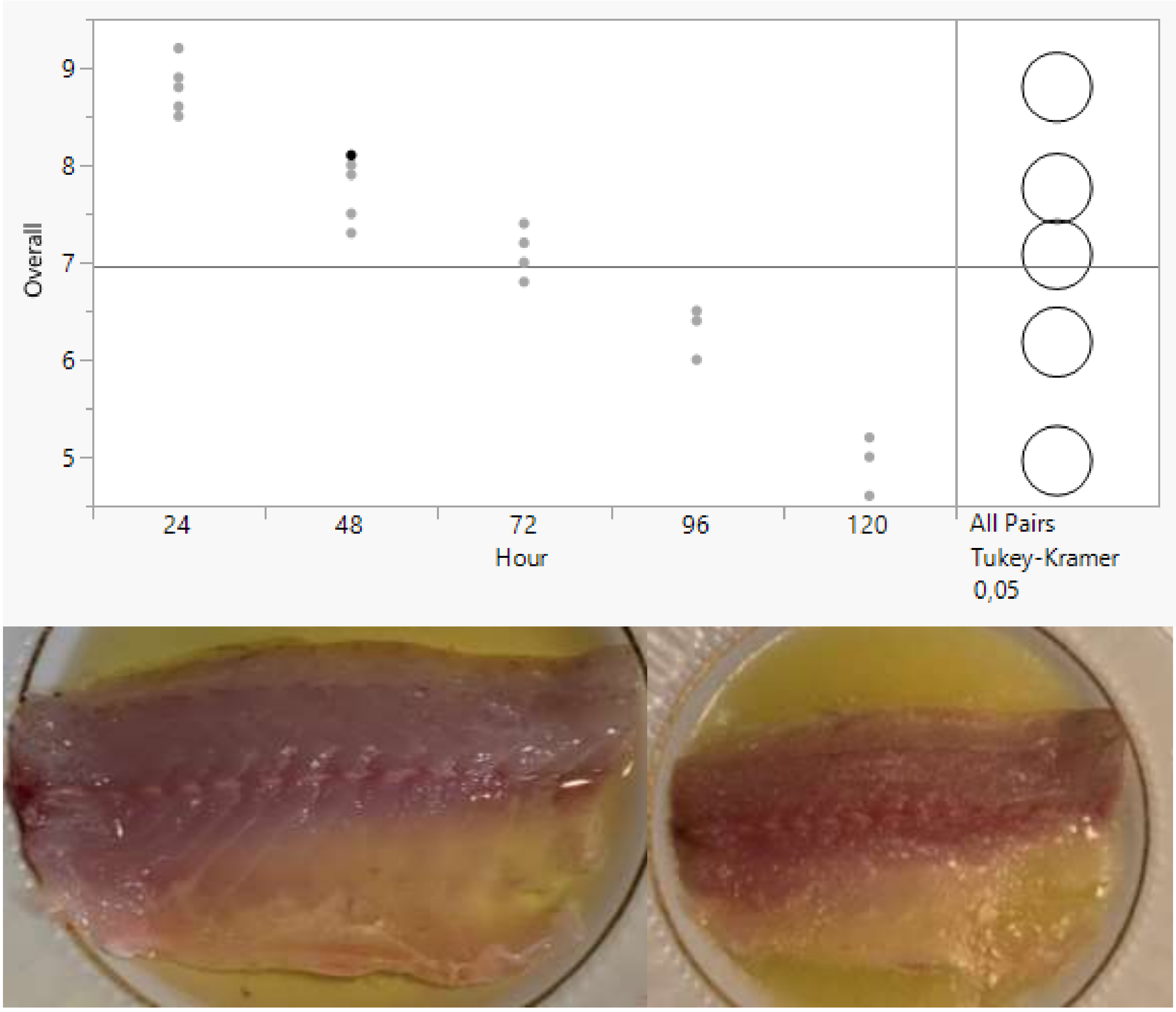
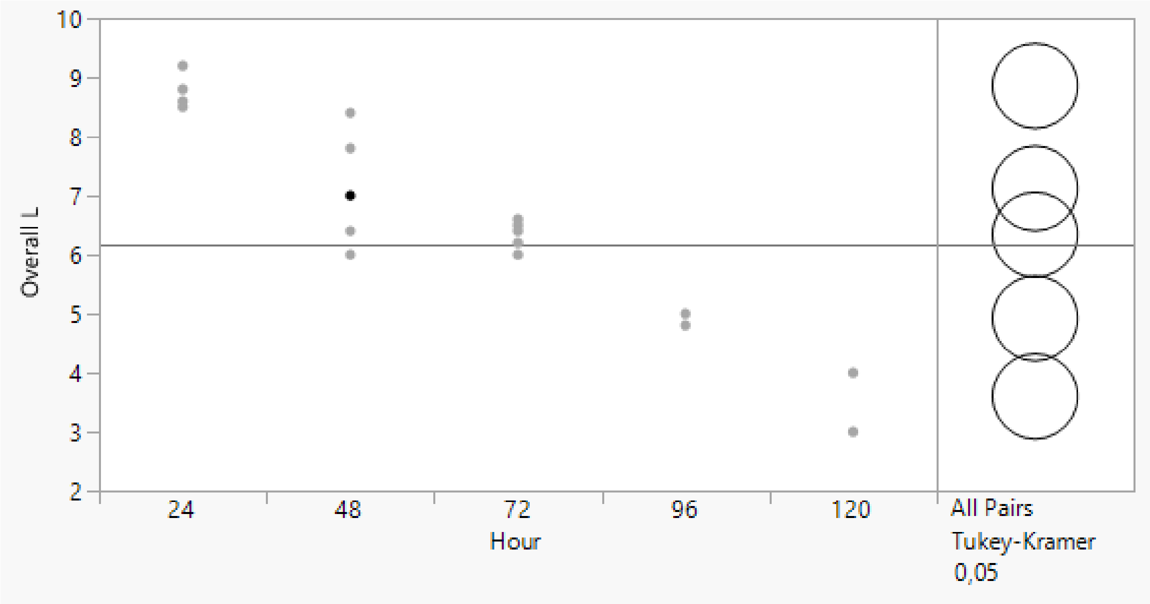


Figure 3: All Pairs Tukey-Kramer test results of SV and visual photographs on initial (left), 120th hour

In summary, results showed the positive effect of apple vinegar and lemon juice on seabass samples, since they provide organoleptically acceptable product up to 120 hours. Apple vinegar and lemon juice added olive oil based marination process may thus provide a means to add aroma, flavor for gastronomic purposes.



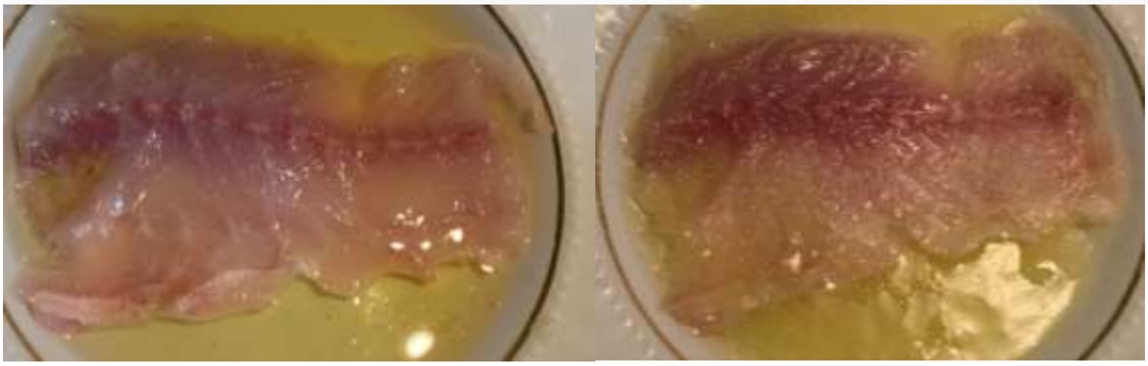


Figure 4: All Pairs Tukey-Kramer test results of SL and visual photographs on initial (left), and 120th hour (right)

CONCLUSIONS

The aim of the present study was to determine the effect of marination on the sensory properties of cold stored beefsteak and seabass fillet for every 24 h. Samples raw marinated in two different olive oil-based liquid containing apple vinegar and lemon juice. Marination with apple vinegar-olive oil was determined more effective treatment as samples with apple vinegar had higher sensory scores. Sensory properties of raw marinated samples were found to be affected by different additives. Results showed that beefsteak had higher scores compared to seabass and was accepted as consumable at 120th hour. However, seabass was evaluated as inconsumable on the 120th hour. Darkening, browning on the surface observed visual photography at the end of storing time for both samples. The marination liquid based on apple vinegar had the higher sensory scores after 96 hours for beefsteak samples and in all storage periods for seabass samples. Apple vinegar was detected as effective in reducing the hardness value of meat on 24th, 48th and 72nd hour compared to lemon juice added group. Color scores of BV was also lower than BL group on 24th hour. Specific color of the apple vinegar may affect the color of marinated meat samples. The usage of lemon or create a significant difference between marinated meat samples in terms of sensory evaluation scores

The results of this study provided an important idea about the shelf life of these two product products. Oxidative and chemical quality deterioration can be delayed in marinated products treated with olive oil-apple vinegar or lemon juice. Vinegar had stronger inhibitory effect on the lipid oxidation and chemical quality loss of marinated product. Marinated seabass fillets treated with vinegar-olive oil had higher overall acceptability score. Further studies should also be focused on determining the effects of fruit vinegars and lemon containing marination liquids on various meat types.

The results of this study demonstrated that the use of apple vinegar or lemon-based marination could be used for improving sensory quality of meat products. Future studies are therefore needed and focus on developing optimal marination formulations/concentrations for the gastronomic applications.

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ASSESSMENT OF ENVIRONMENT EFFECT ON AGRONOMIC AND QUALITY PARAMETERS OF BARLEY (*Hordeum vulgare* L) GENOTYPES UNDER RAINFED CONDITION

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ABSTRACT

This research was carried out to assess the yield and quality parameters of barley genotypes under rainfed conditions. The experiments were set up with 25 barley genotypes in a completely randomized blocks design with four replications during the four consecutive cycles from 2014-2015 to 2017-2018 growing cycle in Edirne location, Turkey. Data on grain yield, plant height, days of heading, 1000-kernel weight, test weight, protein ratio, and grain uniformity was investigated. The results of the study showed that there were significant differences among environment and genotypes based on characters investigated. There was variation in all parameters over the years due to the effect of the environment. It was found various correlations between grain yield and parameters investigated. TKW was positively correlated with test weight, protein ratio and grain uniformity across four environments. In the 2014-2015 cycles, grain yield negatively correlated with plant height and protein ratio. There was a significant positive relation between grain uniformity and TKW, TW and protein ratio. In 2015-2016, grain yield was significantly negatively correlated with plant height and positively correlated with 1000-kernel weight and grain uniformity. Grain uniformity positively significantly correlated with TKW, and negatively correlated with days of heading. In 2016-2017, grain yield was slightly negatively correlated with plant height. A positive correlation was observed between TKW with test weight and grain uniformity. In 2017-2018, grain yield was slightly positively correlated with all parameters investigated in the study. Grain uniformity positively significantly correlated with plant height, TKW, test weight and protein ratio. A positive correlation was found between TKW with test weight, protein ratio and grain uniformity. Protein ratio was significantly correlated with plant height, 1000-kernel weight and test weight. Environment factors had various effects on grain yield, 1000-kernel weight, test weight, protein ratio grain uniformity, earliness and plant height. Various environmental conditions caused different correlations between parameters. Therefore, it is very important in breeding studies to determine stable genotypes.

Keywords: Barley, Genotypes, Yield component, Quality parameters, Environment effect

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the major cereal in many different areas of the Trakya region and is essential for the livelihoods of many farmers. Barley is an annual cereal crop and grown in environments ranging from the many of the areas. Because of various environmental conditions during the growing season (October-June), in weather conditions, some biotic and abiotic stress factors could reduce grain yield (Öztürk, 2019). Climate change presents geographically varied risks to barley production. Due to the large proportion of barley used for animal feed, it is not surprising that the effect of future shocks to supply has been assessed mainly from a food security or feed use perspective (Yawson et al., 2018). Globally, net barley production is projected to fall due to temperature and water stresses, with adverse implications for the malting industry and its downstream industrial chains (Xie et al., 2018). Drought stress can be simply defined as a shortage of water that induces dramatic morphological, biochemical, physiological, and molecular changes. All of these changes reduce plant growth and crop production wheat (*Triticum aestivum* L.) and diploid barley (*Hordeum vulgare* L.) are among the most important cereal crops and large portions of human populations in many parts of the world depend on them as a source of food and animal feed. Both crops can be grown in a wide range of agro-climatic environments, however, many of these environments have drought stress as one of the major challenges to their production and productivity (Anonymous, 2020).

Grain yield in barley is a complex character depending on a large number of environmental, agronomic and physiological characters. Grain yields also depend upon other yield components (Alam et al., 2007; Öztürk et al., 2018). Genetic improvement of grain yield has been marked by the result of empirical selection based on trial and error, with grain yield per se being the dominant selection trait (Loss and Siddique, 1994). Barley grain is used primarily as an energy and protein source for animal feed. The high protein content is desirable for feed production. Variation in weather conditions in the regions, year effect, soil fertility and pest management can affect barley grain quality significantly. Protein quantity in grain varies depending on weather conditions and amount of the nitrogen fertilizer in the region (Öztürk et al., 2017). Reducing plant height has played an important role in improving crop yields. The success of a breeding program relies on the source of dwarfing genes. For a dwarfing or semi-dwarfing gene to be successfully used in a breeding program, the gene should have minimal negative effects on yield and perform consistently in different environments (Wang et al. 2014). The current study investigates and compares yield component and quality parameters under rainfed environment conditions by using four experiments and various genotypes but the same local cultivars.

MATERIAL AND METHOD

The experiments were set up with 25 barley genotypes in a completely randomized blocks design (RCBD) with four replications during the four consecutive cycles from 2014-2015 to 2017-2018 growing cycle in Edirne location, Turkey. Data on; grain yield (kg ha^{-1}), plant height (cm), days of heading, 1000-kernel weight (g), test weight (kg), protein ratio (%), grain uniformity (%) were investigated. The parameter days to heading (DH) was estimated from 1st January to the moment when 50% of main stems in a plot had at least half of emerged ears. The parameter plant height of ten randomly taken plants was measured at harvest maturity from the ground level to the tip of the tallest spike in centimeter and averaged. Thousand kernel weights and test weight (Blakeney et al., 2009), protein ratio (Köksel et al., 2000; Anonymous, 2002; Anonymous, 1990) were investigated.

Table 1. Rainfall and mean temperature scored across four consecutive cropping cycles from 2014-2015 to 2017-2018 in Edirne location

Months	2014-2015		2015-2016		2016-2017		2017-2018	
	RF	MT	RF	MT	RF	MT	RF	MT
October	121.8	15.4	52.6	15.6	44.4	14.3	135.2	13.6
November	43.2	9.3	26.2	13.5	3.2	0.7	71.6	9.5
December	111.3	6.6	0.3	5.5	3.2	0.7	119.6	7.4
January	42.2	3.8	114.8	2.8	67.8	-1.9	55.6	4.3
February	68.6	6.4	91.4	9.2	43.4	5.3	101.8	5.7
Marc	67.8	9.0	54.8	10.2	51.0	10.2	145.6	8.9
April	44.4	13.1	116.1	15.5	65.6	12.5	3.0	16.6
May	45.2	20.4	81.4	17.4	85.0	17.9	18.8	20.3
June	31.0	22.5	10.2	23.9	44.4	21.2	148.4	22.6
Total/Mean	**575.5**	**11.8**	**547.8**	**12.6**	**408.0**	**9.0**	**799.6**	**12.1**

RF: Rainfall (mm), MT: mean temperature (^{o}C)

Summarized meteorological data for the crop cycle from October to June is provided in Table 1. Monthly precipitations and mean temperature was recorded from the weather station of the Institute experimental site. The higher total rainfall was 799.6 mm in 2017-2018 and the lowest amount of rainfall was 408.0 mm in 2016-2017 cycles. The minimum average daily mean temperatures during plant growth stages (from October to June) was 9.2°C in 2016-2017, the absolute maximum mean temperature was 12.6°C in 2015-2016 cycles.

The collected data were subjected to the statistical analysis of variance according to the statistical methods described by Gomez and Gomez (1984), mean performance and LSD of all genotypes were calculated for the comparison of means according to Steel and Torrie (1980). To evaluate significant differences between genotypes, the analysis of variance (ANOVA) was performed. The differences between genotype means of parameters were tested by the L.S.D test (0.05) (Kalaycı, 2005). Letter groupings were generated by using a 5% level of significance. The regression equations were calculated according to Finlay and Wilkinson (1963), and Eberhart and Russell (1969). Regression graphs were used to predict the adaptability of genotypes and the correlations between the quality parameters were determined by Pearson’s correlation analysis.

RESULTS AND DISCUSSION

The results of the variance analysis of the research are presented in Table 2. The combined ANOVA revealed significant differences (P<0.01 and P<0.05) among genotypes for all traits except plant height and, significant differences (P<0.01 and P<0.05) for all traits between years (Table 2).

Table 2. Combined analysis of variance for barley genotypes across four environments for yield and agronomic parameters

Parameters	Genotypes			Year		
	SS	**MS**	**F Ratio**	**SS**	**MS**	**F Ratio**
Grain yield (GY)	382004.0	95501.0	0.65ns	36342925.0	12114308.0	82.20**
Days of heading (DH)	67.30	16.83	4.44*	1107.75	369.25	97.38**
Plant height (PH)	169.00	42.25	1.53ns	318.40	106.13	3.85*
1000-kernel weight (TKW)	118.32	29.58	7.06**	474.21	158.07	37.73**
Test weight (TW)	54.80	8.44	5.20*	73.99	2.63	9.36**
Protein ratio (PRT)	3.94	0.98	6.24**	17.02	5.67	35.88**
Grain uniformity (GU)	1022.85	255.71	4.31*	2647.85	882.622	14.86**

*, ** Significant at p<0.05 and p<0.01 respectively. ns: non-significant. SS: Sum of square, MS: Mean of square

In the study, five same standard varieties, Sladoran, Bolayır, Martı, Harman and Hasat were used in four experiments. There was no difference among genotypes for yield. Harman had higher yield potential among local check varieties. There was a very highly significant variation (p<0.01) in the thousand kernel weights of barley cultivars. The lowest thousand kernel weight was 38.72 g (Martı) and the highest was 45.12 g (Harman) among cultivars and the mean value was 42.0 g across four environments. Comparing the grain uniformity of 5 barley local check cultivars Hasat performed well as compared to other local checks (Table 3). The mean test weight of five cultivars indicated that the highest test weight in cultivar Hasat (73.9 kg), and Harman (73.8 kg), whereas, the lowest test weight was observed in Martı (69.3 kg). There was a significant difference among genotypes for protein ratio. The maximum and lowest protein ratio varied from 11.23% to 10.03% (Table 3). The grain uniformity in barley is another important parameter of malting barley production. The uniformity of grain depends to a large extent on the structure of the genotype of the spikes. There was a highly significant difference among cultivars for grain uniformity and the minimum was 71.25% and a maximum of 89.08%. Cultivars Hasat and Sladoran had a higher ratio of grain uniformity (Table 3).

Table 3. Mean grain yield of the local checks across four environment from 2014-2015 to 2017-2018.

Cultivars	GY	DH	PH	TKW	TW	PRT	GU
Sladoran (G1)	6894[a]	102.3[a]	89.3[a]	42.15[ab]	72.1[a]	10.95[a]	85.46[a]
Bolayır (G5)	6795[a]	104.5[a]	95.8[a]	39.84[bc]	72.7[a]	11.20[a]	71.91[b]
Martı (G10)	6736[a]	101.8[ab]	96.3[a]	38.72[c]	69.3[b]	10.03[b]	71.25[b]
Harman (G15)	7137[a]	99.0[b]	97.0[a]	45.12[a]	73.7[a]	11.23[a]	81.28[ab]
Hasat (G20)	6845[a]	103.3[a]	96.8[a]	44.09[a]	73.9[a]	10.65[a]	89.08[a]
Mean	6881.3	102.2	95.0	42.0	72.3	10.8	79.8
LSD (0.05)	588.9	2.97	8.05	3.12	2.47	0.61	11.80
CV (%)	5.6	1.9	5.5	4.8	2.2	3.6	9.7

GY: Grain yield (kg ha^{-1}), DH: days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GU: Grain uniformity (%),

Table 4. Mean of the parameters across four environment from 2014-2015 to 2017-2018

Years	GY	DH	PH	TKW	TW	PRT	GU
2014-2015	6855[b]	110.2[a]	89.0[b]	46.10[a]	74.36[a]	11.82[a]	92.18[a]
2015-2016	4803[c]	91.6[c]	95.0[ab]	35.96[b]	74.08[a]	10.52[b]	69.70[b]
2016-2017	8538[a]	108.0[a]	95.8[ab]	47.40[a]	71.06[b]	11.46[a]	90.30[a]
2017-2018	7329[b]	98.8[b]	100.2[a]	38.48[b]	69.88[b]	9.44[c]	66.99[b]
Mean	6881.3	102.2	95.0	42.0	72.3	10.8	79.8
LSD (0.05)	526.6	2.67	7.18	2.80	2.21	0.54	10.56

GY: Grain yield (kg ha^{-1}), DH: days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GU: Grain uniformity (%),

Earliness is a favourable character in barley due to rainfall fluctuation. Another reason, followed after early maturating crops farmers are produced second crops, especially for animal feed silage. There was a significant difference among years for days of heading. With the effect of high temperature, there was an early heading in the 2015-2016 cycles. Late heading occurred in 2014-2015. Plant height is an important morphological character directly linked with the productive potential of plants in terms of grain yield (Alam et al., 2007). Stem length and solidness are the most important selection criteria in barley breeding works, being a direct component of lodging resistance. Plant height varied from 89.0 cm to 100.2 cm over years. The shortest plant height was 89.0 cm in 2014-2015 and the tallest was 100.2 cm in 2017-2018 (Table 4). There was a very highly significant difference in 1000-grains weight and test weight of barley genotypes among years ($p<0.05$). Thousand kernel weight ranged from 35.96 g in 2015-2016 to 47.40 g in 2016-2017 among years across four locations. The mean test weight of genotypes indicated that the highest TW was in 2014-2015 (74.36 kg), whereas, the lowest test weight was in 2017-2018 (69.88 kg). There was a significant difference ($p<0.01$) among years for protein ratio. With regard to environmental effects, the lowest protein ratio (9.44%) was in 2017-2018. On the other hand, the highest protein ratio (11.82%) was in 2014-2015

(Table 4). There was a significant difference among years ($p<0.01$) for grain uniformity and the mean was 79.8%. The lowest grain uniformity was in 2017-2018 (696.99%) and higher was 92.18% in 2014-2015 (Table 4).

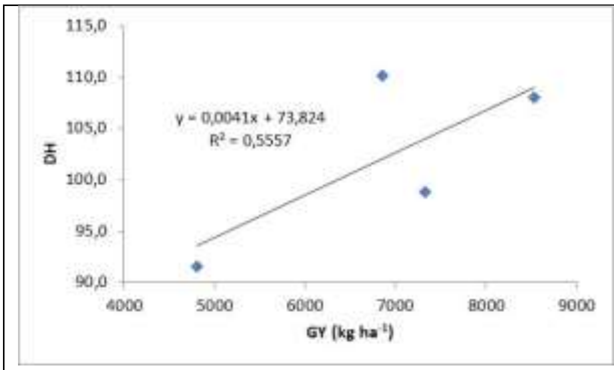
Table 5. Mean grain yield of the genotypes across four environments from 2014-2015 to 2017-2018 cycles

No	Genotype	Cropping Years			
		2014-2015	2015-2016	2016-2017	2017-2018
1	G1 (Sladoran)	6875 c-h	4382 fgh	9141 a	7178 c-f
2	G2	6750 d-h	5293 a-d	9133 a	7030 c-f
3	G3	7330 a-h	4548 efg	8870 ab	7606 a-d
4	G4	7550 a-f	5332 a-d	8789 ab	8140 a
5	G5 (Bolayır)	6532 e-h	4555 efg	8751 abc	7341 b-e
6	G6	8117 a	5601 abc	8633 a-d	7196 c-f
7	G7	7053 a-h	5349 a-d	8571 a-e	6948 def
8	G8	7505 a-f	5260 b-e	8550 a-e	6749 ef
9	G9	8023 ab	5331 a-d	8549 a-e	7173 c-f
10	G10 (Martı)	6316 h	4915 c-f	8525 a-e	7188 c-f
11	G11	6918 b-h	4775 def	8517 a-e	7508 a-d
12	G12	7448 a-g	4804 def	8491 a-e	7076 c-f
13	G13	7543 a-f	4913 c-f	8349 b-e	7276 b-e
14	G14	6388 gh	4550 efg	8326 b-e	7303 b-e
15	G15 (Harman)	7464 a-g	5236 b-e	8301 b-f	7547 a-d
16	G16	7309 a-h	3981 gh	8291 b-f	7945 ab
17	G17	7364 a-h	5117 b-e	8220 b-f	6498 f
18	G18	6925 b-h	5996 a	8197 b-f	7023 c-f
19	G19	6509 e-h	5331 a-d	8084 c-g	5659 g
20	G20 (Hasat)	7088 a-h	4925 c-f	7975 d-g	7390 b-e
21	G21	7683 a-d	5076 b-f	7938 efg	7418 a-e
22	G22	7616 a-e	4794 def	7636 fg	7451 a-e
23	G23	6505 fgh	3743 h	7501 g	7654 a-d
24	G24	6910 c-h	5715 ab	7469 g	7753 abc
25	G25	7945 abc	5318 a-d	7461 g	6914 def
Mean		7187	4994	8331	7238
CV (%)		10.9	10.4	5.8	7.3
LSD (0.05)		110.9	73.1	69.6	74.3

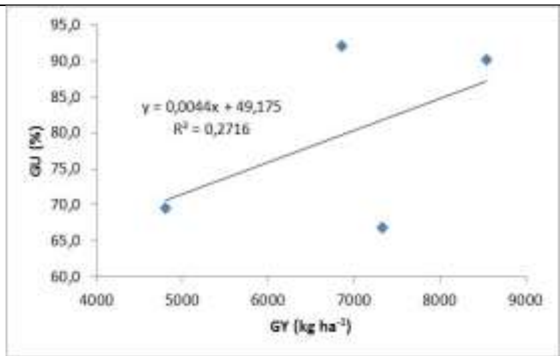
Due to various environmental conditions, grain yield is a complex character depending on a large number of environmental, agronomical and physiological characters (Öztürk et al., 2018). Grain yields are also depending upon other yield components such as spike per square meter and kernel number per spike. Because of various environmental effects, there were significant differences among genotypes based on grain yield. The same standards and different lines took place in the research in 4 growing cycle. The mean yield of the study was 7187 kg ha^{-1} in 2014-2015 cycles and the highest yielding lines were G6, G9 and G25. The highest grain yield was determined with 8117 kg ha^{-1} in G6. The lowest yield was in the 2015-2016 cycle. The mean yield of the experiment was 4994 kg ha^{-1} G18, G24 and G6 were the highest yielding genotypes.

In the study, the highest yielding crop cycle was 2016-2017. The mean yield of the genotypes was 8331 kg ha^{-1}. The highest grain yield was determined in Sladoran, G2, and followed by the G4, G3, and Bolayır. The mean yield of the genotypes was 7238 kg ha^{-1} in the 2017-2018 crop cycles. The highest grain yield was determined in G4, and followed by the G16 and G24 (Table 5).

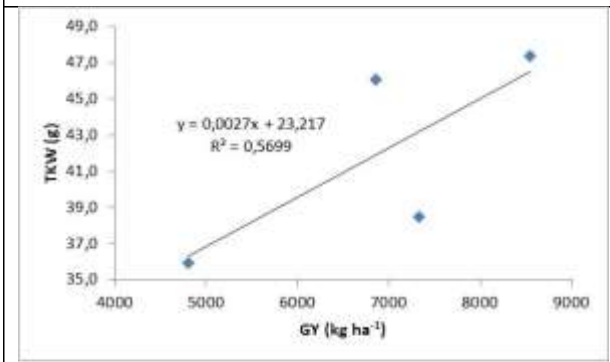
Linear regression was used to determining the association of grain yield, quality and physiological parameters. Grain yield was positively affected by grain uniformity and 1000-kernel weight so it was found positive relation among these parameters. Days of heading had a positive effect on TKW (R^2=0.934) and grain yield (R^2=0.555). It was found a negative relationship between grain yield and test weight (R^2=0.441) (Figure 1). Days of heading (DH) had a positive effect thousand kernel weight (R^2=0.934). Protein ratio and grain uniformity were positively associated (R^2=0.879).



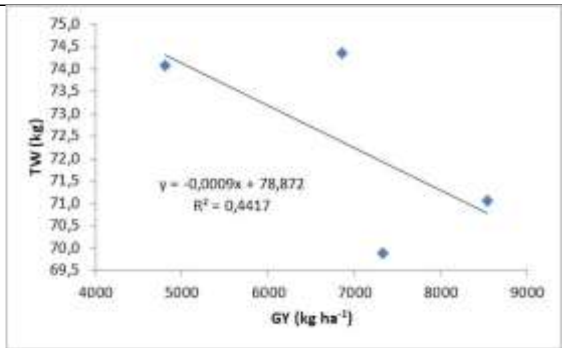
1a. Grain yield and days of heading



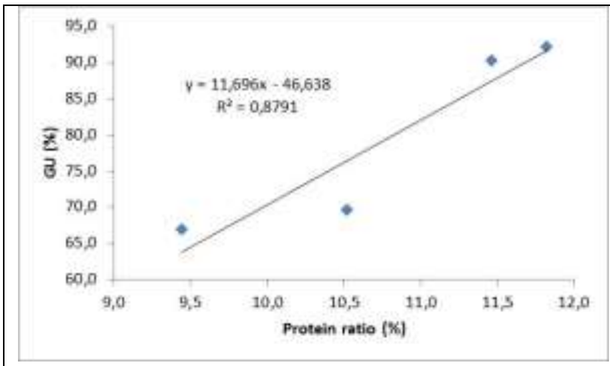
1b. Grain yield and grain uniformity



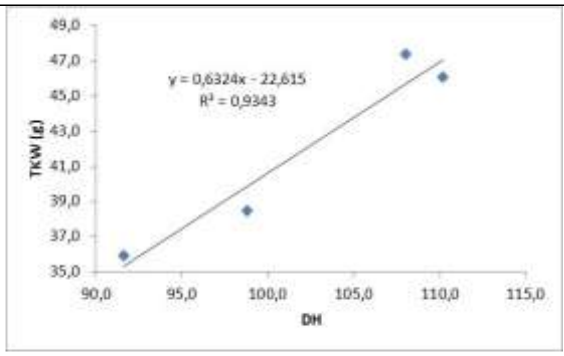
1c. Grain yield and 1000-kernel weight



1d. Grain yield and test weight



1e. Gran uniformity and protein ratio



1f. TKW and days of heading

Figure 1. The comparison of grain yield and some characters investigated in this research

Correlation coefficients based on the investigated parameters were determined by Pearson's correlation analysis. The correlation coefficients determined according to the year averages in the parameters are given in Table 6. Correlation coefficients based on the investigated parameters showed that a negative correlation was determined between grain yield days of heading (r=0.745), 1000-kernel weight (r=0.755), and grain uniformity (r=0.521). The results revealed that test weight was negatively correlated with grain yield (r=-0.665). Days of heading had a positive correlation with 1000-kernel weight (r=0.967*), protein ratio (r=0.713) and test grain uniformity (r=0.907). Plant height had a negative effect on 1000-kernel weight (r=-0.497), test weight (r=-0.845), protein ratio (r=-0.860) and grain uniformity (r=-0.721). Thousand kernel weight was positively associated with protein ratio (r=0.745) and grain uniformity (r=0.745). Protein ratio and grain uniformity was positively associated (r=0.938) (Table 6).

Table 6. Correlation coefficients determined according to four-year averages in the parameters

Parameters	GY	DH	PH	TKW	TW	PRT
DH	0.745					
PH	0.177	-0.514				
TKW	0.755	0.967*	-0.497			
TW	-0.665	-0.024	-0.845	-0.013		
PRT	0.209	0.713	-0.860	0.792	0.574	
GU	0.521	0.907	-0.721	0.953*	0.288	0.938

Significance at *: P<0.05; **: P<0.01; GY. Grain yield (kg ha^{-1}), DH: days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GU: Grain uniformity

The correlations between the parameters in the genotypes in 4 different trials were examined separately according to the years to see the environmental effect and are given in Tables 7, 8, 9, and 10. In 2014-2015 cycles, grain yield negatively correlated with plant height and protein ratio. There was a significant positive relation between grain uniformity and TKW, TW and protein ratio. In the study, a positive correlation was observed between TKW with test weight, protein ratio and grain uniformity.

Table 7. Correlation coefficients among yield and other parameters in 2014-2015

Parameters 2014-2015	GY	DH	PH	TKW	TW	PRT
DH	0.032					
PH	-0.430*	0.103				
TKW	0.034	-0.115	-0.147			
TW	0.046	0.006	0.030	0.425*		
PRT	-0.486*	-0.118	0.408*	0.479*	0.318	
GU	-0.084	-0.351	-0.151	0.840**	0.465*	0.541**

Significance at *: P<0.05; **: P<0.01; GY. Grain yield (kg ha^{-1}), DH: days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GU: Grain uniformity

In 2015-2016, grain yield was significantly negatively correlated with plant height, and positively correlated with 1000-kernel weight and grain uniformity. Grain uniformity positively significantly correlated with TKW, and negatively correlated with days of heading. In 2015-2016 cycles, thousand kernel weight and grain uniformity contributed positively to grain yield. 1000-kernel weight and grain uniformity were highly significantly associated (Table 8).

Table 8. Correlation coefficients among yield and other parameters in 2015-2016

Parameters 2015-2016	GY	DH	PH	TKW	TW	PRT
DH	-0.189					
PH	-0.523**	-0.393				
TKW	0.564**	-0.390	-0.074			
TW	0.212	-0.406*	0.265	0.338		
PRT	-0.047	-0.276	0.366	0.257	0.183	
GU	0.602**	-0.433*	-0.077	0.932**	0.284	0.298

Significance at *: P<0.05; **: P<0.01; GY. Grain yield (kg ha^{-1}), DH: days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GU: Grain uniformity

In the study, correlation coefficients among parameters in the 2016-2017 cycles were given in Table 9. Grain yield was slightly negatively correlated with plant height. A positive correlation was observed between TKW with test weight and grain uniformity. In 2016-2017, correlation coefficients among parameters were generally low compared to other years.

Table 9. Correlation coefficients among yield and other parameters in 2016-2017 cycles

Parameter 2016-2017	GY	DH	PH	TKW	TW	PRT
DH	-0.119					
PH	-0.354	-0.165				
TKW	0.028	-0.167	0.032			
TW	0.150	0.056	0.153	0.403*		
PRT	0.325	0.182	-0.268	0.133	-0.016	
GU	-0.114	-0.169	0.022	0.628**	0.248	-0.123

Significance at *: P<0.05; **: P<0.01; GY. Grain yield (kg ha^{-1}), DH: days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GU: Grain uniformity

In 2017-2018, grain yield was slightly positively correlated with all parameters investigated in the study (Table 10). Grain uniformity positively significantly correlated with plant height, TKW, test weight and protein ratio. A positive correlation was found between TKW with test weight, protein ratio and grain uniformity. Protein ratio was significantly correlated with plant height, 1000-kernel weight and test weight. Tall varieties had higher thousand kernel weight, test weight, protein content, and grain uniformity (Table 10).

Table 10. Correlation coefficients among yield and other parameters in 2017-2018 cycles

Parameters 2017-2018	GY	DH	PH	TKW	TW	PRT
DH	0.173					
PH	0.260	-0.351				
TKW	0.311	-0.075	0.555**			
TW	0.391	0.036	0.333	0.833**		
PRT	0.241	-0.114	0.446*	0.650**	0.679**	
GU	0.217	0.000	0.469*	0.901**	0.793**	0.519**

Significance at *: P<0.05; **: P<0.01; GY. Grain yield (kg ha^{-1}), DH: days of heading, PH: Plant height (cm), TKW: 1000-kernel weight (g), TW: Test weight (kg), PRT: Protein ratio (%), GU: Grain uniformity

For the genotype and environment interaction varieties were evaluated according to GGE biplot analysis across 4 environmental conditions. Obtained GGE biplot analysis results are explained below.

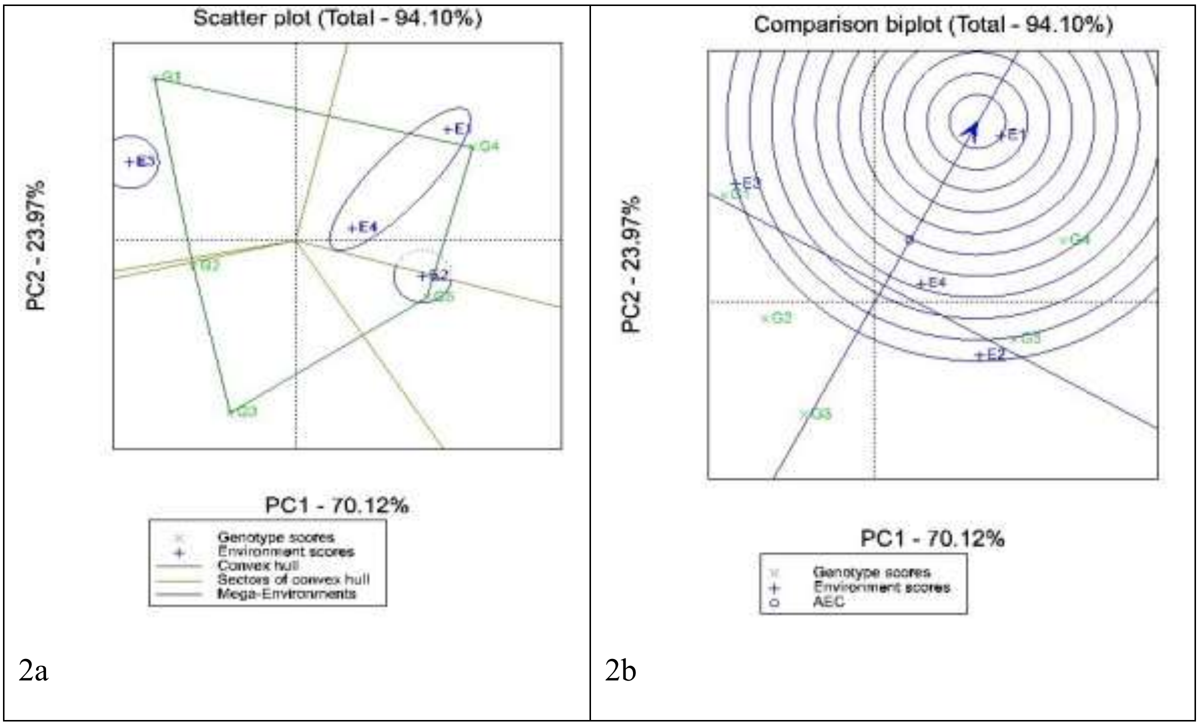


Figure 2. Polygon view of the GGE biplot using symmetrical scaling of 5 bread wheat genotypes across four environments (2a), and GGE biplot with scaling focused on environments, for the evaluation based on the ideal environment of five barley genotypes across four environments (2b).

The polygon view of the GGE-biplot analysis helps one detect cross-over and non-crossover genotype-by-environment interaction and possible mega environments in multiplication yield trials (Yan et al. 2007). G1 (Sladoran), G4 (Harman), G3 (Martı) and G5 (Hasat) were vertex genotypes (Figure 2). They are best in the environment lying within their respective sector in the polygon view of the GGE-biplot (Yan and Tinker 2006), therefore these genotypes are considered specifically adapted. They are best in the environment lying within their respective sector in the polygon view of the GGE-biplot (Yan and Tinker 2006); thus these genotypes are considered specifically adapted (Figure 2a).

The ideal environment is representative and has the highest discriminating power (Yan and Tinker 2006). Similarly to the ideal genotype, the ideal environment is located in the first concentric circle in the environment-focused biplot, and desirable environments are close to the ideal environment. Because it's on the first circle line, Environment E1 (2014-2015 cycles) was the ideal environment. Therefore, it should be regarded as the most suitable to select widely adapted genotypes (2b).

Further information about the discriminating power of environments can be obtained by the environment-vector view of the GGE-biplot. A long environmental vector reflects a high capacity to discriminate the genotypes. Furthermore, the cosine of an angle between vectors of two environments approximates the correlation between them: a wide obtuse angle indicates a strong negative correlation, an acute angle indicates a positive correlation while a close-to-90° angle indicates lack of correlation (Yan and Tinker 2006). With the longest vectors from the origin, environments E1 (2014-2015 cycle) and E3 (2016-2017 cycle) were the most discriminating while E4 was the least discriminating (Fig. 3a).

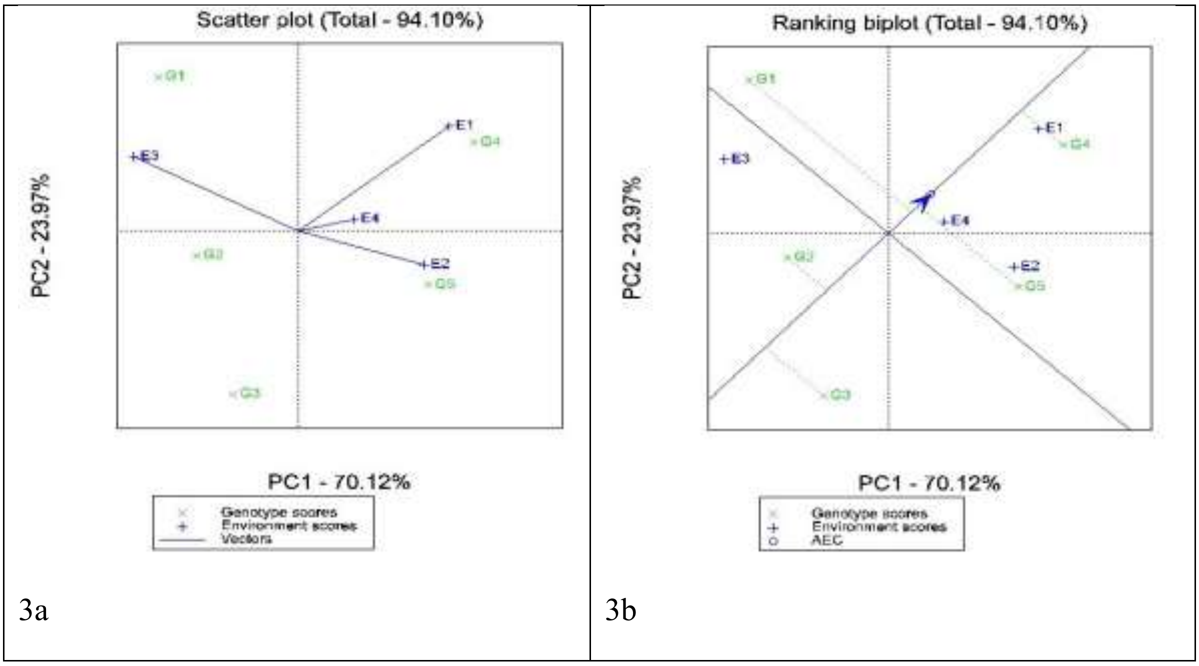


Figure 3. GGE biplot for the evaluation of the relationships among the four environments (3a), average environment coordination (AEC) views of the GGE biplot based on environment-focused scaling for the means performance and stability of genotypes (3b).

The mean yield performance and stability of genotypes was evaluated by an average environment coordination (AEC) method (Yan et al, 2001; Yan and Hunt, 2000; Yan, 2002). The GGE biplot ranks genotypes by their mean yield capacity and stability in a number of environments (Figure 3b). The average tester coordinates X-axis or the performance line passes through the biplot origin with an arrow indicating the positive end of the axis and ranks genotypes according to their performance. The mean yield capacity of genotypes is estimated by the projections of their markers to the ATC X-axis. Genotype G4 (Harman) had the highest mean yield, while G3 (Martı) the lowest mean yield.

CONCLUSION

There was variation in all parameters over the years due to the effect of the environment. In the 2014-2015 cycles, grain yield negatively correlated with plant height and protein ratio. There was a significant positive relation between grain uniformity and TKW, TW and protein ratio. In the crop cycles 2015-2016, grain yield was significantly negatively correlated with plant height and positively correlated with 1000-kernel weight and grain uniformity. Grain uniformity positively significantly correlated with TKW, and negatively correlated with days of heading. In 2016-2017, grain yield was slightly negatively correlated with plant height. A positive correlation was observed between TKW with test weight and grain uniformity. In 2017-2018 cycles, grain yield was slightly positively correlated with all parameters investigated in the study. Grain uniformity positively significantly correlated with plant height, TKW, test weight and protein ratio. A positive correlation was found between TKW with test weight, protein ratio and grain uniformity. Protein ratio was significantly correlated with plant height, 1000-kernel weight and test weight. The highest grain yield, plant height, 1000-kernel weight, test weight and grain uniformity was determined in the 2016-2017 cycle. It was found various correlations between grain yield and parameters investigated. TKW was positively correlated with test weight, protein ratio and grain uniformity across four environments.

Environment factors had various effects on grain yield, 1000-kernel weight, test weight, protein ratio grain uniformity, earliness and plant height. G1 (Sladoran), G4 (Harman), G3 (Martı) and G5 (Hasat) were vertex genotypes. They are best in the environment lying within their respective sector in the polygon view of the GGE-biplot. Therefore these genotypes are considered specifically adapted. With the longest vectors from the origin, environments E1 (2014-2015 cycle) and E3 (2016-2017 cycle) were the most discriminating while E4 was the least discriminating. Environment E1 (2014-2015 cycles) was the ideal environment. Therefore, it should be regarded as the most suitable to select widely adapted genotypes. Various environmental conditions caused different correlations between parameters. The fact that there is a different relationship between the parameters according to the years has shown that the effect of environmental factors is important. Therefore, the study carried out various environmental conditions it is very important in breeding studies to determine stable genotypes.

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FACTOR IN RESISTANCE TO WATER STRESS IN REINA MORA VARIETY OF BEAN (Vicia faba L.)

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ABSTRACT

The present study objectives are to analyze physiological responses to Reina Mora variety water stress of bean, introduced in Algeria, and assess growing substrate importance as an external factor promoting the resistance of the beans to water stress.

Experiments were carried out in a controlled greenhouse at Oran1 University, Algeria. After one month, young seedlings transplanted into pots containing three types well-watered substrates (Sand, Peat and sandy-clay soil) were subjected to moderate water stress at 40% and severe at 10% of the retention capacity for 60 days.

The criteria for resistance to water stress physiological evaluation concerned qualitative morphophysiological parameters (leaf orientation, leaf chlorosis and presence of leaf necrosis) and quantitative morpho-physiological parameters and their sensitivity index such as stem and root length, aerial and root dry weight, number and weight of pod/plant).

The results obtained show that severe water stress had a serious impact on plants cultivated in sandy-clayey soil growth and productivity, probable cause of clay drying out and development high suction pressure for water which can oppose that of roots.

Grown on peat, plants are more predisposed to protect themselves from water stress by lengthening their roots in search water than by changing their leaves orientation to reduce transpiration and maintain a high water potential which is reinforced by organic matter presence very hydrophilic and rich in mineral elements easily assimilated by roots. Water stress negatively affects plant growth and yield on sand. The leaves turn yellow and show necrosis, a response probably due to sandy soil particles which are spaced, dry, poor in nutritive substances and very draining.

Keywords: Water stress, substrate, bean, growth, yield, sensitivity index, resistance

INTRODUCTION

Faba bean (*Vicia faba* L.) is an important legume crop worldwide, ranking as the fourth most important grain legume after dry beans, dry peas and chickpeas (Lopez-Bellido et al., 2005). Faba bean (Vicia faba L.) is cultivated in many areas, mainly located in Mediterranean basin, western and eastern Europe, China, India, South America, Australia, its surface area accounting for: 240,000 ha worldwide (Faostat, 2014). Moreover, *Vicia faba* seeds represent a remarkable energy source, providing 44 Kcal 100 g-1 of fresh seeds, and they are rich in fiber, lysine rich proteins, mineral nutrients, vitamins and antioxidants (Crépon et al., 2010; Amalfitano at al. 2018). Notably, the high fiber concentration is essential for intestinal functions regulation as well as for blood glucose and cholesterol control (Macarulla et al., 2001). Water deficit in faba bean causes a significant reduction in internode length, number and size of leaves, shoot dry matter, number of pods per plant and seed production (Mohamad Zabawi and Dennett, 2010). Water can constrains plant growth also indirectly by influencing how plants subjected to water stress respond to nutrients (Song et al., 2010). Plant growth decline significantly under water stress due to the nutrient deficit that accompanies water shortage (Chapin, 1991). Fertilization provides a practical way to stimulate plant growth: it increases nutrient availability, enhances stress tolerance and encourages more efficient use of limited resources in infertile or dry environments (Wu et al., 2008). Organic manure has multiple benefits due to the balanced supply of nutrients, including micronutrients, increased soil nutrient availability due to increased soil microbial activity, the decomposition of harmful elements, soil structure improvements and root development, and increased soil water availability (Han et al., 2016). Pinitpaitoon et al. (2011) report that cultivation practices with organic manure improve soil properties, balance soil organic matter, *N* and *P* retention and availability (Gong et al., 2011; Meng et al., 2013), and finally, increase soil water use efficiency in dry-land farming (Wang et al , 2018).

To this end, the objective of this study was to analyze plants sensitivity of broad bean variety (Reina mora) on different substrates and at different soil water availability levels and to assess their effects on morpho -physiological characters as well as their productivity.

MATERIAL AND METHOD

Research was carried out on faba bean variety (*Vicia faba* L.) Reina mora grown in Spain and marketed in Algeria, in a greenhouse at Oran 1 university (Algeria). The seeds were disinfected by soaking them in a 5% sodium hypochlorite solution for 5 min and rinsed 3 times with sterile distilled water and sown in washed sand. On tenth day, the seedlings were transplanted individually in plastic pots (18 cm high and 14, 5 cm in diameter) and grown for 1 month, with regular watering before the treatment.

A 3 × 3 factorial experiment with a randomized complete block design, comprising three substrates types and three levels of available soil water, was performed in three replicates. The evaluated treatments included three substrates (1-sandy substrate, 2- sandy-peat mix substrate and 3- sandy clayey soil) and three water levels available in the soil (10, 40 and 100% water completely available).

At the end of the experiment at 100 days after sowing, qualitative morphophysiological parameters (leaf orientation, leaf chlorosis and presence of leaf necrosis) and quantitative morpho-physiological parameters and their sensitivity index such as stem and root length, aerial and root dry weight, number and weight of pod/plant) were measured.

Plant height and root length were measured with meter rod. Shoot and roots were separated and subjected to drying at 80 ºC to take their dry mass. Pods/plant number is evaluated by counting after harvest. Pods/plant was measured using weight balance. The quantitative physiological parameters sensitivity degree to water stress was calculated using the equation described previously (Zombre et al, 1994), as follows:

$$S\% = \frac{\text{(parameter measured on Control- parameter measured on Severe or Moderate)}}{\text{(parameter measured on Control)}} \times 100$$

The average sensitivity index is calculated using the method below:

$$S\% = \frac{(S\%_{Moderate} + S\%_{severe})}{2}$$

The higher it is more sensitive parameter and less resistant it is to water stress (Diallo, 2009).

RESULTS AND DISCUSSION

Qualitative morpho-physiological parameter

Plants do not have same ability to resist water stress. On the sand, we notice a change in leaves direction (vertical position) and chlorosis presence whose size varies with stress intensity. The leaves are moderately necrotic regardless treatment applied (Table1, Figure 1).

Table1: Qualitative morpho-physiological parameters (change leaf orientation, presence of leaf chlorosis and necrosis) for resistance to water stress of bean plants grown in different substrates

Substrate	**Sand**			**(Sandy-Peat) mix**			**Sandy Clay Soil**		
Retention Capacity (RC)	Control	40%	10%	Control	40%	10%	Control	40%	10%
Change leaves orientation	+	++	++	0	++	+++	+	++	0
Chlorosis	++	++	+++	0	0	+	0	0	+++
Necrosis	+	++	++	0	0	0	0	++	+++

0 = no effect; + = Low effect; ++ = medium effect; +++ = important effect.

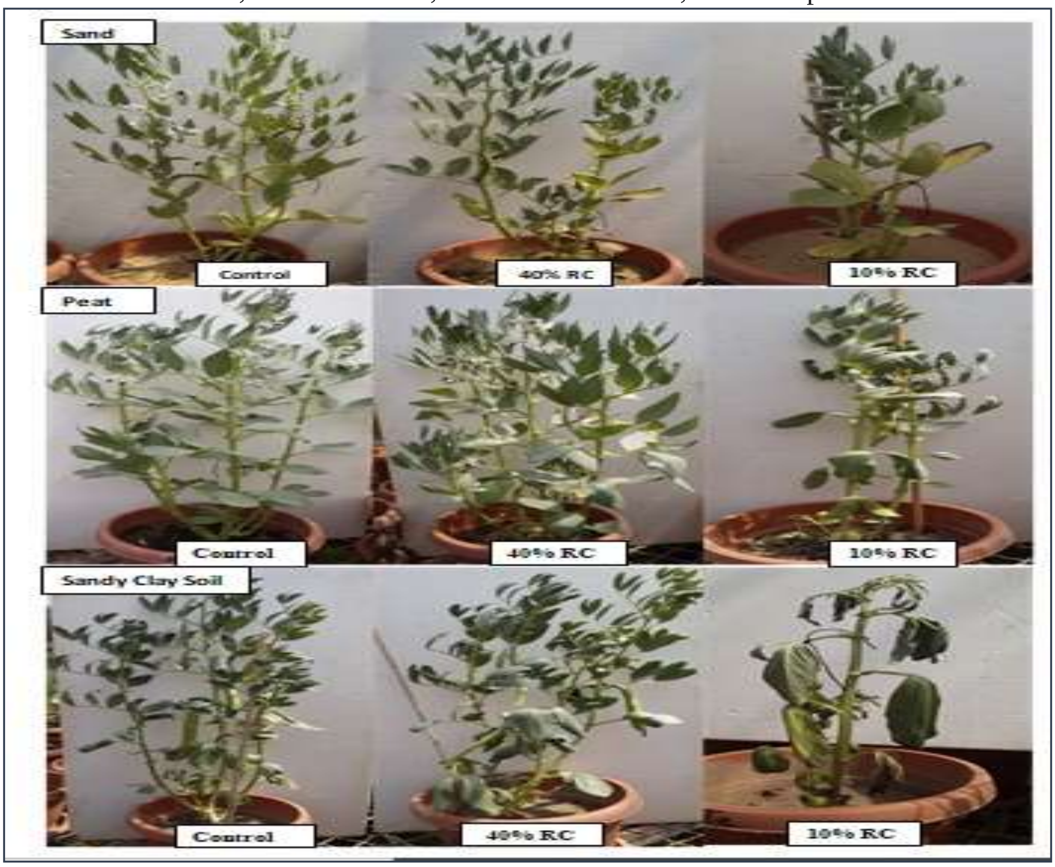

Figure 1: *Vicia faba* L. plants behavior under water stress and different substrates

On the (sandy-peat) mix substrate, stressed plants remained relatively green and little necrotic compared to their respective unstressed controls, which indicates their good resistance to drought probably due to their leaves orientation change to reduce transpiration and maintain high water potential reinforced by organic matter presence very hydrophilic and rich in mineral elements easily assimilated by roots (Table1, Figure 1).

On the sandy clayey substrate, plants leaves exposed to moderate stress (40% RC) have a vertical distribution to reduce transpiration. The green color loss (chlorosis) and necrosis appearance are seen to a moderate degree. Under severe stress (10% RC), plants show no defense reaction, leaves remain exposed to solar radiation, and maintain planophilic distribution which increases transpiration and facilitates water loss leading to plants withering (Table1, Figure 1).

- **Quantitative morpho-physiological parameters**

Plant Height

Figure (2a) explicates the variation in plants height of faba bean related to substrate type and soil moisture. Figure evidenced decrease in plants length with decreasing moisture content at 10% RC. Tallest plants (56.66 cm) were found on well-watered sandy clay substrate (100% RC). A decrease in soil moisture (10% RC) leads to a strong parameter decrease in this parameter which have registered only 26.57 cm against 33 and 40.6 cm recorded respectively on sandy substrate and peat substrate.

Root length

Effect of different substrates at different moisture levels on root length of plant is elucidated in figure (2b). It is illustrated that peat- rich substrate plants treated at 10% RC yielded long roots (31.25 cm) compared to their controls (29.5 cm) and compared to the other two substrates. In the sandy clay substrate, severe water stress (10% CR) strongly affects root proliferation which goes from 39.15 cm in the control to 24.33 cm under treated.
On sand, root length was not affected by water stress. Almost similar values were observed when plants grown with high-watering were compared to plants grown low-watering (Figure 2b)

Aerial and root dry weight

Figure (2c) elucidates the changes in plants aerial dry weight on different substrates and at different soil water content. In each treatment, all plants show a significant reduction in their aerial dry matter where water was maintained at 10% RC. However, this reduction was suggestively greater on peat substrate (6.67 g) than on the two other substrates sandy (3.87g) and sandy-clayey (3.28g). At 40% RC, the variations in dry weight in each treatment are not significant compared to those of control. Peat and sandy clayey Treatments exibited higher values than those under sand. This parameter was also higher on sandy-clayey substrate under well-watered conditions (Figure 2c).

It can be demonstrated from Figure (2d) that in soil with 10% available water, all plants root dry weight on all substrates was significantly decreased. Mean dry weight value of plants cultivated in soil with 100% available water was 5.42; 4.95 and 4.26g in sandy, peat-sandy and sandy clayey substrates; whereas that of plants cultivated in soil with 10% available water were 3.85; 2.66 and 1.75g respectively.

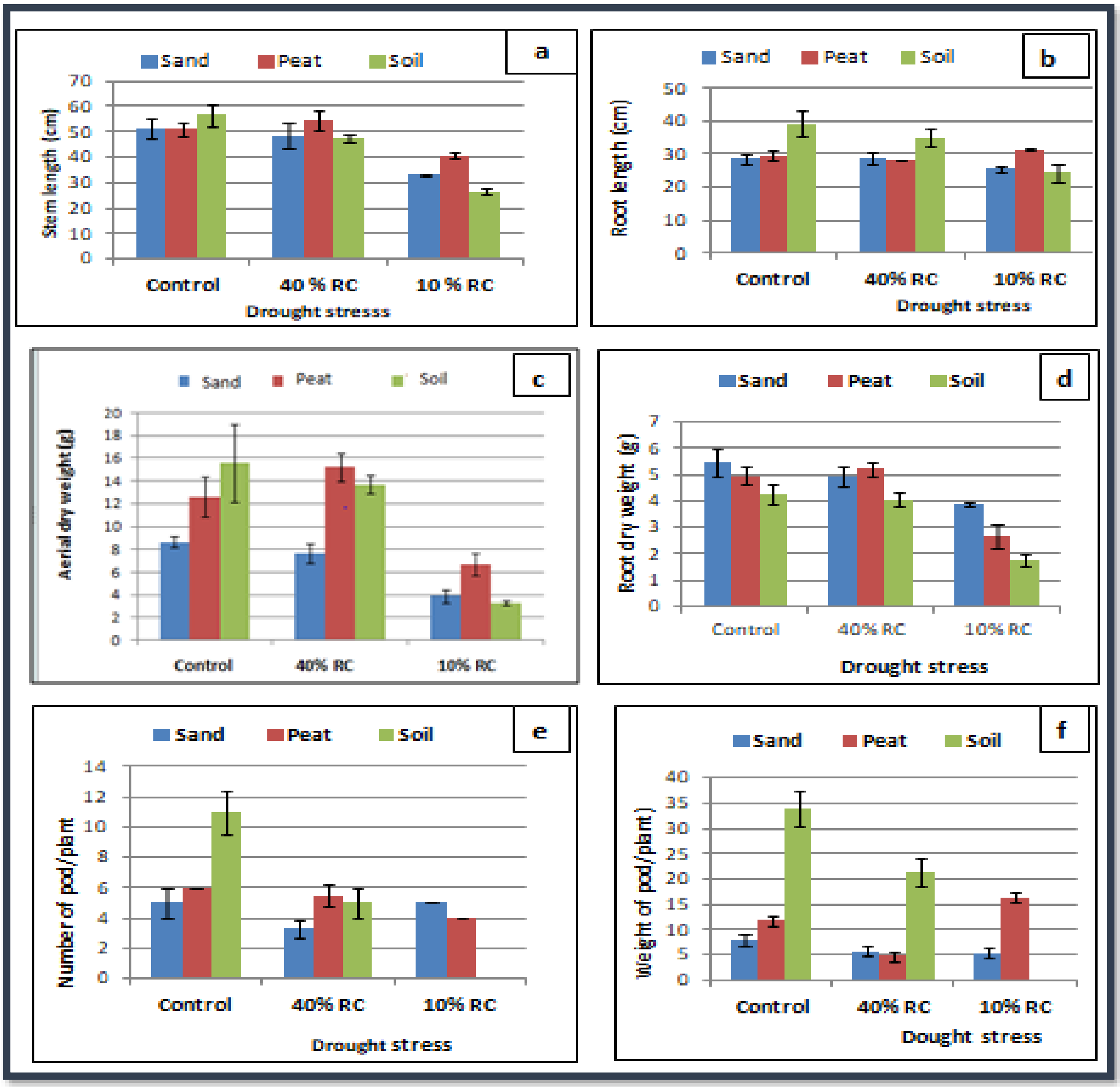

Figure 2: Stem length (a), Root lenght (b), Aerial dry weight (c), root dry weight (d), Number of pod/plant (e) and weight of pod/plant (f) of *Vicia faba* plants.

Number and weight of pod/plant

The pod *Vicia faba* L. plants production is determined at the end of the experience. Soil moisture had an impact on plant production on sandy clayey soil, as 100% water availability yielded 11pods/plant. This value drops to 5 pods/plant under moderate water stress (40% RC), i.e. a reduction in yield of 55% (Treated/Control=0.45). At 10% RC, plants do not produce any pods (Figure 2e).
Compared to previous treatment, plants grown on peat are moderately sensitive to severe water deficit and produce an average of 4pods/plant, while a maximum of 5pods/plant was produced by sand plants under same conditions (Figure 2e).

Figure (2f) examination reveals the variations in pods/plant weight. Compared with other treatments, seedlings grown on sandy clayey soil, under well-watered condition (100% RC) and medium drought stress (40% RC) exhibited the highest pod/plant. The latter was severely declined at 10% RC; the seedlings do not bear fruit.

On peat, severely stressed plants (10% CR) are the most productive and display a high average of 16.3 g compared to medium drought stress (40%) and the control with respectively values of 4.8 g and 11.8 g. Strong decrement (7.9; 5.8 and 5.3g) in pod weight/plant was observed on sand in nontreated and treated soils (Figure 2f).

Mean Sensitivity index to water stress

The Mean Sensitivity index of morpho-physiological parameters is shown in Table 2, a negative percentage means an increase over the control.

Table 2: Mean sensitivity index of quantitative physiological parameters to water stress of *Vicia faba* L. plants grown in different substrates.

Treatment / Parameters (%)	Sand	Peat	Sandy Clay Soil
Stem length	20.42	7.40	34.82
Root length	4.68	-0.68	24.45
Aerial dry weight	33.15	13.23	45.84
Root dry weight	19.05	20.54	32.03
Number of pod/plant	16.67	20.83	77.27
Weight of pod/plante	30.33	10.59	70.46

The mean sensitivity index to water stress shows a variable behavior plants depending on culture medium (Table 2).Indeed, bean plants grown on sandy clayey soil experienced a reduction in all studied parameters compared to other substrates, probable cause of clay drying out and high suction pressure development for the water which can oppose that of plants roots. On the same substrate, yield parameters (number and weight of pods/plant) were the most strongly affected, recording respectively 77.27 and 70.24% reduction compared to control.

On the peat-rich substrate, the stressed plants root length showed a slight increase (0.68%) compared to unstressed controls. This deep root extension may be necessary in search of water. In addition, the peat organic matter, very hydrophilic and rich in mineral elements, has been favorable to growth, production and plants resistance to water stress.

In sand, water stress negatively affects plant growth and yield. This effect is due to soil particles relatively spaced, dry, poor in nutrients and very draining, allowing water to drain quickly under the effect of gravity.

The results obtained, from the experimentation that we carried out on certain qualitative and quantitative plants morpho-physiological parameters of the bean (*Vicia faba* L.) showed responses variation according to different combinations of soil texture and available soil moisture. Sandy-clayey substrate plants are more resistant to moderate stress (40% RC); their leaves are green with little necrosis and show vertical leaves distribution. Under severe stress (10% CR), plants show no defense reaction and maintain planophilic distribution of their leaves. On the (sandy-peat) mix substrate, stressed plants remained relatively green and little necrotic compared to their respective unstressed controls, which indicates their good resistance to drought probably due to their leaves orientation change to reduce transpiration and maintain high water potential. Diallo, (2010) showed that more pronounced rice leaves of Nerica variety curling seems to be a trait linked to plants resistance to water stress. Many authors report that plants adapt their architectural development to the available resources, and differ in plasticity to adapt to abiotic stresses (Ruiz-Ramos et al., 2006). Sunlight interception by plants depends on plant architecture. It is a function of the leaf area index and the angular distribution of leaves (Roujean, 1996). Under water stress, the daily paraheliotropic movement of leaves modify their angular distribution and changes the exponential interception of sunlight inside the canopy (Archontoulis et al., 2011). Leaves change their angular distribution from planophile to vertical position as a result of water stress in soybean (*Glycine max* L. Merr.) (Atti et al., 2005), common bean (Yu and Berg, 1994; Boutraa and Sanders, 2001; Pastenes et al., 2004) and switchgrass (*Panicum virgatum*) (Xu et al., 2012).Using this mechanism, leaves were able to diminish the incidence of direct sunrays and to reduce plant energy load, transpiration and temperature, leading to less sunlight being intercepted (Durigon et al., 2019).

The mean sensitivity index variation of parameters as a function of substrates and water supply levels reveals a resistance polymorphism of bean plants to water stress. Thus, sandy clay substrate plants show a high sensitivity to water stress. All physiological parameters such as plants height, roots length, areal and root dry weight as well as yield parameters (number and weight of pods / plant) underwent a strong reduction under severe water stress. This trend observed in bean plants supports observations made by several authors on different plant species including faba bean (Adid et al., 2017); rice (Diallo et al., 2010) and maize (Shahzad et al., 2019).

Soil texture indirectly affects also plant growth through its influence on soil water supply (Longwell et al., 1963) and on the supply of nutrients such as nitrogen (Wilsie et al., 1944). Plant water deficits probably reduced rates of photosynthesis related in part, to the water capacity and water movement characteristics of the soil (Smith, 1970).

On the peat substrate, measurements made on morphophysiological parameters lead to conclusion that they all show low sensitivity index. This reduction is smaller the more drought tolerant the plants are. This result could be due to organic matter presence rich in hydrophilic substances retaining water. Shahzad et al., (2019) report that organic substrates addition resulted in improved plant vegetative (height, fresh and dry biomass of maize plants) growth. This improvement maybe due to direct nutrient supplementation or by indirect amendment of soil physical characteristics i.e. soil structure and water retention capacity, bulk density, penetration resistance and porosity, infiltration rate (Hati et al., 2008). Positive response of productivity with integrated application of manure and synthetic fertilizers have been reported by many researchers (Bandyopadhyay et al., 2003; Ghosh et al., 2006; Hati et al., 2000). Recently, Wang et al., (2020) indicate that additional organic manure inputs increased yield to a high and sustainable level, due to the improvement of soil water-nutrient uptake when additional organic manure applied (Wang et al., 2011; Hou et al., 2012).

CONCLUSIONS

Drought stress dramatically decreased the growth and yield of *Vicia faba* seedlings. Thus, limited water application in combination with appropriate organic matter supply could improve faba bean cultivation in sandy or clayey soils.

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INVESTIGATION OF LEAF NUTRIENT CONTENT OF SOME APRICOT VARIETIES GRAFTED ON PRUNUS MICROCARPA ROOTSTOCK

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Abstract

This study was carried out to determine the contents of macro and micro nutrients in the leaves of Hacıhaliloğlu, Hasanbey, Kabaaşı and Roksana apricot cultivars grafted on *Prunus microcarpa* seedling. The study was carried out in the field and laboratories of Kahramanmaraş Eastern Mediterranean Transition Zone Agricultural Research Institute between 2011-2016. It was found that leaf phosphorus (0.32%), potassium (4.51%) and magnesium contents (4.58%) were the highest in apricot cultivars grafted on *Prunus microcarpa*, but they were not statistically significant, and there were significant changes in leaf calcium contents compared to rootstock. In the study, in which significant differences occurred in leaf micronutrient contents, it was determined that the iron content of leaf iron (48.91 mg.kg-1) in apricot cultivars grafted on *P.microcarpa* came after the apricot seedling rootstock (56.25 mg.kg-1). It was determined that the leaf manganese, zinc and copper contents of apricot cultivars grafted on *P.microcarpa* were the highest values as 51.32 mg.kg-1, 20.37 mg.kg-1, 17.53 mg.kg-1, respectively. At the end of the study, it was concluded that *P.microcarpa* gave promising results as a rootstock and could be used more intensively in rootstock breeding studies.

Key words: Apricot, *prunus*, rootstock.

INTRODUCTION

Apricot, known for its high nutritional value, is one of the most produced fruits in the world and in Turkey (Özdogru et al., 2015). Although the production of fresh and dried apricots in Turkey varies according to the climate, it shows a continuous increasing trend. As a matter of fact, Turkey's fresh apricot production, which was 75,800 tons in 1963, reached 685,000 tons as of 2018, according to FAO data. Turkey exported 42,157 tons of fresh apricots and 112,590 tons of dried apricots in 2018, generating a total revenue of 355 million USD.

There are more than 250 different species of *Prunus* spp (Rosaceae), many of which are not botanically described. Among these, apricot, peach, cherry, almond, sour cherry and plum are important fruit species produced (Chin et al., 2014). Anatolia, which has a different ecological environment from subtropical to cold climate, is the homeland of many species belonging to the genus Prunus (Ercişli, 2004). Among these species growing in the natural environment are *P.domestica, P.cerasifera, P.divaricata, P.spinosa, P.microcarpa, P.scoparia, P.amygdalus, P.arabica*. Recently, clonal rootstock breeding studies have been started especially in these species grown in natural environments in Turkey (Bolat et al., 2017).

The use of appropriate rootstocks in modern fruit growing is done for purposes such as increasing yield and quality, investing the tree in early yield, creating an appropriate flowering period, and providing resistance to difficult soil conditions (Darikova et al., 2011). This classical production method, which is possible with grafting, has been used for thousands of years. In apricot cultivation also, grafting is used intensively with the spread of clonal rootstocks (Milosevic et al., 2014). Rootstock breeding studies have been carried out for apricots in the world; In these studies, growth vigor (Jimenez et al. 2003), yield (Sosna and Licznar–Małańczuk, 2012), quality (Hernandez et al., 2010), fruit biochemical properties (Gündoğdu, 2019) were examined and important results were revealed. However, as in many fruit trees, the discussions about the appropriate rootstock usage still continues because of the complex structure of rootstock and scion relationship in apricots (Son and Küden, 2003). In recent years, changes and deteriorations in climate and soil conditions have increased the importance of rootstock use. The breeding of rootstocks that adapt well to these difficult conditions, are well compatible with the varieties grafted on, are easily propagated vegetatively, control the growth force, increase the yield and quality of the grafted variety, and have good adhesion to the soil has become increasingly important (Uğur, 2017). Myrobolan rootstocks (*P.cerasifera*) are used as rootstocks in many countries where apricot cultivation is intense (Turkey, Poland, Romania, Russia, Serbia, Czech Republic, France, Hungary, Switzerland). However, these rootstocks are generally not suitable for intensive cultivation due to their strong crown (Milosevic et al., 2014; Sitarek and Bartosiewicz, 2011). In addition, late graft incompatiblity are also observed in some apricot cultivars grafted on Myrobolan rootstocks (Licznar and Sosna, 2005). For this reason, dwarf growing rootstocks are more needed to form more compact trees.

P.microcarpa is a wild *prunus* species that takes attention with its dwarf growing within the hardcore *prunus* genus. It is one of the prunus that grows naturally in the region including Northern Iraq and Western Iran from the eastern, southern and southeastern regions of Anatolia (Forcada et al., 2020), genetically closer to sweety cherry (Nas et al., 2011), and has the smallest carpel structure. Although *P.microcarpa*, where its phytochemical properties were investigated, has spread widely in Anatolia, its rootstock characteristics have not been the subject of sufficient research (Sevgin, 2018). However, recently, studies have been started on the use of *P.microcarpa* as rootstock.

With this study, it is aimed to investigate the uptake of plant nutrients from soil of some apricot varieties grafted on *P.microcarpa* seedling rootstock.

MATERIAL and METHOD

The research was carried out in the orchard and laboratories of Kahramanmaraş East Mediterranean Transitional Zone Agricultural Research of Institute between 2011 and 2017. The experiment was established at 4 x 3 m intervals, and a total of 180 trees took place in an area of approximately 2500 m^2. *Prunus microcarpa* seedlings were used as rootstock, and Hacıhaliloğlu, Hasanbey, Kabaaşı and Roksana apricot cultivars were used as cultivars. Apricot seedling (*P.armeniaca*) and Pixy (*P.institia*) clone rootstock were used as control rootstock.

Collection of Leaf Samples

Between 2012 and 2016, 100-200 leaves were collected from each replication from the leaves that completed their development from the middle part of the shoots of the seedlings in June. The samples taken were numbered and placed on the paper bags. The collected leaf samples were brought to the laboratory without waiting. Here, the numbered sheets were laid on the paper, the unhealthy and worn leaves were extracted. Then the leaves were cleaned by pre-washing. It was washed with 0.1 N HCl solution, then rinsed thoroughly with distilled water. The washed leaves were arranged loosely and left to dry in a drying cabinet at 65°C until their weight did not change (approximately 48 hours). The dried samples were ground, labeled in plastic bags, and stored in the refrigerator until analysis (Steyn, 1961; Lilleland and McCollam, 1961; Sannoveld and Dijk, 1982; Kacar, 2008).

Determination of Nutrient Concentrations

As Miller (1998) reported; Analyzes were made according to the pressure microwave wet digestion method by taking 0.25 g of the ground leaves and burning them with 0.5 ml nitric acid (HNO_3 d= 1.42) and 2 ml hydrogen peroxide (H_2O_2 30%). The amounts of P, K, Ca, Mg, Fe, Cu, Mn and Zn from the filtrates obtained as a result of the analyzes were determined with the Agilent 5100 brand ICP-OES device. Measurements were checked with certified values of relevant minerals in reference plant materials obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Evaluation of Results

Grafted plants from which leaf samples were taken were arranged according to the split plot design in randomized blocks. The experiment was arranged in three replications, and with 5 plants in each plot. Analysis of variance in all data was tested at 5% and 1% significance levels, and multiple comparisons were determined by LSD test. JMP 7 statistical package program was used for statistical analysis.

RESULTS and DISCUSSION

In our study, it was determined that the contents of leaf macro and micronutrients differed significantly according to rootstocks, but these values among cultivars were not statistically significant. As a matter of fact, different researchers have reported that plant leaf components vary significantly according to rootstocks (Jimenez et al., 2018; Yahmed et al., 2020). Therefore, research has been carried out relating to the transmission of plant nutrients of different rootstocks (Shahkoomahally and Chaparro J., 2020; Taaren et al., 2016). Studies on this subject report that the physiological properties of xylem vascular bundles of rootstocks (Tombesi et al., 2011) and root morphology and physiology are highly effective in ion uptake (Nawaz et al. 2011). In this sense, the selection of rootstocks is important in terms of creating healthy gardens and growing techniques (Savvas et al., 2009).

Table 1. Leaf phosphorus and potassium contents of selected and control rootstocks

Rootstock	Cultivar	P (%)		K (%)	
P.microcarpa	Hacıhaliloğlu	0.35 ±0.07	0.32 ±0.04	4.78 ±0.07	4.51 ±0.29 A
	Hasanbey	0.32 ±0.01		4.47 ±0.04	
	Kabaaşı	0.30 ±0.05		4.27 ±0.46	
	Roksana	0.32 ±0.01		4.51 ±0.04	
Pixy (*P.institia*)	Hacıhaliloğlu	0.27 ±0.03	0.29 ±0.06	3.53 ±0.18	3.76 ±0.53 B
	Hasanbey	0.26 ±0.02		3.89 ±0.67	
	Kabaaşı	0.37 ±0.07		3.68 ±0.36	
	Roksana	0.26 ±0.02		3.93 ±0.67	
Seedling (*P.armeniaca*)	Hacıhaliloğlu	0.33 ±0.02	0.32 ±0.16	3.83 ±0.24	3.75 ±0.16 B
	Hasanbey	0.31 ±0.00		3.72 ±0.04	
	Kabaaşı	0.31 ±0.01		3.69 ±0.20	
	Roksana	0.32 ±0.00		3.75 ±0.04	
LSD $_{0.05}$		**Ö.D**	**Ö.D**	**Ö.D**	**0.34****

In the study, in which no statistically significant differences were found between rootstocks and cultivars in leaf phosphorus contents, while the highest leaf phosphorus content was observed in apricot cultivars grafted on *P.microcarpa* apricot seedling rootstocks (0.32%), Pixy rootstock remained in the lower rank with 0.29% (Table 1). Here, it was determined that rootstocks did not show a high difference in leaf phosphorus uptake and they were in the same group statistically. Similar results were also found in leaf magnesium contents (Table 2). It was observed that there were high differences in leaf potassium contents of apricot cultivars grafted on rootstocks (Table 1).

Table 2. Leaf calcium and magnesium contents in selected and control rootstocks

Rootstock	Cultivar	Ca (%)		Mg (%)	
P.microcarpa	Hacıhaliloğlu	2.02 ±0.33	1.75 ±0.29 A	0.64 ±0.03	0.58 ±0.05
	Hasanbey	1.71 ±0.06		0.57 ±0.02	
	Kabaaşı	1.52 ±0.33		0.53 ±0.05	
	Roksana	1.73 ±0.06		0.58 ±0.02	
Pixy (*P.institia*)	Hacıhaliloğlu	1.51 ±0.17	1.61 ±0.27 AB	0.58 ±0.05	0.62 ±0.09
	Hasanbey	1.49 ±0.06		0.60 ±0.05	
	Kabaaşı	1.92 ±0.37		0.67 ±0.15	
	Roksana	1.51 ±0.06		0.61 ±0.06	
Seedling (*P.armeniaca*)	Hacıhaliloğlu	1.44 ±0.20	1.46 ±0.13 B	0.57 ±0.09	0.58 ±0.05
	Hasanbey	1.47 ±0.02		0.59 ±0.01	
	Kabaaşı	1.45 ±0.17		0.58 ±0.07	
	Roksana	1.49 ±0.02		0.60 ±0.01	
LSD $_{0.05}$		**Ö.D**	**0.18***	**Ö.D**	**Ö.D**

The highest leaf potassium content was found in *P.microcarpa* rootstock (4.51%), followed by Pixy and apricot seedling (*P.armeniaca*) rootstocks in the same group with values of 3.76% - 3.75% (Table

1). In a previous similar study with different rootstocks also, leaf potassium contents were reported to be between 0.8-3.5% (Reig et al., 2019). It can be said that these results are suitable with our study. In leaf calcium contents also, it was found that P.microcarpa rootstocks, as in potassium values, received better than other rootstocks with 1.75% (Table 2). When the leaf macronutrient contents are examined in general, it has been determined that the values obtained are also similar to the studies of Shahkoomahally and Chaparro (2020).

Table 3. Leaf iron and manganese contents of selected and control rootstocks

Rootstock	Cultivar	Fe (mg kg^{-1})		Mn (mg kg^{-1})	
P.microcarpa	Hacıhaliloğlu	44 ±1.38	48.91 ±3.38 B	43.96 ±1.99 c	51.32 ±5.08 A
	Hasanbey	48 ±0.51		55.35 ±1.26 a	
	Kabaaşı	53 ±0.97		50.06 ±1.86 b	
	Roksana	49 ±0.52		55.90 ±1.28 a	
Pixy (*P.institia*)	Hacıhaliloğlu	45 ±0.81	47.40 ±2.01 B	32.65 ±1.34 e	37.68 ±3.16 B
	Hasanbey	46 ±1.43		39.55 ±1.57 d	
	Kabaaşı	50 ±0.13		38.59 ±0.90 d	
	Roksana	47 ±1.45		39.94 ±0.90 d	
Seedling (*P.armeniaca*)	Hacıhaliloğlu	57 ±0.90	56.25 ±1.90 A	30.37 ±0.38 e	29.91 ±0.97 C
	Hasanbey	56 ±2.06		29.84 ±1.68 e	
	Kabaaşı	55 ±1.74		29.31 ±0.32 e	
	Roksana	56 ±2.08		30.13 ±0.33 e	
LSD 0.05		**Ö.D**	**4.78****	**3.83****	**1.91****

In the study, in which there were statistically significant differences in the transmission of micronutrients between rootstocks, it was revealed that the differences were realized at the level of 1%, especially in the transmission of iron and manganese. It was found that the highest leaf iron content was determined in the seedling rootstock (56.25 mg kg^{-1}), and *P.microcarpa* rootstock (48.91 mg kg^{-1}) came after this. It was revealed that similar results were seen in the results obtained regarding the leaf manganese content, and the highest leaf manganese content was found in apricot varieties grafted on *P.microcarpa* with a value of 51.32 mg kg^{-1}.

Table 4. Leaf copper and zinc contents of selected and control rootstocks

Rootstock	Cultivar	Cu (mg kg^{-1})		Zn (mg kg^{-1})	
P.microcarpa	Hacıhaliloğlu	17.88 ±0.92	17.53 ±1.21 B	17.28 ±0.20 c	20.37 ± 2.31A
	Hasanbey	16.66 ±0.45		20.32 ±0.29 b	
	Kabaaşı	18.78 ±1.30		23.36 ±1.64 a	
	Roksana	16.82 ±0.46		20.52 ±0.29 b	
Pixy (*P.institia*)	Hacıhaliloğlu	22.31 ±0.40	19.71 ±1.94 A	20.47 ±2.32 b	16.85 ± 2.81B
	Hasanbey	18.74 ±0.96		15.68 ±2.92 c	
	Kabaaşı	18.87 ±2.01		15.41 ±0.38 c	
	Roksana	18.93 ±0.97		15.83 ±0.39 c	
Seedling (*P.armeniaca*)	Hacıhaliloğlu	16.31 ±1.15	16.07 ±0.88 C	16.92 ±0.49 c	16.66 ±0.51 B
	Hasanbey	16.02 ±0.49		16.63 ±0.43 c	
	Kabaaşı	15.77 ±0.84		16.33 ±0.46 c	
	Roksana	16.18 ±0.84		16.79 ±0.46 c	
LSD 0.05		**Ö.D**	**1.00****	**2.51****	**1.25****

When Table 4 was examined, it was found that the difference between rootstocks was significant at the level of 1% in copper and zinc transmissions. It is understood that the best rootstock in copper transmission is Pixy (*P.institia*) (19.71 mg kg^{-1}), while the seedling rootstock is slightly weaker than the others (16.07 mg kg^{-1}, C). In zinc transmission, it is seen that the rootstock of *P.microcarpa* got the best result with 20.37 mg kg^{-1}.

CONCLUSIONS and RECOMMENDATIONS

At the end of the study, it was determined that there were significant differences in the direction of nutrient uptake in all rootstocks, and the leaf nutrient contents in general were remarkably good in apricot cultivars grafted on *P.microcarpa*. Looking at the reference values of plant nutrient contents and the literatures, it was concluded that the rootstock of P.microcarpa used in the study was promising in terms of nutrient transmission. It is thought that *P.microcarpa* can be a potential rootstock for hardstone fruit trees, and a rootstock suitable for dense planting can be developed from *P.microcarpa* species by making more detailed breeding studies.

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THE EFFECT OF OPERATIONAL PARAMETERS ON PHOTOCATALYTIC DEGRADATION OF REACTIVE BLUE 19 DYE USING Cu2O

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ABSTRACT

The rapid progress of modern industry and agriculture is resulted to an extreme amount of wastewater discharging, thus causing a threat for the human health and environmental pollution. The treatment of organic pollutants containing synthetic dyes coming from textile, printing and various industries is direly important before they penetrate to water and end up in soil. A favorable approach of an advanced oxidation process, namely photocatalysis is an alternative and effective technology for the removal of these colored effluents. In this study, the photocatalytic degradation kinetics of Cu_2O nanoparticles on Reactive Blue 19 (RB-19), a commercially important anthraquinone dye were systematically investigated with the use of artificial light source (UV-A). Experiments to study the effects of operational parameters including initial dye concentration and amount of photocatalyst were performed to find out optimum conditions. The photocatalytic decolorization percentage of RB-19 was studied using UV–vis spectrophotometer. The photocatalytic degradation process was well described by pseudo-first order kinetics. The results indicated that the decolorization of RB-19 was increased with decreasing the initial dye concentration.

Keywords: Anthraquinone dye, Cu_2O, decolorization, photocatalysis, Reactive Blue 19.

INTRODUCTION

Clean water accessibility is a vital and mandatory factor for the continuation of life, agricultural and industrial activities. However, the overpopulation and industrialization has induced a water pollution problem resulting a serious threat for environment and human health. Textile industry is one of the major environmental pollutions sources due to the accidental discharge or dumping of dye effluents into natural bodies before application of the treatment procedures. Besides, dye pollutants contribute harm to lands making them unproductive and useless as well (Cinelli et al., 2017; Guedes et al., 2009; Tahir et al., 2019; Yaseen and Scholz, 2019).

The synthetic dyes are more commonly used than natural dyes in textile industry because of their easy production, color variety, and high fastness properties. Synthetic dyes are categorized according to their chemical structure (azo, anthraquinone, sulphur, etc.) and their application field (reactive, direct, disperse, etc.) (Yaseen and Scholz, 2019). Most of these synthetic dyes are toxic, chemically resistant, and nonbiodegradable compounds. Therefore, important effort have been adopted on the elimination of textile dyes from wastewater in recent years (Asghar et al., 2015). Heterogeneous photocatalysis is an effective treatment method to mineralize dye molecules without causing a secondary pollution. The method is based on the formation of highly reactive species such as hydroxyl radicals oxidizing organic pollutants (Rafiq et al., 2021; Tahir et al., 2019). At present, several semiconductors such as TiO_2, ZnO are used to degrade dye molecules (Turkten and Cinar, 2017; Turkten et al., 2021). Cuprous oxide (Cu_2O) attracts much attention as photocatalyst due to its narrow band gap (2 eV) with a high optical absorption coefficient in visible regions. Moreover, Cu_2O is inexpensive, nontoxic and chemical stable. Consequently, these advantages make Cu_2O a good semiconductor candidate for the usage of photocatalytic systems. (Ho et al., 2017; Mosleh et al., 2018; Shoeib et al., 2012; Yadav et al., 2021).

The study was performed to determine the photocatalytic degradation kinetics of Cu_2O nanoparticles on RB-19 under UV light. RB-19 was chosen as the model pollutant used commonly in textile industry. In the current work, the operational parameters of this anthraquinone dye including initial dye concentration and amount of photocatalyst were examined.

MATERIAL AND METHOD

Cu_2O (Riedel) was used as provided by the supplier. All aqueous solutions were prepared with distilled water. The chemical structure of RB-19 is given in Figure 1. Photocatalytic activity testing was achieved by experiments that were carried out in a cylindrical Pyrex reaction vessel illuminated from the top with a 125W black light fluorescent lamp ($\lambda=_{max}$ 365 nm). The light intensity reaching the reaction medium was $I_o=1.65 \times 10^{16}$ quanta/sec as quantified by solution-phase potassium ferrioxalate actinometer (Parker, 1997). Cu_2O nanoparticles were dispersed in a 50 mL of 2.5 mg/L RB-19 dye solution. The experiments were performed without pH adjustment. The solution was filtered immediately after the irradiation process through 0.22 μm cellulose acetate filters to separate the photocatalyst. To investigate operational parameters, three different initial RB-19 dye concentrations (2.5 mg/L, 5 mg/L, 10 mg/L) and four different amounts of photocatalyst (0.25 g/L, 0.50 g/L, 0.75 g/L, and 1.0 g/L) were used. The photocatalytic degradation kinetics of RB-19 was monitored by UV-vis spectroscopy. UV–vis spectroscopic measurements of the photocatalysts were acquired by a Thermo Scientific Genesys 10S double beam spectrophotometer using 1 cm quartz cells.

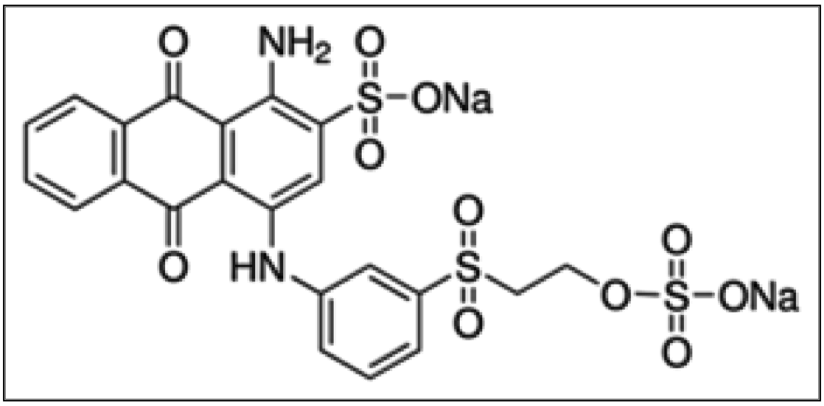

Figure 1. The chemical structure of RB-19 (λ_{max}= 594 nm, MW= 626.54 g/mol).

RESULTS AND DISCUSSION

Effect of Catalyst Concentration

In order to evaluate the optimum amount of photocatalyst, a series of experiments with varied Cu_2O amount from 0.25 g/L to 1.0 g/L was performed in 2.5 mg/L RB-19 dye solution. The removal percentage values of RB-19 were calculated from Equation (1), where C_0 is the initial concentration of the dye and C is the dye concentration at time t and represented in Figure 2.

$$Removal\% = \frac{C_o - C}{C_o} x100 \quad (1)$$

After 30 min irradiation, the degree of photocatalytic degradation of RB-19 increased with an increase in the amount of photocatalyst, and then decreased. This could be attributed to the excessive amount of catalyst leading an enhancement opacity of the suspension. The maximum degradation achieved to be 32.7% at the Cu_2O concentration was equal to 0.50 g/L.

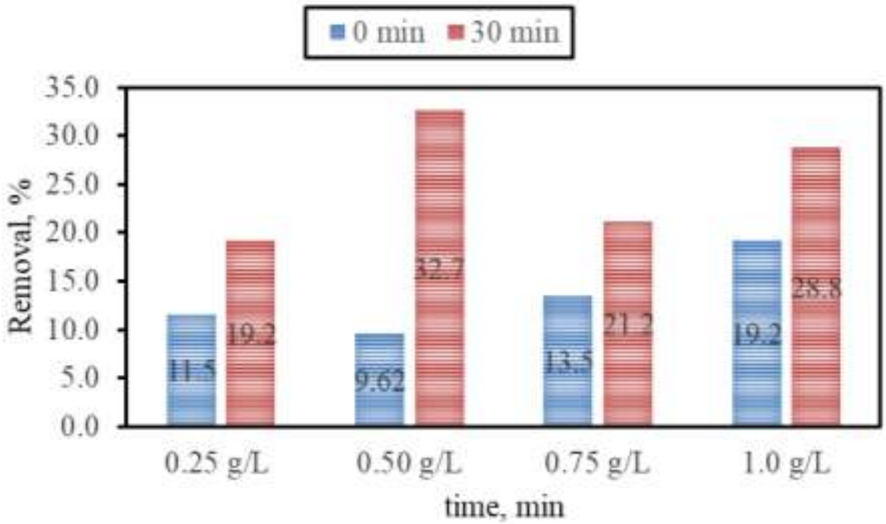

Figure 2. The effect of catalyst dose on the degradation of RB-19.

Effect of Concentration of Dye

The effect of initial dye concentration was examined on the removal percentages of RB-19 with 0.50 g/L photocatalyst dose. The results were presented in Figure 3 and the removal efficiencies of RB-19 were given in Table 1. The maximum degradation occurred at 2.5 mg/L of RB-19 dye solution. As dye concentration was increased, the removal percentage values of RB-19 were decreased after 30 min and 60 min UV-irradiation. The reason of the reduction on the removal efficiency could be explained by the increasing of the adsorption of dye molecules on photocatalyst surface and resulting a decrease in the active sites of the photocatalysts and thereby reducing the generation of hydroxyl radicals (Anju Chanu et al., 2019; Sohrabnezhad, 2011). After 120 min of irradiation, the removal efficiencies were 65%, 35% and 19% at initial RB-19 concentrations of 2.5 mg/L, 5 mg/L and 10 mg/L, respectively.

Table 2. The removal efficiencies of RB-19.

	0 min	**15 min**	**30 min**	**60 min**	**90 min**	**120 min**
2.5 mg/L	9.62	17.3	32.7	40.4	53.8	65.4
5 mg/L	3.57	7.14	10.7	16.7	23.8	34.5
10 mg/L	2.14	4.29	7.14	11.4	14.3	18.6

Kinetic Study

The kinetics model of photocatalytic reaction of RB-19 followed pseudo-first order of kinetics expressed by Equation (2).

$$\ln[A] = -kt + \ln[A_o] \quad (2)$$

where,

A_o: initial absorbance of RB expressed as $A_{,o,}$
A: absorbance of MB expressed as A at time t,

t: irradiation time, min,
k: pseudo first order reaction rate constant, min^{-1}.

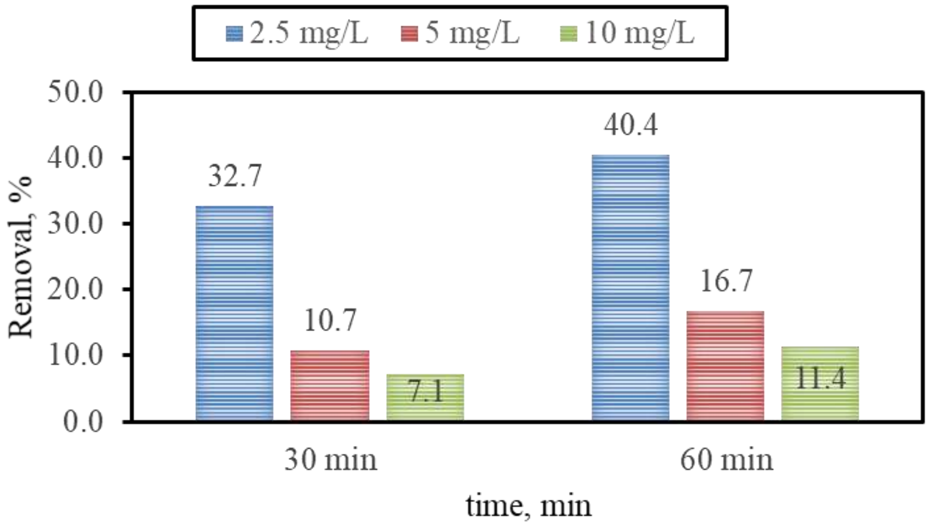

Figure 2. The effect of initial dye concentration on the degradation of RB-19.

Kinetic model parameters (R^2>0.95) were presented in Table 2. Half-life ($t_{1/2}$, min) could easily be calculated by the following equation, $t_{1/2}$=0.692/k. Upon use of Cu_2O, the highest decolorization rate constant of RB-19 was calculated as k=8.28x10^{-3} min^{-1} at 2.5 mg/L dye concentration.

Table 2. Photocatalytic degradation kinetics of RB-19.

	2.5 mg/L	**5 mg/L**	**10 mg/L**
k(x10^{-3}), min^{-1}	8.28	3.26	1.61
$t_{1/2}$, min	83.71	212.6	430.5

CONCLUSIONS

Based on the study, the photocatalytic degradation kinetics of Cu_2O nanoparticles on RB-19 were systematically investigated. The kinetics of photocatalytic degradation of RB-19 followed pseudo-first-order kinetics. The optimum photocatalyst dose amount was determined to be 0.5 g/L of Cu_2O nanoparticles for 30 min decolorization of RB-19. A higher photocatalytic activity for the decolorization of RB-19 was acquired at 2.5 mg/L RB-19 concentration. The removal percentage of RB-19 was decreased with the increasing of initial dye concentration. The removal% of RB-19 (2.5 mg/L) on Cu_2O (0.50 g/L) were estimated to be 65% after 120 min UV light irradiation.

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FRUIT SETTING IN ALMOND (Prunus dulcis)

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ABSTRACT
Almond cultivation has been increasing rapidly in recent years in terms of both production area and production amount in Turkey. Despite the increase, the castings (bud, flower and fruit) during the production season have suffered significant damage potential in some years. Here, the article tries to explain the fruit set in almonds, the castings and their reasons, the precautions that can be taken against these problems and the importance of cultural practices.

Keywords: Almond, flower, fruit, fruit set, Turkey

INTRODUCTION

In the light of latest data gathered all over the world, the global almond cultivation output is 3.497.148 tons in an area of 2.126.304 ha. The major almond production countries respectively are the United States (1,936,840 tons), Spain (340,420 tons), Iran (177,015 ton), Turkey (150,000 tons), and Australia (146,410 tons). These countries are followed by Morocco, Syria, Tunisia, Italy, Algeria, and China. The leading countries with respect to almond production area are Spain (687,230 ha), USA (477,530 ha), Tunisia (225. 453 ha), Morocco (190.612 ha) and Iran (79.597 ha), and Turkey ranks ninth with an area of 47.088 ha. Concerning productivity per unit area, Israel (211.543 hg/ha), Uzbekistan (71.109 hg/ha), Lebanon (60.729 hg//ha), Chili (43.927 hg/ha) and the USA (40.560 hg/ha) occupy top ranks whereas Turkey ranks tenth with 31.855 hg/ha (Anonymous, 2019). Turkey is also in an important position in terms of almond population, as it is located on the gene center of almonds. Despite the fact that it gets a share from the top ranks in the world with regard to almond cultivation, and the cultivation area and the production amount are on the increase, Turkey's productivity per unit area is saliently low (Table 1).

Increasing the yield to be obtained from the unit area; It includes many factors such as the correct selection of the garden location and varieties in suitable ecologies, the precautions to be taken during the flowering-pollination-fertilization periods, the timely and correct application of cultivation techniques and maintenance processes in the management of the gardens. Abundant and/or adequate fruit set in flowers, is the basic factor to get plentiful product from fruit trees. The biological structures of the species, their fertilization biology, flowering, pollination, fertilization, fruit formation characteristics, flower dropping and all factors such as climate, nutrition, irrigation, pruning, disease and pest control affect fruit set.

Fruit set primarily depends on adequate, steady, and healthy development of generative organs in the fruit trees, and occurs as a result of successive flowering, pollination and fertilization in the following process. Adequate number of pollens falling off onto the stigma, the capacity of pollens to germinate and form pollen tube, and the ovary's being alive are the factors having an impact on fruit set.

Table 1. Almond Production, Area and Yield Amounts

PRODUCTION (ton)		AREA (ha)		YIELD (hg/ha)	
USA	1.936.840	SPAIN	687.230	ISRAEL	211.543
SPAIN	340.420	USA	477.530	UZBEKISTAN	71.109
IRAN	177.015	TUNISIA	225.453	LEBANON	60.729
TURKEY	150.000	MOROCCO	190.612	CHILI	43.927
AUSTRALIA	146.410	IRAN	79.597	USA	40.560
MOROCCO	102.185	SYRIA	71.520	JORDAN	38.815
SYRIA	80.258	LIBYA	64.132	AUSTRALIA	38.628
TUNISIA	80.000	ITALY	52.040	KYRGYZSTAN	35.810
ITALY	77.300	TURKEY	47.088	CHINA	35.126
ALGERIA	72.412	AUSTRALIA	37.903	TURKEY	31.855
CHINA	45.000	ALGERIA	35.380	IRAN	22.239
WORLD	**3.497.148**	**WORLD**	**2.126.304**	ALGERIA	20.467
				AZERBAIJAN	15.958
				GREECE	14.508
				WORLD	**16.447**
Anonymous 2019					

1. Formation of flower buds in almond trees

When almond trees ripen this year's fruit, they also form flower buds that will produce the next year's fruit. In almond, flower buds occur on annual shoots and main branches during previous year. Starting from the fruit set, changes called “Physiological Differentiation Phase” occur within the vegetative buds which grow on the shoots of different age with the effect of a number of physiological and biochemical events. As of this phase, the meristem goes through a process of expansion, growth, and changes, in a period called “Morphological Differentiation Phase”, which can be monitored via microscopic methods in the flower buds. In the morphological differentiation phase, the flower primordia inside the buds transform and the formation and growth of flower’s organs start. The flower buds which complete the morphological process already formed all parts of a flower at the beginning of the winter dormancy. In order to exit the bud dormancy, the chilling requirement for the buds should be met at the different periods for various species and varieties. The buds which complete the winter dormancy, start flowering in spring depending on the air temperature. The total temperature in the spring is also effective on the beginning of flowering. In this period, the buds engage in gametogenesis and with flower bloom, the pollination, and fertilization processes occur (Özbek, 1978; Ağaoğlu et al., 2013).

The morphological differentiation phase is characterized in August, the microsporogenesis between December and January, and the macrosporogenesis just before flowering in almond. The beginning of flower bud differentiation within buds of almond changes according to varieties, but it begins when the green hull starts to splite off; the period covers August and the first week of September in northern hemisphere. In some varieties (such as Nonpareil, Ferragnes), the primordia formation starts nearly three weeks after the begins green hull splite off. It is stated that flower buds are located on short and longer spur branches and long shoots, while ovarium abortion is observed in flowers on long shoots depending on the variety, while this situation is rarely encountered in flowers on spur branches (Serafimov, 1981; Rugini and Monastra, 2003; Soylu, 2003; Socias i Company et al., 2017).

The climatic events (temperature, rains, etc.) and cultural practices (irrigation, fertilization, pruning) during the differentiation phases observed in a mature almond tree, affect the density and quality of the flowers that will be open in the subsequent flowering period. In other words, as it is the case in all fruit species, the amount of flower buds which will yield that year's crop, and the quality of the flowers (thorough formation of all organs in flowers), are affected by the previous year's climate conditions, and maintenance conditions such as irrigation, fertilization, and pruning.

The growth and development of generative organs in fruit trees is more complex and require more nutrients and photosynthesis products (Faust, 1989). If the level of nutritive items is less than the levels needed by the plants, the formation of flower buds and consequently the trees' productivity decrease. Similarly, in the period the formation of flower bud starts, the fruit trees' need for irrigation must be met at optimum level. The flower buds goes through winter dormancy by completing their development on trees which are irrigated or grown in humid areas; but on trees which are not irrigated, the flower buds stop their development and complete their growth in autumn where the water is sufficient (Özbek, 1978; Ağaoğlu et al., 2013).

It is stated that some fruit species and varieties that form flower differentiation early are tolerant to water deficiency in the post-harvest period, in other words, water stress experienced in the post-harvest period in these species does not have a significant negative effect on fruit set and yield in the following year. Yet, the almond trees are reportedly very sensitive to lack of water in the period after the harvest because the flower bud differentiation period starts late. In period after the harvest, the lack of water harms flower primordia which are being formed in that period, leading to low-quality flower formation, and dropping next year's fruit set, and consequently the fruit productivity. Also, severe lack of water in autumn reportedly leads to early and rapid development of pistil and shorten the period to accept pollens. The consequent productivity loss is between 48% and 88%. This is less important in orchards which are established in climates with relatively cooler autumns and which at the same time have deeper soil, but it is of big importance in orchards established in climates with warm autumns and have shallow soil. The irrigation after the harvest allows leaves to be active and functional until normal defoliate (by the end of November), that is, the leaves continue to produce nutrients. The carbohydrates produced daily by the leaves rather than the carbohydrates stored within the body of the tree are used to maintain the growth and development of the flower primordia. Nitrogen is required for the leaves to produce nutritive item (carbohydrate). The almond trees consume significant amount of the nitrogen for fruit growth and their own development until the harvest period. That's why, they do not have sufficient amount of nitrogen for formation of new flower bud primordia after the harvest. For this reason, nitrogen fertilization is advised after the harvest, provided that it does not exceed 20% of the nitrogen to be applied to the tree annually. As a result, because the formation of flower bud primordia starts late in almond trees, conscious irrigation and nitrogen fertilization practices will positively affect the following years' productivity (Çağlar, 2012; Socias i Company et al., 2017).

Besides macroelements, microelements such as boron, manganese, iron, and zinc are reportedly required for flower bud formation, fruit set, and fruit development. (Hanson, 1997; Nyomora et al., 1997; Lamp et al., 2001; Perica et al., 2001a; et al., 2001b; Rezaee et al., 2008; Nezami, 2012; Amiri et al., 2016).

2. Flowering, pollination, and fertilization in almond trees

Because the almond's need for chilling is much less than other temperate climate fruit species, flowering occurs early with the disappearance of compulsory resting conditions. That's why it is a strain especially limited to regions with late spring frost. It is reported that in order to regulate the flowering period for almonds, the temperature required for flowering, as well as the chilling period of varieties, plays a decisive role (Tabuenca et al., 1972; Westwood, 1978; Felipe, 2000; Yi et al., 2003; Samani et al., 2005; Arús et al., 2009; Dicenta et al., 2017).

The flowering time in almond differs with respect to varieties and growing regions The flowering period occurs from the end of winter (February) to spring depending on the varieties in the United States, one of the leading almond producers. In our country, the flowering on trees starts early spring in almond cultivation areas. The flowering of the varieties cultivated in the Southeastern Anatolian region, starts approximately as of the second week of March and continues until the first week of April. Also, since the total temperature in the spring is also effective on the flowering, the flowering time of the same variety will also differ according to the weather conditions of the year, the latitude and altitude of the place. The flowering period as well as the start of the flowering time is important. The period differs from 4 days to 30 days according to the climate conditions and the varieties, and it can be longer in the Mediterranean climate belt. In continental climate, the flowering of the varieties occur almost in the same period. (Rugini and Monastra, 2003; Soylu, 2003). The climate factors during the blossoming period (especially warmth and humidity) significantly affect flowering period, and consequently the productivity. Because a long and efficient flowering period will have positive impulse on pollination and fertilization, it ensures desired level of fruit set. Shorter flowering periods cause low productivity levels.

In some almond varieties, boric acid and zinc applications carried out when 10% of the flower buds are open, extend the flowering period and increase the fruit set (Castro and Sotomayor, 1998).

As it is known, because the almond fruit is in fact the seed itself, the fertilization must necessarily occur following an efficient pollination in the period after the flowering to ensure fruit set on almond trees. Abundant number of flowers on almond trees and high rates of pollination increase number of fruits although they are small, and thus increase total productivity. That’s why, fruit yield from almond trees is directly related to all applications carried out to ensure efficient pollination and fertilization in almond orchards. This situation may also decrease the level of drop which is caused by inadequate fertilization.

The period when pollination ends with fertilization is called “Efficient Pollination Period”. The efficient pollination period defines the rate of pollen tubes that reach stigma with a combination of events such as the stigma vitality, the ovary longevity, and the time required for the pollen tube to reach the ovary. Thanks to the studies carried out concerning almond, it has been seen that efficient pollination period and flower density define the fruit set, and the length of the period ensures maximum success for fertilization. That’s why, these factors should be taken into account in efforts to ensure fruit set and productivity (Nyomora et al., 2000; Kızıldemir, 2006; Boyacı and Çağlar, 2009).

Almond flowers are hermaphrodite and have typical *Rosaceae* figures (five sepal-, and five petal in pink, red, or white, one female organ and 20-40 male organs). There are two ovules inside the pistil. It is an entomophilous species. In almond, the number of chromosomes is n=8, and triploidy is not seen as well as nutrition-induced pollen infertility. Most almond varieties are self-incompatible, excluding a few varieties, and also, cross-incompatibility between varieties can also be seen. That’s why, almond plantations should be established with at least two varieties that bloom in overlapping times and periods, fertilize each other and do not show cross incompatibility. In addition, the plantation shape of the varieties in the orchard to ensure the cross pollination, presence, strength, and placement of bee colony in the orchard, and the weather conditions during the flowering period are factors which are influential on efficient pollination and consequently fruit set.

The fruit formation process starts following fertilization after an efficient pollination during the flowering period. The speed of reaching the ovary, which maintains its vitality, of a pollen tube belonging to a pollen which germinates after it lands on a reseptive stigma is important with respect to fertilization. For higher rates of productivity, longer vitality periods are important for male and female organs on flowers, and these periods change according to the varieties. The stigma on almond flowers receives pollens three or four days after the flowering. The stigma receptiveness is high in the first one or two days, stable on the third and fourth days, and decrease rapidly in three or five days after the pollination. The temperature is an effective agent in this period, and cool weather extend the period for a period whereas higher temperatures decrease the stigma receptiveness. Temperatures below 1 ^{o}C lead petals to fall and cause death of styles and ovules (Ortega et al., 2004; Rugini and Monastra, 2003).

Depending on the varieties, the temperature should be between 15-23 ^{o}C for higher germination rates of pollens and development of pollen tubes. The dehiscence of almond flower anthers at temperatures higher than 18 ^{o}C, releases the pollen grains inside the anthers. But continuous rains during the flowering-pollination period can delay the anther dehiscence, and the dispersed pollen grains can be washed away or the liquid at the stigma surface can become thinner, obstructing germination of pollen grains (Özbek, 1978; Rugini and Monastra, 2003).

Nearly one hour after the pollen is fall off on the stigma, the pollen tube enters the style, and elongates rapidly inside the style at 20-30 ºC. At temperatures lower than 15 ºC, the extension of the pollen tube gets slower. And it receives damage at temperatures higher than 30 ºC. The pollen tube reaches the ovary between 96 or 120 hours. Thus the fertilization is completed nearly eight days after the flowers are pollinated. Usually, the shorter is the pollination period after the flowering, the higher is the chance to ensure the fertilization and the fruit set. And this case also means a decrease in castings which stem from lack of fertilization (Westwood, 1978; Tromp et al., 2005).

Self-fertility of almond varieties is an important factor which affects development of the pollen tube inside the style. Self-infertile varieties and incompatible combinations prevent pollen germination on the stigma surface or development of the pollen tube inside the style (Okay and Ayfer, 1994). Indeed, during self-pollination applications on almond, which usually have self-incompatible varieties, it is confirmed that pollen tube development is prevented at the 1/8 or 6/8 part of the style, and the pollen tube penetrates the style completely and reaches the base of style nearly in 72 hours in case of foreign pollination (Önal, 1993; Zeybekoğlu, 1993).

During pollination and fertilization processes of almond trees, some plant nutrition elements reportedly have a positive effect on pollen germination and pollen tube growth, increasing the fruit set. Applying boron (B) through leaves in autumn on almond trees which do not show symptoms of lack of B in vegetative tissues, reportedly increases the B content in the tissues, increasing the pollen germination rate and the speed of pollen tube extension (Nyomora et al., 2000; Bolat and Pırlak, 2003). This feature is important with regard to completion of fertilization, and thus, fruit formation. Indeed, exogenous B applications reportedly affects fruit set in almonds (Nyomora et al., 1997; Sotomayor et al., 2002; Rufat and Arbonés, 2006). Similarly, boron applied on leaves of some almond varieties at different doses in autumn, has increased in succeeding spring in vitro pollen germination and pollen tube length, leading to an increase of approximately 10% fruit set compared to control trees (Boyacı and Çağlar, 2009).

Plant growth regulators are also stated as application to be used by determining their impact on increasing pollination-fertilization and fruit set with respect to their different effects such as delaying bloom, or extending bloom period. The plant growth regulators can also be used to minimize the stress factors created by various factors like turn of the season, or excessively high temperatures (Eriş, 2007; Karaat, 2020).

With completion of the fertilization, the fruit formation and growth process starts. On almond trees, there are two ovules inside the ovary of the female organ. Usually, only one of these is fertilized, develops, and forms the seed. Thus, a single kernel is formed. Sometimes both ovules are fertilized and develop, and two seeds are formed within the shell, resulting in a double kernel. In some varieties, twin kernel are formed as a result of the formation and development of two embryos in a seed ovule. They have no commercial value. Almond is botanically a stone fruit. The difference from other stone seeds is that the green hull dries towards maturity and becomes leathery and cracks. While the fruits are still small (before the fleshy part dries up and the endocarp, which forms the hard shell, becomes petrified), it is possible to eat it as green almond. In the third stage of the fruit growth curve in almonds, the mesocarp completes its development and becomes hard by losing water during fruit ripening. From March to June, almond kernels mature and grow to full size, with the shell hardening around it-both protected by a fuzzy outer hull. Aproximetely in July, almond hulls split open, exposing the almond shell and allowing it and the kernel inside to dry. Shortly before harvest, the hulls turn a straw-yellow color and open completely (Anonymous 2021). The fruits are in different sizes (small, medium, large, extremely large) and shapes (round, ovate, oblong, cordate, extremely narrow) according to the varieties. In addition, shell color intensity, marking of outher shell, suture opening of the shell, shell retention, softness of shell characteristics are also evaluated in the identification of the varieties. Kernel color intensity (extremely light, light, intermediate, dark, extremely dark), shriveling of kernel (slightly wrinkled, intermediate, wrinkled), kernel pubescence (low, intermediate, high, extremely high), the percentage of sound kernels and double and twin kernel percentages are the other characteristics that vary according to the varieties. Kernel taste is sweet, intermediate or bitter (Özbek, 1978; Gülcan, 1985; Rugini and Monastra, 2003; Soylu, 2003; Küden et al., 2014; Anonim, 2021).

3. Flower and nut drops on almond trees

One of the most important problems encountered in almond cultivation areas which rapidly increase in our country, is the drops seen during and after the bloom period. The problem which causes significant crop losses for the producers in some years, is not peculiar to almond and can be seen also in several other fruit species with different rates of severity.

As known, harvesting fruits with high rates of productivity and of good quality is the basic in fruit cultivation. This will be possible if the tree carries enough fruit load that it can feed and turn it into quality fruit in terms of features such as size, shape and color. Not all of the flowers that open on fruit trees bear fruit. For example; it is considered a good fruit set when 15-20% of the flowers opened on apples, 8-5% on pears, and 3.5-4.5% of the flowers opened in some plum varieties remain as fruit on the tree. It is expected that an average of 20-25% of its flowers will set fruit in optimum conditions during the yield period of an almond tree that is self-sterile, that is, in need of a pollinator. This percentage can range between 15-40%. Most orchards, however, set between the 20-30% range, with average orchards around 25%. This percentage varies year-to-year, and is dependent on flower density, temperature at bloom and post-bloom, and tree health. The increase or decrease of this ratio, as explained above; It depends on the quality of the organs in the flower, the climatic conditions during the flowering period and the intensity of bee activity needed for effective pollination (Doll, 2016; Miarnau et al., 2017).

Flower and fruit dropping seen in fruit species is a mechanism that starts with the formation of flower buds in fruit trees and includes flowering, pollination and fertilization processes, and is affected by the climate, irrigation and nutritional factors in these periods.

In general, fruit trees; flowers and small fruits, which have defective female organs and do not fertilize, fall from the tree during 'blossom drop' which occurs very shortly after flowering and 'flower and small fruit drop' period which takes place after approximately two weeks or a little longer, depending on the species.

However, the 'June drop', which occurs approximately one month after the second drop, and which takes place in the fruits that will form the product of that year, are caused by the lack of water and nutrients and should be carefully monitored in terms of productivity. In this sense, it can be said that the natural flower and small fruits dropping seen in fruit trees, especially in the flowering period, are actually a natural thinning mechanism of the tree. However, the increase in drop rates in these periods due to climatic factors, inadequate nutrition and maintenance conditions will adversely affect the product amount of that year (Özbek, 1978).

Dropping encountered in almond cultivation starting from the flowering period are common in three different periods. These periods are grouped as 'flower drop', 'small fruit drop' and 'June drop' as in other fruit species (Figure 1).

Flower drops occurs during the flowering period. It happens in the form of shedding of some of the flowers that are fully opened during the flowering period, and it has been observed that the ovaries of the spilled flowers failed to develop fully. In other words, the flowers that failed fertilization function are shed. It is possible to name the factors affecting these drop as internal and external factors. The chilling required for the awakening of flower buds before flowering is an intrinsic factor affecting flower drop. When the chilling period required by the varieties for effective flowering is not met, the flowering period is short and the quality of the blooming flowers may be low. These reasons, especially after the ineffective winter cold, cause the casting of flowers that are formed when the buds that cannot meet the need for cooling open with the warming of the weather. It has even been observed in the Southeastern Anatolian region that this problem causes the buds to shed even before flowering in some cultivars. The other internal factor affecting the flower drop is; the quality and amount of nutrients stored by the plant before flowering, depending on the post-harvest and pre-flowering nutrition program. If the plant enters the season without storing enough storage nutrients, it will suffer more by the stress factor to be experienced. It should not be forgotten that unconscious nitrogen applications made before flowering also increase flower dropping.

Climatic factors such as the amount, frequency, intensity of precipitation besides temperature and wind during flowering are external factors affecting flower dropping. Sudden high and low temperatures, precipitation and strong winds experienced at the beginning and during the flowering period significantly increase the flower dropping.

Among these factors, only the nutrition factor is the factor that can be affected by the practices of our producers, and the other factors are purely variety character and climatic factors. Considering these factors; It will be possible to be affected by the flower dropping at a minimum level by carefully examining the climate data in the region where the cultivation will be carried out, choosing the variety and adhering to the analysis results that will be made regularly in the plant nutrition programs.

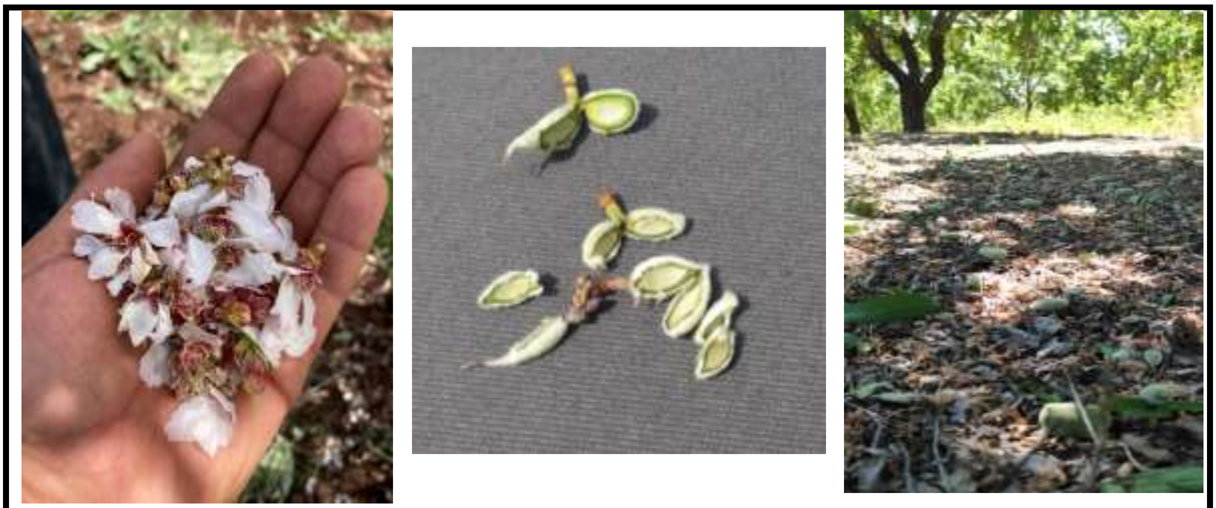
The second dropping after flowering in almond cultivation occur during the small fruit period. In this period, the fruit started to come out of the flower sheath and the embryo has not yet formed. Small fruitlets consisting of unfertilized flowers and/or unfertilized flowers drop. The most important factor affecting in this period is the absence of effective pollination and fertilization. All fruits that do not fertilize are shed during this period. Although there are enough bees in the gardens, fertilization does not occur during periods when the necessary climatic conditions for maximum bee activity are not met, and the unfertilized fruits are drops during this period. All applications for effective pollination and fertilization in almond orchards should be carried out in order to prevent these drops caused by lack of pollination and fertilization. In addition, care should be taken to apply pesticides during flowering (such as *Monilia laxa* control) in the evening hours so that they do not adversely affect bee activities. It should not be forgotten that in applications made during the day, the flowers, which pollination is adversely affected, are also drop during the small fruit period.

The last dropping in almond cultivation is called the June drop, which takes place in about 2-6 weeks after the small fruit period. Although it varies according to regions and years, some fruits that reach 2-3 cm in size at the beginning of May stop growing, their colors turn yellow and they fall off before they reach their full size. It indicates the lack of water and/or nutrition of the trees. It happens because the fruits on the tree compete with each other for water and/or nutrients to grow. Therefore; the most important factor affecting these drops is the irrigation and feeding program. Thanks to the feeding made depending on the age of the tree after flowering and taking into account the results of the soil analysis carried out before the season, these drops remain at a minimum level. However, in the orchards that are not nourished enough, these drops are more severe. Likewise, regular irrigation programs in the spring and summer periods according to precipitation and soil moisture are other important factors in preventing or reducing the severity of June drop. Another factor affecting the June drop is the effective struggle against diseases and pests. Dropping can also occur with sudden low and high temperatures, after heavy rains and as a result of unsuccessful struggles against fungal diseases and kernel worm damage. In this period, yellowing of the fruit color is seen, and green and full fruits that have been exposed to diseases and pests may also drop.

Climate factors also affect the June drops. Especially in the dry seasons or in the seasons with sudden changes in air temperatures, June drops can be seen intensively. Especially in the orchards that cannot reach sufficient soil temperature, as a result of the ineffectiveness of root activities, the uptake of water and nutrients by the trees cannot be done sufficiently, and due to the lack of water and nutrients, dropping may occured or be exacerbated.

Depending on the climatic characteristics that may change over the years, in the orchards where the flowering period lasts longer, the fruits formed by the last flowers may remain smaller than the other fruits. It was observed that this type of fruit was poured at a higher rate than the fruits of other sizes in the June drop. It is obvious that this may be due to the fact that small fruits cannot compete with fruits that are larger than themselves in terms of water and nutrients. In order to reduce the dropping of these fruits, it is necessary to pay attention to the fertilization program to be made from both leaves and soil. In the June period, with the effect of strong winds blowing at a speed of 30-40 km/h, larger fruits may also drop.

Adverse climatic factors such as strong winds, heavy rains and hail occurring during both flowering and fruit set periods may increase the dropping rates (Figure 2). Unfortunately, these factors are unpredictable and unavoidable natural conditions. Especially in orchards where the above-mentioned issues that can be managed by the producers such as effective pollination, adequate water and nutrient provision are not taken into consideration, the damage caused by dropping will be even greater.



Flower drop Small fruit drop June drop

Figure 1. Flower, small fruit and June drops in almond

Figure 2. Flower and fruit casting in almond orchards because of wind and frost

Deficiencies or excesses of nitrogen (N) and potassium (K) in macro elements and boron (B), zinc (Zn), magnesium (Mg), iron (Fe) and manganese (Mn) in micro elements are the most important nutrients that affect these drops. Nitrogen and potassium are important in terms of affecting the photosynthesis ability of the plant to cope with the stress factors to be experienced after flowering. In addition, they are the most needed nutrients after flowering and fruit filling period. They have a positive effect on cell division and growth of young tissues thanks to the plant's high photosynthetic ability after flowering and fruit set. Therefore, nitrogen and potassium elements are important during flowering and fruit set periods. The amount to be applied in nitrogen and potassium fertilization should be planned considering the planned almond yield during the season. Excessive nitrogen fertilizer applications will not only impair the internal fruit nutrition quality, but also cause a decrease in resistance against diseases and pests as it will cause excessive vegetative growth (Brown and Uriu, 1996; Geisseler and Horwath, 2019).

Boron, one of the microelements, is the most important nutrient affecting the development of flower organs, pollen germination and fruit set. Zinc is effective in cell development and growth, chlorophyll synthesis, and also fruit set. Manganese, iron and magnesium, on the other hand, are nutrients that have a significant effect on the formation of the next year's flower bud and fruit development , as they affect the photosynthesis activity in the leaves (Lamp et al., 2001; Muhammad et al., 2007; Rezaee et al., 2008; Nezami, 2012; Amiri et al., 2016; Denizhan et al., 2021).

CONCLUSIONS

In fruit cultivation, sufficient fruit set forms the pillar of increasing fruit productivity per unit area. As in the other fruit species, the fruit set in almond is also a process which starts with formation of adequately healthy, well developed reproductive organs, followed by efficient pollination and fertilization, and affected by castings. The biological peculiarities of the species, the climate conditions of the region, the orchard maintenance procedures, and some exogenous practices have an effect on fruit set directly and indirectly. Consequently, in the almond cultivation as it is the case in all fruit species, higher rates of fruit set can be ensured, the severity of castings can be reduced and/or managed, and thus, desired amount of almond crop can be obtained from an almond tree which is capable of turning 20-30% of the bloomed flowers into fruits, provided that the orchards are established in suitable climate conditions; at right spots with selection of right rootstock and varieties; and in appropriate directions; and by suitable plant spacing and taking measures to ensure growth of flower buds which are able to create sufficient amount of good quality flowers, and also taking measures to ensure an efficient pollination and fertilization process; and by timely and efficient practices of cultivation (nutrition, irrigation, pruning, and fight with disease and pest control).

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CREDIT USE AND SUSTAINABILITY IN APPLE PRODUCTION IN ISPARTA

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ABSTRACT

In this study, it has been tried to determine the producer's views on the use of credit and the sustainability in apple growing enterprises in Isparta Province. In the enterprises examined within the scope of the study, demographic characteristics, people who are influential in decision making, reasons for agricultural production, factors that will help to continue agricultural activities, future goals and objectives, information sources, credit sources, purposes of using credit, evaluations for credit use, evaluation of agricultural income etc. topics are included. The material of the study consisted of data obtained from primary and secondary sources. In terms of representing the province of Isparta, the data collected from the agricultural enterprises in Eğirdir district through a questionnaire is the primary source of the study. In the research, a questionnaire was applied to the producers in 50 apple growing enterprises. The data obtained by the survey application were evaluated using the SPSS package program, and the results were summarized in the tables. According to the research results, the average experience period of producers is 25,84 years. The average family size in the surveyed farms is 4,22 people. Making profit and owning land are the most important factors among the reasons for the producers to make agricultural production. 18% of the youth in the surveyed enterprises do not want to continue agricultural activities in the future. In the research, it was determined that Ziraat Bank took the first place among loan sources.

Keywords: Apple growing, Credit, Sustainability, Decision-making, Ziraat Bank, Isparta

INTRODUCTION

Agricultural production activities constitute the most important source of income for the population living in rural areas around the world. The agricultural sector is one of the most protected and supported sectors, due to its low income, difficult to manage and low social welfare of the population in the sector compared to other sectors in the economy. The continuation of the use of imported inputs instead of the inputs produced in the enterprises, the increase in the size of agricultural enterprises and the increasing specialization in agriculture have led to an increase in the capital and loan needs of today's farmers (Semerci, 2021).

While a significant part of the financing demand for the agricultural sector is met by organized agricultural credit institutions, especially in developed countries, it is met by unorganized credit institutions in developing countries and Turkey. Agricultural loans in Turkey and in the world are given by local banks and/or credit institutions specialized in this field, cooperative banks, which are generally supported by the state and know the characteristics of such loans (Taskiran and Ozudogru, 2010).

The differences in the activity characteristics of agricultural enterprises cause agricultural finance to show its own characteristics. Dependence of agricultural production on natural conditions, small scale of agricultural enterprises and insufficiency of savings in enterprises, low capital turnover rate, high share of fixed assets in capital structure and production factors, subjective characteristics of agricultural financing (devotion to traditions, customs and practices, inadequacy of credit use, etc.) affects the shaping of agricultural finance and credit use in agriculture and sustainability in agriculture.

Although there are many national and international studies on the use of credit in agriculture, agricultural financing and sustainability, no research has been found on this subject in the research area. Some of the studies on agricultural financing and credit use; Berk (2019), Ozvardar (2019), Topuzoglu and Artukoglu (2019), Rad and Aydogdu (2019), Hayran and Gul (2018), Kasık (2015), Kocturk et al. (2013), Tercan et al. (2012), Aksoy et al. (2010), Ozcan (2009), Adıguzel (2006).

Sustainable agriculture is expressed as a set of systems that include production systems that protect human health and the environment, as well as balanced use of technology and correct business management. The effects of sustainable agriculture are at a global level and have three main components: economic, social and environmental. Economic sustainability; profitability of agricultural enterprise, operating costs, financial risk and investments, social sustainability; the wages of the employees of the enterprises, the quality of life of the producers and the ethical issues in the enterprise, while the environmental sustainability includes energy efficiency, soil and water quality, protection of wildlife, food and feed safety and operational safety. By maintaining the balance between the components of sustainable agriculture, the damage caused by agricultural activities to the environment will be reduced, while at the same time, the contribution of agricultural production to the country's economy will be ensured. In this way, the population that makes a living from agriculture will be employed on-site and the characteristic of the agricultural population as a rural society will be preserved (Eryilmaz et al., 2019).

Studies on sustainability in agriculture are examined under three headings: social, economic and ecological sustainability. Chopra (1993), Hansen (1996), Webster (1997), Smith and McDonald (1998), Von Wirén-Lehr (2001), Bongıovannı and Lowenberg-Deboer (2004), Nelson (2007), Akcaoz ve Kizilay (2009), Akcaoz et al. (2009), Akcaoz et al. (2010), Dillon et al. (2010), Karim et al. (2015), Latruffe et al. (2016), Muhamadi (2016), Bachev (2017), Janker et al. (2019), Janker and Mann (2020) examined economic and social sustainability in their work.

In this study, it was tried to determine the producer's views on the use of credit and the sustainability of the activity in apple growing enterprises in Isparta Province. Demographic characteristics of the enterprises examined within the scope of the study, people who are influential in decision making, reasons for making agricultural production, factors that will help to continue agricultural activities, future goals and objectives, information sources, credit sources, purposes of using credit, evaluations for credit use, evaluation of agricultural incomes, etc., topics are included.

MATERIAL AND METHOD

The material of the study consisted of data compiled from primary and secondary sources. In terms of representing the province of Isparta, the data compiled through face-to-face survey from 50 apple producing agricultural enterprises in Egirdir district is the primary source of the study. The survey application covered the production period of 2016. The data obtained by the survey application were evaluated using the SPSS package program, and the results were summarized in the tables. The secondary sources of the research are similar studies conducted in the national and international arena and institutions and organizations related to the subject.

RESEARCH FINDINGS

Demographic Characteristics of the Surveyed Farms

In the farms within the scope of the research, the experience period was found to be minimum 7 years, maximum 40 years, and the average experience period was 25,84 years. The average family size in the examined businesses was found to be 4,22 people. The number of individuals in the farms included in the research was found to be a minimum of 1 person, a maximum of 6 people, and the total number of individuals was 212 people. In the farms, 48,6% of the total population is female and 51,4% is male. In the farms examined, 66,98% of the total population consists of individuals between the ages of 15-49. 35,3% of the farmers in the farms within the scope of the research are primary school graduates. In the farms examined, 76% of the family population works in their own enterprises. In the research, it was determined that 89,7% of the family members did not work in other agricultural enterprises other than their own farms, and 64,6% did not work in non-operational agricultural works (Table 1).

Table 1. Demographic Characteristics of the Investigated Farms

	Person	**%**
Distribution of Population by Gender		
Female	103	48,6
Male	109	51,4
Total	***212***	***100,0***
Distribution of Population by Age Groups		
0-6	1	0,47
7-14	6	2,83
15-49	142	66,98
50+	63	29,72
Total	***212***	***100,00***
Educational Status of the Farmer		
Primary School Graduate	18	35,3
Secondary School Graduate	13	25,5
High School Graduate	15	29,4
College/University	4	7,8
Total	***50***	***100,00***
Educational Status of the Family Members*		
Literate	2	0,94
Primary School Graduate	43	20,28
Secondary School Graduate	42	19,81
High School Graduate	60	28,30
College/University	62	29,25
Total	***209***	***100,00***
Working Status of Family Members in the Own Farm		
Working	161	76,00
Not Working	51	24,00
Total	***212***	***100,00***
Working Status of Family Members in Agricultural Jobs Outside the Own Farm		
Working	22	10,30
Not Working	190	89,70
Total	***212***	***100,00***

Working Status of Family Members in Non-Agricultural Jobs		
Working	75	35,40
Not Working	137	64,60
Total	***212***	***100,00***
Average Family Size (person)	***4,24***	
Average Experience of Farmers (years)	***25,84***	

***** 3 people are not yet of school age.

In Table 2, the evaluation of agricultural incomes in the surveyed farms are given. In the examined farms, 10% of the farmers stated that they deposited the income in the bank, 68% bought gold, 2% bought foreign currency, 2% stocks, bonds, etc. stated that they bought land, 26% of them bought land, 78% of them bought tools and machinery, and 98% of them used it for their family needs.

Table 2. The Evaluation Status of Agricultural Incomes in the Inspected Farms (N=50)

	Person	**%**
I deposit it in the bank	5	10,00
I'm buying gold	34	68,00
I'm buying stocks, bonds etc.	1	2,00
I'm getting a currency	1	2,00
I'm buying land	13	26,00
I'm buying a machine	39	78,00
I use it for family needs	49	98,00

***** More than one answer has been received.

In 50 farms within the scope of the research, the total farm width was found to be 1.332 decares and the average farm width was found to be 26,64 decares. The average number of parcels in the farms was 2,38 and the average parcel width was 11,19 decares. Considering the total land widths of the farms in the research, it was determined that 93,47% of them consisted of property and 6,53% of them consisted of rental land. According to the topographic structure of the enterprises, 67,94% of the lands are flat land, according to the soil structure, 42,27% of the lands are base land, 62,76% of the lands according to the soil fertility are medium fertile lands, 48,27% of the lands according to the land type it is dry field land (Table 3).

Table 3. Land Situation in the Investigated Enterprises

	Total Area (da)	**%**
Property Status		
Propert	1.245	93,47
Rent	87	6,53
Parner	-	-
Total	***1.332***	***100,00***
Topographic Condition		
Straight	905	67,94
Slightly bumpy	186	13,96
Moderately bumpy	54	4,05
Mountainous	187	14,04
Total	***1.332***	***100,00***

Soil Structure		
Rocky	270	20,27
Base	563	42,27
Silty	43	3,23
Barren	436	32,73
Sandy	20	1,50
Total	***1.332***	***100,00***
Soil Fertility		
High	251	18,84
Middle	836	62,76
Low	245	18,39
Total	***1.332***	***100,00***
Type of Land		
Irrigated Field	81	6,08
Dry Field	643	48,27
Fruit	560	42,04
Meadow Pasture	28	2,10
Forage Crops	20	1,50
Total	***1.332***	***100,00***
Average Number of Parcels (unit/farm)	***2,38***	
Average Parcel Width (da/farm)	***11,19***	

Sustainability of Agricultural Activity

Decision-making is choosing the most appropriate form of action from various possible forms of action in order to achieve a goal. Decision is the choice of one among two or more possible behavior patterns (Akcaoz, 2001). In the research, the people who are influential in making decisions about agricultural activities are examined and the results are given in Table 4. In the farms examined within the scope of the research, investment, business expansion, marketing and financing decisions are mostly made by the farmer. The family evaluates together in decisions about saving, future and non-operational work. It has been stated that agricultural engineers are effective in the decisions about the production pattern in the farms.

Table 4. Influential Persons in Decision-Making Related to Agricultural Activities (%, N=50)

Decision Type	**Farmer**	**Spouse**	**Agricultural Engineer**	**Dealer**	**Family**
Investment Decisions	72	2	4	-	54
Savings Decisions	22	4	-	-	92
Financing Decisions	52	2	2	-	68
Future Decisions	22	-	2	-	90
Business Growth Decisions	66	-	32	-	28
Marketing Related Decisions	90	-	4	2	4
Decisions Regarding Production Pattern	50	-	62	6	-
Non-Business Decisions	36	-	2	-	88

***** More than one answer has been received.

Farm records are kept in 98% of the farms surveyed in the research. 78% of the farmers stated that they are afraid of failing in their work. For the sustainability of agricultural activity, farmers were asked whether they would continue agricultural activity when a fixed fee was paid. In 76% of the surveyed farms, the farmer stated that they would not give up on agriculture in return for a fixed fee. 92% of the farms within the scope of the research are registered in the farmer registration system. It was determined that 76% of the farms were not members of a cooperative for agricultural purposes (Table 5).

Table 5. Information on General Structure in Enterprises

	Yes		**No**		**Total**	
	Person	**%**	**Person**	**%**	**Person**	**%**
Do you keep records of your business?	49	98	1	2	50	100
Are you afraid of failing at what you do?	39	78	11	22	50	100
If you are paid a fixed wage, would you quit farming?	12	24	38	76	50	100
Are you registered in the farmer registration system?	46	92	4	8	50	100
Are you a member of an agricultural cooperative?	12	24	38	76	50	100

The reasons for dealing with agricultural activities in the examined farms and the subjects of continuing agricultural activities were also examined (Table 6). The reasons for dealing with agricultural activities in the examined farms were determined using the Likert Scale. The most important reason among the reasons for dealing with agricultural activities in the examined farms was to make a profit, followed by having land for agricultural activity, being the boss of one's own business, and others.

Table 6. Reasons for Agricultural Production

	Average	**Standard Deviation**
Make a profit	1,020	0,141
Having land for agricultural activity	1,040	0,197
Being my own boss	1,120	0,520
I enjoy farming	1,260	0,564
It is very difficult to change jobs	1,500	0,909
It's hard for me to do another job	1,600	0,925
Lack of good opportunities outside of agriculture	2,040	1,049
My family helping me with this job	2,160	0,997

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

The factors that will help the farmers in continuing the agricultural activities in the farms within the scope of the research are determined with the Likert Scale and shown in Table 7. Among the factors that will help the farmers to continue their agricultural activities in the examined farms, the factors stated as very important; there is not much debt (1,080), there is government support (1,100), product prices are guaranteed (1,160), there are no difficulties in recruiting workers, there is support against natural disasters (flood, hail, frost, etc.) (1,200), and in advance about product prices to have knowledge (1,200). The expansion of contracted production and the availability of childcare services were found to be unimportant among the factors that will help to continue agricultural activities.

Table 7. Factors that will Help to Continue Agricultural Activities

	Average*	**Standard Deviation**
Don't have too much debt	1,080	0,340
Have state support	1,100	0,364
Guaranteeing product prices	1,160	0,509
No difficulties in recruiting workers	1,180	0,560
Providing support against natural disasters (flood, hail, frost, etc.)	1,200	0,494
To have information about product prices in advance	1,200	0,494
Finding a loan when needed	1,220	0,648
Agricultural engineers at drug dealers visit the plant	1,520	0,677
Increasing credit opportunities	1,520	0,886
Visit of agricultural engineers in agricultural establishments	1,580	0,730
Having/increasing storage services	1,640	0,827
Having non-agricultural income	1,900	0,974
Increasing the number of agricultural cooperatives	2,040	1,194
Making extension programme	2,240	0,743
Have training in marketing	2,280	1,010
Having transport services	2,420	0,882
Providing additional employment opportunities for family members	2,560	1,033
Information on packaging	2,680	0,819
Packaging, etc. convenience in matters	2,780	0,736
Improvement of infrastructure facilities (road, communication, etc.)	2,880	0,798
Family members working outside the business	2,920	0,965
Increasing non-agricultural job opportunities	3,080	0,723
Increasing/improving educational opportunities	3,300	0,931
Making agricultural education programs for young people	3,320	0,913
Having childcare (home economics) services	3,640	0,597
Expansion of contract manufacturing	3,800	0,857

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

The opinions of young people about continuing agricultural activities in the examined farms are given in Table 8,82% of young people in farms consider continuing their agricultural activities. This is an important social indicator in terms of the sustainability of businesses' activities.

Table 8. The Opinion of Youth to Continue Agricultural Activities in the Investigated Farms

The Thought of Youth to Continue Agricultural Activity	**Person**	**(%)**
Yes	41	82
No	9	18
Toral	***50***	***100***

In Table 9, the factors affecting these decisions of young people who do not want to continue agricultural activities are given with a Likert scale. Among the reasons why young people do not want to continue agricultural activities in farms, the most important ones are that agriculture does not provide sufficient income, agricultural activity is risky, they do not like to deal with agriculture and there are more educational opportunities in sectors other than agriculture.

Table 9. Reasons for Young People's Unwillingness to Continue Agricultural Activities in the Inspected Farms

Reasons	**Average***	**Standard Deviation**
Insufficient income of agriculture	1,200	0,421
The risk of agricultural activity	1,200	0,421
Dislike of farming	1,300	0,674
More education opportunities in sectors other than agriculture	1,400	0,699
Lack of knowledge and skills in agricultural matters	1,900	0,994
Better living standards in sectors other than agriculture	2,000	0,816
Low government support for agriculture	2,100	1,370
Difficulties in obtaining/obtaining a loan	3,200	1,229

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

In the study, the goals and objectives of the farmers for the future were also examined according to the Likert scale and the findings are given in Table 10. Among the future goals and objectives of the farmers, the most important ones, respectively, are to obtain sufficient income, to obtain high-quality products, to produce products of the quality that the market demands, to produce the amount desired by the market, to increase the income, to improve the standard of living, to reduce the risks, and to protect the standard of living.

Table 10. Future Goals and Objectives of the Farmers in the Inspected Farms

Future Goals and Objectives	**Average***	**Standard Deviation**
Earn enough income	1,000	0,000
Obtaining high quality products	1,020	0,141
Producing products of the quality demanded by the market	1,020	0,141
Improving our standard of living	1,040	0,197
Increase my income	1,040	0,197
Producing the amount of product the market wants	1,040	0,197
Reducing risks	1,060	0,313
Maintaining my standard of living	1,060	0,239
Investing in the business	1,080	0,444
To be able to sell the product at the highest price in the market	1,080	0,444
Grow the business	1,120	0,479
Saving money for retirement	1,140	0,571
Having no problems in marketing products	1,160	0,467
Protecting the environment while producing	1,200	0,404
Saving money for children's education	1,200	0,699
Being open to innovations	1,660	0,847
Reducing my workload	1,760	0,846
Enabling children to continue farming in the future	1,860	1,106
Recognition among other farmers	1,880	1,081
Making time for activities outside of work	2,160	0,888
No problems with storage	2,240	0,822
Having no problems with product transport	2,380	0,805
Having no problems with packaging	2,660	0,592
Being a member of an agricultural cooperative	3,120	0,961
To be able to produce on contract	3,880	0,824

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

Information Resources

Information sources related to agricultural activities in the farms within the scope of the research were also examined (Table 11). In case the operators encounter a problem related to agricultural activities, 62% of them receive support from agricultural input dealers. In the surveyed farms, 42% of the farmers stated that they met with experts once every three months and 32% once a month. According to 54% of the farmers surveyed in the research area, the pioneers in bringing innovations are agricultural engineer/extension staff. Television (80%) ranks first among the communication tools used in agricultural matters in farms.

Table 11. Information Sources in the Surveyed Farms

	Person	**%**
People Who Benefit from their Knowledge to Cope with Problems*		
Myself	27	54,00
Other Farmers	23	46,00
Agriculture Provincial/District Directorate Staff	12	24,00
Agricultural Engineer/veterinarian	13	26,00
Dealer	31	62,00
Frequency of Meeting with Experts		
Once a week	1	2
Every fifteen days	1	2
Once in a month	16	32
Quarterly	21	42
Twice a year	7	14
I'm not meeting	4	8
Total	***50***	***100***
Pioneers in Bringing Innovations*		
Agricultural Engineer / Extension Worker	27	54
Agriculture Provincial/District Directorate Staff	10	20
Dealer	23	46
Communication Tools Used Regarding Agriculture*		
TV	40	80
Internet	7	14
Brochures	13	26
Magazine/Newspaper	3	6

* More than one answer was received.

Agricultural Credit Utilization in the Investigated Farms

The issues related to the use of credit in the farms within the scope of the research are examined in this section. Credit is not used in 20% of the examined farms (Table 12). On the other hand, it has been determined that credit has been used for 6 years on average per farm in farms using credit. It is seen that 47,50% of the farms that use loans obtain loans from Ziraat Bank (Table 13).

Chart 12. Credit Utilization in the Examined Farms

Credit Availability	**Person**	**%**
Using credit	40	80
Not using credit	10	20
Total	***50***	***100***

Table 13. Agricultural Credit Resources in the Investigated Farms (N=40)

Credit Resources	**Person**	**%**
Ziraat Bank	19	47,50
People	2	5,00
Relatives	16	40,00
Private Banks	6	15,00

* More than one answer was received.

Within the scope of the research, the purposes of credit usage in the farms that have used credit in the last five years are given in Table 14. It has been determined that the purpose of credit use in all farms is to complete the working capital deficiency and to provide agricultural inputs, while 97,5% of them use credit for family needs.

Table 14. Agricultural Credit Usage Purposes in the Investigated Farms in the Last Five Years (N=40)

	Person	**%**
To make up for the working capital shortage	40	100,0
For the supply of agricultural inputs (chemicals, fertilizers, feed, seeds, etc.)	40	100,0
Land acquisition	4	10,0
Machinery investment	22	55,0
Building investment	2	5,0
Irrigation investment	13	32,5
Animal purchase	2	5,0
For family needs	39	97,5

* More than one answer was received.

The issues that the farmers using credit in the examined farms pay attention to in the use of agricultural credits are evaluated in Table 15. According to the results obtained from the survey data applied in the research, the farmers stated that the interest rate, maturity structure, the status of the collateral, the time of lending and the transactions in the loan process are very important in the use of agricultural loans and they stated that they pay attention to these the most.

Table 15. Considerations for Agricultural Credit Usage in the Inspected Farms (N=40)

	Average*	**Standard Deviation**	**%**					
			1	**2**	**3**	**4**	**5**	**Total**
To the interest rate	1,000	0,000	100,0	-	-	-	-	100,0
To maturity structure	1,000	0,000	100,0	-	-	-	-	100,0
Status of guarantees	1,000	0,000	100,0	-	-	-	-	100,0
Time of loan	1,025	0,158	97,5	2,5	-	-	-	100,0
Transactions in the loan process	1,200	0,563	87,5	5,0	7,5	-	-	100,0
Family friend etc. to his advice	2,225	1,120	42,5	2,5	45,0	10,0	-	100,0

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

In Table 16, the factors affecting the choice of bank while the farmers use agricultural loans are examined. According to the farmers, all of the factors affecting the choice of bank were expressed as very important. These factors are interest rates, maturity status, the attitude of the bank in case of difficulties, ease of obtaining loans, the cost of the loan, the consultancy service offered by the bank, the size of the bank, and its expertise in the sector. The results obtained here show that the operators are very careful and insecure in obtaining credit.

Table 16. Factors Influencing Bank Selection in Agricultural Credit Utilization in the Investigated Farms

	Average*	Standard Deviation	%					
			1	**2**	**3**	**4**	**5**	**Total**
Interest rates	1,000	0,000	100	-	-	-	-	100
Maturity conditions	1,000	0,000	100	-	-	-	-	100
The bank's attitude in case of difficulty	1,000	0,000	100	-	-	-	-	100
Ease of obtaining loans	1,000	0,000	100	-	-	-	-	100
Cost of the loan	1,080	0,276	92	8	-	-	-	100
Consulting service offered by the bank	1,080	0,276	92	8	-	-	-	100
Industry expertise	1,080	0,276	92	8	-	-	-	100
The size of the bank	1,160	0,553	92	-	8	-	-	100

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

47,50% of the farmers using loans in the research obtained loans from Ziraat Bank. Ziraat Bank ranks first in the use of agricultural loans in Turkey. Table 17 includes the evaluations of the farmers using loans from Ziraat Bank. All of the farms using loans from Ziraat Bank used the loan they received in agricultural production. The rate of farms who cannot pay their loan debts on time is 21,05%. All of the farms using loans from Ziraat Bank stated that they were satisfied with the bank employees. All of the farmers agree that subsidized farmer loans are also given by other banks.

The reasons why the operators using loans from Ziraat Bank within the scope of the research prefer this bank are given in Table 18. The most important reason for choosing Ziraat Bank by the operators is that it is the only bank that provides subsidized loans to the farmer, the feeling of trust in the bank and the appropriate interest rates.

Within the scope of the research, 15% (6 farms) of the farmers using loans used loans from banks other than Ziraat Bank, and 5 of them could not pay their loan on time. The payment methods of the farms that cannot pay their debts on due date are given in Table 19. All of the farmers who could not pay their debts, gold etc. by selling its assets, 60% by borrowing from individuals.

Table 17. Evaluations of Credit Utilization in Enterprises Using Credits from Ziraat Bank for Agricultural Purposes

	Yes		No		Total	
	Person	**%**	**Person**	**%**	**Person**	**%**
1. Do you pay your debt to another bank with a subsidized loan from Ziraat Bank?	-	-	19	100,00	19	100,00
2. Do you use the loan you received in your non-agricultural activities?	-	-	19	100,00	19	100,00
3. Are you taking out the loan on behalf of someone else?	-		19	100,00	19	100,00
4. Have you ever experienced any foreclosure on the loans you have received from banks?	2	10,53	17	89,47	19	100,00
5. Have you ever been unable to pay your loan debt on time?	4	21,05	15	78,95	19	100,00

6. Do you pay your loan debt to Ziraat Bank with the loan you use from other banks?	2	10,53	17	89,47	19	100,00
7. Do you usually pay off your debts with another debt?	1	5,26	18	94,74	19	100,00
8. Can you say that you have paid without any difficulties while closing your credit accounts?	15	78,95	4	21,05	19	100,00
9. Do you use Ziraat Bank loan from Ziraat Bank and other consumer loans from different banks?	8	42,11	11	57,89	19	100,00
10. Are you satisfied with Ziraat Bank employees?	19	100,00	-	-	19	100,00
11. Is the agricultural lending process slow at Ziraat Bank?	11	57,89	8	42,11	19	100,00
12. Do you think it is correct that subsidized farmer loans are also given by other banks?	19	100,00	-	-	19	100,00
13. Should subsidies be made to farmers in other matters such as vehicle and housing loans?	18	94,74	1	5,26	19	100,00
14. Does the low value of the title deed at the bank affect your loan demand negatively?	8	42,11	11	57,89	19	100,00
15. Do you think that you sign contracts for bank loans too much?	19	100,00	-	-	19	100,00
16. Are there any agricultural enterprises in your area that went bankrupt by using agricultural loans from banks?	12	63,16	7	36,84	19	100,00
17. Do you have any information about the resources that public and other financial institutions offer to farmers?	7	36,84	12	63,16	19	100,00
18. Are the advertisements offered by Ziraat Bank to the farmers sufficient?	17	89,47	2	10,53	19	100,00
19. Are the advertisements offered to farmers by other banks sufficient?	2	10,53	17	89,47	19	100,00

Table 18. Reasons for Preferring Ziraat Bank in the Inspected Farms (N=19)

	Average*	**Standard Deviation**	**%**					
			1	**2**	**3**	**4**	**5**	**Total**
Being the only bank that provides subsidized (discounted) loans to the farmer	1,000	0,000	100,00	-	-	-	-	100,00
Sense of trust in the bank	1,052	0,229	94,74	5,26	-	-	-	100,00
Having good relations with the bank	1,526	0,841	68,42	10,53	21,05	-	-	100,00
Appropriate interest rates	1,263	0,653	84,21	5,26	10,53	-	-	100,00
Because family members want	3,421	1,017	10,53	5,26	15,79	68,42	-	100,00

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

Table 19. Payment Methods of the Farmers Who Could Not Pay the Agricultural Loans Taken from the Banks in the Inspected Farms within the Due Date (N=5)

	Person	**%**
By selling tools, machinery or animals	2	40
Using a loan from another bank or credit institution	1	20
Friends, relatives, tradesmen, moneylenders, etc. taking money from	3	60
Gold etc. selling my assets	5	100

* More than one answer has been received.

Within the scope of the research, the reasons for the farmers to borrow from individuals were examined and the data obtained are given in Table 20. All of the farmers borrowing from the individual preferred because there is no mortgage and they can adjust the maturity as they wish.

Table 20. Reasons for Borrowing from a Person (Lender, Tradesman, Merchant or Friend) in the Inspected Farms (N=18)

	Average*	**Standard Deviation**	**%** 1	2	3	4	5	**Total**
No mortgage	1,000	0,000	100,00	-	-	-	-	100,00
Ability to set the maturity as desired	1,000	0,000	100,00	-	-	-	-	100,00
To be familiar	1,136	0,639	94,44	-	-	5,56	-	100,00
Faster debt collection	1,136	0,639	94,44	-	-	5,56	-	100,00
Low interest rate	1,227	0,751	88,89		5,56	5,56	-	100,00
No official actions	1,318	0,838	88,89	-	5,56	5,56	-	100,00
Banks' lack of confidence	2,863	1,424	38,89	-	22,22	27,78	11,11	100,00

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

All of the farmers who borrowed from the individual stated that they paid interest below the bank interest rate. 2 of the 18 farmers who borrowed from individuals could not pay their debts on time. Payment was made in these businesses by selling tools and machinery and borrowing from other persons. In the farms examined within the scope of the research, it has been tried to determine whether there are farms that borrow from the individual and go bankrupt. It has been determined on the basis of declaration that approximately 33% (6 businesses) of borrowers from individuals in the examined businesses are bankrupt.

CONCLUSION

One of the important problems of the agricultural sector in Turkey and one of the areas in need of solution is financing. Due to the unique structure of the agricultural sector, agricultural financing affects sustainability in agriculture. In the farms included in the research, 36% of the farmers are primary school graduates. Farmers have an average of 26 years of experience. In the surveyed farms, 67% of the farmers are between the ages of 15-49, and 30% are aged 50 and over. 24% of farmers stated that they could quit farming if a fixed fee was paid. In the farms examined in the research, farmers are engaged in agriculture in order to have lands for agricultural activities, to be the boss of their own business and to make profit. 18% of young people in farms do not want to deal with agriculture. The reasons for this are; the risk of agricultural activities, the inability to provide sufficient income from the income obtained from agriculture, the high educational opportunities in sectors other than agriculture, the good standard of living and their dislike of dealing with agriculture. In the farms included in the research, 80% of the farmers use loans.

According to the results obtained from the research, 47% of the farmers prefer Ziraat Bank. The most important reasons for choosing Ziraat Bank are; being the only bank that provides subsidized loans to the farmer, the feeling of trust in the bank and the appropriate interest rates are listed as. Issues that farmers pay attention to in the use of agricultural credit; interest rate, maturity structure and collateral status.

The results obtained from the research, it has revealed the necessity of encouraging young people for the continuity of agricultural production activities and giving importance to extension and policy studies for this purpose. In order to solve the problems faced by the farmers on various issues in agricultural activities; effective use of government support, taking measures against uncertainty in product prices and providing support against natural disasters are stated as the most important factors that will help farmers to continue their agricultural activities.

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RISK AND SUSTAINABILITY IN OVINE LIVESTOCK ACTIVITY: ANTALYA PROVINCE CASE

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ABSTRACT

In this research, it is aimed to determine the risk sources and the risk management strategies they apply in agricultural production of the producers engaged in ovine breeding in Antalya. In addition, in the study, the future thoughts of producers in ovine breeding and whether they will continue their activities were examined in terms of sustainability. The research area is the Döşemealtı district of Antalya province, and the data obtained from the questionnaires applied to 47 ovine breeders were used in the evaluations. In the study, the data obtained from the questionnaire application were evaluated in the SPSS package program and summarized in the tables. Factor analysis was applied to the risk sources and risk management strategies encountered in ovine breeding activities, reducing the number of variables and grouping them under meaningful headings. According to the results obtained from the research, the most important sources of risk in ovine breeding are the changes in the policies implemented and the changes in the country's economy. In addition, the difficulties encountered in finding foreign labor in the examined enterprises and the changes in the presence of animals were determined as risk sources that are not considered very important. The risk management strategies that the producers stated as important within the scope of the research are financial management tools such as saving and finding capital resources.

Keywords: Ovine breeding, Risk sources, Risk management, Sustainability, Antalya.

INTRODUCTION

Considering the geographical structure and large pastures of Turkey, it is seen that it has an important potential for low cost and quality livestock. Ovine breeding is an important sector in terms of adapting to lands where agricultural production is unproductive in a short time and transforming these areas into products such as meat, milk, fleece, hair, mohair and leather. Sheep and goat breeding, in particular, is the cheapest animal husbandry that can be done in the country. Sheep is an animal that can make the most of the pasture, make the best use of the pasture, and use the pasture at any time of the year. In addition, the vast majority of pastures in the country are low-yielding and more suitable for small cattle breeding (Aksoy and Yavuz, 2012).

Although Turkey is one of the leading countries in the world in terms of sheep and goats, it is far from meeting expectations in terms of yield per animal. Ovine breeding in Turkey; it has an extensive structure whose population is mostly composed of low-yielding domestic breeds, feeding conditions are mainly based on grazing and production is targeted with limited input. These features of the sector; it can be added that the enterprises have a small and weak structure, the lack of input supply, product marketing and evaluation opportunities, accordingly the producer's low share of the market price, the production being mostly subsistence (Sahinler and Demir, 2016).

Sustainable animal husbandry can be expressed as preserving the resources necessary for the existence of animal husbandry and transferring them to future generations, and trying to ensure the continuity of animal supply and demand. Sustainable development has three dimensions: economic, social and environmental. Environmental sustainability; business activities are sensitive to environmental impacts. Social sustainability; it is the responsibility of the company to its employees, society and all its stakeholders. On the other hand, economic sustainability can be explained as the effects of the activities of the enterprise on the national and international economy (Ozalp and Sayin, 2018).

It is a priority for small cattle breeding enterprises to have sufficient working capital in order to continue production and develop themselves in the future. The reasons such as the small scale of most of the small cattle breeding enterprises, the low profitability, the structure against the breeders in market conditions and the inadequacy of the breeders in the organization constitute a structure that prevents the changes that can be made in the development of the enterprises and the production systems. The importance of the livestock sector in terms of creating permanent employment and providing raw materials to agriculture-based industries is undeniable. However, those living in rural areas, mainly young people, and especially those dealing with small cattle breeding have started to show no interest in this field of activity. This trend is one of the most important problems for the future of sheep and goat breeders and the sector (Cedden et al., 2020).

There are many studies on sheep and goat breeding in Turkey, some of which are; Gursoy (2009), Sahinli (2011), Aksoy and Yavuz (2012), Karakus and Akkol (2013), Tufekci and Olfaz (2015), Semerci and Celik (2016), Sahinler and Demir (2016), Yilmaz (2016), Turkan (2017), Element (2018), Ozalp and Sayin (2018), Bakir and Mikail (2019), Simsek (2019), Yilmaz (2019), Tasdemir (2019), Acibuca (2020), Satar (2021). Küçükbaş hayvancılık faaliyetinde risk yönetimi konusunda ülkemizde benzer çalışmaya rastlanmamıştır. In general, some of the national and international studies on risk management in livestock activities; Hazneci (2009), Gebreegziabher and Tadesse (2013), Nasir et al. (2014), Hayran (2015), Kizilay and Akcaoz (2016), Waithira (2017), Bishu et al. (2018), Ozsayin (2019), Gokgoz and Kayahan (2021).

In this research, it is aimed to determine the risk sources and the risk management strategies they apply in agricultural production of the producers engaged in ovine breeding in Antalya. In addition, in the study, the future thoughts of producers in small cattle breeding and whether they will continue their activities were examined in terms of sustainability.

MATERIAL AND METHOD

The material of the study consisted of data compiled from primary and secondary sources. In terms of representing the province of Antalya, the data collected from 47 agricultural enterprises in the district of Dosemealti by questionnaire is the primary source of the study. The survey application covered the production period of 2015. By going to the district of Dosemealti, where the research will be conducted, the questionnaire forms, which were prepared in accordance with the purpose of the research, were filled in by mutual interviews with the agricultural business owners. The data obtained by the survey application were evaluated using the SPSS package program, and the results were summarized in the tables. The secondary sources of the research are similar studies conducted in the national and international arena and institutions and organizations related to the subject.

Factor analysis, which is among the multivariate analysis techniques, will also be used in the study. Factor analysis; it aims to find the factors between the observed variables. If there are too many variables, this analysis is applied to reduce the number of variables and to interpret them easily.

Factor analysis can be summarized in three stages;

1. A correlation matrix is created for all variables.
2. The factors are extracted from the correlation matrix based on the correlation coefficients of the variables.
3. Factors are rotated to maximize the relationship between some of the factors and the variables.

Model (Ozdamar, 2010);

- $X_1-M_1=L_{11}F_1+L_{12}F_2+.................L_{1k}F_k+ \varepsilon_1$
- $X_2-M_2= L_{21}F_1+L_{22}F_2+.................L_{2k}F_k+ \varepsilon_2$
- $X_P-M_P= L_{P1}F_1+L_{P1}F_2+................L_{Pk}F_k+ \varepsilon_P$

Lij: Coefficient of factors (factor load); i: Variable; j: Specifies the load (weight) of the variable on the factor.

- ✓ The new variables derived in the analysis are called **factors**.
- ✓ **Analysis;** It aims to reveal the random factors reflecting the classification, which are observed and not observed from the variable in the data matrix with correlations between them, but emerge with the combination of the variables.
- ✓ The areas where it is used; they are mostly attitude and behavior measurements in fields such as social sciences, psychology, sociology, educational sciences, medicine.
- ✓ In this study, factor analysis will be used to reduce the number of variables expressed as risk sources and risk management strategy in agricultural production and to make them easier to understand.

Reliability measurement was made for the variables used in factor analysis in the research. Reliability refers to the fit of a measurement. It is a measurement that should be considered especially in studies where behavioral measurements are made. It is not desirable for the reliability value found in behavioral studies to fall below 80%. Cronbach's alpha coefficient, which is among the internal fit measurement methods, is the most widely used reliability coefficient. This coefficient enables the series of variables to be measured in one dimension. Cronbach alpha is low when the data has a multidimensional structure. Cronbach's alpha is not a statistical test, it is a reliability or fit coefficient. The Cronbach alpha coefficient can be interpreted as the correlation coefficient. This coefficient takes values between 0 and 1. Negative values indicate that there is no positive relationship between the factors. In such a case, the reliability model is invalid. If the coefficient value is close to 1, the model is quite reliable. On the other hand, as the coefficient value approaches 0, reliability decreases (Akcaoz, 2001).

RESEARCH FINDINGS

Demographic features

The demographic characteristics of the farms surveyed in the research area were examined and the data obtained are given in Table 1. The average experience period of the farmers in the farms within the scope of the research is 23,96 years, the minimum experience period is 7 years, and the maximum experience period is 50 years. The average family size in the examined farms was found to be 3,38 people. The number of person in the farms is minimum 2 people, maximum 7 people and the total number of family member is 159 people. It has been determined that 51,57% of the population in farms is female and 48,43% is male population. The educational status of the population in the examined farms was also evaluated. According to the results obtained, 52,83% of the population is primary school graduate, 17,61% is high school graduate, 10,69% is secondary school graduate, and 14,47% is university and college graduate. In the farms included in the research, 59,11% of the family population works in their own farms. While there is no population working in agricultural jobs other than their own farms, the rate of those not working in non-agricultural jobs is 98.74%.

Table 1. Demographic Characteristics of the Investigated Farms

	Person	**%**
Distribution of the population by sex		
Female	82	51,57
Male	77	48,43
Total	***159***	***100,00***
Distribution of farmers by gender		
Female	3	6,38
Male	44	93,62
Total	***47***	***100,00***
Educational status of the farmer		
Primary school graduate	34	72,34
Secondary School Graduate	7	14,89
High school graduate	6	12,77
Total	***47***	***100,00***
Educational status of the population*		
Literate	4	2,56
Primary school graduate	84	52,85
Secondary School Graduate	17	10,90
High school graduate	28	17,95
College/University Graduate	23	14,74
Total	***156***	***100,00***
Employment status of the family population in the farm		
Working	94	59,12
Not working	65	40,88
Total	***159***	***100,00***
Employment status of the family population in non-agricultural jobs		
Working	2	1,26
Not working	157	98,74
Total	***159***	***100,00***
Average family size (person)	***3,38***	
Average experience of the farmer (years)	***23,96***	

* 3 people are not yet of school age.

In 47 farms within the scope of the research, the total farm width was found to be 844 decares and the average enterprise width was found to be 17,96 decares. In the farms examined in the study, data on parcel width, parcel number, ownership status, topographic condition of the lands, soil structure, soil fertility and land type were obtained and presented in Table 2. Soil fertility in farms is classified as high, medium, low and very low. It has been stated that all of the farm lands are of medium productivity.

Table 2. Land Characteristics of the Investigated Farms

	Total Area (da)	%
Ownership status		
Property	578	68,48
Rent	255	30,21
Partner	11	1,31
Total	***844***	***100,00***
Soil structure		
Rocky	20	2,37
Base	824	97,63
Total	***844***	***100,00***
Land type		
Irrigated Field	466	55,21
Dry Field	378	48,79
Total	***844***	***100,00***
Product pattern in businesses		
Barley	293	34,71
Wheat	516	61,14
Clover	5	0,59
Oat	5	0,59
Olive	25	2,97
Total	***844***	***100,00***
Average number of parcels (unit/enterprise)		***1,64***
Average parcel width (da/business)		***10,96***

Ovine breeding is carried out in the pasture in the examined farms. The total number of sheep and goats in the farms surveyed in the research was 11.624, with 13,44% sheep, 2,58% rams and 83,98% goats (Table 3).

Table 3. The Presence of Ovine Animals in the Farms

	Number	**%**
Sheep	1.562	13,44
Ram	300	2,58
Goat	9.762	83,98
Total	***11.624***	***100,00***

Risk Sources and Risk Management Strategies

Sources of Risk

There are many risk factors affecting agriculture. Therefore, those who deal with agricultural production often face these risk factors, no matter how much experience they have in agriculture. In agriculture, businesses should try to prevent risks as much as possible or try to minimize their effects in order to continue their activities successfully. It is important to know the risk sources encountered in agriculture and to take various precautions against them.

In this study, the sources of risk faced by the farmers in the district of Döşemealtı in Antalya province were tried to be revealed based on the data obtained from 47 farmers interviewed in the region. In the study, the sources of risk faced by farmers are grouped under 59 titles. A 5-point Likert scale was used to determine the extent to which these risk sources affect agricultural production. Since there are no similar studies on ovine breeding in the studied region and Turkey, it was not possible to analyze the results obtained for the region comparatively. The most important risk factors in the farms examined within the scope of the research are the changes in the policies implemented by the government and the changes in the economic situation of the country (Table 4).

Table 4. Sources of Risk in the Investigated Farms

Risk Sources	Average*	Standard Deviation	%					
			1	2	3	4	5	Total
Changes in government policies	1,3830	0,60982	68,1	25,5	6,4	-	-	100,0
Changes in the country's economic situation	1,4468	0,80240	72,3	12,8	12,8	2,1	-	100,0
Production cost changes of animal products	1,9362	1,27525	48,9	34,0	-	8,5	8,5	100,0
Instability in the price of meat	2,1702	1,71082	59,6	14,9	-	-	25,5	100,0
Indebtedness status	1,7660	0,72869	38,3	48,9	10,6	2,1	-	100,0
Transmission of epidemic diseases	2,3191	1,14410	27,7	29,8	34,0	-	8,5	100,0
Changes in interest rates	2,7021	1,38179	29,8	17,0	12,8	34,0	6,4	100,0
Changes in climatic conditions	1,8298	0,81612	36,2	51,1	6,4	6,4	-	100,0
Instability in milk price	2,8085	1,39312	21,3	29,8	8,5	27,7	12,8	100,0
Health status of the farmer family	2,3191	1,32051	25,5	51,1	4,3	4,3	14,9	100,0
Problems in animal nutrition	1,6383	1,13109	61,7	29,8	-	-	8,5	100,0
Low productivity due to animal diseases	1,6383	1,13109	61,7	29,8	-	-	8,5	100,0
Health/injury/death of owner/worker	1,5532	1,19434	76,6	8,5	6,4	-	8,5	100,0
Worker accidents in the farm	1,8511	1,50331	68,1	12,8	2,1	-	17,0	100,0
Problems/disputes in family relationships	2,2979	1,53105	44,7	15,5	14,9	-	14,9	100,0
Changes in animal husbandry policies	2,0851	0,88046	27,7	42,6	23,4	6,4	-	100,0
Changes in milk yield	3,3191	1,56186	12,8	31,9	4,3	12,8	38,3	100,0
Insufficient family workforce	2,6383	1,38980	29,8	17,0	25,5	14,9	17,8	100,0
Difficulties in finding foreign labor	3,7872	1,35030	12,8	-	25,5	19,1	42,6	100,0
Lack of government support	3,0426	1,60105	25,5	19,1	8,5	19,1	27,7	100,0
Insufficient tools/machines for livestock	2,7234	1,19241	25,5	8,5	36,2	27,7	2,1	100,0
Theft	2,7234	1,48474	34,0	12,8	10,6	31,9	10,6	100,0
Insufficient technological development	2,7021	1,14046	21,3	17,0	34,0	25,5	2,1	100,0
Changes in animal presence	3,5957	1,27960	8,5	10,6	25,5	23,4	31,9	100,0
Increase in animal accidents	2,9149	1,28249	17,0	17,0	40,4	8,5	17,0	100,0
Failure to keep farm records	2,8085	1,19124	14,9	27,7	27,7	21,3	8,5	100,0
Lack/absence of artificial insemination	2,7234	1,31384	27,7	14,9	19,1	34,0	4,3	100,0
Adverse events at birth	2,4894	1,26615	29,8	19,1	31,9	10,6	8,5	100,0
Instability in feed price	2,8936	1,30607	19,1	23,4	14,9	34,0	8,5	100,0
Distance to point of sale	2,4255	1,17482	31,9	17,0	27,7	23,4	-	100,0
Insufficient farm capital	2,2553	0,98837	21,3	48,9	12,8	17,0	-	100,0
Lack of funding	2,4043	0,97042	10,6	61,7	4,3	23,4	-	100,0
Disease transmission during milking	2,7021	1,55919	36,2	17,0	-	34,0	12,8	100,0
Non-compliance with hygiene rules	3,0638	1,59352	23,4	23,4	4,3	21,3	27,7	100,0
Animals are not insured	2,7021	1,38179	31,9	12,8	12,8	38,3	4,3	100,0
Changes in the prices of herbal products	3,3404	1,37171	19,1	4,3	19,1	38,3	19,1	100,0
Changes in crop yields	3,2979	1,44335	19,1	8,5	21,3	25,5	25,5	100,0
Frost event	2,8936	1,06799	6,4	38,3	19,1	31,9	4,3	100,0
Too much precipitation	2,7660	0,98274	6,4	36,2	38,3	12,8	6,4	100,0
Insufficient rainfall	2,6170	0,79545	6,4	38,3	42,6	12,8	-	100,0
Low yield due to plant diseases	2,7021	0,80528	4,3	38,3	40,4	17,0	-	100,0
Low yield due to plant pests	2,4043	0,61360	4,3	53,2	40,4	2,1	-	100,0
Changes in land prices	2,4894	1,06061	21,3	27,7	34,0	14,9	2,1	100,0
No contract manufacturing	3,0851	1,05973	8,5	17,0	40,4	25,5	8,5	100,0
Insufficient agricultural tools and machinery	2,9362	1,13068	10,6	23,4	38,3	17,0	10,6	100,0
Damage due to fire	3,0000	1,28537	12,8	27,7	21,3	23,4	14,9	100,0
Damage to the product due to flooding	2,6170	1,26066	29,8	10,6	31,9	23,4	4,3	100,0
Landslide	2,7660	1,47755	31,9	14,9	8,5	34,0	10,6	100,0
Failure to carry out accounting transactions	2,4468	1,15737	31,9	12,8	34,0	21,3	-	100,0
Changes in sales periods of animal products	1,8085	0,82458	44,7	29,8	25,5	-	-	100,0
Inflation	1,5106	0,68754	59,6	29,8	10,6	-	-	100,0
Capacity/availability of credit resources	2,3830	0,94531	14,9	48,9	19,1	17,0	-	100,0
Debt structure (maturity, source)	2,0851	0,90481	29,8	38,3	25,5	6,4	-	100,0

Non-equity financing	1,8723	0,76944	36,2	40,4	23,4	-	-	100,0
Increase in drug and veterinary fees	1,7234	1,05711	63,8	8,5	19,1	8,5	-	100,0
Barn etc. insufficiency	1,6809	1,23546	63,8	25,5	-	-	10,6	100,0
No division of labor	2,7021	1,62741	29,8	31,9	4,3	6,4	27,7	100,0
Non-epidemic diseases	2,9362	1,14975	10,6	31,9	14,9	38,3	4,3	100,0
Difficulties in getting to the vet	2,6809	1,32051	19,1	31,9	27,7	4,3	17,0	100,0

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

Factor analysis was applied in order to collect the factors that emerged as risk factors for ovine livestock activities in the Dosemealti district of Antalya province under the main headings. The reliability of the data obtained before applying the factor analysis was calculated and the Cronbach alpha value was found to be 0,886 (Table 5). Having this value close to 1 is important for the reliability of the test. It states that there is a positive relationship between the factors.

The factor weights obtained as a result of factor analysis are given in Table 5. Considering the factor weights, it was predicted that 6 factors would be sufficient according to the purpose of the research. As a result of factor analysis, 6 factors explain 70,19% of the variance. While naming the factors, variables with factor weights above 0,40 were taken into account. Factor 1, changes in the production cost of animal products (0,443), the transmission of epidemic diseases (0,716), the health status of the farmer family (0,739), low productivity due to problems in animal nutrition (0,641), illness/injury/death status of the owner/employees (0,682)), insufficiency of family workforce (0,734), theft (0,881), increase in animal accidents (0,816), negativities at birth/low birth rate (0,837), instability in feed price (0,431), insufficiency in farm capital (0,654), lack of financing (0,670), the increase in drug and veterinary fees (0,765), barns etc. inadequacy (0,684), lack of division of labor (0,653), non-epidemic diseases (0,719), difficulties in reaching the veterinarian (0,697) were positively related and named as ***"personal risk and production risk"***. Factor 2 is expressed as ***"financing and environmental risks".*** Factor 2, meat price instability (0,524), indebtedness (0,421), changes in interest rates (0,659), lack of government support (0,521), shortage of tools and machinery for livestock (0,799), insufficient technological development (0.804), changes in animal availability (0,581), failure to keep business records (0,637), lack of insurance on animals (0,807), damage to crops due to floods (0,727), landslides (0,792), lack of accounting (0,603), capacity/availability of credit resources (0,535) and self-employment with capital-free financing (0,675) variables were positively correlated.

Factor 3 was determined as ***"marketing risk"*** as it was positively related to meat price instability, milk price instability, lack/absence of artificial insemination, distance to sales location and changes in land prices. Factor 4 has been named as ***"production cost risk"*** since it is associated in the same direction with changes in the production cost of animal products, changes in interest rates, instability in feed prices, disease transmission in milking and damage due to fire. Factor 5 has been named as ***"credit risk"*** since it is related in the same direction with farm capital insufficiency, financing insufficiency, inflation, capacity/availability of credit resources, debt structure (term, source) and non-equity financing variables. Factor 6 has been named ***"political risk".*** Factor 6, includes changes in the policies implemented by the government (general and agriculture), changes in the economic situation of the country, changes in the sales periods of animal products, changes in climatic conditions, distance from the sales place, lack of contracted production, inadequacy of agricultural tools and machinery, and changes in the sales periods of animal products, is positively related.

Table 5. Factor Analysis Results for Risk Sources in Investigated Farms

Variables	Factors					
	1	2	3	4	5	6
Changes in government policies	-,195	**-,400**	-,017	,094	,103	**,563**
Changes in the country's economic situation	,107	-,206	,062	-,042	-,059	**,866**
Production cost changes of animal products	**,443**	-,123	-,217	**,528**	-,219	,390
Instability in the price of meat	,294	**,524**	**,496**	,144	,099	-,044
Indebtedness status	,020	**,421**	,060	-,251	,289	,267
Transmission of epidemic diseases	**,716**	,274	-,381	,346	,104	-,197
Changes in interest rates	-,114	**,659**	,260	**,402**	,145	,287
Changes in climatic conditions	-,174	,185	,035	,105	,392	**,464**
Instability in milk price	-,001	,143	**,743**	-,149	-,219	,178
Health status of the farmer family	**,739**	-,081	-,197	-,209	-,224	-,226
Problems in animal nutrition	**,641**	-,160	**-,602**	,073	-,060	-,274
Low productivity due to animal diseases	**,769**	-,115	-,306	,306	-,097	-,247
Health/injury/death of owner/worker	**,682**	-,189	**-,533**	,193	,088	-,237
Worker accidents in the farm	,292	,017	**-,797**	-,090	-,131	-,281
Problems/disputes in family relationships	,251	,039	**-,836**	-,067	-,062	-,071
Changes in animal husbandry policies	,101	,125	**-,660**	,130	**-,488**	,105
Changes in milk yield	,350	-,008	,229	,380	**-,651**	,048
Insufficient family workforce	**,734**	-,157	,053	,236	-,016	,015
Difficulties in finding foreign labor	**-,618**	,357	,238	-,079	-,088	,128
Lack of government support	,078	**,521**	-,036	**-,574**	-,191	,346
Insufficient tools/machines for livestock	,033	**,799**	,245	-,077	-,027	,206
Theft	**,881**	,178	,144	,110	,203	-,036
Insufficient technological development	-,101	**,804**	,376	-,021	-,073	-,016
Changes in animal presence	,093	**,581**	,054	,200	**-,479**	-,065
Increase in animal accidents	**,816**	,032	-,209	-,280	-,135	,029
Failure to keep farm records	-,199	**,637**	-,014	-,318	,193	-,163
Lack/absence of artificial insemination	-,216	,010	**,753**	,304	,037	,091
Adverse events at birth	**,837**	-,215	,161	,188	-,089	,057
Instability in feed price	**,431**	,033	,237	**,673**	-,105	-,216
Distance to point of sale	,203	,246	**,665**	,068	,131	**,467**
Insufficient farm capital	**,654**	,128	,094	,047	**,550**	,131
Lack of funding	**,670**	,204	-,285	,064	**,492**	-,076
Disease transmission during milking	,255	,071	-,160	**,866**	,020	,103
Non-compliance with hygiene rules	,174	**,447**	**-,583**	,310	-,372	-,046
Animals are not insured	-,042	**,807**	-,155	,103	-,088	-,076
Changes in the prices of herbal products	**,607**	,212	,099	-,193	,091	,196
Changes in crop yields	,215	**,540**	,391	-,227	,040	,019
Frost event	,279	,345	**,598**	-,110	,050	-,191
Too much precipitation	**,447**	,251	-,017	**-,517**	,237	,040
Insufficient rainfall	,277	,345	-,104	**-,577**	,330	,152
Low yield due to plant diseases	-,061	**,428**	-,255	**-,562**	-,268	,142
Low yield due to plant pests	,063	,265	,283	-,019	-,148	-,100
Changes in land prices	-,189	-,015	**,702**	,222	,349	-,147
No contract manufacturing	**-,413**	,264	,296	-,124	-,025	**,422**
Insufficient agricultural tools and machinery	-,151	,047	,365	,060	,110	**,737**
Damage due to fire	**,458**	,233	-,057	**,454**	,032	,344
Damage to the product due to flooding	,171	**,727**	-,013	-,041	,055	,054
Landslide	-,023	**,792**	-,122	-,279	,259	,025
Failure to carry out accounting transactions	-,265	**,603**	-,140	,054	,214	,014
Changes in sales periods of animal products	-,106	,273	-,068	-,227	-,130	**,606**
Inflation	,102	,189	,187	-,056	**,831**	,089
Capacity/availability of credit resources	,082	**,535**	**,440**	-,123	**,526**	-,105
Debt structure (maturity, source)	,230	-,028	,250	,199	**,672**	-,005
Non-equity financing	,089	**,675**	,186	-,164	**,539**	-,246
Increase in drug and veterinary fees	**,765**	,122	-,104	,185	,153	**-,415**

Barn etc. insufficiency	**,684**	-,112	-,256	,244	,005	**-,445**
No division of labor	**,653**	,328	,004	,048	,177	-,375
Non-epidemic diseases	**,719**	,151	,163	-,114	-,073	,146
Difficulties in getting to the vet	**,697**	-,084	-,297	-,248	-,320	,311
Cumulative Variance	***19,399***	***33,627***	***46,366***	***54,503***	***62,397***	***70,199***
Eigen value	***13,161***	***10,176***	***6,361***	***4,473***	***4,161***	***3,086***
Reliability coefficient (Cronbach alpha)			***0,886***			

Risk Management Strategies

The methods applied to partially eliminate or reduce the risk factors in agricultural production are called risk strategies (Akcaoz, 2001). In this section, the farmer's views on the risk management strategies applied against the risk sources discussed in the previous section are examined (Table 6). According to the farmers, the most important risk management strategies in the farms surveyed in the research are to save and find capital resources.

Table 6. Risk Management Strategies in Investigated Farms

Risk Management Strategies	Average*	Standard Deviation	%					
			1	2	3	4	5	Total
To produce at the lowest possible cost	1,9787	1,35918	59,6	6,1	19,1	6,4	8,5	100,0
Employees are insured	3,5745	1,28104	12,8	2,1	27,7	29,8	27,7	100,0
To comply with hygiene rules	3,1277	1,26176	4,3	38,3	19,1	17,0	21,3	100,0
To have information about the market where the product will be sold	1,8298	1,14814	53,2	25,5	12,8	2,1	6,4	100,0
Planning spending	1,8298	1,23920	57,4	21,3	10,6	2,1	8,5	100,0
Investing outside the farm	2,5106	1,38112	40,4	2,1	29,8	21,3	6,4	100,0
Family members working outside the farm	2,6596	1,08901	17,0	25,5	36,2	17,0	4,3	100,0
Farmer working outside the business	3,1702	1,47912	23,4	10,6	10,6	36,2	19,1	100,0
Debt management by experts	2,2553	1,67420	59,6	4,3	6,4	10,6	19,1	100,0
Regulating overuse of available resources	2,1915	1,09620	19,1	66,0	-	6,4	8,5	100,0
Reducing borrowing	2,8298	0,73186	36,2	44,7	19,1	-	-	100,0
Animal life insurance	1,9149	1,05973	44,7	34,0	6,4	14,9	-	100,0
Fighting against diseases	2,7021	0,90686	14,9	14,9	55,3	14,9	-	100,0
Keeping farm records regularly	2,2128	1,15976	38,3	21,3	21,3	19,1	-	100,0
Contract manufacturing (marketing, production)	2,9574	1,45897	27,7	8,5	19,1	29,8	14,9	100,0
To diversify the farm (to include more than one type)	3,3617	1,35816	14,9	2,1	46,8	4,3	31,9	100,0
Making differentiation in the farm (involving more than one product)	3,4468	1,26506	10,6	2,1	51,1	4,3	31,9	100,0
Increasing your solvency	1,7872	0,65727	34,0	53,2	12,8	-	-	100,0
Lowering the family's standard of living	2,0213	1,05273	40,4	27,7	23,4	6,4	2,1	100,0
Spreading product sales over time	1,6809	0,88726	48,9	42,6	2,1	4,3	2,1	100,0
Being a member of the cooperative	1,5532	0,82905	59,6	29,8	-	8,5	2,1	100,0
Saving to money	1,2766	0,57868	78,7	14,9	6,4	-	-	100,0
Finding capital resources	1,3191	0,51526	70,2	27,7	2,1	-	-	100,0
Making financial analyzes	1,7021	0,6888	42,6	44,7	12,8	-	-	100,0
Selling capital assets	2,1915	1,15417	38,3	23,4	19,1	19,1	-	100,0
Using a consultant	2,3404	1,16613	38,3	6,4	38,3	17,0	-	100,0

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

Factor analysis was applied in order to gather the risk management strategies applied by the producers against risks in the ovine livestock farms within the scope of the research under the main headings. The Cronbach alpha value for risk management strategies was found to be 0,797. Having this value close to 1 is important for the reliability of the test. It states that there is a positive relationship between the factors. The factor weights obtained as a result of the factor analysis applied for risk management strategies are given in Table 7. Considering the factor weights, it was deemed appropriate to collect the variables under 6 factors. As a result of factor analysis, 6 factors explain 86,73% of the variance.

Factor 1 was named ***"off-farm income and financial management".*** Factor 1 investing outside the farm, family members working outside the farm, farmer working outside the farm, debt management by experts, animal life insurance, contract production (marketing, production), diversification in the business (including more than one type), differentiation in the farm (including more than one product), financial analysis, selling capital assets and using consultants were positively related to variables. Factor 2 is named as ***"cost management"*** because the variables of producing at the lowest possible cost, planning expenditures, working outside the farm, regulating the excessive use of existing resources, fighting against diseases, saving and finding capital resources are related in the same direction. Factor 3 was named ***"organization/ cooperativeness".*** Spreading product sales over time, being a member of a cooperative, making savings and making financial analyzes are positively related to Factor 3. Factor 4 was named as ***"debt management"*** because the variables of reducing debt, lowering the family's living standard and finding capital resources are positively related.

Table 7. Factor Analysis Results for Risk Management Strategies in Investigated Farms

Variables	Factors					
	1	**2**	**3**	**4**	**5**	**6**
To produce at the lowest possible cost	**-,440**	**,775**	-,021	-,033	-,095	,325
Employees are insured	,370	**-,427**	-,216	,158	,030	**,735**
To comply with hygiene rules	**-,725**	,044	-,079	-,199	,248	**,493**
To have information about the market where the product will be sold	-,156	-,097	,100	,059	**,901**	-,009
Planning spending	-,068	**,943**	-,013	,097	,074	-,150
Investing outside the farm	**,861**	,170	,240	,182	,254	,007
Family members working outside the farm	**,820**	-,091	,323	-,094	-,060	,180
Farmer working outside the business	**,675**	**,568**	-,050	-,110	,012	-,284
Debt management by experts	**,603**	-,080	,375	**-,518**	-,184	-,087
Regulating overuse of available resources	-,056	**,890**	-,024	-,024	-,172	-,042
Reducing borrowing	-,044	,248	-,324	**,679**	,327	,198
Animal life insurance	**,640**	-,176	,359	**-,402**	-,120	,050
Fighting against diseases	,157	**,599**	,056	,069	**-,462**	**,542**
Keeping farm records regularly	,245	-,051	**-,442**	,362	**,693**	-,004
Contract manufacturing (marketing, production)	**,902**	-,146	-,025	,103	,139	-,233
To diversify the farm (to include more than one type)	**,854**	-,343	-,026	,105	-,275	,125
Making differentiation in the farm (involving more than one product)	**,801**	**-,479**	-,005	,023	-,074	,045
Increasing your solvency	,294	-,269	,304	,068	**,423**	**-,430**
Lowering the family's standard of living	,041	-,150	,199	**,926**	-,046	-,105
Spreading product sales over time	,131	-,030	**,921**	,114	,099	-,168
Being a member of the cooperative	,242	,377	**,768**	-,089	-,132	-,009
Saving to money	-,157	**,833**	**,425**	,140	-,025	-,012
Finding capital resources	,347	**,513**	,252	**,607**	,199	,132
Making financial analyzes	**,755**	,231	**,447**	,092	,243	,113
Selling capital assets	**,908**	-,057	,054	-,262	-,118	,095
Using a consultant	**,877**	,002	-,089	,128	,126	,096
Cumulative Variance	***31,829***	***50,735***	***61,606***	***71,398***	***80,139***	***86,731***
Eigen value	***8,789***	***5,116***	***3,365***	***2,442***	***1,577***	***1,262***
Reliability coefficient (Cronbach alpha)			***0,797***			

Factor 5 was named as ***"collecting market information and keeping records".*** This factor was found to be related to the variables of having information about the market where the product will be sold, keeping the business records regularly and increasing the solvency. Since the variables of having insurance of the employees, complying with the hygiene rules and fighting against the diseases with drugs are positively related to Factor 6, this factor was named as ***"following the health and hygiene rules".***

Sustainability of Agricultural Activity

In the survey study conducted in the research, according to the decision types, the people who are influential in making decisions about agricultural activities are given in Table 8. It is seen that the farmer is effective in all decisions in the examined farms. In the decisions of investment, savings, future and business growth, the farmer's spouse also actively participates in the decisions.

Table 8. Influential Persons in Decision-Making Related to Agricultural Activities in the Inspected Farms (%, N=47)

Decision Type	Farmer	Farmer's spouse	Children	Agricultural engineer	Agricultural extension worker	Family
Investment	100,00	76,59	-	-	2,13	
Saving	100,00	78,72	-		-	
Financing	100,00	44,68	-	27,66	8,51	4,25
The future	95,74	59,57	-	4,25	8,51	23,40
Growing the business	100,00	57,45	-	12,77	4,25	12,77
Marketing	100,00	10,64	2,13	6,38	29,79	2,13
Production pattern	100,00	19,15	-	23,40	-	-
Off-farm business	100,00	44,68	-	-	-	23,40

* More than one answer was received.

70,21% of the farms included in the research keep records. The ratio of farms registered to the farmer registration system is 46,81%. 42,55% of the surveyed farms have membership in an agricultural cooperative. 42,55% of the farmers stated that they can give up agricultural activity provided that a fixed wage is paid (Table 9).

Table 9. Registration and Membership Status in the Surveyed Farms

	Yes	%	No	%	Total	%
Do you keep records of your business?	33	70,21	14	29,79	47	100,0
Are you registered in the farmer registration system?	22	46,81	25	53,19	47	100,0
Are you a member of an agricultural cooperative?	20	42,55	27	57,45	47	100,0
If you are paid a fixed wage, would you quit farming?	20	42,55	27	57,45	47	100,0

The reasons for dealing with agricultural activities in the examined farms and the subjects of continuing agricultural activities are mentioned. In the study, the reasons for dealing with agricultural activities in the farms were determined using the Likert Scale and the results were summarized in Table 10. Farmers in the examined farms stated that making profit from agricultural production and the lack of good opportunities outside of agriculture as important reasons.

The factors that will help to continue agricultural activities in the farms within the scope of the research are shown in Table 11. Among the factors that will help the farmers to continue their ovine livestock activities in the examined farms are the improvement of infrastructure opportunities, guaranteeing product prices, providing support against natural disasters, and increasing credit opportunities.

Table 10. Reasons for Agricultural Production in the Inspected Farms

Reasons	Average*	Standard Deviation
Having land for agricultural activity	1,9149	0,97423
I enjoy farming	2,2340	0,98274
My family helping me with this job	1,8298	0,84233
Make a profit	1,6383	1,00921
Lack of good opportunities outside of agriculture	1,7872	0,95408
It's hard for me to do another job	1,8936	0,89038
It is very difficult to change jobs	2,2766	1,42497
Being my own boss	2,2766	1,09747

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

Table 11. Factors That Will Help Continuing Agricultural Activities in the Inspected Farms

	Average*	Standard Deviation
Making extension programs	2,1277	0,87519
Don't have too much debt	1,9787	1,05273
Finding a loan when needed	1,8936	0,81385
Having non-agricultural income	2,4894	1,19550
Have state support	2,0851	1,26542
No difficulties in recruiting workers	2,2128	1,31771
Agricultural engineers at dealers visit the plant	2,2128	1,02015
Visit of agricultural engineers in agricultural establishments	2,1702	1,02828
Family members working outside the farm	2,7447	1,43660
Making agricultural education programs for young people	2,2766	1,15537
Having childcare (home economics) services	2,3830	1,29469
Increasing/improving educational opportunities	1,8936	0,84014
Improvement of infrastructure facilities (road, communication, etc.)	1,6383	1,03052
Increasing non-agricultural job opportunities	1,9787	0,92052
Providing support against natural disasters (flood, hail, frost, etc.)	1,8085	0,94727
Providing additional employment opportunities for family members	1,8723	1,01332
Guaranteeing product prices	1,7447	1,01012
Increasing credit opportunities	1,8511	0,97755
Increasing the number of agricultural cooperatives	2,3191	1,36901
Have training in marketing	2,9149	1,41159
Expansion of production contract	2,8936	1,32261
To have information about product prices in advance	2,0426	1,19705
Having/increasing storage services	3,0426	1,31806
Having transport services	2,8936	1,20206
Information on packaging	4,2340	1,38647
Providing convenience in matters related to packaging supply	4,2553	1,39047

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

All of the farmers in the examined farms are considering quitting ovine breeding activities in the future. The reasons why the farmers do not want to continue their ovine breeding activities are given in Table 12. Among the reasons why the farmers surveyed within the scope of the research do not want to continue this activity, the most important ones are the low state support to agriculture and the insufficient income of agriculture.

In the central district of Van, farmers have come to the point of quitting their business due to the difficulties of finding a shepherd in ovine breeding farms, the inadequacy of veterinary services, the low demand of the young population for this branch of employment, and the rapid increase in migration to big cities (Yıldız ve Aygun, 2021).

Table 12. Reasons for Unwillingness to Continue Agricultural Activities in the Inspected Farms

Reasons	Average*	Standard Deviation
Low government support for agriculture	1,2340	0,69822
Insufficient income of agriculture	1,3191	0,69490
Lack of knowledge and skills in agricultural matters	2,4043	1,63733
Difficulties in obtaining a loan	2,5319	1,79190
The risk of agricultural activity	1,5745	1,03723
Dislike of farming	2,8723	1,39279
More education opportunities in sectors other than agriculture	1,6809	0,75488
Better living standards in sectors other than agriculture	1,7234	0,77184

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

In the study, the future goals and objectives of the farmers were examined and the data obtained are given in Table 13. Among the future goals and objectives of the farmers surveyed in the farms included in the research, it was found very important to reduce risks, increase income, and earn sufficient income.

In order to develop small cattle breeding in Sırnak, 99,22% of the producers stated that support policies for forage crops should be developed. In the study, 92,97% of the producers stated that the support for ovine breeding should be increased, 62,50% of them stated that reductions in input prices should be applied to the producers, and 50,00% of them stated that the constant changes in the policies for animal husbandry negatively affected the production Ögel (2018).

Table 13. Future Goals and Aims of the Producers in the Inspected Farms

Goals and Aims	Average*	Standard Deviation
Reduce the workload	1,5745	0,61661
Reducing risks	1,3191	0,51526
Recognition among other farmers	2,1915	1,19124
Being open to innovations	2,2340	0,91397
Making time for activities outside of work	3,0000	1,74456
Protecting the environment while producing	3,2979	1,36597
Ensuring that children continue to farm in the future	3,7021	1,24961
Maintaining my standard of living	2,5106	1,13965
Improving our standard of living	1,7447	0,64160
Raise income	1,3830	0,60982
Earn enough income	1,4681	0,50437
Investing in the business	2,0000	0,72232
Grow the business	1,8085	1,03500
Saving money for retirement	1,5957	0,90071
Obtaining high quality products	1,6170	1,29469
Saving money for children's education	2,1064	1,00508
Able to produce on contract	2,9574	1,04168
Being a member of an agricultural cooperative	2,3191	1,30395
Producing products of the quality demanded by the market	1,9149	0,90481
Producing the amount of product the market wants	1,8085	1,19124
To be able to sell the product at the highest price in the market	1,5745	1,09831
Having no problems in marketing products	1,5957	1,05624
Having no problems with product transport	3,0426	1,39810
Having no problems with packaging	4,1064	1,10794
No problems with storage	4,2553	0,89608

*Scale: 1: Very important, 2: Important, 3: Neutral, 4: Not important, 5: Not important at all

Information Resources

In the farm within the scope of the research, the data obtained about the people whose knowledge is used in case of any problems related to agricultural activities, the frequency of meeting with experts on agricultural activities, the pioneers in bringing innovations related to agricultural activities, and the communication tools used in relation to agriculture are shown in Table 14. Agricultural/engineer/veterinarians take the first place among the people whose knowledge the farmers benefit from in the examined farms. It was stated that 61,7% of the surveyed farms were permanent agricultural engineers. The pioneers who bring innovations to the village are the headman and the cooperative, respectively. While television was the first among the communication tools used in agriculture, it was followed by the internet and the newspaper.

Table 14. Information Sources on Agricultural Activities in the Investigated Farms

	Person	**%**
People whose knowledge is used		
Farmer	1	2,13
Headman	15	31,91
Relative	2	4,26
Neighbor	1	2,13
Other farmers	5	10,64
Agriculture district directorate staff	4	8,51
Agriculture consultant	2	4,26
Agriculturist/Veterinarian	17	36,17
Total	***47***	***100,00***
Frequency of meeting with experts		
Once a week	2	4,26
Every fifteen days	9	19,15
There is always an agricultural engineer in the business.	29	61,70
Once in a month	4	8,51
Quarterly	3	6,38
Total	***47***	***100,0***
Pioneers in bringing agricultural innovations		
Farmer	1	2,13
Headman	21	44,68
Agriculturist / Extension Person	1	2,13
Veterinary	1	2,13
Cooperative	20	42,55
Chemical, fertilizer, seed dealer	3	6,38
Total	***47***	***100,00***
Communication tools used in agriculture		
TV	19	40,43
Newspaper	13	27,66
Internet	14	29,79
Brochure	1	2,13
Total	***47***	***100,00***

CONCLUSIONS

In this study, the sources of risk and the risk management strategies applied by the farmers of ovine breeding in the province of Antalya were determined in agricultural production. The thoughts of the farmers for the future and whether they will continue their activities in ovine breeding have been examined in terms of sustainability. According to the farmers; changes in the government's policies and the country's economic situation are very important sources of risk. Saving and finding capital resources are very important risk management strategies. In the farms included in the research, it is important to make profit and the lack of good opportunities outside of agriculture among the reasons for the farmers to make agricultural production. Among the factors that will help to continue agricultural activities in the examined farms, the improvement of infrastructure facilities and the guarantee of product prices were found important by the producers. All of the farmers surveyed in the farms want to leave the small cattle breeding activity. The low level of government support and the inability to obtain sufficient income were considered very important reasons for the farms to want to quit their agricultural activities. According to the results obtained from the research, similar problems experienced in the livestock sector in Turkey were also seen in the examined enterprises. In this production activity, the farmers' inability to obtain sufficient income, insufficient government support, difficulties in finding credit and the problems experienced threaten the sustainability of ovine breeding.

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INVESTIGATION OF SOME QUALITY PARAMETERS OF HOMEMADE YOGURT WITH NETTLE POWDER

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ABSTRACT

In this study, it was aimed to determine the effect of powdered nettle addition at the rate of 1% (A1), 2% (A2) and 3% (A3) on some quality parameters in traditional yoghurt made using homemade yoghurt yeast. Physical-chemical [pH, titration acidity, syneresis, viscosity, colour (L*, a*, b*)] and sensory properties were investigated on the 1st, 7th, 14th and 21st days of storage. The titration acidity (% lactic acid), pH values and syneresis values were determined respectively as between 0.71-1.21%, 3.86-4.40 and 2.85-9.50 ml/25 g in yoghurt samples. The average viscosity value of the yogurt samples containing nettle powder was higher than the control yogurt. The highest viscosity value was found in the A3 sample group on the 21st day and the lowest viscosity value was found in the control group on the 14th day. Differences between L*, a* and b* values of yoghurt samples according to yoghurt types and storage times were statistically significant ($p<0.01$). Panellists, who evaluated the sensory properties of yoghurts such as appearance, taste, colour, odour, consistency, acidic taste and general acceptability, scored the 1% (A1) group as close to the control group. Sensory evaluations have shown that 1% and 2% nettle powder can be used in the production of yoghurt. Colour is an important parameter that determines the acceptability of a product, and the intensity of the green colour reduced panellists' appreciation. It is thought that the development of alternative dairy products with the addition of nettle, a plant with high nutritional and therapeutic properties, will bring an innovative understanding to the functional food market.

Keywords: Nettle, Homemade yogurt, Sensory properties, Viscosity, Syneresis

INTRODUCTION

Nettle (*Urtica*) is a herbaceous plant in the nettles (Urticaceae) family, found up to 1800 meters altitude, growing in moist soils, meadows and shady places of abandoned fields (Karpuz et al., 2017, Grauso et al., 2020). In different regions in Anatolia, it is named as stingray, çızlagan, cızgan, dalagan, cinçar, stinging nettle and stinging nettle (Baytop, 1999; Ayan et al., 2006). When stinging nettle feather is touched, it has a burning effect due to the effect of acetylcholine, histamine and 5-hydroxytryptamine (serotonin) (Fu et al., 2006).

Stinging nettle contains high levels of chlorophyll, flavonoids, plant sterols, plant enzymes, phenylpropanes, coumarins, terpenoids, potassium salts, vitamin C, polysaccharides, plant lignans, formic acid and small molecular weight lectin (*Urtica dioica* agglutinin (UDA)) in its roots (Gözüm et al., 2003; Weber, 2003; Akbay et al., 2003; Tello et al., 2008). Nettle powder is a rich source of antioxidants. The most important antioxidants compounds are α-tocopherol (vitamin E), ascorbate (vitamin C), flavonoids (chemferol, quercetin, rutin), carotenoids (β-carotene, xanthophyll, retinoic acid, retinol), vitamin K, catechins, tannins, and caffeic acid, coumaric acid and ferulic acid from phenolic compounds (Wetherilt, 1989; Palozza and Krinsky, 1992; Tsuchiya et al., 1992; Seven and Candan, 1996; Şener et al., 2010). Stinging nettle has been used in traditional medicine since ancient times due to its components with a wide range of activities such as antioxidant, antimicrobial, antiulcer, analgesic (Gülçin et al., 2004; Mitrović et al., 2020). Nettle leaves are rich in minerals, chlorophyll, amino acids, lecithin, carotenoids, flavonoids, sterols, tannins and vitamins (Taylor, 2005). It is also known to provide anti-aging and antioxidant effects with the presence of phenolic compounds such as quercetin and ursolic acid in its extract (Bourgeois et al., 2016; Bayrami et al., 2020).

This study was planned to investigate the interaction of a plant with valuable nutrients such as nettle with a nutrient-rich fermented product, product quality, nutritional and sensory properties. For this purpose, it is aimed to determine the best quality and acceptable yoghurt product in terms of chemical, physical and sensory during storage by using nettle plant powder.

MATERIAL AND METHOD

Materials

Cow milk obtained from a local producer in Erzincan province was used in the production of homemade yogurt with the addition of nettle powder. The milk was prepared for production right after the morning milking in the Nutrition Principles Application Laboratory of the Nutrition and Dietetics Department of Erzincan Binali Yıldırım University. The traditional yoghurt yeast used in the research is natural and additive-free yoghurt yeast produced by traditional fermentation. The nettle plant was collected from the village of Büyük Çakırman, formerly Vank, in the province of Erzincan on 05.05.2021.

Preparation of Nettle Plant

In the study, after the stinging nettle was collected, the weeds were removed and dried in a shaded environment after being thoroughly washed. Only the leaves of the plant were used in the study. After grinding, it was sieved and the powder obtained from under the sieve was used in production. Samples without nettle powder were prepared as the control group (0%).

Yogurt production method with nettle powder

In yoghurt production, milk with nettle powder added at 65 °C was divided into 3 groups as 1% nettle powder (A1), 2% nettle powder (A2) and 3% nettle powder (A3). All mixes were homogenized by Ultra-Turrax (Daihan Scientific, Co., Ltd) for 5 minutes and pasteurized at 90 °C for 10 minutes and cooled to the fermentation temperature of 45°C with 3% traditional yoghurt culture was added and incubated for approximately 3.5-4 hours. All yoghurt groups were kept at 4°C and analysed on the 1st, 7th, 14th and 21st storage days.

Figure 1. Yogurt with nettle

Physico-chemical Analysis

Titratable acidity (% lactic acid) of yogurt samples was made according to the Kurt et al. (2007). The pH value of the trial samples was determined with a Eutech PH 150 Model pH meter (Cemeroğlu 2013). The viscosity determination of yoghurts was analysed by using Brookfield brand viscometer (Model DV-1; Brookfield Engineering Laboratories, Inc., MA, USA) and the results were determined as cP (Gassem et al., 1991). Serum separation of trial samples was determined by evaluating the serum separated from 25 g yogurt sample filtered using filter paper at +4°C in millilitres (Atamer and Sezgin, 1986). HunterLab (Colorflex-EZ, Hunterlab, Virginia, USA) device was used to determine the colour values in yogurt samples and colour values were given as L* (brightness), a* (+red, -green) and b* (+yellow, -blue) (Cueva and Aryana, 2008).

Sensory Analysis

The sensory evaluation of the samples was made by a panellist team of 10 people according to 7 different parameters, including appearance, taste, colour, odour, texture (consistency), acidic taste and general acceptability during storage (Bodyfelt et al., 1988).

Statistical Evaluation

Variance analysis of the data was performed the IBM SPSS 22 package program. Comparison of means of samples and storage was made Duncan's multiple comparison test.

RESULTS AND DISCUSSION

Chemical properties of cow's milk used in yoghurt production were determined as pH 6.68±0.12, titration acidity 0.18±0.00%, dry matter 13.34±0.91 and fat 3.82±0.13%. Some physico-chemical properties of yoghurt with nettle are given in Table 1.

It was determined that the effect of nettle powder addition on pH and acidity (%) values of yogurt samples was statistically significant ($p<0.01$). It was determined that the pH values of the samples decreased during the storage period due to the nettle powder effect, and these values varied between 3.86 and 4.40, while the highest pH value was found in the A3 sample on the 1st day of storage, and the lowest value in the control group on the 21st day of storage. Akbal (2013) found the pH values of yogurts with thyme extract to be between 3.95 and 4.39 and between 3.91 and 4.34 for plain yogurts. It was determined that the acidity values of the samples varied between 0.71% and 1.21% and the acidity values of the samples increased in direct proportion to the amount of nettle powder during storage (Table 1).

Viscosity in yogurt is an important parameter, especially in terms of acceptability. When the viscosity and serum separation values between the samples were examined, it was determined that the highest viscosity value was in the A3 sample and the lowest value was in the control group. Serum separation was found as 2.85 to 7.68 mL/25 g in nettle powder samples. It was observed that serum separation values in yoghurts with nettle powder during storage were lower than the control sample, and serum separation values decreased due to the increase for powder added to yoghurts.

Colour analysis results of yogurt samples with nettle powder are given in Table 2. Significant changes were detected in the colour values of yoghurts during storage. The highest L* value in the control sample was determined as 96.19 and it was determined that the L* value decreased as the nettle powder concentration increased. It was found that there was a decrease in a* value and an increase in b* values in yoghurt samples depending on the storage time. Felfoul et al. (2017) reported that there was a decrease in the L* (brightness) values and an increase in the a* and b* values of yoghurts produced by adding powdered ginger.

Table 1. Some physico-chemical analysis results of nettle yoghurts

		Yogurt Samples			
Analysis	Storage time (days)	C	A1	A2	A3
pH	1	$4.19 \pm 0.01^{c,A}$	$4.31 \pm 0.01^{c,B}$	$4.38 \pm 0.01^{d,C}$	$4.40 \pm 0.00^{d,D}$
	7	$4.03 \pm 0.02_{b,A}$	$4.29 \pm 0.01^{c,B}$	$4.32 \pm 0.01^{c,BC}$	$4.37 \pm 0.01^{c,C}$
	14	$4.00 \pm 0.00^{b,A}$	$4.24 \pm 0.07^{b,B}$	$4.28 \pm 0.01^{b,C}$	$4.30 \pm 0.00^{b,D}$
	21	$3.86 \pm 0.01^{a,A}$	$4.00 \pm 0.01^{a,B}$	$4.06 \pm 0.01^{a,C}$	$4.09 \pm 0.01^{a,D}$
Titration acidity (%)	1	$0.95 \pm 0.02^{a,C}$	$0.93 \pm 0.01^{a,C}$	$0.83 \pm 0.01^{a,B}$	$0.71 \pm 0.02^{a,A}$
	7	$1.06 \pm 0.01^{b,A}$	$0.91 \pm 0.01^{a,B}$	$0.98 \pm 0.01^{a,C}$	$0.81 \pm 0.01^{b,A}$
	14	$1.10 \pm 0.01^{c,D}$	$1.03 \pm 0.00^{b,C}$	$1.00 \pm 0.00^{b,B}$	$0.84 \pm 0.01^{b,A}$
	21	$1.21 \pm 0.01^{d,D}$	$1.08 \pm 0.01^{c,C}$	$1.07 \pm 0.00^{c,B}$	$0.90 \pm 0.01^{c,A}$
Serum separation (mL/25 g)	1	$9.50 \pm 0.70^{b,C}$	$7.68 \pm 0.07^{b,B}$	$4.60 \pm 0.14^{b,A}$	$3.40 \pm 0.01^{c,A}$
	7	$7.75 \pm 0.35^{a,B}$	$7.55 \pm 0.07^{a,B}$	$4.15 \pm 0.21^{b,A}$	$3.61 \pm 0.02^{d,A}$
	14	$7.64 \pm 0.07^{a,C}$	$7.52 \pm 0.03^{a,C}$	$3.85 \pm 0.20^{b,B}$	$3.06 \pm 0.01^{b,A}$
	21	$7.45 \pm 0.07^{a,C}$	$5.10 \pm 0.14^{a,C}$	$3.35 \pm 0.21^{a,B}$	$2.85 \pm 0.01^{a,A}$
Viscosity (cP)	1	$1395 \pm 176.77^{a,A}$	$1638 \pm 138.59^{a,A}$	$1705 \pm 388.90^{a,A}$	$2195 \pm 21.21^{a,A}$
	7	$1593 \pm 492.14^{a,A}$	$1688 \pm 214.36^{a,A}$	$1994 \pm 200.81^{a,A}$	$2265 \pm 134.35^{a,A}$
	14	$1366 \pm 19.79^{a,A}$	$1695 \pm 530.33^{a,A}$	$2455 \pm 35.35^{a,A}$	$2580 \pm 483.80^{a,A}$
	21	$1385 \pm 3.535^{a,A}$	$2220 \pm 367.69^{a,A}$	$2805 \pm 35.34^{a,A}$	$3240 \pm 56.56^{a,A}$

C: Control, A1: 1% Nettle powder yogurt, A2: 2% Nettle powder yogurt, A3: 3% Nettle powder yogurt.
$^{a-d}$The difference between the values shown with different letters on the same line is statistically significant ($p<0.05$).
$^{A-D}$The difference between the means shown with different letters in the same column is significant at the $p<0.05$ level.

Table 2. Colour values of yogurt samples with nettle powder

Yogurt Samples					
Parameters	Storage time (days)	C	A1	A2	A3
L*	1	$87.16\pm0.23^{a,D}$	$64.22\pm0.01^{a,C}$	$53.21\pm0.01^{a,B}$	$41.77\pm0.02^{bc,A}$
	7	$86.94\pm0.00^{a,D}$	$66.25\pm2.11^{ab,C}$	$53.32\pm0.02^{a,B}$	$36.24\pm0.01^{a,A}$
	14	$96.19\pm0.27^{c,D}$	$71.25\pm0.35^{c,C}$	$58.09\pm0.32^{b,B}$	$41.40\pm0.04^{b,A}$
	21	$94.71\pm0.40^{b,D}$	$68.36\pm0.51^{bc,C}$	$58.24\pm0.34^{b,B}$	$42.24\pm0.33^{c,A}$
-a*	1	$4.16\pm0.02^{d,B}$	$3.44\pm0.01^{d,A}$	$4.23\pm0.10^{d,B}$	$5.90\pm0.01^{a,C}$
	7	$3.95\pm0.02^{c,C}$	$2.80\pm0.01^{c,A}$	$3.14\pm0.01^{c,B}$	$4.25\pm0.01^{b,D}$
	14	$0.44\pm0.01^{a,A}$	$0.99\pm0.01^{b,B}$	$2.05\pm0.05^{b,C}$	$2.56\pm0.01^{d,D}$
	21	$1.36\pm0.01^{b,C}$	$0.92\pm0.01^{a,A}$	$1.32\pm0.01^{a,B}$	$2.20\pm0.01^{c,D}$
b*	1	$10.97\pm0.02^{a,A}$	$11.36\pm0.01^{b,C}$	$11.09\pm0.01^{a,B}$	$13.21\pm0.02^{a,D}$
	7	$10.85\pm0.01^{a,C}$	$11.57\pm0.01^{c,B}$	$11.65\pm0.01^{c,AB}$	$13.91\pm0.01^{a,A}$
	14	$13.26\pm0.07^{b,B}$	$13.18\pm0.01^{d,B}$	$12.05\pm0.04^{d,A}$	$16.34\pm0.01^{a,C}$
	21	$11.28\pm0.39^{a,A}$	$11.15\pm0.01^{a,A}$	$11.59\pm0.01^{b,A}$	$14.66\pm0.02^{a,B}$

C: Control, A1: 1% Nettle powder yogurt, A2: 2% Nettle powder yogurt, A3: 3% Nettle powder yogurt.
$^{a\text{-}d}$The difference between the values shown with different letters on the same line is statistically significant ($p<0.05$).
$^{A\text{-}D}$The difference between the means shown with different letters in the same column is significant at the $p<0.05$ level.

Sensory Analysis Results

Evaluations of sensory analyses of yoghurts with nettle powder during storage are given in Table 3. The appearance scores of the control group were found to be higher than the nettle powder added samples, followed by A1>A2>A3 samples, respectively. The taste scores of the yoghurt samples varied between 8.50% and 1.87%. The panellists liked samples with 1% and 2% nettle powder, but samples with 3% added were not liked and received the lowest scores. The colour values of the samples received low scores from the panellists depending on the nettle powder concentration. The panellists also stated that they did not like the colour of the A3 sample, as they considered it a mossy colour. According to the odour scores of the samples, the control group got the highest score on the 21st day of storage (8.25), while the A3 sample got the lowest score on the 14th day of storage (2.30). According to the consistency scores, the control group received the highest values during storage, followed by nettle powder groups A1>A2>A3 respectively. On the 21st day of storage, it was observed that the consistency of the A3 sample was more fluid.

Table 3. Sensory evaluation results of yoghurts with nettle powder

Yogurt Samples					
Parameters	Storage time (days)	C	A1	A2	A3
Appearance	1	$8.00\pm0.46^{a,C}$	$7.0\pm0.00^{a,BC}$	$6.21\pm1.11^{a,B}$	$2.37\pm0.17^{a,A}$
	7	$8.10\pm0.14^{a,C}$	$5.50\pm1.27^{a,B}$	$4.50\pm1.69^{a,AB}$	$2.80\pm1.55^{a,A}$
	14	$8.65\pm0.49^{a,C}$	$6.25\pm0.07^{a,B}$	$3.30\pm0.00^{a,A}$	$2.05\pm0.63^{a,A}$
	21	$8.35\pm0.91^{a,B}$	$7.10\pm0.14^{a,AB}$	$4.75\pm1.48^{a,A}$	$4.0\pm1.27^{a,A}$
Taste	1	$8.33\pm0.35^{a,C}$	$5.83\pm0.71^{ab,BC}$	$5.04\pm0.41^{a,AB}$	$1.87\pm0.64^{a,A}$
	7	$7.80\pm0.14^{a,B}$	$5.50\pm1.41^{a,AB}$	$4.40\pm2.26^{a,A}$	$3.0\pm1.97^{ab,A}$
	14	$8.50\pm0.42^{a,C}$	$5.45\pm0.91^{a,B}$	$3.50\pm0.00^{a,A}$	$2.35\pm0.49^{ab,A}$
	21	$8.35\pm0.77^{a,C}$	$7.05\pm0.35^{b,BC}$	$4.65\pm1.06^{a,AB}$	$4.20\pm1.13^{b,A}$
Colour	1	$8.24\pm0.23^{a,C}$	$6.58\pm0.12^{a,BC}$	$5.66\pm1.42^{a,B}$	$2.62\pm0.06^{a,A}$
	7	$8.10\pm0.14^{a,B}$	$5.55\pm0.91^{a,AB}$	$4.75\pm2.19^{a,A}$	$3.30\pm0.98^{a,A}$
	14	$8.40\pm0.42^{a,D}$	$6.30\pm0.28^{a,C}$	$3.60\pm0.42^{a,B}$	$2.45\pm0.21^{a,A}$
	21	$8.40\pm0.70^{a,B}$	$6.75\pm0.49^{a,AB}$	$4.25\pm1.34^{a,A}$	$4.20\pm1.83^{a,A}$
Odour	1	$8.12\pm0.06^{a,C}$	$5.87\pm0.41^{a,BC}$	$4.87\pm0.99^{a,AB}$	$2.42\pm0.35^{a,A}$
	7	$7.40\pm0.42^{a,B}$	$4.90\pm1.41^{a,AB}$	$4.20\pm2.54^{a,A}$	$2.95\pm1.76^{a,A}$
	14	$8.20\pm0.28^{a,C}$	$6.05\pm0.77^{a,B}$	$3.90\pm0.14^{a,A}$	$2.30\pm0.56^{a,A}$
	21	$8.25\pm0.77^{a,B}$	$7.33\pm0.47^{a,AB}$	$5.50\pm0.42^{a,AB}$	$4.30\pm1.69^{a,A}$
Consistency	1	$8.25\pm0.24^{a,B}$	$6.87\pm0.17^{b,B}$	$5.71\pm1.82^{a,B}$	$2.12\pm0.77^{a,A}$
	7	$7.80\pm0.14^{a,B}$	$6.05\pm0.21^{a,AB}$	$4.60\pm2.12^{a,AB}$	$3.0\pm1.55^{a,A}$
	14	$8.50\pm0.56^{a,D}$	$6.95\pm0.21^{b,C}$	$3.55\pm0.07^{a,B}$	$2.25\pm0.35^{a,A}$
	21	$8.35\pm0.77^{a,B}$	$6.90\pm0.28^{b,AB}$	$4.85\pm0.91^{a,A}$	$4.95\pm0.21^{b,A}$
Acidic Taste	1	$8.25\pm0.24^{a,C}$	$6.21\pm0.52^{a,BC}$	$4.96\pm1.24^{a,AB}$	$2.50\pm0.46^{a,A}$
	7	$7.95\pm0.91^{a,C}$	$5.70\pm1.97^{a,BC}$	$5.15\pm2.75^{a,AB}$	$3.25\pm1.76^{a,A}$
	14	$8.15\pm0.07^{a,B}$	$5.25\pm1.48^{a,A}$	$3.10\pm0.42^{a,A}$	$2.95\pm0.63^{a,A}$
	21	$8.35\pm0.77^{a,B}$	$7.40\pm0.84^{a,AB}$	$5.50\pm0.70^{a,AB}$	$4.75\pm1.34^{a,A}$
General acceptability	1	$8.29\pm0.05^{a,B}$	$6.33\pm0.00^{a,B}$	$5.54\pm1.11^{a,B}$	$2.37\pm0.53^{a,A}$
	7	$7.80\pm0.28^{a,B}$	$5.20\pm2.26^{a,AB}$	$4.40\pm2.54^{a,A}$	$3.15\pm1.76^{a,A}$
	14	$8.40\pm0.28^{a,C}$	$5.35\pm1.90^{a,B}$	$3.55\pm0.63^{a,AB}$	$2.05\pm0.49^{a,A}$
	21	$8.40\pm0.84^{a,B}$	$7.05\pm0.07^{a,AB}$	$5.65\pm0.91^{a,A}$	$2.75\pm2.47^{a,A}$

C: Control, A1: 1% Nettle powder yogurt, A2: 2% Nettle powder yogurt, A3: 3% Nettle powder yogurt.
[a-d]The difference between the values shown with different letters on the same line is statistically significant ($p<0.05$).
[A-D]The difference between the means shown with different letters in the same column is significant at the $p<0.05$ level.

While the acidic taste of the samples was highly appreciated by the panellists during the storage period in the control group, the samples with nettle received lower values depending on the nettle powder concentration. After the control group, especially the A1 coded sample was liked more in terms of acidic taste. Considering the general acceptability scores, the control group got the highest scores (8.40) followed by samples containing 1% (7.05), 2% (5.65) and 3% (3.15) nettle powder, respectively. In addition, yoghurts containing 1% nettle powder were highly appreciated by the panellists but some panellists stated that they do not prefer the intense nettle aroma too much. For this reason, yoghurts containing 1% and 2% nettle powder were preferred more by the panellists.

CONCLUSIONS

In this study, a product that is both beneficial to health and slightly different from the tastes that consumers are used to was produced. While the serum separation and pH values of the samples to which nettle powder was added during the storage period decreased, viscosity and titration acidity values were found to increase. In yoghurt samples, the highest L* value was detected in the control sample, while a* values decreased and b* values increased in nettle powder samples during storage. In terms of physico-chemical properties and sensorial results, 1% and 2% additives are usable as a new production method in the production of yoghurt with nettle powder. In addition, it is recommended to carry an industrial dimension the production of a new product with nettle plant that will contribute to the immune system by enriching the bioavailability of the fermented milk product, which is known to be beneficial for health and consumed by the people in their daily lives.

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THE USE OF MAPLE SYRUP AND Chlorella vulgaris IN YOGHURT PRODUCTION

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ABSTRACT

Today, food science and technology strives to establish a new fundamental field in the light of nutritional guidelines. The enrichment of yogurt, which is an important culture of Turkish cuisine, with additive components in terms of both nutritional and health is one of these applications. At the same time, because of yogurt is loved by most people, it is accepted as a good medium to provide and improve the daily intake of nutrients that can prevent diseases and have positive effects on consumer health. As a result, the studies on enriching the functional and nutritional values of yogurt are increasing day by day. As an example, we can give the development of a new product by combining these two valuable products, since algae contain bioactive compounds or phytochemicals that can benefit health and have valuable nutrients. Microalgae have been an important part of human nutrition for centuries. Especially *Spirulina* and *Chlorella* are produced on a large scale around the world. *Chlorella vulgaris* contains essential amino acids, proteins, ascorbic acid and B complex from vitamins, mineral substances, β-carotene, chlorophyll and other important compounds necessary for health. The consumers have changed their preferences instead of synthetic additives and turned to natural additives has led to an increase in studies on the use of microalgae-based components in foods. However, considering the effects of consumption of sugary products on chronic diseases such as diabetes and obesity, it is extremely important to examine the importance of using natural sweeteners and the production of a new functional and healthy microalgae fermented product in terms of consumer expectations. The purpose of this review was to examine the use of natural sweeteners maple syrup and *Chlorella vulgaris* in yogurt production in the light of scientific data in terms of the sustainability of our health and a protective/preventive diet.
Keywords: Microalgae, *Chlorella vulgaris*, Maple syrup, Yogurt

INTRODUCTION

It is a fact that the population will reach approximately 9.8 billion in 2050 due to the increase in the world population and that together with food-related problems; energy crises will also reveal serious risk factors such as global warming, toxic gas emissions, greenhouse effects and climate changes (Aratboni et al., 2019). Today, sudden fluctuations in the population, various environmental parameters and chronic diseases have compelled researchers to work on alternative food sources. Algae, which take their place in studies in the field of food, are among the most comprehensive food chain links living on Earth. The reasons why they are recommended as an alternative food source are that they can increase up to 2-3 times their weight in a day under certain conditions, their growing environment is easy and economical, and they do not show any side effects when consumed (Alçay et al., 2017). Algae that can grow in fresh or salt waters are among alternative food sources with their rich biodiversity and large biomass (Singh and Sharma, 2012).

Maple syrup is a natural sweetener produced by boiling the sap of the sugar maple (*Acer saccharum* L.) (Perkins and van den Berg, 2009). Maple syrup is produced commercially by concentrating the sugar sap using the evaporation method (approximately 40 L of sap yields 1 L) (Ball, 2007; Stuckel and Low, 1996; Gad et al., 2021). Maple syrup is a widely consumed product in food products. Compared to other natural sweeteners, maple syrup is seen as a superior option over other natural sweeteners due to its high mineral content, high antioxidant capacity, and compounds with antiproliferative (anti-cancer) effects (Thériault et al., 2006, González-Sarrías et al., 2012; Perkins and Van den Berg, 2009, Phillips et al., 2009, Singh et al., 2014; Aytaç, 2017).

Functional foods have recently constituted an important group for conscious consumers looking for nutritious and safe foods, in terms of contributing to the protection and development of health by being obtained from natural sources (Helkar and Sahoo, 2016; Bimbo et al., 2017). This review has been prepared to emphasize the importance of use of microalgae, which is rich in bioactive compounds, together with maple syrup that is one of the natural sweeteners in the preparation of a valuable product such as yoghurt.

COMPOSITION OF ALGAE AND NUTRITIONAL CONTENT

It is known that the number of algal species is between 30,000 and 1 million, and according to Guiry (2012), there are an estimated 350 million identified species, and even over 150,000 species in the internet database AlgBase system (Guiry and Guiry 2018; Ścieszka and Klewicka, 2019, Anonymous, 2021). They are divided into two groups as prokaryotic and eukaryotic, according to their structures. While cyanobacteria (e.g. *Cyanophyta* and *Prochlorophyta* are containing chlorophyll-a) are in the prokaryotic group, eukaryotes consist of a wide variety of algal classes (Tomaselli, 2004; Uzuner and Haznedar, 2020). Depending on the pigment substances they carry, they are grouped as brown, red, green algae and cyanobacteria (Ak, 2015; Alçay et al., 2017). In addition, algae are also divided into “Microalgae” and “Macroalgae” according to their general classification.

a. Macroalgae; large-sized, visible to the naked eye, having multiple cells that can grow very quickly, divided into groups as brown (*Phaeophyta*), green (*Chlorophyta*) and red algae (*Rhodophyta*),

b. Microalgae are microscopic and form the eukaryotic group such as cyanobacteria (*Chloroxybacteria*) or prokaryote with green algae (*Chlorophyta*) (Khan et al., 2018).

USAGE AREAS OF ALGAE

From the archaeological data, it has been learned that algae were used in the nutrition of the people who lived in Chile first in the year 14000 BC. It has been a source that has been used in the nutrition of many cultures for thousands of years and is still used in the nutrition of different communities today (Wells et al., 2017). Although there are more than 200,000 different types of algae known, about 200 of them are successfully used in industrial areas (Akyıl et al., 2016; Figure 1).

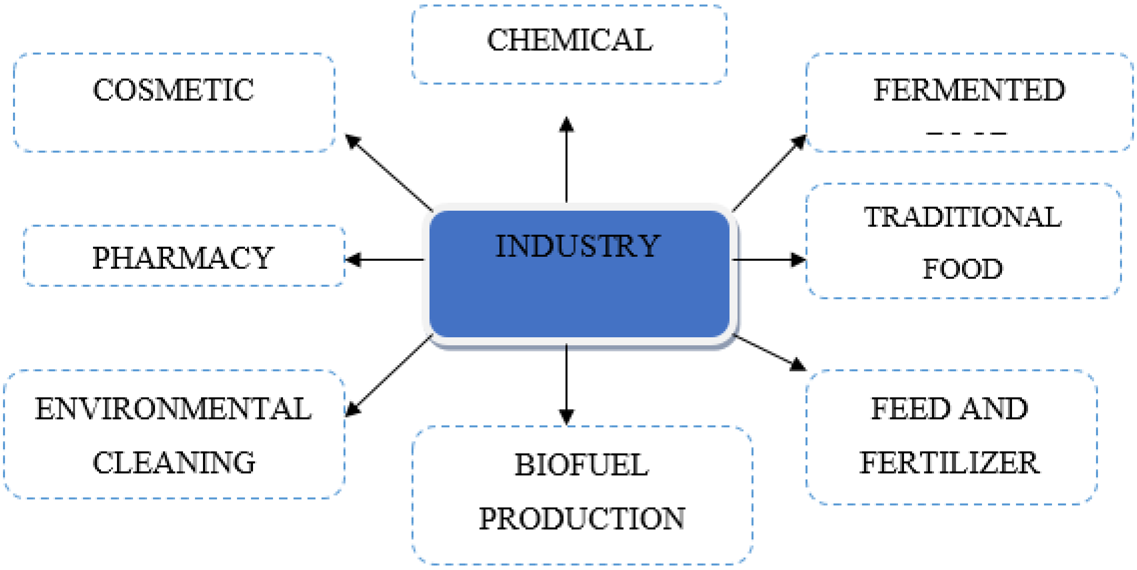

Figure 1. Use of algae in various industries

Algae are suitable for use in the food industry, as they contain components such as protein, polysaccharides, lipids, vitamins, minerals, amino acids, fatty acids, carotenoids and produce bioactive compounds (Ranga Rao and Ravishankar, 2018). Algae, known by different names as "Asaksanori, Suschi, Amanori, Tjintiow, Kanten, Kombu" as a ready-made food in Japan, is also used as tea. Studies are carried out on the use of algae species such as Ulva, Porphyra, Gelidium, Rhodymedia, Laurencia and Polysiphonia, which are more common in the seas of our country in different food applications (Casal et al., 2009; Aktar and Cebe, 2010).

MICROALGES

More than 50.000 various microalgal species are found in oceans and fresh waters (Milledge, 2011). In addition, microalgae contain 40-70% protein, 4-20% lipids, 12-30% carbohydrates, 8-14% carotene and significant amounts of vitamins B1, B2, B3, B6, B12, D, E and K (Ejike et al., 2017). The protein, carbohydrate and fat contents of some microalgae and foods are given in Table 1.

Table 1. General composition of algae and various foodstuffs (Hudson, 2008).

Component	*Chlorella*	*Spirulina*	*Haematococcus*	*Dunaliella*	Milk	Meat	Egg
Protein (%)	64.5	63	23.6	7.4	26	43	47
Carbohydrate (%)	15.0	17.8	38.0	29.7	38	1	4
Fat (%)	10.0	4.3	13.8	7.0	28	34	41

Microalgae most commonly used in the food industry are: *Bacillariophyceae* (diatoms), *Chlorophyceae* (green algae), *Chrysophyceae* (golden algae) and *Cyanophyceae* (blue-green algae) (Carlsson et al. 2007). The most common known microalgae containing foods can be shown in two main groups in general:

1. Dried microalgae, mainly *Chlorella* and *Spirulina* species. These products can be marketed directly as dietary supplements or used in the manufacture of other innovative products (Wells et al., 2017; Bhalamurugan et al., 2018).

2. Products containing microalgae biomass or a specific compound derived from microalgae. These products include products enriched fatty acids including omega-3 with docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA), pigments such as astaxanthin and β-carotene and antioxidants (Lafarga, 2020).

Annual production of microalgae is approximately 7.5 million tons and its market value is known to be approximately 6.5 billion dollars. Of this, 2.5 billion dollars is food and health, 1.5 billion dollars is docosahexanoic acid (DHA, C22:6n-3) and 700 million dollars is aquaculture (Hemantkumar and Rahimbhai, 2019). The traditional microalgae growing system is given in Figure 2 (De Vree et al., 2015; Koller, 2015; Vo et al., 2018; Li et al., 2019).

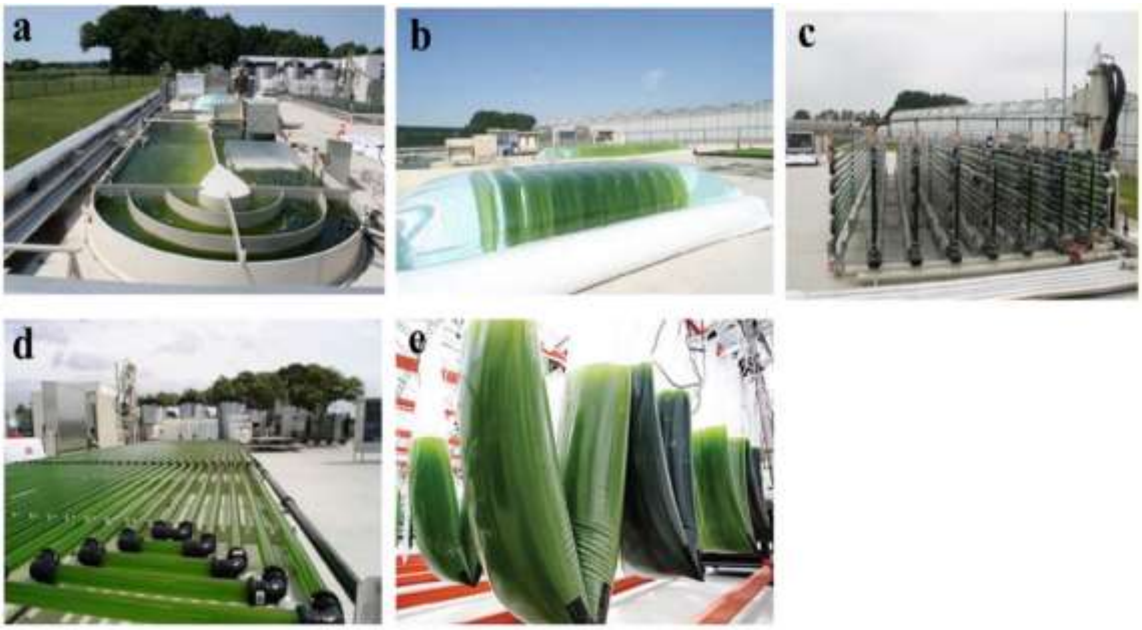

Figure 2. Commonly used conventional microalgae growing system: (a) Ponds; (b) Flat panel photobioreactors; (c) standpipe photobioreactors; (d) horizontal tube photobioreactors; (e) soft-frame photobioreactors

A schematic view of potential microalgal "cell factory" production, which is microalgae products and derivative compounds containing pigments, lipids, protein, polysaccharides and bioactive compounds related to various application areas such as food technology, chemicals, pharmaceuticals, nutritional and agriculture are presented in Figure 3 (Koller, 2014; Matos, 2017).

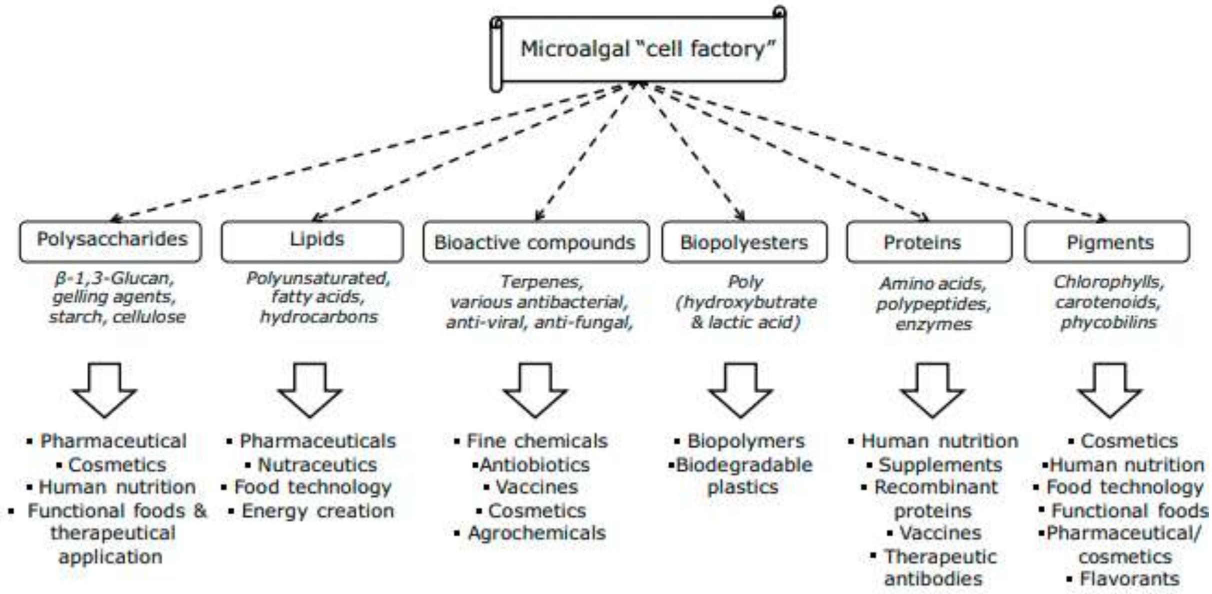

Figure 3. Products synthesized by microalgae and potential applications

Studies are continuing rapidly to determine the effects of bioactive compounds produced by microalgae and their potential benefits to consumers (Shahidi, 2004). Grossmann et al. (2020) reported that immulina and immurella polysaccharides, which they isolated from Spirulina platensis and Chlorella pyrenoidosa, respectively, could provide biological activities against cancer. They provide an important raw material source in the cosmetic industry with their antioxidant and anti-inflammatory bioactivities. Microalgae pigments are also used as non-radioactive fluorescent markers in clinical applications (Jacob-Lopes et al., 2019). In Table 2, different bioactive compounds of microalgae species and their health benefits are given.

Table 2. Bioactive compounds in different microalgae species and their health benefits

Microalgae species	Bioactive compounds	Benefits
C.vulgaris	santaxanthin	Color agent and antioxidant
	lutein	Antioxidant, anticancer, prevent heart ailments
	Sulfonated polysaccharide	Antiviral, Anticancer, antioxidant
	EPA	nutritional supplement
	glycoprotein	anti-inflammatory
	Vitamin C	It prevents heart ailments.
H.pluvialis	Astaxanthin	Strong antioxidant property, anti-inflammatory effect, anticancer
Dunaliella spp	β-carotene	Food colorant, Antioxidant property, anti cancer
	glutathione	Antioxidant, heart attack reducing effect, anticancer activity, anti Parkinson's disease
Spirulina spp	γ-linolenic acid (GLA)	nutritional supplement
	Phycocyanin	Natural food coloring, antioxidant

There are only a few species of microalgae that the FDA considers to have GRAS status. These algae are *Arthrospira platensis*, *Auxenochlorella protothecoides*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Dunaliella bardawil* and *Euglena gracilis*. For an algae species to be considered GRAS, some safety tests are required (Torres-Tiji et al., 2020).

Chlorella vulgaris

Chlorella is derived from the the Latin ella and the Greek chloros which is meaning green and small. Chlorella microalgae constitute the microalgae species found on earth since the pre-Cambrian period 2.5 billion years ago. Today, Japan is the world leader in Chlorella microalgae consumption (Andrade et al., 2018). Chlorella vulgaris is a species of green algae that contains astaxanthin, cantaxanthin and small amounts of colorants including β-carotene and lutein (Zielke et al., 1978; Beheshtipour et al., 2012).

Chlorella plays a health-promoting role for many diseases such as stomach ulcers, wounds, constipation, anemia, hypertension and diabetes (Mello et al., 2013). It has been determined that Chlorella exhibits immunological properties such as various antibacterial and antiviral effects. In addition, Chlorella administration is known to prevent cadmium-induced toxicity, toxin-induced oxidative stress and cellular damage (Karlan and Valdivia, 2011). Chlorella is also generally considered safe for human consumption, although approved strains of Chlorella vary by country.

Use of Chlorella vulgaris in yogurt production and studies

Yogurt is a well-known food product that stands out as one of the most consumed fermented milk products in the world. It can be a good tool in providing daily intake of nutrients that can prevent diseases and have positive effects on health, because it is a product that is consumed with love (Gahruie et al., 2015). When the studies on the use of microalgae in dairy products were examined, it was determined that the enrichment process was mostly done with yogurt and drinkable fermented milk products.

Dubey and Kumari (2011) the physicochemical properties of low fat and high protein frozen yogurt with papaya fruit and Spirulina, Beheshtipour et al. (2012) viability and biochemical properties of probiotic bacteria in yogurt in C. vulgaris and S. platensis supplementation, Güldaş and Irkin (2010) their effects of yoghurts with S. platensis on yogurt bacteria and Lb.acidophilus, Fadaei et al. (2013) their effects of probiotic yoghurt with spinach and the powder form of S. platensis on Lb. delbrueckii subsp. bulgaricus, S. thermophilus, and Lb. acidophilus, Agustini et al. (2016) the physical and sensory properties of the samples of different concentrations of S. platensis (0%, 0.6% and 1.2%) in ice cream production and different concentrations (0%; 0.5%; 1% and 1.5%) in soft cheese production, Silva et al. (2019) various properties of S. platensis microencapsulated using maltodextrin in yogurt production,, Agustini et al. (2017) physico-chemical, microbiological and sensory properties of yogurt samples produced using 0.5%, 0.75%, 1%, 2% and 3% concentrations of S. platensis and Aydemir (2019) physicochemical and sensory properties of S. platensis in yogurt samples produced using different concentrations (0.25%, 0.50%, 0.75% and 1%) were investigated.

MAPLE SYRUP

Maple syrup is a natural sweetener obtained from various Acer (*Acer saccharum*) tree species and is grouped as extra white (grade AA), white (grade A), amber (grade B) and dark (grade C) (Perkins and van den Berg, 2009). Sucrose (approximately 60-66%) mainly contains carbohydrates, with minor amounts of glucose, fructose, complex carbohydrates and high molecular weight polysaccharides (Storz et al., 1986). In addition, maple syrup contains minerals such as K, Ca, Mg, Na, Mn, Al, Zn, Fe etc., vitamins riboflavin, niacin, thiamine etc., amino acids arginine, threonine, proline etc., organic acids fumaric acid, malic acid, etc. and phytohormones abscisic acid, phaseic acid and their metabolites (Ball, 2007; Perkins and van den Berg, 2009; Zhang et al., 2014). Currently, there are maple-based food products in food markets that contain sterilized or pasteurized maple juice (i.e., maple sap that is ca. 98% water) (Yuan et al., 2013). Although the amount of effect is not yet known in the pure maple group, the presence of more than 50 antioxidant compounds associated with human health with anti-cancer, anti-bacterial and anti-diabetic properties has been determined (Li and Seeram 2010, Legault et al. 2010). Maisuria et al. (2015) in their study examining the antimicrobial effects of maple syrup extract, which is rich in phenolic substances, reported that *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa* bacteria increase the permeability of the outer membrane and provide an inhibitory effect.

CONCLUSION AND RECOMMENDATIONS

It is a fact that algae, which provide positive contributions to the environment without the need to use a lot of land for the prevention of hunger and a sustainable nutrition program, are considered as an alternative source. Bioactive substances such as antioxidant vitamins, polysaccharides, phenolic compounds and lutein they contain are widely used in the fields of medicine and food in terms of preventing diseases and promoting health. Projects are needed to increase the organoleptic properties and social acceptance of algae as food. The use of the undesirable distinctive flavors of algae, which is already limited in our country by consumers, can be expanded by using natural sweeteners in products with rich nutritional content such as yogurt.

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CONSTRAINTS OF LIVESTOCK MARKETS IN THE REPUBLIC OF BENIN AND SUGGESTIONS

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Abstract

Livestock and livestock markets are important foundations of the rural economy, food and nutrition security and regional integration in West Africa. The livestock sub-sector is characterized by a low-yield production system, a pastoral and agro-pastoral type, a large number of low-productivity animals and the marketing of live animals. Livestock marketing generates income for pastoralists and agro-pastoralists and for other actors in the value chain (traders, transporters, butchers, etc.). Despite these multiple benefits in terms of food and nutritional security, wealth creation, etc., livestock marketing still faces several challenges. To address these challenges that undermine the livestock marketing system, it is important to conduct scientific research on the subject. This study was conducted to identify the main constraints that undermine the development of livestock markets in the Republic of Benin and make suggestions for its improvement. Primary data were collected from face-to-face surveys of a random sample of 600 respondents consisting of livestock farmers (300) and livestock traders (300) participating in self-managed livestock markets (MBA) and traditional livestock markets (MT). SEPO analysis was also used to evaluate the Successes, the problems or Failures, the Potentials and the Obstacles of livestock markets in the study area. The results of the study showed that the poor road infrastructure, the multiple taxes collected on the livestock markets, the lack of financial support for market actors, and the poor market infrastructure are the first major constraints of the livestock markets. To address these problems, the government should improve road infrastructure, regulate the taxes, and facilitate access to credit for market participants. Addressing these constraints will not only allow actors in the livestock sub-sector to improve their income, but will also contribute to the effective improvement of the livestock marketing system, to the development of the rural economy and thus to the reduction of poverty in rural areas.

Keywords: constraints, SEPO analysis, self-managed livestock markets, traditional livestock markets, Republic of Benin, pastoralists

Introduction

Along with agriculture, livestock is one of the economic pillars of West African countries. It contributed 44% of the GDP of the Economic Community of West African States (ECOWAS) countries and ranked first in terms of trade in West Africa (Fabien, 2019). This economic importance of livestock in economic growth derives from the marketing of live animals.

Livestock marketing is therefore a powerful vector for regional economic integration and food and nutritional security. The organization of the livestock trade in West Africa is changing rapidly due to the growing demand for meat. It is characterized by large circuits, illustrating the fact that livestock is an important factor in the integration of regional trade. There has been a net increase in livestock trade between countries in the region over the past two decades. This is due to the growth of the population in the region and the increase of their income.

In Benin, the contribution of the livestock sub-sector to GDP was 5.82% and its share in the Gross Agricultural Production value was 15.55% (FAO, 2016). It ranks second after crop production. Most of pastoralists and agro-pastoralists live on livestock farming since it’s their main income activity.

For their monetary needs, livestock farmers market their animals and animal products mainly in livestock markets. In this study two types of livestock markets were considered. These are self-managed livestock markets (MBA) which are modern market and traditional livestock markets (MT). In Benin, as in most African countries, livestock markets generate significant revenues for its stakeholders and for the local government. This allows local government to finance its budgets. Today, some livestock markets, especially MBAs, have become local development pillars.

Despite its multiple roles, the livestock trade faces significant policy, technical and organizational challenges that limit its performance (FAO, 2017). Most of these challenges have remained unaddressed due to inadequate agricultural policies (Akouegnonhou and Demirbas, 2019). This study was conducted to identify the constraints that undermine livestock markets in the Republic of Benin and make proposals for its improvement. It is important to bring new elements on the constraints of the marketing system in Benin to attract the attention of actors and policy makers for sustainable solutions. Sustainable solutions are needed to improve the income of pastoral and agro-pastoral populations in order to reduce poverty and thus finance rural development in the Republic Benin.

Material and Method

This research was carried out in the Republic of Benin. Two different livestock markets were concerned in the study area. These are traditional livestock markets (MT) and self-managed livestock markets (MBA).

MT are the oldest livestock trading system in the local areas. The marketing systems in this type of market is traditional and they are somewhat further away from city centers. The MBA are the modern livestock markets where the transaction controls are more rigorous. The trading system in the MBA market is much more organized and modernized compared to MT markets.

The main material for this study was obtained from face-to-face surveys with 600 respondents consisting of livestock farmers (300) and livestock traders (300), randomly selected from livestock markets of Gogounou, Nikki, Bassila, Matéri, Savè and Iwoyé (Kétou). SEPO methodological tool - Successes, Failures, Potentials and Obstacles (PPR-AO, 2007; DDC, 2017; FAO, 2018) was used to analyze the constraints and then make proposals.

Results and Discussion

African livestock markets are experiencing similar problems (Onibon, 2004; Jabbar et al., 2008; Chabi, 2016; FAO, 2017). In most of these markets, the marketing system is limited by many constraints. This study addresses the main constraints of the livestock trade, which are listed below. The constraints cited in this document are the most commonly discussed by livestock market actors in the study area.

Constraints of livestock markets

SEPO analysis

Table 1 presents the diagnostic elements of the livestock marketing chain identified using the SEPO (in French: Succès, Echecs, Potentialités, Obstacles) methodological tool - Successes, Failures, Potentials and Obstacles. It allowed to analyze the constraints (causes of failures and unresolved barriers), while the successes and potentials will contribute to the development of evidence-based policy and action proposals (PPR-AO, 2007; DDC, 2017; FAO, 2018).

Table 1. Diagnostic elements of livestock marketing in the Republic of Benin according to the SEPO method

Success 1. Adoption of community regulations favourable to the production and marketing of livestock; 2. Emergence of socio-professional organizations of livestock farmers and traders at the communal, departmental and national levels such as: ALGMB, ANOPER, UDOPER, UCOPER; 3. Creation of self-managed livestock markets that reduces the number of intermediaries and increases the profit margin of livestock farmers and traders; 4. Creation of an autonomous MBA management committee.	**Potentials** 1. Important size of the domestic ruminant herd 2. Meat demand growth of 4% per year driven by population growth, urbanization and rising incomes 3. Renewed interest of technical and financial partners (USAID, World Bank, Swiss Cooperation) in the livestock marketing sector. 4. Existence of hardy animal species well adapted to African production systems, different ecosystems and socio-economic conditions of the Country 5. Existence of large areas of natural pasture
Failures 1. Sale of animals on a random basis resulting in an inequitable distribution of profit margins; 2. Poorly equipped livestock markets that limit their effectiveness and efficiency; 3. A multitude of parasitic intermediaries resulting in reduced profit margins for	**Obstacles** 1. Lack of specific regulations on the transport of animals; 2. Weak application of trade texts ; 3. Illicit payments that can increase the price of livestock and reduce the profit margin of livestock producers and traders;

herders and livestock traders, especially in traditional livestock markets 4. Low level of banking of commercial transactions with high risks of theft and losses during exchange; 5. Weak Market Information Systems (MIS), MIS with less or no functionality, resulting in weak data for political (policy makers) and operational (professionals) decision making; 6. Poor governance of the marketing system (lack of contractualization, sale on credit, etc.) 7. Gender-insensitive livestock marketing system. Very few women are involved in the livestock trade in the study area. 8. The non-existence of an annual program of activities for livestock markets. 9. Non identification of animals traded	4. High costs and poor conditions of transport by truck; 5. Lack of funding for the livestock marketing system; 6. Insecurity on the roads; 7. Poor access to market information; 8. Low transparency of animal transactions; 9. Inadequate price formation system; 10. Scarcity of forage resources 11. Insufficient training of livestock farmers and traders 12. Lack of scientific research in livestock trade 13. Inadequate legislative and regulatory framework for the livestock trade; 14. The low level of private sector participation in the livestock trade 15. Lack of livestock identification facilities 16. Low farmer's education level 17. Lack of animal passageways 18. Lack of feed and water for livestock in the markets 19. Credit sales, especially in traditional markets 20. Lack of security (risk of losing animals or money along trade routes).

Existence of intermediaries or brokers in the livestock markets

The intermediaries also called brokers or *« Dilalï »* (Haoussa name) influence the transactions between buyers and sellers (Okike et al, 2006). They manage to impose a domination on the pricing of animals. They have progressively conquered and concentrated a substantial part of the margin to their own profit. This reduces profits especially for sellers and lengthens the value chain. In MT markets, intermediaries still exist and are key players in the marketing chain (Onibon, 2004). But in MBA markets they are converted into witnesses and their role is limited to witnessing the transaction and certifying animals. MBA markets were created as a result of revolts against the exploitation of livestock farmers by middlemen. Their existence in the marketing chain is a real problem for the development of livestock markets.

Lack of infrastructure (Poor market and road infrastructure)

Infrastructure involves both the physical (communication, transport, market committee offices, and roads) and institutional infrastructure (market information, security and animal disease control). The distance between the production area and established markets requires transportation or "trekking" of livestock. Given the poor condition of the roads and the inadequate means of transport, the cost of animal's transport is high. The most commonly used means of transporting animals are foot conveyance, trucks, tricycles and two-wheeled motorcycles. The roads are mostly gravel and severely eroded, making access to livestock markets difficult. Transport is the most important weakness in the livestock marketing system in most African countries (Bailey et al., 1999; Sartorius von Bach, 1992). Extension services are limited due to poor road networks (Coetzee et al., 2004). Transport is the most recurrent problem that constantly comes in all studies and research on animal trade in Benin and most African countries (Okike et al., 2006), as the poor condition of roads makes the cost of transporting animals always high (PACBAO, 2017) and the animal marketing system inefficient. Ruijs et al. (2004) and Jari and Fraser (2009) reported that farmer participation and access to markets are positively impacted by good road conditions.

Marketing infrastructure is one of the serious constraints of livestock markets in the study area and is a common problem to most African countries (Mahabile et al., 2002; Dinku et al., 2021). Poor marketing infrastructure limits farmers to supply animal to formal livestock markets and buyer to purchase. This is the reason why some farmer prefers close markets though it is not benefit for them in terms of price and the formal markets are poorly supplied by farmers (USAID, 2003). Poor infrastructure often increases such market transaction costs (Takeshima, 2008).

Insecurity

Thefts and losses of animals are caused by the lack of security in rural areas. Robberies are also frequent on the roads linking markets to homes. The insecurity linked to the military-political and economic crises that many countries in the West African sub-region are experiencing. To reduce insecurity, the MBAs hire public security (police, gendarmes) to ensure the security of the market-on-market day. Only the MT are left at the mercy of total insecurity.

Lack of Credit

There are no specialized livestock banking services in Benin. Existing banks interest rates are high, up to 24%. Those banks require big guarantee to grant loans. Unfortunately, rural market actors are generally not able to give out the guarantee. This limits them in developing their activities. In the study area, commercial banks and some government institutions give loan to very few people. The mode of financing the livestock sub-sector is a central issue, which is not sufficiently taken into account in most existing analyses. It is the focal point of the relationships between actors and the relationships of dependence within the sector. It partly determines the marketing strategy of farmers and the purchasing capacity of traders (PACBAO, 2017).

High Taxes

Too much tax kills tax. This finds its explanation in the livestock markets of Benin. In addition to the taxes collected by local authorities and management committees, there are those made by brokers and sometimes by individuals posing as tax collectors. All these taxes are usually paid by the farmer. Which reduces its profit margin. The taxes collected in livestock markets are not supposed to be high but because of corruption, many illicit taxes are collected on the markets, especially on MT markets where there is no control (Jabbar et al., 2008; PACBAO, 2017). In MBA, taxes are fixed but still high because of some abnormalities in the tax collection. Some extra taxes are also collected for other services in the market. This makes the taxes high (Okeke-Agulu and Ochelle, 2019).

Lack of marketing information

In the study area, radio, mobile phone and personal communication are used as main source of information but the farmers remain uninformed in terms of market prices, trends, etc. Access to televisions and internet by farmers is still limited. In most cases information is broadcasted and written in French and rarely in local languages. This makes the information irrelevant to the majority of communal farmers who only understand their local language. Insufficient information is a big constraint to livestock farmers and traders. It can also be the cause of low or non-participation in livestock markets. Since livestock markets are held weekly, it is much easier to process the data collected and make it accessible to everyone. Weak information systems contribute to the development of informal networks in the marketing system in rural areas (Bailey et al., 1999). The lack of information usually results in price discovery informed by judging body conformation rather than weight of livestock (Coetzee et al., 2004). A good access to market information positively impact the farmer's participation and their access to markets (Ruijs et al., 2004; Jari and Fraser, 2009).

Veterinary services

Veterinary services are inefficient and insufficient to control animal health. There is a big gap of qualified vets in the rural areas. The services are corrupted too. One can bribe to avoid the control of his animals. This affects the quality of the animal sold in the livestock markets. The lack of veterinary staffs and equipment are the biggest constraints to animal health in the livestock markets (Damango et al., 2004; FAO, 2017).

Proposals for measures and actions to improve livestock marketing

According to the SEPO analysis table, the following solutions were proposed: (i) Strengthen the organizational capacities of existing structures such as ALGMB, ANOPER, UDOPER, UCOPER to enable them to offer better services to their members (contract, access to credit, consideration of youth and women, etc.); (ii) support the modernization and equipment of livestock markets, including equipment for weighing animals for sale by live weight; (iii) Promote self-managed livestock markets (MBA) to reduce the number of intermediaries and improve profit margins of livestock farmers and traders; (iv) Promote and develop a functional Market Information System (MIS) for livestock at national and regional levels; (v) support the construction and/or modernization of private slaughterhouses for livestock farmers and especially for rural livestock farmers' organizations, in order to add value to the livestock sub-sector and make the marketing system competitive; (vi) Improve the transparency of commercial transactions for the knowledge of the structure of price formation; (vii) Facilitate access to credit for livestock market actors to improve business transactions and cash sales; (viii) To sensitize and accompany the actors of the livestock to the banking of transactions by the use of the varied range of banking products and other means of money transfer available to avoid thefts, robberies and losses; (ix) Promote fodder production and create fodder markets in rural areas to enable farmers to cope with the scarcity of fodder resources and climate change; (x) Regulate land laws to allow easy access to land for pastoralists and agro-pastoralists; (xi) Development of annual management programs by the self-managed livestock market management committee; (xii) Improve livestock productivity through programs aimed at optimizing feeding, health protection and the development of the genetic heritage of local breeds; (xiii) Strengthen the capacities of farmers' organizations and interprofessions; (xiv) Support for improved livestock statistics and trade, including support for livestock MIS; (xv) Prioritize road infrastructure projects to reduce transportation costs; (xvi) Promote livestock identification facilities; (xvii) Training programs should be focused on visual aid materials and adequate illustration by weighing animals and applying current market information (prices per kilogram); (xviii) The unique tax proposal seems to be the best and most accepted one. Its level will be decided by the market management staffs in consultation with the other beneficiaries and a distribution key will be established; (xix) The texts are not well respected by the actors of the livestock markets. It is therefore necessary to advocate at the level of the authorities for an effective application of these texts. But before that, it is necessary that the different actors appropriate the content of these texts. Information seminars could be organized for them. (xx) Strictly apply sanctions in case of non-compliance with the texts.

Conclusion

Considering the multiple human, material and organizational investments that would be required to set up a livestock marketing system, the live animal trade still has long way to go. Livestock marketing as a value-added activity for live animals, plays a very important role in the economy of the Sahel region, and livestock markets, as the meeting place for most of the actors involved in this activity, make a significant contribution to it. To improve this contribution and strengthen the role of livestock markets in the promotion of livestock production with a view to a more comprehensive revitalization of the livestock sector, there is a need for genuine involvement of all stakeholders, mainly livestock farmers, livestock traders and also local authorities. It can be noted that most of the constraints to the livestock market have been studied in the context of improving the marketing system for live animals and animal products in Benin.

The study concluded that marketing of livestock in Benin is constrained by several factors such as lack of access to credit by farmers and traders, illegal taxation, poor veterinary services, inadequate market information as well as inadequate provision of marketing infrastructure, poor transport infrastructure, etc (Mohammed et al, 2006; ILRI, 2009; Dinku et al., 2021). These constraints are reflected in the livestock trade.

In addition to these constraints, new constraints are likely to hamper this animal trade, namely the growing insecurity in the region and climate change. The livestock marketing system in Benin would be effective with an integrated approach.

The constraints identified in this study are practical in nature and their resolution would require a joint effort by the government, livestock market stakeholders, livestock organizations and private sector actors. Moreover, to improve livestock marketing efficiency, attention should be paid to institutional arrangements and their relationship to the market, the effects of climate change on the environment. Taking these proposals into account in future livestock marketing support programs will improve livestock trade, with medium- and long-term impacts on the incomes of livestock market actors, and thus on poverty reduction, food and nutrition security, local development and regional economic integration.

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COMPARISION OF FERTILIZER USE EFFICIENCY IN BALKAN COUNTRIES

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ABSTRACT

Changes in the ecological system due to climate change and the negative effects caused by the increase in the demand for agricultural products have increased the tendency of imbalances in agricultural products markets all over the world. These developments have made agricultural production efficiency more important than ever. In order to meet the rapidly increasing food demand of human beings, it has become necessary to increase productivity. The main way to achieve this is through the conscious and effective use of modern agricultural techniques. Chemical fertilizers play an important role in increasing productivity in agricultural production. The use of chemical fertilizers has increased rapidly all over the world, but a quality and sustainable production model has not been reached.

In the study, it was aimed to determine the efficiency of chemical fertilizer use in crop production and to compare the differences between Balkan countries. These countries are Albania, Bulgaria, Bosnia and Herzegovina, Croatia, Greece, Montenegro, North Macedonia, Romania, Serbia and Turkey. Data Envelopment Analysis was used in the efficiency analysis under the assumption of variable returns to scale. The input variables used in the analysis were determined as the crop production areas of the countries, the total amount of nitrogen, phosphorus and potassium and the output variable as the total crop product income of the countries. The data covers the years 2015-2018 and were obtained from the FAO database.

As a result of the study, the average fertilizer use efficiency score was found to be 0.808 in the Balkan countries. Accordingly, the same crop production value is possible to achieve by using 19.2% less input. Albania, Montenegro, North Macedonia, Greece and Turkey were determinate as efficient countries. While efficient countries do not need to make any changes in their inputs, other countries need to reduce their input use by up to 50% or include products that will provide higher returns in their production patterns.

Keywords: data envelopment analysis, crop production, economic efficiency

INTRODUCTION

Changes in the ecological system due to climate change and the negative effects caused by the increase in the demand for agricultural products have increased the tendency of imbalances in agricultural products markets all over the world. These developments have made agricultural production efficiency more important than ever. In order to meet the rapidly increasing food demand of human beings, it has become necessary to increase productivity. The main way to achieve this is through the conscious and effective use of modern agricultural techniques. Chemical fertilizers play an important role in increasing productivity in agricultural production. The use of chemical fertilizers has increased rapidly all over the world, but a quality and sustainable production model has not been reached.

In crop production; It is important to use all inputs effectively in the development of quality, economic and sustainable management strategies. In the study, the efficiency of agricultural input use will be discussed in the context of chemical fertilizers.

The concept of efficiency in the literature is based on the study by Koopsman (1951) in which he explains the concept of technical efficiency. Koopsman defined technical efficiency as an input/output vector in which it is not technologically possible to increase any output without simultaneously reducing the other output. Later, Farrell (1957) developed the concept of technical efficiency as the measure of the maximum radial decrease in all inputs compatible with the production of observed outputs (Okursoy and Tezsürücü, 2014).

The aim of the study is to determine the efficiency of chemical fertilizers used in crop production in the Balkan countries and to compare the differences between the countries. Data Envelopment Analysis (DEA) was used in the efficiency analysis.

Balkan countries consist of Albania, Bulgaria, Bosnia and Herzegovina, Croatia, Montenegro, Kosovo, North Macedonia, Romania, Serbia, Slovenia, Greece and Turkey. Turkey; due to the fact that the Thrace Region is located in this region, it is among the Balkan Countries.

When the general economic structure of the Balkan countries is examined, it is seen that they are not among the major economies in the world. It is seen that agriculture and tourism incomes come to the fore in these countries. Table 1 shows the per capita national income of countries according to purchasing power parity, the share of agricultural national incomes in gross domestic product (GDP) and agricultural employment rates. The country with the highest per capita income in purchasing power parity is Slovenia. Romania, Croatia and Greece follow Slovenia, respectively. The country with the lowest per capita income is Albania.

When the share of agricultural income in GDP in the Balkan countries is examined, it is seen that Albania takes the first place with 19.3%. Albania is followed by North Macedonia, Turkey, Serbia and Montenegro, respectively. The countries with the lowest share of agriculture in GDP are Slovenia, Croatia and Bulgaria, respectively.

In Table 1, it is seen that agricultural employment rate of the Balkan countries. Albania has the highest share with 36% agricultural employment rate. Slovenia has the lowest share of agricultural employment with 2%.

The distribution of agricultural land in the Balkan Countries is given in Table 2. It is seen that the largest agricultural land is in Turkey with 37.7 million hectares, and the smallest one is Montenegro with 257 thousand hectares. Considering the share of agricultural land in the total surface area, it is seen that Romania is in the first place with 60%. Romania is followed by North Macedonia with 50%, Turkey with 49% and Greece with 47.4%. The country with the lowest ratio of agricultural lands to total surface area is Montenegro with 19.1%.

Table 1. Some Economic Indicators in Balkan Countries

	GDP per capita PPP (current international $)	Agriculture, forestry, fishing value added (% of GDP)	Employment in agriculture (% of total employment - ILO estimate)
Albania	13818	19.3	36
Bosnia and Herzegovina	15612	6.2	18
Bulgaria	24367	3.4	7
Croatia	28504	3.3	6
Greece	28464	4.1	12
Montenegro	20567	6.4	7
North Macedonia	16927	9.1	14
Romania	31946	3.8	21
Serbia	19231	6.5	16
Slovenia	39593	2.0	4
Turkey	28119	6.6	18

Source: World Bank, 2021

Table 2. Distribution of the Agricultural Land in Balkan Countries

1000 ha	Albania	Bosnia and Herzegovina	Bulgaria	Croatia	Greece	Montenegro
Agricultural land	1174	2216	5037	1504	6103.6	257
Cropland	696	1121	3629	897	3221.6	15
Meadows and past	478	1095	1408	607	2882	243
Forest land	789	2188	3880	1937	3902	827
Land area	2740	5120	10856	5596.4	12890	1345
Agr. land %	42.8	43.3	46.4	26.9	47.4	19.1
1000 ha	Romania	North Macedonia	Serbia	Slovenia	Turkey	
Agricultural land	13826	1265	3482	612.28	37716	
Cropland	9378	460	2785	234.46	23099	
Meadows and past	4448	805	675	377.82	14617	
Forest land	6929	1001	2722	1240	22064	
Land area	23008	2522	8746	2013.64	76963	
Agr. land %	60.1	50.2	39.8	30.4	49.0	

Source: FAO, 2021

In Tables 3a, 3b and 3c, the yield per decare of some important herbal products in the Balkan countries is given. It is seen that each country has a yield advantage for certain products. The products with the highest yield per decare of the countries can be listed as follows. Greece in apple, cucumbers and maize production, Croatia in barley, wheat and tobacco production, Turkey in tomato, potato, rice and green bean production, North Macedonia in fig production, Montenegro olive production, Albania orange production, Slovenia sugar beet production, Serbia sunflower production.

Table 3a. Yields of Some Important Crops in Balkan Countries (kg/da)

	Apples	Barley	Beans, green	Cucumbers and gherkins	Figs
Albania	2403.74	289.64	1249.8	4771.36	1479.11
Bosnia and Herzegovina	317.66	356.14	323.59	1188.79	196.00
Bulgaria	1053.62	502.68	508.7	3559.51	200.00
Croatia	1380.81	519.19	647.62	3333.33	192.86
Greece	2821.89	276.52	1058.87	7881.05	494.49
Montenegro	677.27	253.85	193.07		300.23
North Macedonia	910.31	315.09	83.13	4924.61	1647.37
Romania	934.21	418.80	496.1	1716.79	-
Serbia	1914.9	372.90	160.24	983.81	-
Slovenia	2390.75	484.77	550.00	1721.43	400.00
Turkey	2074.51	265.65	1363.95	4980.11	594.83

Source: FAO, 2021

Table 3b. Yields of Some Important Crops in Balkan Countries (kg/da)

	Maize	Olives	Oranges	Potatoes	Rice, paddy
Albania	705.29	208.57	4959.66	2564.55	-
Bosnia and Herzegovina	644.08	177.57	50.38	1113.67	-
Bulgaria	723.79	-	-	2124.97	600.00
Croatia	898.17	178.51	1020.00	1843.98	
Greece	1068.07	135.99	2800.40	2948.03	739.89
Montenegro	439.06	221.43	982.43	1639.51	
North Macedonia	427.02	211.59	-	1435.88	611.26
Romania	649.99	-	-	1508.61	532.03
Serbia	763.40	-	-	2058.30	-
Slovenia	926.85	-	-	2355.71	-
Turkey	940.34	173.46	2263.29	3537.66	791.02

Source: FAO, 2021

Table 3c. Yields of Some Important Crops in Balkan Countries (kg/da)

	Sugar beet	Sunflower seed	Tobacco	Tomatoes	Wheat
Albania	3814.29	230.75	173.00	4497.51	406.80
Bosnia and Herzegovina	2137.50	220.43	127.15	1261.55	383.92
Bulgaria		237.53	183.05	2815.73	527.22
Croatia	6119.00	296.16	200.00	6881.25	561.14
Greece	4929.81	296.82	158.11	5387.54	279.39
Montenegro			198.26	3412.87	281.82
North Macedonia	2973.20	142.00	157.29	2771.48	348.48
Romania	4035.02	278.25	134.44	1835.79	474.88
Serbia	5419.30	332.30	113.80	1415.30	438.90
Slovenia	6266.67	242.42	-	4095.45	523.05
Turkey	5832.16	279.37	86.38	7075.94	278.11

Source: FAO, 2021

MATERIAL AND METHOD

In the study, the efficiency of fertilizer use in the Balkan countries was analyzed. Data Envelopment Analysis (DEA) was used in the efficiency analysis. DEA, which is a non-parametric method, uses linear programming to determine the points on the curve obtained by using the inputs and outputs of the most efficient firm, instead of using any production function (Fanchon, 2003). In DEA, which is a multivariate productivity analysis model used to measure the relative efficiency of homogeneous decision units, the measurement of efficiency is calculated as the Weighted Sum of Outputs / Weighted Sum of Inputs (Talluri, 2000).

In DEA, technical efficiency is divided into two disjoint components, pure technical efficiency and scale efficiency. This separation allows understanding the reason for the inefficiency in resources. A measure of pure technical efficiency can be found by estimating the efficient frontier under the assumption of variable returns to scale. It is a measure of technical efficiency without scale efficiency and reflects managerial performance to regulate inputs in the production process. The ratio of technical efficiency to pure technical efficiency gives scale efficiency. The measurement of scale efficiency provides management competence to choose the optimum resource size (Kumar and Gulati, 2008). The level of success shown in the realization of production at the appropriate scale level can be expressed as scale efficiency (Gökgöz, 2009). Returns to scale, increasing returns to scale IRS (Increasing Returns to Scale), constant returns to scale CRS (Constant Returns to Scale), decreasing returns to scale DRS (Decreasing Returns to Scale), which is an economics definition showing that an increase in the amount of input will affect the potential production capacity) in three different ways (Wang and Cui, 2010).

There are two DEA models commonly used in the literature. These models are the CCR model introduced by Charnes, Cooper and Rhodes (1978) with the assumption of constant returns to scale, and the BCC model, which is based on the assumption of variable returns to scale, introduced by Banker, Charnes and Cooper (1994) (Okursoy and Tezsürücü, 2014).

In the study, the input-oriented BCC model, which is based on the assumption of varying returns to scale, was used. The input variables used in the analysis were determined as the crop land of the countries, the total nitrogen, phosphorus and potassium usage amounts, and the output variable as the total crop product income of the countries. The data were analyzed by taking the average of the years 2015-2018 in order to minimize the effect of yield differences over the years. The data used in the analysis was obtained from the FAO (2021) database.

In Table 4, fertilizer active ingredients used per decare and agricultural income per decare are given. When the fertilizer use is examined, it is seen that the country with the highest fertilizer use per unit area is Slovenia with 11.58 kg/da N. Slovenia is followed by Croatia with 9.86 and Bulgaria with 9.53. When the crop production value per decare is analyzed, Montenegro takes the first place with 504 US$/da. The country with the lowest income is Bosnia and Herzegovina with 89.56 US$/da.

Table 4. Fertilizer Active Ingredients and Crop Production Value in Balkan Countries

	N (Nitrogen) (kg/da)	P (Phosphorus) (kg/da)	K (Potassium) (kg/da)	Gross Product Value (US$/da)
Albania	5.15	3.51	0.17	174.69
Bosnia and Herzegovina	7.85	0.88	1.00	89.56
Bulgaria	9.53	2.01	1.01	111.63
Croatia	9.86	3.10	3.84	181.30
Greece	5.39	1.64	1.40	239.99
Montenegro	8.47	3.76	3.76	503.98
North Macedonia	4.45	1.04	0.81	213.87
Romania	4.28	1.63	0.57	122.86
Serbia	6.81	1.77	1.64	160.38
Slovenia	11.58	3.89	4.76	152.30
Turkey	7.09	2.82	0.52	182.45

Source: FAO. 2021

RESULTS AND DISCUSSION

Data Envelopment Analysis was applied to measure the input use efficiency of chemical fertilizers used in crop production in Balkan countries. Table 5 includes DEA results. As a result of the analysis involving 11 countries, the average pure technical efficiency score was calculated as 0.808. Accordingly, while the Balkan countries reach the same agricultural product value, average fertilizer use will be reduced by 19.2%. On the other hand, the mean scale efficiency score was calculated as 0.846. In general, it can be said that the Balkan countries are able to realize agricultural production at the appropriate scale level.

When country-based analysis is made, it is seen that Albania, Greece, Montenegro, North Macedonia and Turkey have full efficiency (pure technical efficiency). According to this result, it is seen that the use of inputs in agricultural production in countries with full efficiency is administratively perfect. The scale effectiveness scores of Greece and Turkey, which have full efficiency, were calculated as 0.878 and 0.906, respectively. This means that although the production management has been successful in terms of input usage in these two countries, the appropriate scale level has not been reached.

Albania, Montenegro and North Macedonia are the countries with the highest scores in terms of both technical efficiency and scale efficiency. When the return to scale situation is examined in countries that have not achieved scale efficiency, it is seen that all of them have decreasing returns to scale. This means that the increase in the amount of input in these countries will reduce the potential production capacity.

The countries with the lowest efficiency score (pure technical efficiency) are Bosnia and Herzegovina with 0.507, Bulgaria with 0.515 and Slovenia with 0.568. These countries will achieve the same agricultural income when they reduce their use of fertilizers and land by an average of 49.3%, 48.5% and 43.2% respectively. Although the efficiency scores of Bosnia and Herzegovina and Bulgaria were low, the scale efficiency scores were 0.980 and 0.903, respectively. It can be said that efficiency cannot be achieved in terms of input use in these countries, but the agricultural production of the countries is maintained at an appropriate scale.

Table 5. Results of the Data Envelopment Analysis

	Total Technical Efficiency (CRS)	Pure Technical Efficiency (VRS)	Scale Efficiency	Return to Scale
Albania	1.000	1.000	1.000	-
Bosnia and Herzegovina	0.497	0.507	0.980	drs
Bulgaria	0.465	0.515	0.903	drs
Croatia	0.415	0.741	0.560	drs
Greece	0.878	1.000	0.878	drs
Montenegro	1.000	1.000	1.000	-
North Macedonia	1.000	1.000	1.000	-
Romania	0.660	0.895	0.738	drs
Serbia	0.547	0.666	0.822	drs
Slovenia	0.292	0.568	0.514	drs
Turkey	0.906	1.000	0.906	drs
Average	0.696	0.808	0.846	

CONCLUSIONS

As a result of the study, it was concluded that the countries with full efficiency in the use of fertilizers are Albania, Greece, Montenegro, North Macedonia and Turkey. Although the country with the lowest efficiency is Bosnia and Herzegovina, it has a very high efficiency score in terms of scale efficiency. Similarly, although the efficiency score of Bulgaria is low, the scale efficiency is quite high. Slovenia has low scores for both technical efficiency and scale efficiency.

In the analysis, crop land and active ingredients of fertilizers were used as input variables; agricultural income was used as output variable. When the input use of the countries with low efficiency scores is analyzed, it is seen that there is a very high amount of fertilizer use per unit area in Slovenia and Bulgaria. These countries should reduce their use of fertilizers in order to increase the efficiency of fertilizer use. Reducing the use of fertilizers will contribute to sustainable agricultural activities as well as providing input use efficiency.

In countries such as Bosnia and Herzegovina and Bulgaria, although the use of inputs is not high, the efficiency score is low. The reason for this is the low agricultural income of these countries. In order to increase agricultural income, giving priority to products with high added value, as climatic conditions allow, will increase the output and increase the efficiency of fertilizer use.

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MOLECULAR AND MORPHOLOGICAL IDENTIFICATION IN THRIPS (THYSANOPTERA: THRIPIDAE) SPECIES IN ARTVIN (TURKEY)

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ABSTRACT
Molecular techniques as a basic and correct approach identifying thrips species, when the morphological approach is unsure or even impossible. Several thrips species (Insecta, Thysanoptera) are universally known as crop pests and vectors of viral diseases, but their identification is hard because of their minor body size and hidden morphological differences. Additionally, DNA barcoding can be considered a valuable alternative to the classic morphology method for identification of major thrips. Sequencing variation in the mitochondrial cytochrome c oxidase I (COI) region has been shown to be useful for identification not only thrips but also many insect pests. DNA barcoding based on nucleotide sequencing analysis of the mitochondrial cytochrome oxidase I (COI) and Internal transcribed spacer (ITS) gene were shown to provide sufficient variation to be used within the genus *Thrips* and *Frankniella*. In that study, DNA barcoding for molecular identification was used to support the thrips species characterization by two gene regions. This study also aimed to construct a molecular and morphological identification key for several thrips species including (*Thrips physapus* Linnaeus, *T. meridionalis* (Priesner), *Frankliniella tenuicornis* (Uzel), *F. occidentalis* (Pergande) and *F. intonsa* (Trybom)). Individuals were collected from Yusufeli (Artvin) located northeast of Turkey between 2016 and 2017.

Key words: Thrips, COI, ITS, Morphological identification, Artvin

INTRODUCTION

Thrips (Thysanoptera) are very small insects, widespread throughout the world as in Turkey. Of the approximately 6,000 species so far diagnose, only a few hundred are crop pests, causing important damage or transmitting diseases to growing crops and harvestable product in most countries. Their minute size makes them difficult to notice either in the field or in food transported during international trade of vegetables, fruit and ornamental flowers. As a pest, thrips have been studied seriously for over a century Parker et al. (2013); (Wiki, 2014).

Thrips Linnaeus is one genus of Thysanoptera which has shown common species in most parts of the world, and includes many species of economic importance (Bhatti, 1980). *Thrips tabaci* Lindeman, known as an onion thrips and a vector of virus like tomato spotted wilt virus (TSWV), Iris yellow spot virus (IYSV), is a highly polyphagous species and is distributed widely throughout the world (Zawirska, 1976; Doi et al., 2003). *Thrips meridionalis* (Priesner) (peach thrips), is a species widely distributed in the warmer parts of Europe and live in the flowers of almond, apple, apricot, cherry, grapevine, nectarine, pear, peach, plum and other Rosaceae species (Alford, 2007; Hazır et al., 2011; Uzun et al., 2015; Kaplan et al., 2016).

The western flower thrips, *Frankliniella occidentalis* (Pergande)(Thysanoptera: Thripidae), has become a global invasive and dominant pest of agricultural in the last 30 years (Kirk, 2002). It can cause direct damage to vegetable, fruit and ornamental crops, and transport tospoviruses to a wide variety of plant hosts (Mound, 1997; Ullman et al., 2002). The flower thrips *Frankliniella intonsa* (Trybom) is polyphagous pest affecting widely diverse ornamental and vegetable crops in the world (Mound and Lewis, 1997). After sucking cell fluids by adults and nymphs, there are silvery scars and leaf chlorosis on the leaves, stems, flowers, and fruit surfaces. *Frankliniella intonsa* is also known as a vector of Tomato spotted wilt virus (Inoue et al., 2004). *Frankliniella tenuicornis* (Uzel) (Thysanoptera: Thripidae) is widespread and an important thrips species especially on Gramineae (Köppä, 1970; Michael and Lima, 2020). It causes injuries on grain malformations or complete sterilization (Przybylska et al., 2016).

DNA barcoding is a system designed to provide quick and direct species diagnose by using short, standardized gene regions as interior species labels. The barcoding system can make the Linnaean taxonomic system more reachable for the control of pests, invasive species, and food safety by rapidly assigning known or new specimens and highlighting divergent taxa (Hebert and Gregory, 2005).

In present study, DNA barcoding for molecular identification was used to support the thrips species characterization by two gene regions (COI and ITS). This study also aimed to construct a molecular and morphological identification key for several thrips species including (*Thrips physapus*, *T. meridionalis*, *Frankliniella tenuicornis*, *F. occidentalis* and *F. intonsa*) collected from Yusufeli (Artvin) located northeast of Turkey (2016- 2017).

MATERIAL AND METHOD

The samples were collected in 70% ethanol and stored at + 4°C. The Hoyer's medium preparation methods were used in the morphological diagnoses. For the Hoyer preparation to dehydrate the samples were used to Phenol + Lactic Acid (1: 1) solution after the samples were stayed in hoyer medium. The samples were diagnosed to follow zur Strassen (2003) book .

After the pre-diagnosis step, individually for DNA isolation the 'CTAB' protocol was used (Doyle and Doyle, 1987). The COI deg primers was used for the mitochondrial "Cytochrome Oxidase Subunit I" (COI) gene region (~350 bp) (Timm et al., 2008). The ITS primers (~650 bp) were set up by Assoc. Dr. Cengiz IKTEN. The PCR cycling annealing conditions consisted 53°C for the COI and 60°C for the ITS primers. the sequencing results were taken from BM Labosis.

After taking sequencing results, the samples individually were analyzed in the NJ (Neighbor Joining) methods using Mega X program (using the Kimura 2-parameter model (Kimura, 1980)).

RESULTS AND DISCUSSION

In present study also aimed to construct a molecular and morphological identification key for *T. physapus*, *T. meridionalis*, *F. tenuicornis*, *F. occidentalis* and *F. intonsa*, collected from Yusufeli (Artvin) located northeast of Turkey between 2016 and 2017.

Molecular Results

The COI and ITS results were inferred using the Neighbor-Joining method theorized by Saitou and Nei (1987) (Figure 1 and 2). The COI optimal tree with the sum of branch length = 0.68816421 is shown. The ITS optimal tree with the sum of branch length = 0.24506179 is shown. Both genes of the evolutionary distances were computed using the p-distance method constructed by Nei and Kumar (2000) and are in the units of the number of base differences per site. The COI analysis involved 9 nucleotide sequences. Codon positions included were 1^{st} +2^{nd} +3^{rd} +Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 284 positions in the COI final dataset. The ITS analysis involved 16 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 664 positions in the ITS final dataset. Evolutionary analyses of both genes were conducted in MEGA X conducted by Kumar et al. (2018). Molecular data indicate that different species in the genus *Thrips* are located in distinct groups in phylogenetic tree. The COI overall distance (0.19) (Table 1.) and the ITS overall distance (0.50) (Table 2.) results show that molecular keys can be a useful on thrips identification for pest management and plant quarantine purposes.

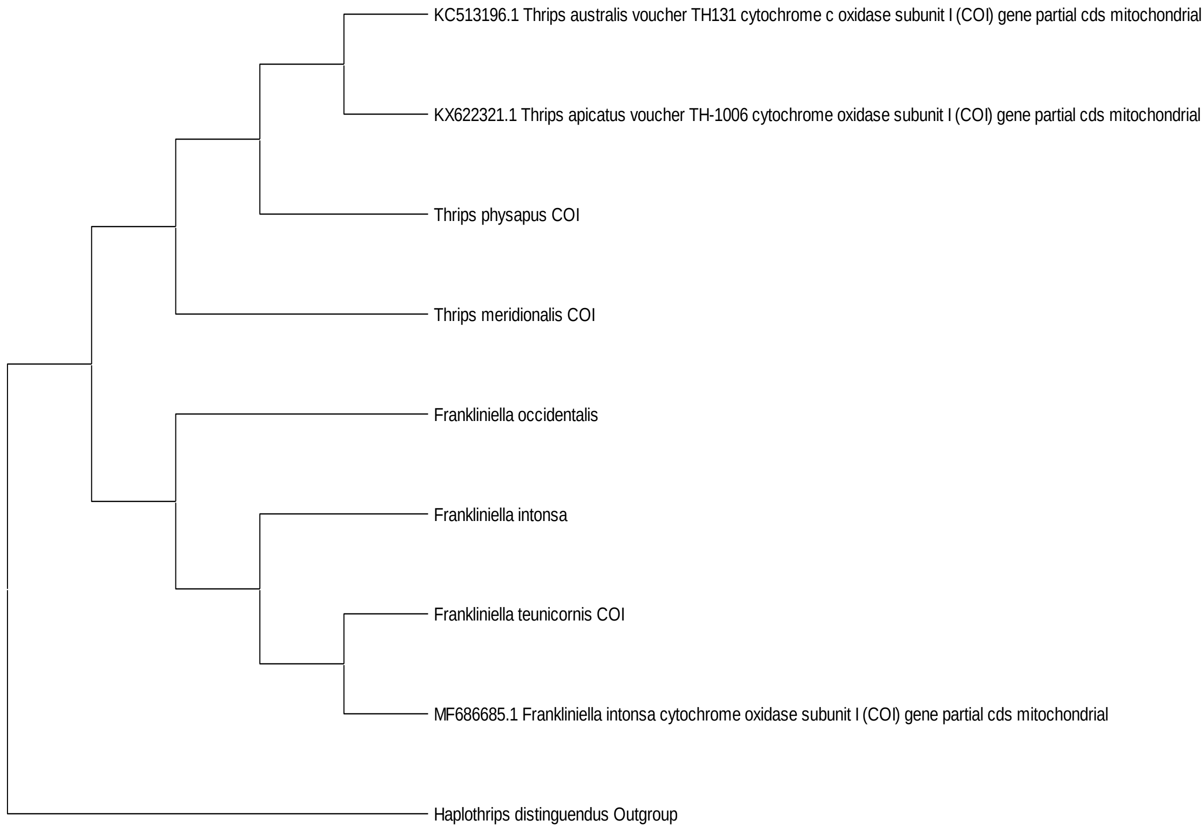

Figure 1. The COI gene of Neighbor-Joining phylogenetic tree, outgroup: *Haplothrips distinguendus* (Uzel)

Table 1. The COI overall distance (0.19)

		1	2	3	4	5	6	7	8	9
1	*Thrips physapus*_COI									
2	*Thrips_meridionalis*_COI	0.14								
3	KC513196.1_*Thrips australis*	0.17	0.15							
4	KX622321.1_*Thrips_apicatus*	0.15	0.13	0.14						
5	*Frankliniella occidentalis*_COI	0.20	0.17	0.20	0.15					
6	*Frankliniella_intonsa*_COI	0.18	0.21	0.19	0.19	0.19				
7	*Frankliniella_teunicornis*_COI	0.17	0.21	0.19	0.19	0.19	0.00			
8	MF075957.1_*Frankliniella schultzei*	0.18	0.18	0.16	0.16	0.20	0.17	0.18		
9	MF686685.1_*Frankliniella_intonsa*	0.19	0.21	0.19	0.21	0.19	0.00	0.02	0.19	
10	*Haplothrips distinguendus*_outgroup	0.30	0.28	0.28	0.31	0.29	0.33	0.33	0.31	0.32

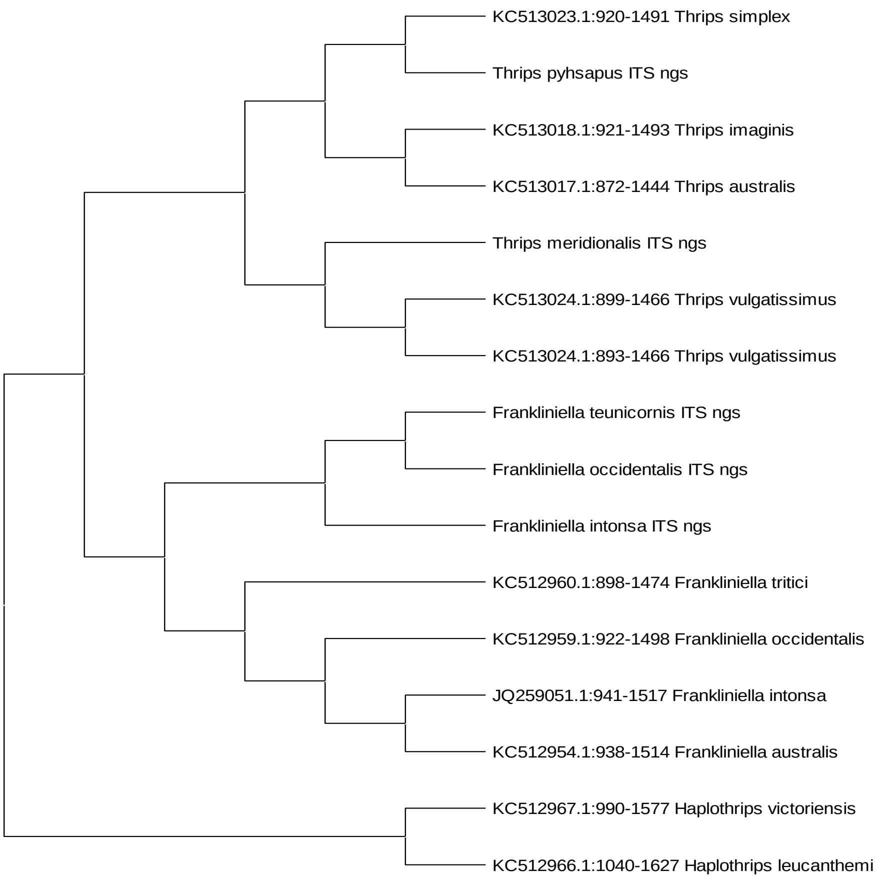

Figure 2. The ITS gene of Neighbor-Joining phylogenetic tree, outgroup*: Haplothrips victoriensis* Bagnall and *H. leucanthemi* (Schrank)

Table 2. The ITS overall distance (0.50)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Thrips_pyhsapus*_ITS																
2	*Thrips_meridionalis*_ITS	0.7															
3	KC513024.1:*Thrips_vulgatissimus*	0.8	0.7														
4	KC513024.1:*Thrips_vulgatissimus*	0.2	0.7	0.7													
5	KC513023.1:*Thrips_simplex*	0.2	0.7	0.8	0.2												
6	KC513018.1:*Thrips_imaginis*	0.6	0.8	0.8	0.6	0.6											
7	KC513017.1:*Thrips_australis*	0.6	0.8	0.8	0.6	0.6	0.0										
8	KC513013.1:*Tenothrips_frici*	0.2	0.7	0.7	0.1	0.2	0.5	0.5									
9	KC512960.1:*Frankliniella_tritici*	0.5	0.7	0.8	0.5	0.6	0.6	0.6	0.5								
10	KC512959.1:*Frankliniella_occidentalis*	0.5	0.7	0.8	0.5	0.6	0.6	0.6	0.5	0.0							
11	JQ259051.1:*Frankliniella_intonsa*	0.5	0.7	0.8	0.5	0.6	0.6	0.6	0.5	0.0	0.0						
12	KC512954.1:*Frankliniella_australis*	0.5	0.7	0.8	0.5	0.6	0.6	0.6	0.5	0.0	0.0	0.0					
13	*Frankliniella_teunicornis*_ITS	0.5	0.7	0.7	0.5	0.6	0.6	0.6	0.5	0.0	0.0	0.0	0.0				
14	*Frankliniella_occidentalis*_ITS	0.5	0.7	0.8	0.5	0.6	0.6	0.6	0.5	0.0	0.0	0.0	0.0	0.0			
15	*Frankliniella_intonsa*_ITS	0.5	0.7	0.8	0.5	0.6	0.6	0.6	0.5	0.0	0.0	0.0	0.0	0.0	0.0		
16	KC512967.1:*Haplothrips_victoriensis*	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	
17	KC512966.1:*Haplothrips_leucanthemi*	0.6	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.0

Morphological Results

The zur Strassen book was used for the morphological identification key for *T. physapus*, *T. meridionalis*, *F. tenuicornis*, *F. occidentalis* and *F. intonsa*. *Thrips physapus* has 7-segmented antennae, metanotum campaniform sensilla absent. Fore wing first vein with 3 setae on distal half (Figure 3a-d). Besides the fore wing first vein of *T. meridionalis* with 2 setae on distal half, antennae 8-segmented, metanotum campaniform sensilla present (Figure 3e-h).

Frankliniella genus species has three pairs of ocellar setae, both vein of the fore wing with 2 complete rows of setae, complete posteromarginal comb on VIII. abdominal tergit etc. but there were some differences between them; *F. occidentalis* body is mainly yellow and metanotum campaniform sensilla usually present. When *F. intonsa*'s body colour brown and its metanotum has not campaniform sensilla. In additions *F. tenuicornis* female dark brown and metanotum campaniform sensilla is absent (Figure 4a-m).

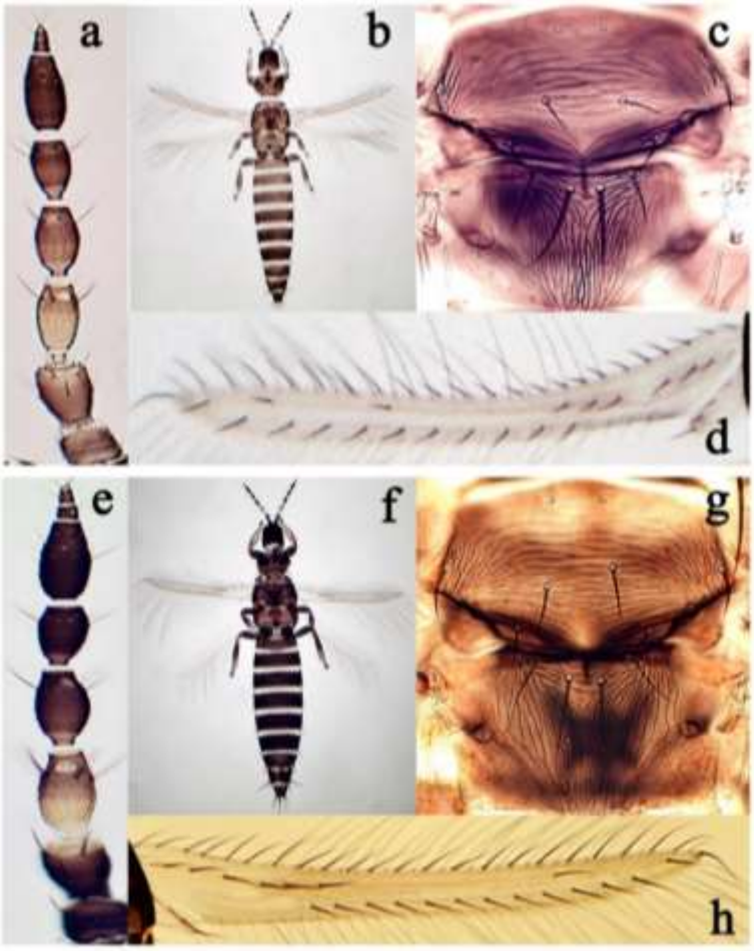

Figure 3. *Thrips physapus* a.antennae b.body c.metanotum d. fore wing; *Thrips meridionalis* e.antennae f.body g.metanotum h.fore wing.

Research on thrips has been focused generally on a few economically important pest species (Mound, 2001). Systematics of thrips remain based entirely on morphology. Generally poly classifications have been suggested (Bhatti, 1988; 1992; Zherikhin, 2002; Bhatti, 2006). Crespi et al. (1996) studied cytochrome oxidase I and ITS gene regions for the thrips' phylogenetic relationships in eight ingroup taxa. Buckman et al. (2013) phylogenies study was constructed based upon 70 thrips genera were sequenced from five genetic loci. The study on molecular diagnoses Thysanoptera has raised in recently. Therefore this, the benefit of molecular data can be investigate deeply and the thrips phylogeny needs new extensive studies as mentioned by Buckman et al. (2013).

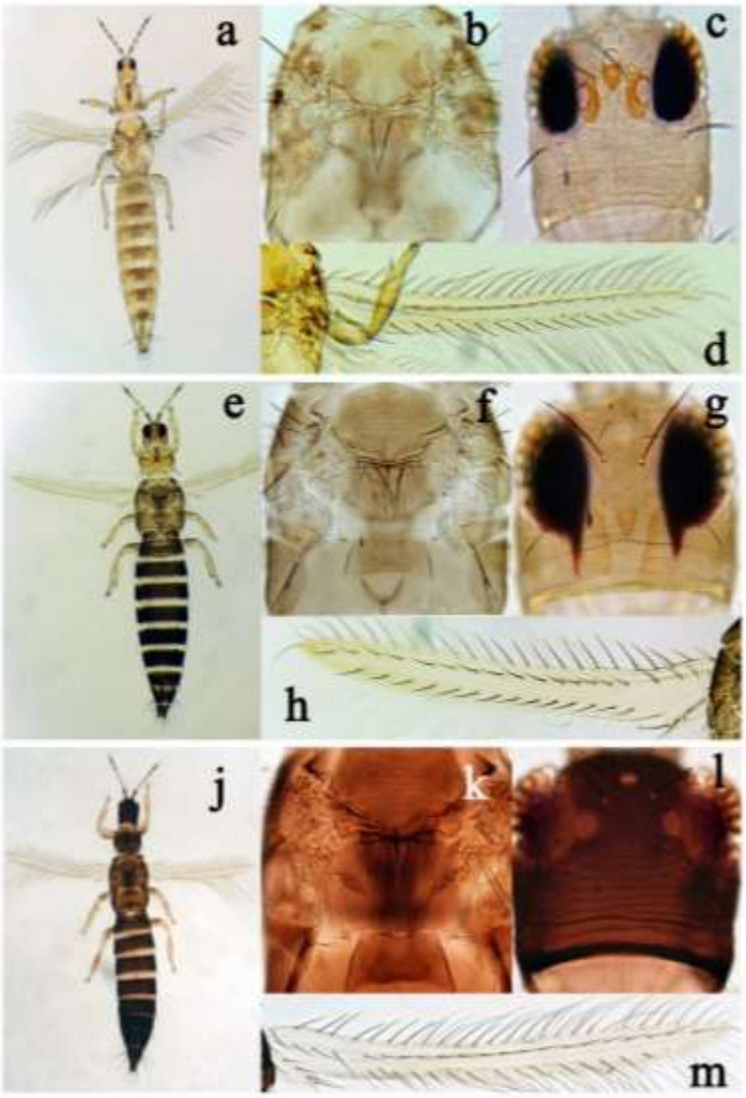

Figure 4. *Frankliniella occidentalis* female a.body b.metanotum c.head d.fore wing; *Frankliniella intonsa* female e.body f.metanotum g.head h.fore wing; *Frankliniella tenuicornis* female j.body k.metanotum l.head m.fore wing

CONCLUSIONS

Based on the study, molecular data indicate that the two species in the genus *Thrips* and three species in the *Frankliniella* genus are located in distinct groups in phylogenetic tree. The COI overall distance (0.19) and the ITS overall distance (0.50) results show that molecular keys can be a useful on thrips identification for several purposes. Maybe the study will be study in large scales samples to perform all thrips species too use both methods for diagnose.

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THE EFFECTS OF CORN AND SUNFLOWER STRAW ADDED TO A SOIL WITH HIGH CLAY CONTENT ON WATER HOLDING AND MODULUS OF RUPTURE VALUE OF SOILS

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ABSTRACT

As in the whole world, corn and sunflower are among the main food sources in Turkey. Due to the fact that both products have high nutritional value, the storage, transportation and processing process is easier than many products, and they adapt to the environment very quickly, their cultivation has increased in our country in recent years. However, corn and sunflower straw have the capacity to contribute high organic matter to soils. In this study, an incubation experiment was established in 5 kg pots by mixing 0.5%, 1, 2 and 4 % corn and sunflower straw in a soil with high clay content. Sampling was made after the 30th day of the established trial, and field capacity and wilting point and modulus of rupture values of the soils were analyzed. As a result of the analysis, it was found that the field capacity value increased from 37.99% to the highest 43.17%, and there was no statistically significant difference in the wilting point value. The modulus of rupture value, which was measured as 2910 mbar in the control soil, decreased as the dose rates increased, and it was measured as 552 and 993 mbar at the highest doses of corn and sunflower straw, respectively. According to the results of this short-term incubation, it has been observed that corn and sunflower straw have a great effect on the physical properties of the soils, and it is anticipated that it will shed light on other field studies.

Keywords: Corn straw, Sunflower straw, Field capacity, Modulus of rupture

INTRODUCTION

As in the whole world, corn and sunflower are among the main food sources in Turkey. It has been ensured that both products have become a food source cultivated all over the world due to reasons such as having high nutritional value, easier storage, transportation, and processing compared to many products, and adapting to the environment very quickly. In addition, it is a product that has strategic value in terms of social and cultural aspects, as well as being used in livestock activities, economy, field agriculture (Li, 2017). Turkey is one of the world's leading grain producer countries. Among the cereal crops, maize, whose production has been increasing rapidly in recent years, draws attention. According to 2020 data in our country, corn and sunflower plants are grown in 6.388287 and 7.526318 decares, respectively (TÜİK 2020). Turkey's estimated annual plant stem production is 56 million tons, of which 36 million tons of wheat straw, 8 million tons of barley straw, 2.5 million tons of corn straw, 3 million tons of cotton straw, 2.0 million tons of sunflower straw. Wheat straw is mostly used as animal feed, compost material, as well as many agricultural and industrial materials. However, sunflower and corn stalk cannot be used as animal feed due to their high fiber structure. In today's conditions, corn straw is used as animal litter in cattle farms. Sunflower stalks are mostly dried and still used as fuel. Corn and sunflower straw are a residual by-product of the production of these products at harvest. The total biomass of this product depends on various factors such as the variety selected, soil structure, nutrient management, and weather conditions. At harvest, maize and sunflower straw are collected or spread over the field using stationary threshers or self-propelled combines, depending on harvesting threshing methods (Sutherland vd. 2001; Dexter, 1997; Li vd. 2002). The amount of straw removed from the field depends mainly on the cutting height (ie the height of the stubble remaining in the field). The straw left in the field after harvest can be collected, burned, or mixed with the soil and left to decompose. In addition, the uncut portion of both crops remains in the fields after harvest and can be incinerated or incorporated into the soil to prepare the next crop. However, it is thought that due to the high carbon and nitrogen content of the harvest residues of materials such as corn and sunflower that constitute high harvest residues, they will provide an organic contribution to our soils.

It has been determined by literature studies that the organic matter content of most of the soils in the Central Anatolia region is low. Considering that the organic matter content has an effect on all general properties of the soil, it is thought that the straw mixture can be a source of organic matter by decomposing in large quantities. In addition, the most important agricultural potential in our region, which is in the arid and semi-arid climate zone, is to increase the storage capacity of water in the soil, both in irrigated farming areas and in fallow areas. In addition, another problem encountered in our region's soils due to the low organic matter content is the crusting layer. For this reason, in this study, the effects of sunflower and corn straw on soil water retention and modulus of rupture values were investigated in a short-term incubation study with different doses of sunflower and corn straw due to the problems in physical soil properties.

MATERIAL AND METHOD

For the establishment of an incubation experiment, soil samples were taken from the area where irrigated farming is carried out with high clay content and where corn-sunflower and wheat cultivation is made intensively. The sample, which was taken through a 4 mm sieve in the field, was laid in the laboratory in order to bring it to air dry condition. In October-November, corn and sunflower straw were obtained from the farmers grown in our region. The supplied straw was dried in the open place until the air came to dry conditions and then dried in an oven at 70 °C until it reached a constant weight. After the dried samples were ground with the help of a grinder, they were stored in airtight storage containers until the beginning of the brewing. The incubation experiment was carried out in 5 kg pots and 3 replications. After the moisture determination was made for the air-dry soils, the pots were filled to a 5 kg oven dry weight. The ground straw mixtures were weighed 1-2% and 4% into the pots and mixed. The mixed materials will be irrigated with distilled water to reach 80% of the previously determined field capacity and the incubation trial will be started. Incubation continued for 30 days, irrigation and mixing were done throughout the incubation. At the end of the incubation, field capacity, wilting point and breaking value analyzes were made in the soils (Cassel and Nielsen, 1986; Reeve,1965).

RESULTS AND DISCUSSION

When the results of the short-term incubation carried out under laboratory conditions were examined, the applications caused significant differences in the physical properties of the soil. With global warming and increasing the protection of water resources becoming important, it is important to improve the water holding capacity of soils, especially in arid and semi-arid areas (Şeker and Karakaplan 1999). When the effect of the applications on the field capacity value was examined, the field capacity of the control soil, which was measured as 37.99 g g^{-1}, increased as the application doses increased, and a statistically significant difference was obtained ($p<0.05$). It is observed that the highest increase occurred in the 4% dose of corn straw application (Table 1). When corn and sunflower straw were compared, it was found that corn straw application had a higher effect on field capacity value.

When the wilting point values were examined, although there was a statistically significant difference between the values ($p<0.05$), high differences were not found between the values. The wilting point value is mostly the textural properties of the soils and the effect of the water held in their micropores. The study showed that the effect on micropores in short-term incubation is less than expected.

When the available water capacity, which is the most important of the irrigation parameters, was examined at the end of the experiment, it was calculated that it increased depending on the increase in field capacity. While the available water capacity was calculated as 16.07 g g^{-1} in the control soil, this value increased by 35 and 27%, respectively, at the highest dose of corn and sunflower application.

Table 1. The effect of corn and sunflower wastes ground and applied in different doses on the water holding capacity of the soil.

Practices	**Field Capacity (g g^{-1})**	**Permanent wilting point (g g^{-1})**	**Available water capacity (g g^{-1})**
C	37.99 ± 0.36^{d}	21.93 ± 0.21^{a}	16.07 ± 0.48^{d}
S0,5	39.81 ± 0.18^{cd}	21.73 ± 0.06^{a}	18.08 ± 0.22^{cd}
S1	39.75 ± 0.23^{cd}	20.90 ± 0.31^{b}	18.85 ± 0.19^{bc}
S2	40.03 ± 0.38^{c}	19.88 ± 0.34^{c}	20.15 ± 0.28^{abc}
S4	40.51 ± 0.57^{bc}	20.10 ± 0.27^{c}	20.41 ± 0.81^{ab}
Co0,5	39.73 ± 0.35^{cd}	21.64 ± 0.10^{a}	18.09 ± 0.30^{cd}
Co1	39.26 ± 1.29^{cd}	19.68 ± 0.27^{c}	19.56 ± 1.56^{abc}
Co2	41.95 ± 0.44^{ab}	21.55 ± 0.23^{ab}	20.40 ± 0.37^{ab}
Co4	43.17 ± 1.01^{a}	21.57 ± 0.25^{ab}	21.60 ± 1.16^{a}

C: control; S0.5: Sunflower 0.5% application dose; S1: Sunflower 1% application dose; S2: Sunflower 2% application dose; S4: Sunflower 4% application dose; Co0.5: corn 0.5% application dose; Co1: corn 1% application dose; Co2: corn 2% application dose; Co4: corn 4% application dose.

The problem of crusting layer in the soils of the region with low organic matter and poor structural properties has become the most important problem after planting. The added corn and sunflower straw caused a significant decrease in the crust resistance of the soils (Figure 1). The fracture value measured as 2910 mbar in the control soil decreased as the dose rates increased. It is seen in the results that corn straw application is the most effective application in terms of modulus of rupture value.

When all the results are examined, it is revealed that both applications are effective in the examined features. Corn straw application was determined to be more effective than sunflower straw. Due to the more fibrous structure of sunflower straw, it is thought that the effect in a short time is not fully revealed. Faster disintegration of the corn straw mixture had a faster effect on the results.

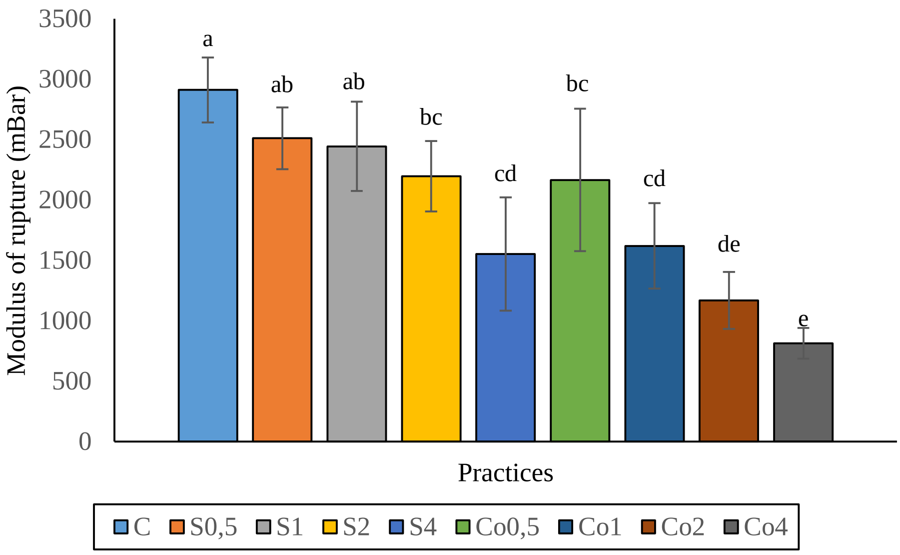

Figure 1.

CONCLUSIONS

In this study, the effect of different straw residues on the important physical properties of soils as a result of short-term incubation in our country was evaluated. When the results were examined, it was determined that both straw applications had a significant effect on the water potential and modulus of rupture values of the soils, albeit in a short time. In particular, it is considered appropriate to recommend the use of corn straw in our soils with problematic physical properties. These results should be supported by long-term incubation studies and field trials to be established as a result of this preliminary study.

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EFFECT OF DIFFERENT HARVEST PERIODS AND NITROGEN DOSES ON THE YIELD AND QUALITY PROPERTIES IN SWEET CORN

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ABSTRACT

In this study, it was aimed to determine the effects of nitrogen dose and harvest period on yield and quality characteristics of sweet corn. The study was carried out in Isparta University of Applied Sciences, Faculty of Agriculture, Field Crops Department, in the trial areas in 2020. The experiment was set up in randomized complete blocks split plot design with 3 replications. Nitrogen doses (15, 20, 25 kg/da) were placed on the main plots and harvest periods [early milk (Z71-73), late milk (Z77-79), dough (Z79-83)] on the sub plots. In the study, ear diameter, ear yield, kernel number in cob, dry matter content, water soluble dry matter content, total soluble sugar content, ash ratio, crude protein ratio and color parameters (L* and b*) were investigated. According to results of research, with the increase of nitrogen doses and harvest periods, ear diameter, ear yield, kernel number in cob, dry matter ratio, water soluble dry matter content, L* and b* values were increased, total soluble sugar content, ash and crude protein ratio were decreased. As a result, it can be suggested to use 25 kg/da of nitrogen fertilization in Kompozit Şeker variety, and to harvest it in dough period based on yield, and harvesting in the late milk period based on quality.

Keywords: Sweet corn, Nitrogen doses, Harvest Periods, Yield, Quality

INTRODUCTION

Corn is one of the hot climate cereals rich in terms of nutritional content. In addition to being an important food product in human and animal nutrition, it has an important place in the industry due to its different usage areas (Burcu and Akgün, 2018). Sweet corn is one of the subspecies of corn and differs from other corn subspecies in terms of grain structure and chemical composition. In sweet corn varieties, the sugar content of the grains is high thanks to the 'su', 'sh-2' and 'se' genes (sugary gene) that prevent the conversion of sucrose transported to the endosperm into starch (Ndiaye et al., 2017). Compared to other corn varieties, sweet corn with larger embryos has more fat and protein content (Sade, 2002). It was determined 15% protein and 64% carbohydrates (more than 30% starch and 9% fat) in the dry matter of the sweet corn kernel (Sevov, 2017). It is also rich in phosphorus, magnesium, iron, zinc and vitamin content (Keerthi et al., 2017). It increases the balanced nutrition of consumers and the profitability of the producer, with the harvesting of sweet corn in the period when the nutrient content and yield are highest (Öktem et al., 2010). However, the chemical composition of sweet corn is related to cultivation techniques, maturity and storage methods (Salunkhe and Kadam, 1998).

Sweet corn production in the world is mostly made in America, followed by Nigeria, Mexico, Indonesia and Peru (FAO, 2020). Although there is not enough statistical information about the cultivation area and production amount of sweet corn in our country, the cultivation areas are increasing from year to year, especially in Çukurova, Aegean and Marmara regions (Özata et al., 2016). In addition, the development of sweet corn harvest and food industry, with increase in exports and consumption in coastal regions, the cultivation area also increases (Tezel et al., 2020). Sweet corn production in the world does not meet the market demand and one of the reasons for this is its low yield (Laksano et al., 2018). One of the important factors in increasing sweet corn production is fertilization and/or adding organic matter to the soil (Irshad et al., 2013; Roeswitawati et al., 2021).

Nitrogen, one of the most common elements, is part of amino acids, proteins, nucleic acids, chlorophyll and many other metabolites necessary for plant survival (Bolat and Kara, 2017). Nitrogen is one of the most important macronutrients in corn agriculture and plays an important role in the vegetative and generative periods (Yürürdurmaz and Tansı, 2021). The response of the maize plant to increasing nitrogen doses is different, some varieties showed an increase in terms of protein content and grain yield with the increase of nitrogen doses (Pollmer et al., 1979), while in some corn varieties, number of cobs, length of cobs and number of kernels per cob increased (Russel and Balko, 1980). Since commercial fertilizers are not used in a balanced and economical way in our country, it increases the cost, and also, a significant amount of foreign currency is paid for fertilizer raw materials (Çokkızgın and Çölkesen, 2006; Can and Akman, 2014).

Since the production and consumption of sweet corn in our country has increased in recent years, it is important to determine fertilization programs suitable for the variety and ecology. In this study, it was aimed to determine the effects of different nitrogen doses (15, 20, 25 kg N/da) and harvesting periods (early milk, late milk, dough formation) on yield and quality characteristics of sweet corn in Isparta conditions.

MATERIAL AND METHOD

The study was carried out in Isparta University of Applied Sciences, Faculty of Agriculture, Education Research and Application Farm trial fields in 2020. Kompozit Şeker, which is a type of sweet corn, was used as the material in the study. The soil analysis results of the experimental area were found to have a clayey loam structure, medium level in terms of organic material, low lime content, salt-free and slightly alkaline (Şenol et al., 2020). In the period of the experiment, the average temperature (May-September 2020) (22.7^{o}C) is higher than the long-term average (20.16^{o}C), the total precipitation (162.4 mm) is higher (139.7 mm) than the long-term total, and the relative humidity average (45.6%)) was lower (51.3%) compared to long years (Anonymous, 2020).

The research was carried out according to completely randomized blocks the split plot design with 3 replications. Nitrogen doses (15, 20, 25 kg/da) in main plots, harvest times in sub plots [early milking (Z71-73), late milking (Z77-79) and dough formation (Z79-83)] (Meier, 1997; Anonymous, 2021)]. The area of each plot was 14 m^2 (5 m x 4 rows, 70 cm between rows, 20 cm above rows). All plots were fertilized with 8 kg P_2O_5 per decare and different nitrogen levels (15, 20, 25 kg N/da). All of the phosphorus and half of the nitrogen fertilizers (in the form of ammonium sulfate) were given at planting, and the other half when the corn plants were 45-50 cm. Sowing was done by hand on May 6, 2020, with two seeds per quarry. After the germination is completed, both weed control and a plant are left in each quarry with manual hoeing processes. The first irrigation was done immediately after planting in order to ensure homogeneity of the output. The drip irrigation method, one of the pressurized irrigation systems, was used in irrigation and irrigation was done in a way to prevent the plants from entering moisture stress.

In the research, ear diameter, ear yield, kernel number per cob, dry matter ratio (Ülger et al., 1997), water soluble dry matter amount (Erbaş et al., 2015), total soluble sugar content

(Dubois et al., 1956), ash ratio (Yılmaz, 2005), crude protein ratio (Fowler and Brydon, 1989) and color parameters (L* and b*) were examined. The data of the properties examined in the study were subjected to analysis of variance by using the TOTEMSTAT package program in accordance with the experimental design of split plots in random blocks. The differences between the means were determined according to the LSD test.

RESULT AND DISCUSSION

Cob diameter (cm) and Cob Yield (%)

In the study, the effect of nitrogen dose and harvesting periods on cob diameter in Kompozit şeker corn was found to be statistically significant. The mean cob diameter increased in parallel with the increase in nitrogen doses, and the highest average cob diameter was determined in the plots where 25 kg/da nitrogen dose was applied. According to harvest periods, the mean cob diameter varied between 4.00-4.78 cm. The highest mean cob diameter was determined in the dough formation period, and there was no statistical difference between the late milking period (Table 1).

The vegetative organs of plant develop better and produce more photosynthesis products depending on the increase in nitrogen doses. For this reason, in parallel with the increase in the amount of nutrients transported to the corn cob, diameter of cobs also increases (Özata, 2013). It has been stated by many researchers that cob diameter increases due to the increase in nitrogen fertilizer doses in maize (Alıcı 2005; Saruhan and Şireli, 2005; Çarpıçı, 2009). On the other hand, cob diameter has a high relationship with the yield, and the larger cob diameter increases the marketing value (Öktem and Öktem, 2006). Tezel et al., (2020), Tezel et al., (2020), stated that the diameter of the cob of seven different types of sweet corn varies between 43.9-48.5 mm during the milk production period. According to these results obtained in different studies, cob diameter can be affected by environmental conditions and cultural practices as well as genetic structure.

Table 1. Effects of different nitrogen doses and harvesting period on means of cob diameter (cm) and cob yield (%) in sweet corn

	Cob Diameter				**Cob Yield**			
Harvest periods	**Nitrogen Doses (kg/da)**				**Nitrogen Doses (kg/da)**			
	15	**20**	**25**	**Mean**	**15**	**20**	**25**	**Mean**
Early milking	4.00	4.00	4.00	***4.00 B[1]***	61.33 c	66.00 c	68.00 c	***65.11 C[1]***
Late milking	4.00	4.67	5.00	***4.56 A***	67.33 b	71.33 b	73.33 b	***70.67 B***
Dough formation	4.33	5.00	5.00	***4.78 A***	74.00 a	76.33 a	76.33 a	***75.56 A***
Mean	**4.11 B[1]**	**4.56 B**	**4.67 A**		**67.56 B**	**71.22 A**	**72.56 A[1]**	
F values: N doses (A): 14.00*; Harvest period (B): 19.50** AXB: 3.00 CV(A)= %5.3, CV(B)= %6.12; **p≤0,01					**F values**: N doses (A): 54.30** ; Harvest period (B): 553.00** AXB: 9.25** CV(A)= % 1.5, CV(B)= % 0.95 ; **p≤0.01			

[1]The difference between the means given with the same letter in the same column and row is not significant.

The nitrogen dose, harvest periods and interaction of nitrogen dose x harvest periods on cob yield was found to be significant (p≤0.01). Mean cob yield varied between 67.56% and

72.56% in nitrogen dose applications. The highest mean cob yield was determined at 25 kg/da nitrogen dose, and it was statistically in the same group with 20 kg/da nitrogen dose. According to the harvest periods, the mean cob yield was determined at the highest (75.56%) dough formation period, and the lowest (65.11%) in early milking period. When the interaction of nitrogen dose x harvest periods was examined, it was determined that sweet corn harvested during the dough formation period had the highest cob yield in all nitrogen dose applications (Table 1).

The cob yield is obtained by dividing the husked cob weight to the unhusked cob weight. That is, it expresses how much of the weight of the cob when harvested can be used for fresh consumption (Şahin, 2021). Cob yield is important in terms of consumption and marketing, and it is desired to be high (Burcu, 2016). In the study, cob yield increased in parallel with increase in applied nitrogen doses and progress of harvest periods. Cob yield varies depending on cultivation techniques, cultural practices, climate and environmental conditions, as well as the genetic structure of the variety. As a matter of fact, Eşiyok and Bozokalfa (2005) determined the ear yield as 68.6-75.4%; Başçifçi et al. (2013), 69-81.7%; Kul (2012) determined that it varies between 60.7-71.9%.

Grain Number Per Cob and Dry Matter Rate (%)

In the study, effect of nitrogen dose, harvest periods and interaction of nitrogen dose x harvest periods on grain number per cob was statistically significant. Grain number per cob increased with the increase in nitrogen doses, and the highest grain number per cob was determined in the application of 25 kg/da nitrogen dose (663.11). According to harvest periods, grain number per cob varied between 557.78-643.44, the highest in the dough formation period, and the lowest in the early milking period. Depending on nitrogen dose applications, grain number per cob increased with progression of harvest periods and the highest grain number per cob was found in the dough formation period. In addition, the highest grain number per cob was determined in the 25 kg/da nitrogen dose application of the dough formation period (716.00) (Table 2). The grain number per cob is an important parameter that affects cob yield and unit area yield (Kul, 2012). In maize, although grain number per cob is mostly under the control of genetic structure (Kırtok, 1998), environmental factors can be effective on fertilization (Akgün et al., 2017). On the other hand, as amount of nitrogen dose increases, plant growth and development are better, and this development positively affects the yield components (cob length, ear diameter, etc.) and thus grain number per cob (Turgut, 2000). As a matter of fact, it has been reported in many studies that increasing nitrogen doses increase the grain number per cob, and these results are similar to the findings of the study (Turgut, 2000; Gökmen et al. 2001; Presterl et al., 2003; Yürürdurmaz and Tansı, 2021). In addition, Ağaçkesen and Öktem (2020) determined the highest grain number of 6 different sweet corn varieties in the Z77 (late milking) period.

Table 2. Effects of different nitrogen doses and harvesting period on mean grain number per cob and dry matter rate (%) in sweet corn

	Grain Number Per Cob				**Dry Matter Rate**			
Harvest periods	**Nitrogen Doses (kg/da)**				**Nitrogen Doses (kg/da)**			
	15	**20**	**25**	**Mean**	**15**	**20**	**25**	**Mean**
Early milk	501.00 c	548.00 c	624.33 c	***557.78 C[1]***	15.33 c	16.67 c	19.00 c	***17.00 C[1]***

Late milk	552.33 b	595.00 b	649.00 b	***598.78 B***	24.33 b	32.33 b	33.33 b	***30.00 B***
Dough formation	590.67 a	623.67 a	716.00 a	***643.44 A***	41.67 a	44.67 a	45.67 a	***44.00 A***
Mean	**548.00 C[1]**	**588.89 B**	**663.11 A**		**27.11 C[1]**	**31.22 B**	**32.67 A**	
F values: N doses (A): 2934.298** ; Harvest period (B): 1253.098** AXB: 25.719** CV(A)= % 0.54, CV(B)= % 0.61 ; **p≤0.01					**F values**: N doses (A): 79.176**; Harvest period (B): 2606.294** AXB: 17.118** CV(A)= % 3.20, CV(B)= % 2.62 ; **p≤0.01			

[1]The difference between the means given with the same letter in the same column and row is not significant.

The nitrogen doses, harvest periods and nitrogen dose x harvest periods interaction on dry matter rate was found to be significant. In the study, dry matter rate increased with the increase in nitrogen doses and harvest periods. As a matter of fact, the mean dry matter rate according to the nitrogen doses varied between 27.11-32.67% and the highest values were determined in the 25 kg/da nitrogen application and the lowest values in the 15 kg/da nitrogen application. In different harvest periods, the highest dry matter content was determined in the dough formation period (44.00%) and the lowest in the early milking period (17.00%). When the interaction of nitrogen dose x harvest periods was examined, it was determined that the sweet corn harvested during dough formation period had the highest dry matter rate in all nitrogen dose applications. In addition, the highest dry matter rate (45.67%) was determined during the dough formation period of 25 kg/da nitrogen dose application (Table 2).

Nitrogen increases the leaf area in the vegetative period of the plant, so dry matter accumulation is more (Tolenaar et al. 1997). As a matter of fact, Iskender (2020) determined that increasing nitrogen doses in different sweet corn varieties increased the dry matter ratio up to 22.5 kg/da, and after this dose, the dry matter ratio decreased. Kantarci et al. (2016), stated that as a result of the harvesting of corn varieties planted at three different times in different periods (milking, yellowing and pulping), total dry matter amount increased with the delay of harvesting periods. Ağaçkesen and Öktem (2020) stated that grain dry matter rates of sweet corn varieties are different from each other and grain dry matter rates increase with increase in harvest time. In addition, the researcher determined the lowest dry matter rate in the early milking period (17.7%) and the highest dry matter rate in the early yellowing period (29.4%) according to the means of two years. The results of these literature studies examined are in agreement with the study.

Crude Ash Rate (%) and Crude Protein Rate (%)

It has been determined that the effect of harvesting periods on crude ash ratio and interaction of nitrogen dose x harvest periods are important, but the effect of nitrogen doses is insignificant. According to the harvest periods, crude ash rate of sweet corn varied between 1.89-3.99%. With the delay of harvest periods, crude ash ratio decreased and the highest crude ash ratio was determined in the early milking period. Although effect of nitrogen doses (15, 20, 25 kg/da) on crude ash ratio was not statistically significant, crude ash ratio decreased with increase of nitrogen doses (2.89%, 2.80%, 2.67%, respectively). Crude ash content decreased with delay in harvesting periods at all nitrogen doses. (Table 3).

Crude ash is in the structure of nucleoproteins that play an important role in the cell and consists of minerals that have functions in many events. It also includes non-combustible inorganic materials remaining after the combustion of dry matter (Geren, 2000). As a matter of fact, Çarpıcı (2009) reported that nitrogen fertilizer applications generally did not affect the crude ash content of maize plants. On the other hand, İnceer (2011) stated that crude ash content decreased during ripening in all maize subspecies. The researcher determined that sweet corn had less crude ash content in the physiological maturity period (2.12%) than the dough formation period (2.37%). These reviewed studies support the results obtained from this study.

In the study, the nitrogen dose, harvest periods and interaction of nitrogen dose x harvest periods on crude protein ratio was found to be significant. The mean protein ratio increased with the increase of nitrogen doses (15, 20 and 25 kg/da) (11.48%, 12.11% and 12.20%, respectively). However, nitrogen doses of 20 and 25 kg/da were statistically in the same group. The protein rate decreased with the delay of harvest periods, the highest protein rate was found in the period of early milking (15.30%), and the lowest protein rate was found in the dough period (9.42%). When the interaction of nitrogen dose x harvest periods is examined, the protein ratio of all nitrogen doses decreased with the progress of harvest periods, and the highest crude protein ratio was determined in the early milking period of 20 kg/da nitrogen dose (Table 3).

Table 3. Effects of different nitrogen doses and harvesting period on mean crude ash and crude protein rate (%) in sweet corn

	Crude Ash Rate				**Crude Protein Rate**			
Harvest periods	**Nitrogen Doses (kg/da)**				**Nitrogen Doses (kg/da)**			
	15	**20**	**25**	**Mean**	**15**	**20**	**25**	**Mean**
Early milk	4.00a	3.97 a	4.00 a	***3.99 A[1]***	14.98 a	15.96 a	14.98 a	***15.30 A[1]***
Late milk	2.67 b	2.77 b	2.00 b	***2.48 B***	10.19 b	11.21 b	11.80 b	***11.07 B***
Dough formation	2.00 c	1.67 c	2.00 c	***1.89 C***	9.28 c	9.17 c	9.81 c	***9.42 C***
Mean	**2.89**	**2.80**	**2.67**		**11.48 B**	**12.11 A**	**12.20 A[1]**	
F values: N doses (A): 1.255 ns; Harvest period (B): 139.533** AXB: 3.472** CV(A)= % 10.77 CV(B)= % 9,88 ; **p≤0.01, ns: insignificant					**F values**: N doses (A): 12.877* ; Harvest period (B): 891.115** AXB: 10.404** CV(A) = % 2.72, CV(B)= % 2.56 ; *p≤0.05 **p≤0.01			

[1]The difference between the means given with the same letter in the same column and row is not significant.

Nitrogen is an element in the structure of proteins and amino acids. Many researchers have stated in their study that the crude protein ratio increases as the nitrogen dose increases in sweet corn (Koçak, 1991; Altıparmak, 2001; Can and Akman, 2014). On the other hand, as the grains of sweet corn mature, they form a wrinkled surface due to the decrease in moisture content and volume. Therefore, the ratio of sugar and protein decreases, while the ratio of starch increases (İnceer, 2011). As a matter of fact, Ağaçkesen and Öktem (2020) determined the lowest protein rate of sweet corn varieties in the medium yellow maturity period (13.2%), and the highest protein rate in the early milking period (16.9%). In addition, many researchers have stated that the protein ratio decreases with the delay in the harvest period, similar to the findings in the study (Bressani and Conde, 1961; Thornton et al., 1969; Elgün et al., 1990) and support the results obtained from this study.

Water Soluble Dry Matter Amount (%) and Total Soluable Sugar Content (mg/g)

The nitrogen dose, harvesting periods and interaction of nitrogen dose x harvesting periods were found to be significant on the amount of water soluble dry matter (WSDM). In parallel with the increase in nitrogen doses, the amount of WSDM also increased. The highest amount of WSDM was determined in 25 kg/da nitrogen application (26.67%), and the lowest in 15 kg/da nitrogen application (22.89%). The mean amount of WSDM according to the harvest periods varied between 14.67-32.11%, and amount of WSDM increased with the delay of the harvest periods. On the other hand, the highest amount of WSDM at all nitrogen doses was determined in the dough formation period. In addition, the application of 25 kg/da nitrogen dose to Kompozit Şeker variety and the amount of WSDM in the harvesting period were the highest (Table 4).

Sugars constitute the majority of water-soluble dry matter, and it has been stated that the amount of water-soluble dry matter can be used to compare the taste of sweet corn (Flora and Wiley 1974). On the other hand, Su (1989), in his study on sweet and super sweet corn varieties, and the amount of grain WSDM in harvested at different times, reported that the amount of WSDM increased with the prolongation of the harvest time, due to the decreasing moisture content. Agackesen and Öktem (2020) determined that the amount of WSDM increased with the delay of the harvest time in sugar corn varieties

Table 4. Effects of different nitrogen doses and harvesting period on averages of water soluble dry matter amount (%) and total soluable sugar content (mg/g) in sweet corn

	Water Soluble Dry Matter Amount				**Total Soluble Sugar Content**			
Harvest periods	**Nitrogen Doses (kg/da)**				**Nitrogen Doses (kg/da)**			
	15	**20**	**25**	**Mean**	**15**	**20**	**25**	**Mean**
Early milk	14.00 c	14.00 c	16.00 c	***14.67 C***[1]	10.10	10.18	10.33	***10.20***
Late milk	24.67 b	26.33 b	29.00 b	***26.67 B***	10.06	10.12	10.09	***10.09***
Dough formation	30.00 a	31.33 a	35.00 a	***32.11 A***	10.00	10.05	10.02	***10.02***
Mean	**22.89 C**	**23.89 B**	**26.67 A**[1]		**10.05**	**10.11**	**10.15**	
F values: N doses (A): 232.750** ; Harvest period (B): 9678.50** AXB: 26.75** CV(A)= % 1.57. CV(B)= % 1.11 ; **p≤0.01					**F values**: N doses (A): ; Harvest period (B): AXB: CV(A)= %. CV(B)= %; ns: insignificant			

[1]The difference between the means given with the same letter in the same column and row is not significant.

The nitrogen dose, interaction of harvest period and applications on total soluble sugar content was determined to be insignificant. Parallel to the increase in nitrogen doses, the total soluble sugar content increased, although statistically insignificant, and the highest total soluble sugar content was found at 25 kg/da nitrogen dose (10.15 mg/g). On the other hand, the total soluble sugar content varied between 10.02-10.20 mg/g according to the harvest periods, and the total soluble sugar content decreased statistically insignificantly as the harvest periods progressed (Table 4).

Sweet corn has more sugar than other corn subspecies. It has been stated that the total sugar ratio, which is 1-3% in other corn types, varies between 4-12% in sugar corn (Tracy, 2001). In addition, it has been reported that environmental factors are also effective on sugar content, as well as the sweetness gene in the endosperm of the cultivars (Szymanek, 2009). Different results were found in studies examining the effect of nitrogen fertilizer applications on sugar ratio. As a matter of fact, Can (2014) reported that the effect of nitrogen doses on sugar content is insignificant and the sugar ratio varies between 10.6% and 11.5%. Akgün and Siyah (2015), determined that nitrogen is important and biofertilizer application is insignificant on the total sugar amount. On the other hand, Ağaçkesen and Öktem (2020) determined the lowest sugar rate in the middle yellow maturity period, and the highest sugar rate in the middle milking. Similar to the findings in the study, many researchers have determined that the sugar content decreases with the delay of the harvest period (Khanduri et al., 2011; Kantarcı et al., 2016).

Grain colour (L* and b* values)

Grain color is an important quality criterion in sweet corn. In the study, the interaction of nitrogen doses x harvest periods, nitrogen dose and harvest periods were found to be important on grain color characteristics (L* and b*). The mean color values (L* and b*) increased in parallel with the increase in nitrogen doses. As a matter of fact, the highest color values (L* and b*) were determined at 25 kg/da (73.33 and 46.67, respectively). On the other hand, the mean color values increased with the progress of the harvest periods. According to the harvest periods, the highest L* and b* values were determined in the dough formation period, and the lowest L* and b* values were determined in the early milk setting period. At the same time, it was determined that harvest of 25 kg/da nitrogen dose during the dough formation period gave the highest result in all color parameters (Table 5).

One of the grain color parameters, the L value represents the brightness of the material, and b represents the yellow-blue colors (McGuire, 1992). As a matter of fact, it was determined that the grain brightness (L*) and yellow color intensity of Kompozit şeker corn variety increased with the increase of nitrogen doses and delayed harvesting periods in the study. Yellow color is a desirable feature in sweet corn (Hashim, 2011). Singh et al. (2009) expressed the L* value of sweet corn as 82.3 and the b* value as 25.8. İnceer (2011) stated that L* and b values increase with the progression of the maturity period in sweet corn. The effect of genotype, genotype x environment interaction and cultural practices on color parameters is high (Esiyok et al., 2004; Esiyok and Bozokalfa, 2005).

Table 5. Effects of different nitrogen doses and harvesting period on averages of L* and b* values in sweet corn

	L* values				b* values			
Harvest periods	**Nitrogen Doses (kg/da)**				**Nitrogen Doses (kg/da)**			
	15	**20**	**25**	**Mean**	**15**	**20**	**25**	**Mean**
Early milk	67.00 B	69.67 C	70.67 C	***69.11 C[1]***	31.00 c	37.33 c	38.33 c	***35.56 C[1]***
Late milk	73.33 A	71.00 B	73.00 B	***72.44 B***	45.67 b	45.00 b	49.67 b	***46.78 B***
Dough formation	73.00 A	74.00 A	76.33 A	***74.44 A***	47.67 a	50.00 a	52.00 a	***49.89 A***
Mean	**71.11 B[1]**	**71.56 B**	**73.33 A**		**41.44 C[1]**	**44.11 B**	**46.67A**	
F values: N doses (A) : 11.20* ; Harvest period (B) : 141.12** AXB: 12.84** CV(A)= % 1.46. CV(B)= % 0.95 ; **p≤0.01, *p≤0.05					**F values**: N doses (A): 70.511**; Harvest period (B) :531.269** AXB:10.019** CV(A)=% 2.12, CV(B)=%2.23 ; **p≤0.01			

[1]The difference between the means given with the same letter in the same column and row is not significant.

CONCLUSIONS

In the study, the effects of different nitrogen doses on harvest periods were determined. In the reseach, the interactions of all applications were found to be statistically significant except for cob diameter and total soluble sugar content. In the study, with the increase in nitrogen doses, cob diameter, ear yield, kernel number per cob, dry matter content, L* and b* values increased, crude ash content, crude protein content and total soluble sugar content decreased. As a result, it can be suggested that 25 kg/da nitrogen fertilization should be done in Kompozit şeker sweet corn variety, and harvesting in the dough formation period based on yield and in early milk production period based on quality.

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DETERMINATION OF TRACTOR USAGE PREFERENCES IN AGRICULTURAL ENTERPRISES

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ABSTRACT
Agricultural mechanization is a complementary production technology that increases efficiency, provides economic efficiency and improves working conditions in agricultural production. The use of tractors with features that will meet the needs of agricultural enterprises is important for enterprises to make an economical production. In the study, a survey is conducted with 113 farms producing sunflower and wheat in the Trakya region. The aim of the study is to determine the size and tractor assets of farms and tractor usage preferences of the farmers. In addition, the mechanization indicators of the farms are calculated and compared with Turkey. When the relationship between tractor ownership and land size is examined, 51,16% of the enterprises with a land size of less than 150 decares and 84.29% of the enterprises with a land size of more than 150 decares consider the size of the land is adequate to own a tractor. 98.23% of the enterprises do not rent their tractors to other enterprises and 97.35% do not rent tractors from other enterprises. The number of tractors per 1000 hectares and the tractor power per 1 hectare were calculated as 61.68 units and 5.27 hp/ha, respectively. Indicators show that the number of tractors and tractor power in the Trakya region is higher than Turkey's' average. Shared use of tractors and agricultural tools-machines is not common in the region. 60.18% of farmers think that it would be beneficial to start a cooperative for the use of shared tools - machines and shared tractors. Since shared use is not common, farmers buy tractors and agricultural tools-machines regardless of the farm size. This situation causes farms to allocate more capital than they need for mechanization. With the widespread of shared use, the profitability of farms can be increased by using the capital allocated for mechanization to other production factors.

Keywords: mechanization indicators, farmer preference, farm tractor, Trakya Region

INTRODUCTION

Agricultural mechanization is the use of agricultural tools, machinery and systems working with mechanical power sources in an agricultural enterprise, region or country instead of human and animal power (Gökdoğan, 2005). Agricultural mechanization is an agricultural production technology as a complementary element that increases the efficiency of other agricultural inputs, provides economic efficiency and improves working conditions (Altundaş and Demirtola, 2004). Mechanization in agricultural enterprises is applied at different levels depending on the technical and economic conditions of the enterprise (Zeren et al., 1995). Tractor has the most important position among agricultural tools and machinery used in agricultural mechanization (Liljedahl et al., 1989). For this reason, the selection of tractors that will meet the needs of the farmers is the main factor for the enterprises to make an economical production (Işık, 1996). In agricultural management, suitable tractor and agricultural equipment-machine selection for the production is an important situation in terms of planning and optimization. Land type, land size and crop pattern are the most important parameters in this selection. In agricultural production, making the selection in accordance with the purpose and importance of the work to be done increases agricultural efficiency, productivity and quality (Sağlam and Çetin, 2017).

Among the most important indicators that determine the agricultural mechanization level of a country are criteria such as the quality/quantitative status of the tractor stock, its growth over the years, density and power level per agricultural unit area (Evcim et al., 2005). In the literature, there are studies on farmers' tractor assets and tractor usage preferences (Akdemir and Sağlam, 1998; Aytuğ and Karadibak, 1998; Durgut and Arın, 2005; Sağlam and Polat, 2005; Baran et al., 2014; Özmen, 2014; Altıntaş, 2015, Kayhan et al., 2017; Oğuz et al., 2017; Kipritçi, 2018; Aksoy et al., 2019). In addition, the mechanization indicators on a country basis (Gunjal and Heady, 1983; Sharabiani and Ranjbar, 2008; Rasouli et al., 2009; Gökdoğan, 2012; Iqbal et al., 2015) and regionally (Yazıcı, 2006; Olaoye and Rotimi, 2010; Mehta et al., 2014) are also available. The aim of this study is to determine the tractor usage preferences of the farmers in the Trakya region and to calculate the mechanization indicators of the enterprises.

MATERIAL AND METHOD

Material

The material of the research are the data obtained through surveys from agricultural enterprises producing sunflower and wheat in Tekirdağ, Edirne and Kırklareli provinces. Survey data belong to the 2018/2019 production period. Information such as the number and size of the agricultural enterprises is obtained from Provincial Directorates of the Ministry of Agriculture and Forestry in Edirne, Kırklareli, and Tekirdağ.

Method

In the sampling method, first of all, the number of villages to be included in the sample is determined, and then the number of enterprises from the villages included in the sample is reached. The stratified random sampling formula of Neyman Method (Equation 1) is used to determine the number of agricultural enterprises to be surveyed (Yamane, 2001). Villages are divided into strata according to the number of enterprises, and enterprises are divided into strata according to their size. Equation 2 is used to determine the number of agricultural enterprises in the strata.

$$n = \frac{(\sum N_h * S_h)^2}{N^2 * D^2 + \sum(N_h * {S_h}^2)} \qquad \text{(Equation 1)}$$

$$n_i = \frac{N_h * S_h}{\sum N_h * S_h} * n \qquad \text{(Equation 2)}$$

$$D^2 = \frac{d^2}{Z^2} \qquad \text{(Equation 3)}$$

N_h : Number of agricultural enterprises in strata

S_h : Standard deviation of the strata

N : Population size

d : Sampling error

Z : Table value of confidence level

S_h^2: Variance of strata

n_i : Number of samples per strata

n : Sample size

The number of enterprises in the villages of the Trakya region is divided into three strata: 0-50, 51-100, 101 and above. The number of villages in the first, second and third strata are 9, 9 and 20 respectively. The surveyed villages are chosen randomly.

Agricultural enterprises operating in 38 villages included in the sample are divided into two strata, 20-150, 150 decares and above, according to their land size. The number of agricultural enterprises to be surveyed is 113 agricultural enterprises in total, 43 agricultural enterprises in the first strata and 70 agricultural enterprises in the second strata.

RESULTS

General Information About Agricultural Enterprises

The agricultural enterprises are divided into two groups according to the land size as between 20 and 150 decares (group I) and 150+ decares (group II). The results are given separately for both groups and all agricultural enterprises.

The land assets and land properties of agricultural enterprises are given in Table 1. The average land size of group I is 90.85 da, group II is 305.50 da and all agricultural enterprises is 223.82 da. In all enterprises, 74.93% of the total land is owned land and 25.07% is leased land. When the average parcel number and average parcel size are examined according to the size groups, the average parcel number is 6.56 and the average parcel size is 13.85 da/lot in group I, while average parcel number is 12.51 and the average parcel size is 24.41 da/lot in group II. These results are above the Turkey average (5.9 plots and 12.9 da/lot) for both groups.

Table 1. Land assets and land properties of agricultural enterprises

	Group I (20-150 da)		Group II (150 da +)		All agricultural enterprises	
	da	%	da	%	da	%
Owned land size (da)	75.15	82.72	224.55	73.50	167.7	74.93
Leased land size (da)	15.7	17.28	80.95	26.50	56.12	25.07
Total agricultural enterprise land size (da)	90.85	100.00	305.5	100.00	223.82	100.00
Average number of parcels	6.56		12.51		10.25	
Average parcel size (da/lot)	13.85		24.41		21.84	

The average age of farmers is 55.07 in group I, 51,84 in group II and 53,07 in all agricultural enterprises. The ratio of farmers between 50-64 years of age is quite high (49.56%) in the average of enterprises.

The ratio of farmers who graduated from primary school is the highest for both groups. The rate of primary school graduates is 62.79% in group I and 47.14% in group II. The lowest rate in all groups belongs to college/university graduates. The agricultural experience of farmers varies between 3 years and 70 years, with an average agricultural experience of 33 years.

Average number of tractors is 1.07 in group I, 1.57 in group II and 1.38 for all enterprises. Average tractor power is 72.76 hp in group I, 90.67 hp in group II and 85.39 hp in all agricultural enterprises (Table 2). In previous studies, tractor powers were 59.28 hp in Turkey (Sabancı et al., 2003), 47.48 hp in Isparta (Gökdoğan, 2005), 62.13 hp in Erzurum

(Yıldız and Erkmen, 2006), 71 hp in Aydın (Cankurt, 2008), 60 hp in the Harran Plain (Mutlu, 2011) and 69.89 hp in Eskişehir (Altıntaş, 2015). This shows that large and powerful tractors are used more in the Trakya region.

Average tractor age is 19.65 for group I, 13.00 for group II and 14.90 for all agricultural enterprises. Total number of tractors is 46 in group I, 110 in group II and 156 in all agricultural enterprises.

Table 2. Tractor assets of agricultural enterprises

	Group I (20-150 da)			Group II (150 da +)			All agricultural enterprises		
	Min.	Max.	Avr.	Min.	Max.	Avr.	Min.	Max.	Avr.
Number of tractors	1	2	1.07	1	3	1.57	1	3	1.38
Tractor power (hp)	40	112	72.76	45	160	90.67	40	160	85.39
Tractor age	0	43	19.65	1	52	13.00	0	52	14.90
Total number of tractors		46			110			156	

Information on Tractor Usage of Agricultural Enterprises

The distribution of the answers given to the question "Do you think your land size is big adequate to own a tractor?" is given in Table 3. 51,16% of group I, 84.29% of group II and 71.68% of all agricultural enterprises consider the size of the land adequate to own a tractor. Nearly half of the enterprises in the first group have tractors even though they do not consider their land size adequate to purchase tractors. It is thought that this situation is related to the difficulties experienced by the farmers in the use of shared tractors.

Table 3. Opinions of business owners on whether or not the size of the land is adequate to own a tractor

	Group I (20-150 da)		Group II (150 da +)		All agricultural enterprises	
	Number	%	Number	%	Number	%
Adequate	22	51.16	59	84.29	81	71.68
Inadequate	21	48.84	11	15.71	32	28.32
Total	43	100.00	70	100.00	113	100.00

Whether or not the farmers consider the size of the land adequate to buy tractors and the land sizes of the farms are compared with the cross tables (Table 4). Farmers with low land size think that the land size is inadequate to buy tractors ($p=0.00<0.05$, phi= -0.357). 51.2% of the enterprises with a land size of 20-150 decares and 84.3% of the enterprises with a land size of 150 decares or more consider the land size is adequate to own a tractor. 27.2% of the enterprises that consider the land size adequate are in the group I and 72.8% are in the group II.

Table 4. Whether or not the farmers consider the size of the land is adequate to own a tractor

		Total land size (da) 20 – 150	150 <	Total
Adequate	Number	22	59	81
	By opinion	%27.2	%72.8	%100.0
	By land size	%51.2	%84.3	%71.7
Inadequate	Number	21	11	32
	By opinion	%65.6	%34.4	%100.0
	By land size	%48.8	%15.7	%28.3
Total	Number	43	70	113
	By opinion	%38.1	%61.9	%100.0
	By land size	%100.0	%100.0	%100.0

The distribution of the answers given to the question “Do you have a tractor that you rent out?” is given in Table 5. All enterprises in group I and 97.14% of the group II enterprises stated that they do not rent their tractors. The rate of those who do not rent their tractors in all enterprises is 98.23%.

Table 5. The farmers whether or not their tractors to other farmers

	Group I (20-150 da)		Group II (150 da +)		All agricultural enterprises	
	Number	%	Number	%	Number	%
Yes	0	0.00	2	2.86	2	1.77
No	43	100.00	68	97.14	111	98.23
Total	43	100.00	70	100.00	113	100.00

When the reasons for the farmers not to rent their tractors are examined, 42 of farmers who do not rent their tractors did not give any reason (Table 6). 33.80% of the farmers stated that they thought the tractor would not be used well, 29.58% stated they did not trust the person who would rent their tractor, 29.58% stated that the tractor can only meet his farm’s needs and 19.72% stated that they did not need to rent their tractor.

Table 6. Reasons for farmers not to rent their tractors

	Group I (20-150 da)		Group II (150 da +)		All agricultural enterprises	
	Number	%	Number	%	Number	%
Only meets the needs of the farmer	8	38.10	13	26.00	21	29.58
Thinks they will abuse/does not trust	5	23.81	19	38.00	24	33.80
Doesn't need to rent his tractor	5	23.81	9	18.00	14	19.72
All farmers have their own tractor/no demand	3	14.28	9	18.00	12	16.90
Total	21	100.00	50	100.00	71	100.00

4.65% of the group I, 1.43% of the group II and 2.65% of all enterprises stated that they rent tractors from other enterprises (Table 7).

Table 7. Tractor rental status of farmers from other enterprises

	Group I (20-150 da)		Group II (150 da +)		All agricultural enterprises	
	Number	%	Number	%	Number	%
Renting	2	4.65	1	1.43	3	2.65
Not renting	41	95.35	69	98.57	110	97.35
Total	43	100.00	70	100.00	113	100.00

95.35% of group I, 97.14% of group II do not use shared tractors (Table 8). 32.56% of the group I enterprises, 27.14% of group II enterprises and 29.20% of all enterprises use shared tools and machinery.

Table 8. Shared tractor and shared tool-machine usage of enterprises

		Group I (20-150 da)		Group II (150 da +)		All agricultural enterprises	
		Number	%	Number	%	Number	%
Tractor	Yes	2	4.65	2	2.86	4	3.54
	No	41	95.35	68	97.14	109	96.46
	Total	43	100.00	70	100.00	113	100.00
Tool - machinery	Yes	14	32.56	19	27.14	33	29.20
	No	29	67.44	51	72.86	80	70.80
	Total	43	100.00	70	100.00	113	100.00

60.18% of farmers think that it would be beneficial to start a cooperative for the use of shared tools - machinery and shared tractors (Table 9). According to agricultural enterprise groups, 67.44% of group I and 55.71% of group II think that the shared use cooperative will be beneficial.

Table 9. Approaches of farmers to start cooperatives for shared use

	Group I (20-150 da)		Group II (150 da +)		All agricultural enterprises	
	Number	%	Number	%	Number	%
Feasible	29	67.44	39	55.71	68	60.18
Not feasible	14	32.56	31	44.29	45	39.82
Total	43	100.00	70	100.00	113	100.00

The reasons for 35 of the farmers that do not find beneficial to start a cooperative for shared use are given in Table 10. Other farmers have no comment. 37.14% of the farmers think that there will be a disagreement between the partners, 22.86% think that the machines will break down quickly due to usage mistakes and will not be repaired, 22.86% think that the process cannot be done on time and the tractor will not be on time when it is needed.

Table 10. Reasons of farmers who do not find the cooperative beneficial

	Group I (20-150 da)		Group II (150 da +)		All agricultural enterprises	
	Number	%	Number	%	Number	%
Disagreement between partners	5	45.45	8	33.33	13	37.14
Machines break down quickly due to usage mistakes / broken machine cannot be repaired	2	18.18	5	20.83	8	22.86
Process is not done on time / not everyone gets a turn	3	27.27	7	29.17	8	22.86
Cooperatives are not working effectively	1	9.10	4	16.67	6	17.14
Total	11	100.00	24	100.00	35	100.00

Spearman correlation coefficient is used to determine the relationship between the size of the enterprises and the tractor power. As a result of the analysis, it is determined that there is a strong relationship (ρ=0.687; p=0.00) between the size of the agricultural enterprise and tractor power. According to this, it is understood that the farmers take the size of the farm into account in the selection of tractors.

The number of tractors per 1000 ha agricultural area, which is one of the mechanization indicators, is 117.75 tractors/1.000 ha for group I, 51.44 tractors/1.000 ha for group II and 61.68 tractors/1.000 ha for all agricultural enterprises. Tractor power per hectare is 8.57 hp for group I, 4.66 hp for group II and 5.27 hp for all enterprises (Table 11). As the size of the farm increases, the cultivated area per tractor increases, the number of tractors per 1,000 ha agricultural area and the tractor power per 1 ha agricultural area decrease. In Turkey, the number of tractors per 1000 ha agricultural area is 61.37 tractors/1.000 ha, and the tractor power per one ha area is 3.21 hp/ha in 2020 (TUIK, 2021). When the research findings are compared with Turkey in general, it is seen that the number of tractors and tractor power in the Trakya region is higher than the average of Turkey.

The tractor capital of the agricultural enterprises and the share of the tractor capital per hectare in the total capital are shown in Table 11 according to the groups. The average tractor capital per decare is calculated as 471,02 TL/da. The tractor capital per decare is higher in group I. The reason for this is that the land size of enterprises in group I is less.

The status of using loan of farmers when purchasing tractors is given in Table 12. 54.87% of farmers stated that they used loan while purchasing tractors. The ratio of farmers who have existing tractor loan payments is 34.51%. Loan utilization rate is calculated as 34.88% in group I, and 67.14% in group II. The average loan debts of agricultural enterprises per decare is 86.56 TL/da.

Table 11. Mechanization indicators of agricultural enterprises

	Group I (20-150 da)	Group II (150 da +)	All agricultural enterprises
Number of tractors per 1000 ha agricultural area (Number of tractors / 1000 ha)	117.75	51.44	61.68
Tractor power per 1 ha agricultural area (hp/ha)	8.57	4.66	5.27
Tractor capital per decare	593.86	448.58	471.02
Share of tractor capital in active capital	5.07	2.95	3.21

Table 12. Status of using credit of farmers when purchasing tractors

	Group I (20-150 da)		Group II (150 da +)		All agricultural enterprises	
	Number	%	Number	%	Number	%
Agricultural enterprises using loan	15	34.88	47	67.14	62	54.87
Enterprises with existing loan debt	5	11.63	34	48.57	39	34.51
Loan debts of enterprises (TL/da)	70.01		89.59		86.56	

CONCLUSIONS

In this study, the general characteristics of 113 agricultural enterprises producing sunflower and wheat in the Trakya region are determined and the factors affecting the tractor usage and tractor preferences of the enterprises are revealed.

Average number of tractors is determined as 1.07 in group I and 1.57 in group II. When the average tractor power of the enterprises is examined, it is seen that average tractor power is 72.76 hp in group I and 90.67 hp in group II. When compared with the studies carried out in different times and regions in Turkey (Gökdoğan, 2005; Cankurt, 2008; Altıntaş, 2015), it is seen that tractor horsepower per enterprise is higher in the Trakya region.

Average tractor age is calculated as 19.65 years for I. group enterprises and 13 years for II. group enterprises. Assuming that the economic life of a tractor in Turkey does not exceed 15 years (Mutaf, 1984; Eren, 1991; Akıncı, Topakçı, & Çanakçı, 1997; Sabancı et al., 2003; Altıntaş, 2015), 40.38% of the tractors in the enterprises are not economically viable. Long-term use of tractors whose mechanical life has expired will lead to an increase in repair and maintenance costs, as well as may cause the tractor to encounter malfunctions when it is needed to use. Changing old tractors to new ones is not easy for every agricultural enterprise and requires high capital.

54.87% of the enterprises stated that they used loan while purchasing tractors and 34.51% of the enterprises have existing tractor loans. Enterprises that do not have sufficient equity capital take out loans to buy tractors, and when they cannot pay the loan, they try to pay off the loan by selling their fields. Thus, while farmers renew their tractors, they reduce the size of their enterprise. This situation is one of the factors that negatively affect the profitability of the enterprise.

In group I, 48.84% of the farmers stated that they do not consider the land size is adequate to purchase tractors. This rate is quite high and this is due to the fact that small-scale enterprises have to buy tractors due to problems related to shared use.

96.46% of farmers stated that they do not use shared tractors and 70.80% of them do not use shared tools-machines. Agricultural enterprises need tractors and agricultural tools-machines in order to make agricultural production. In the absence of shared use, all enterprises buy tractors and agricultural tools and machines, regardless of the size of the enterprise. This situation causes enterprises to allocate more capital than they need to mechanization.

Although farmers state that they do not use shared tractors at a high rate, 60% of farmers think that it would be beneficial to start a cooperative for the use of shared tractors and tools. However, in case of starting a cooperative for the use of shared tractors and machinery by nearly 40% of the farmers think that a cooperative cannot be beneficial due to reasons such as there will be disagreement between the partners, the machines will break down quickly and the broken machines will not be repaired in a short time, everybody cannot benefit from the tractor because of busyness and the work will not be done on time when the production activities are intense. Elimination of these concerns of farmers depends on the professional management of the cooperative to be started and the well-organized rental system. In order to reduce the negative thoughts of the business owners that the machines will break down quickly due to unconscious use and that the broken machines will not be repaired, the probability of malfunction of the machines can be reduced by ensuring that the machines belonging to the cooperative are used by certain people who have received the necessary training and are competent. In addition, with this method, when the machine fails, it will be possible to repair it as soon as possible. In this context, research findings reveal that it is possible to implement shared tractor and tool-machine use cooperatives.

Whether the size of the agricultural enterprise is adequate to own a tractor or not, there is at least one tractor in every enterprise. This situation increases the investment capital and operating costs and reduces the profitability of the enterprise. Enterprises that do not have enough capital to buy tractors have difficulties in obtaining loans and repaying the loan. In addition, the maintenance and repair costs of the tractor bring an extra cost to the enterprise. Agricultural enterprises with low profitability are also under these burdens. With the successful implementation of the shared use of tractors and tools-machines, businesses can be relieved of these burdens to a large extent.

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SAFFRON (Crocus Sativus L.) CULTIVATION IN TURKEY

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ABSTRACT
Turkey is one of the countries with a rich flora, which contains many medicinal and aromatic plants. The reason for this is that it is located at the intersection of three phytogeographic regions and is the origin and differentiation centre of many genera and sections. Our country is among the leading countries in the trade of medicinal aromatic plants, as it is in a position to supply raw materials to important sectors such as herbal medicine, plant chemicals, food additives, cosmetics and perfume industry and paint industry. Saffron is one of the most expensive medicinal plants in the world. The demand for saffron (*Crocus sativus* L.) is increasing worldwide due to its use as a spice and flavouring to flavour and colour desserts in the kitchen, its use in the manufacture of medicines in medicine, and its interesting role in cosmetics. Successful experiments on saffron cultivation have been carried out in all seven regions in Turkey. Karabük province with Safranbolu district is the main centre of saffron production. In this study, a review was made about the current situation of saffron cultivation in our country and the importance of plant nutrition in saffron.

Keywords: Saffron (*Crocus sativus* L.), Cultivation, Plant Nutrition, Medicinal and Aromatic Plants,

INTRODUCTION

Saffron is obtained from the red stigmas of violet flowers (Figure 1.) of *Crocus sativus* L. Saffron is a monocot plant, perennial, herbaceous, and triploid (2n = 3x = 24 chromosomes) belonging to the plant family of Iridaceae (Cardone et al., 2020; Kahriz, 2020). Extracts from saffron stigmas are widely used. Known for more than 4000 years, the colour of saffron has symbolical importance for Hinduism and Buddhism, as it is thought to represent salvation and piety (Shokrpour, 2019). Saffron has long been cultivated in many regions and countries. For example; Australia, Azerbaijan, Iran, Kashmir, Greece, Pakistan, Italy, Morocco, Spain, France, China, Turkey, Afghanistan, and the United States. Iran has a share of more than 80% of the world's total saffron production and therefore, all other countries are considered small producers of saffron (Kahriz, 2020).

Saffron is known as one of the most expensive herbs among medicinal plants. That's why it is called 'red gold'(Leone et al., 2018). The demand for saffron is increasing worldwide due to its use as a spice and flavouring to flavour and colour desserts in the kitchen, its use in the manufacture of medicines in medicine, and its interesting role in cosmetics (Cardone et al., 2020; Kahriz, 2020). It is also used in dyeing wool, fabric and carpets in some regions spices and functional foods are used in the daily diet to prevent chronic diseases or cancer. Especially, saffron is good for human health thanks to its bioactive components of picrocrocin, crocin and safranal (Cardone et al., 2020).

Saffron contains approximately 150 chemical components (sugars, minerals, vitamins, anthocyanins, carotenoids, flavonoids and terpenes. The red colour of saffron (Figure 1) is generally due to the crocin component (Sharma et al., 2020). Crocin is the main active component of saffron and has multiple pharmacological effects. Crocin protects cells from oxidative damage. Saffron has anti-inflammatory, antioxidant, anticancer, hepatoprotective and cardioprotective effects and these have been proven by pharmacological studies. (Godugu et al., 2020). It is picrocrocin that gives saffron its bitter taste, and the aromatic aldehyde, which contains about 70% of the essential oil obtained from saffron flowers, is safranal (Sharma et al., 2020).

Figure 1. Stigmas of saffron (Shokrpour, 2019)

Saffron Cultivation:

Saffron is cultivated in a wide area between 0–90° east longitude and 30–40° north latitude in the world. With its stigmas, taste and colouring properties, in the food industry; It is widely used in pharmacy due to its therapeutic properties. Saffron is not a fast-growing species. The corms used in saffron production constitute the most expensive input. Since the saffron plant is infertile, it can only be produced vegetatively (Yıldırım et al., 2017). One of the cultivation maintenance processes applied to increase yield during saffron production is fertilization. One study has been compared the effects of various combinations of nitrogen, phosphor, potassium and cow manure on saffron cultivation. According to the results obtained, it has been determined that the most effective nutrient element in increasing flower yield is nitrogen mineral. Phosphorus and potassium fertilizers added in addition to nitrogen fertilizer were not statistically significant; flower yield has been slightly positive affected. In the case of organic carbon deficiency in soil content, it has been stated that the most important factor positively affecting saffron cultivation is cow manure (Ünal & Çavuşoğlu, 2005).

Climate and soil requirement:

Saffron is a plant that grows easily in Mediterranean climate conditions. It is ideal for the development of hot summers and mild winters. Saffron prefers loose, neutral pH, stone-free, well-drained and sandy soils. The soil to be planted must be thoroughly cleaned of weeds and seeds. Pest control after planting is quite difficult. For the yield to be obtained from the saffron plant in the first year to be high, the diameter of the onions should be at least between 15 and 20 mm. The increase in the diameter of the onion increases the flower yield in the plant and the amount of saffron to be obtained in direct proportion with it.

Sowing-time and sowing process:

Saffron planting should be done in August to get the best yield from the plant. Sowing should be done in early September at the latest. Planting saffron bulbs at the beginning or middle of the true dormancy period results in a decrease in flowers due to the shortened storage period. Growth inhibitory hormones are given to the saffron bulbs during the dormant period, helping the farmers to lift these bulbs from the soil to reuse them for planting in the next period. The best time to plant baby onions is between the saffron bulbs harvest and the actual dormancy period (early July). Flower yield decreases in planting towards the end of summer (Cardone et al., 2020; Godugu et al., 2020; Kara, 2019).

Saffron bulbs (Figure 2) are planted in the fields prepared for planting, at a distance of 30-45 cm between rows and at a depth of 10-12 cm. After planting, burnt cattle manure is covered (8-10 cm) so that they are covered, and after a few cm of soil is thrown on the manure, the planting process is completed. Approximately 150-600 kg of saffron bulbs is required for one decare of cultivation area (Cardone et al., 2020; Kara, 2019).

In saffron cultivation, it was observed that the deeply planted corms became larger, and when they were removed every two years, both saffron yield and corm yield increased. It was observed that the large size corms planted on the surface formed more juvenile corms and the number of corms increased. When all the results are evaluated in general terms, it is seen that it is not appropriate to remove the saffron plant every year. It is very important to leave the plants without harvesting in the field for at least two years after planting, both in terms of the flower and saffron yield and the number of corms and yields obtained (Yıldırım et al., 2017).

Figure 2. Saffron bulbs (corms) and reproduced saffron bulbs (Şahin, 2021)

Harvest and storage:

In October and November, the flowers are plucked every morning without opening and after they are left in the shade to open, the stigmas are plucked by hand, scissors or tweezers

(Figure 3). The detached stigmas are dried at a temperature between 30-45 °C and the dried products are placed in wooden boxes or dark coloured bottles and kept in cool (5-15 °C), out of light and moisture-free (humidity between 5-23%) areas. Saffron bulbs are stored at 23-27 °C (Cardone et al., 2020).

Figure 3. Saffron harvest (Gümüş, 2021)

Saffron Trade in Turkey:

Saffron can grow and adapt to a wide variety of soils in our country, except agricultural areas with gravelly, stony and clayey soil structures. Turkey is located at the intersection of Africa, Asia and Europe. For this reason, there are climatic differences between regions and this ensures a high plant diversity. Especially after 2010, the prevalence of saffron cultivation in Turkey has increased rapidly that shown in Table 1 (Şahin, 2021).

Successful practices related to saffron cultivation have been carried out in Turkey. Experimental scale cultivation of saffron has been reported in the provinces of İstanbul, Tekirdağ and Tokat (Koç, 2012). Safranbolu is the major place of saffron cultivation in Turkey. The first studies in Turkey were made with Karaarslan, a local culture plant of the Transit region Agricultural Research Institute in Eskişehir, saffron plant (*C. sativus*) in 2014 as a consequence of genetic improvement and breeding studies carried out since 2002 for the protection of local plant genetics. The bulbs obtained in consequence of these breeding studies were reproduced to be distributed to the public. Safranbolu cultivation in the greenhouse has been tried in Safranbolu Municipality (Kara, 2019).

Table 1. Cities where saffron is harvested in Turkey in 2019-2020 (Şahin, 2021)

Province	District	Province	District	Province	District
Denizli	Honaz Kale Acıpayam Pamukkale	İzmir	Bayındır Menderes	Düzce	Kaynaşlı
Hatay	Kırıkhan Reyhanlı Yayladağı Dörtyol	Eskişehir	Seyitgazi Sarıcakaya	Osmaniye	Hasanbeyli Bahçe
Çanakkale	Bayramiç Ayvacık Küçükkuyu	Yozgat	Sorgun	Van	Özalp
Isparta	Aksu Uluborlu Eğirdir	Adana	Kozan	Konya	Derbent
Ankara	Kazan Polatlı Çubuk Kızılcahamam	Burdur	Bucak	Bursa	Harmancık Yenişehir
Muğla	Ula Seydikemer Menteşe	Amasya	Göynücek	Balıkesir	Dursunbey
Mersin	Toroslar Aydıncık Mut	Karaman	Sarıveliler	Gümüşhane	Kelkit
Şanlıurfa	Bozova Hilvan Harran	Edirne	City center	Kütahya	Simav
Antalya	Gazipaşa Manavgat Korkuteli	Aydın	Buharkent	Bartın	City center
Karabük	Safranbolu	Tokat	Zile	Kahramanmaraş	Afşin
Adıyaman	City center				

CONCLUSION

In this review, information about the climate and soil characteristics required for the cultivation of the saffron plant, which is produced in some countries of the world and our country. the production amount of saffron is limited, but the usage area is quite wide and suitable for food and industry and used both as a spice and medicinally, is given. And also, with this study, the value of saffron was emphasized and attention was drawn to various aspects of the plant. The economic importance of saffron stems from the fact that it has such a wide range of uses in various industries in the world. The added value provided by the processing of saffron, which is fully integrated into the EU agricultural policy; emerges as an agricultural product that attracts attention due to its sustainable and ecological production methods, optimum use of water resources due to its perennial nature, reducing erosion and being a suitable product for cultivation in poor rural areas and empty areas.

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THYME (Thymus vulgaris L.) CULTIVATION IN TURKEY

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ABSTRACT

Turkey is one of the most important countries in the world with the production and export of thyme plant, which is collected from natural flora or cultivated. Considering the production and export values of the thyme (*Thymus vulgaris*), along with its many uses in the spice, medicine, chemical and cosmetic sectors, it is an important medicinal and aromatic plant for our national economy. Our country has a very important place in the world thyme trade with a 60-70% share. According to the data obtained in 2016, 14,724 tons of production was realized from an area of 121 thousand decares. Denizli, which is in the first place, has a share of 90% in terms of cultivation area and 85% in terms of production. Following that, Manisa, Kütahya and Uşak provinces are other important provinces that make up the production share. In this study, a review was made about the current situation of thyme cultivation in our country and the relationship of plant nutrition in thyme.

Keywords: Thyme (*Thymus vulgaris*), Cultivation, Plant Nutrition, Medicinal and Aromatic Plants,

INTRODUCTION

Thyme (*Thymus vulgaris*) is a medicinal aromatic plant known since ancient times, has been many bushy leaves and evergreen, growing 15-30 cm tall, in the form of a bush with a woody stem, aromatic and blooming purple or pink flowers in early summer (Akbar, 2020). Thyme has elliptical and linear leaves. These leaves are 3-8 mm long and 0.5-2.5 mm wide. *T. vulgaris* can be highly variable in leaf, branching, and flowering in its wild populations or cultivated forms. The genus *Thymus*, which belongs to the *Lamiaceae* family, consists of 215 species. *Thymus vulgaris* L., also known as garden thyme, is the most widely used commercial species. The *Coridothymus* (single species), *Tymbra* (12 species worldwide, 2 species and 4 taxa in Turkey), *Satureja* (30 species worldwide, 13 species and 14 taxa in Turkey), *Thymus* (220 species in the world; 39 species and 58 taxa in Turkey) and *Origanum* genus is the most important plant of the Lamiaceae family in Turkey (Bozdemir, 2019).

T. vulgaris was used by the Egyptians for mummification, by the Greeks in baths, and by the Romans for the purification of rooms. At the same time, the Greeks placed incense made from the thyme plant in coffins, believing that it provides the transition to the next life (Bozdemir, 2019; Vouillamoz & Bastien, 2020). It is now used as an aromatic herb to flavour foods, liqueurs and herbal blends. Thanks to the antimicrobial and antioxidant properties of its essential oil, its use in the treatment of dental plaque, dermatophyte infections, bronchitis, cough, inflammatory skin disorders and gastrointestinal disorders are the benefits of being a medicinal plant. In addition, it has analgesic, carminative, aphrodisiac properties and is also effective in the treatment of diseases such as epilepsy, asthma, gonorrhoea. *Oregano* oil and extract obtained from the plant are used in disinfectant production, shampoos, toothpaste, hair creams, colognes, soaps, detergents, creams and foods due to the phenolic components it contains. The thyme plant contains carvacrol or thymol as the main component in the essential oil it contains (Akbar, 2020; Dauqan & Abdullah, 2017; Vouillamoz & Bastien, 2020). The nutritional values it contains are given in Table 1.

Table 1. The nutritional values of thyme (Ibrahim, 2014)

Nutrient	Value	Nutrient	Value	Nutrient	Value
Fe	274.1	Cu	8.83	Cr	1.24
Mg	2380	Ni	1.87	Zn	19.56
Ca	20160	Pb	1.36	Mn	22.29
K	14185	Cd	0.120	Ba	12.67
Na	937.2	Sb	0.465	Co	nd
Se	1.08	Al	496.8	Ba	12.67

Thyme cultivation:

Plants need adequate and balanced nutrition to survive, grow and reproduce. The presence of too little or too many nutrients in the growth medium adversely affects the development of the plant. Macro and micronutrients are taken from the soil through the roots of the plant under suitable conditions such as pH, humidity, temperature and soil structure (Bradley & Hosier, 1999). In a study, the effect of nitrogen (N) fertilization on N, iron (Fe) and magnesium (Mg) nutrient contents of thyme plant was investigated. It was observed that the iron content also changed in direct proportion with the N fertilizer applied at different concentrations. As a result of fertilization, there was a significant increase in both fresh and dry matter weight of the plant (Golcz & Politycka, 2009). In another study conducted to investigate the effect of micronutrient application on the yield and content of the plant in thyme and some other medicinal plants, it was found that the carvacrol and thymol ratio, stem number, height, root, shoot, dry and fresh matter amount has increased with increasing doses in *Thymus vulgaris* L.. It has been observed that it gives good results at 400 ppm Fe, Cu, Zn and Mn concentrations (Yadegari, 2015). And also, It was observed that thyme plants with N and phosphorus (P) applications had the highest amount of essential oil content and growth compared to control plants (Sharafzadeh, 2011).

Ecological demands

- It is a plant that likes well-drained, light-textured, calcareous, neutral (pH 6.0 -8.0) soils and sunny places.
- Although it is not picky about soil, it grows best in loamy, clayey and alluvial soils. Sandy soils are not good for their development.
- The quality and content of the essential oil component obtained from thyme are affected by the soil content of the plant, the climatic conditions in which the plant is grown, the time the plant is harvested, the geographical structure and the post-harvest processes.
- It can be easily grown in high altitude plateau conditions.

Production and Harvesting:

It attracts a large number of bee and insect populations during the flowering phase, as it is largely foreign pollinated (Figure 1). The plant's ability to regenerate (re-development of the plant after cutting) is high, and there may be a chance to take three or more forms in the same vegetation. Its seeds are quite small and its thousand-grain weight is 0.2-0.3 g. Seed colour is brown, the shape is round-oval, and seeds retain their germination ability for 2-3 years. Germination takes place in 1-2 weeks at 20°C. Immature seeds in the seed pod reduce the germination power. Germination can take place in the light and the dark.

Figure 1. Thyme plant (T.C. Tarım ve Orman Bakanlığı Bitkisel Üretim Genel Müdürlüğü, 2020a)

The following should be considered while preparing the seedlings, planting the seedlings and harvesting:

- The mixture prepared with soil, sand and burnt barn manure is filled into the containers in which the seedlings will be planted and prepared for sowing seeds by pressing lightly on them.
- Seeds are covered with 0.5–1 cm thick soil.
- When the seedlings reach approximately 10 cm in length, they are transferred to the field.
- Transfer to the field, that is, diversion should be carried out at the beginning of the spring season.

- The seedlings are planted in the field with 70 cm planting distance and 30 cm planting distance. An average of 5000 seedlings can be planted on 1000 m^2 (decare).
- Irrigation is carried out after each cutting process and during the flowering period. Plant growth slows down when irrigation is not done during the dry months. Plant yield and quality can be increased by performing timely maintenance procedures during thyme cultivation.
- Harvest; If possible, thyme should be harvested by hand/machine at a time when 50% of flowering is completed.
- An average of 5 cm above the soil level is recommended for harvesting.
- While one harvest can be obtained from the plant in the first harvest year, it is possible to get 2-4 harvests from irrigated agricultural areas and 1-2 harvests from dry agricultural areas after the second year. If the thyme planted area is well cared for, 5-6 years can be economically benefited from a planting. In the following years, the yield gradually decreases. In the first year 100-150 kg/da in irrigated conditions, 40-50 kg/da in dry conditions, in the second and third years 400-500 irrigated, 150-200 kg/da dry herb yields can be obtained (Bagdat, 2018; T.C. Sanayi ve Teknoloji Bakanlığı, 2020).

Processing of thyme and oil extraction:

To keep the yield and quality of thyme at a high level, the drying process should be dried quickly on days that are not very humid and rainy, and in a shaded environment. During this process, weeds and other substances that are involved in the harvest should also be cleaned. The harvested thyme is dried in the natural drying area in the field within 3-4 days, and the thyme to be used for the spice industry is separated from its stems and filled into sacks. Dried thyme to be used for essential oil is distilled to obtain essential oil (Figure 2). An average of 3-4 kg of essential oil can be obtained from 100 kg of dried thyme (T.C. Sanayi ve Teknoloji Bakanlığı, 2020).

Figure 2. Dried thyme (*Thymus nummularius* L.) and its oil (T.C. Tarım ve Orman Bakanlığı Bitkisel Üretim Genel Müdürlüğü, 2020b)

Thyme trade in Turkey:

Turkey is one of the most important countries in the world with the production and export of thyme plant collected from natural flora and cultured (Table 2), and considering the production and export values of thyme plant with many usage areas such as spice, medicine, chemistry and cosmetics sector. It is an important medicinal and aromatic plant for our national economy. While Turkey exports most of the thyme plant it produces to the USA, it also exports it to countries such as Germany, Netherlands, Poland, France, India, Austria, Italy, Japan, Brazil, Canada, South Africa and Australia.

Table 2. Thyme production in Turkey (TÜİK, 2021)

Years	Production amount (tons)
2021	27300
2020	23866
2019	17965
2018	15895
2017	14477
2016	14724

The essential oil obtained from thyme, which is also an oil plant, is one of the most demanded and traded essential oils in the world. Unit export prices of thyme essential oil vary according to years and countries. It has been calculated that an average of 3 kg of essential oil can be obtained from 100 kg of dried thyme. The thyme plant, whose essential oil has been removed, also has commercial value in the feed and similar food industry. To maintain and improve our position in the world markets and to make exports continuous, the production of spices and essential oils following the standards, quality and by the market demands should be made and encouraged. (T.C. Sanayi ve Teknoloji Bakanlığı, 2020).

Our country has a very important place in the world thyme trade with a 60-70% share. According to the data obtained in 2016, 14,724 tons of production was realized from an area of 121 thousand decares. Denizli ranks first with a 90% share in cultivation area and 85% in production amount, while Manisa, Kütahya and Uşak provinces have created the other important production amount (Table 3).

Table 3. Thyme yield in 2020 by the city in Turkey (TÜİK, 2020)

City	Yield (kg/da)	City	Yield (kg/da)
Adana	200	Eskişehir	250
Afyon	137	Hatay	150
Ankara	167	Isparta	105
Antalya	384	Karaman	250
Aydın	128	Konya	88
Balıkesir	143	Kütahya	129
Burdur	288	Manisa	330
Bursa	286	Muğla	226
Denizli	124	Samsun	262
Düzce	143	Uşak	186
		İzmir	133

CONCLUSION

In adapting to climate change and protecting the environment, it is necessary to expand product alternatives and to ensure sustainable use of resources by protecting soil, water, gene resources, biodiversity and the environment. Producing the right product at the right time and in the required amount is very important in terms of sustainability. When the production process is started by using labour, knowledge and material resources, there is an increase in the values such as agriculture profit, added value of essential oil and added value of thyme extract in the production of thyme made with varieties suitable for their usage areas. It is predicted that an increase in thyme production can occur when the correct methods are applied in the production process, suitable climatic conditions and sustainable use of resources are provided. In this study, information was given about the promotion of thyme, which is among the medicinal aromatic plant species, its production conditions and its production share in our country. Consequently, with this information, it has been tried to make information for the producers who will cultivation thyme.

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SIMULATING FUTURE STATUS OF AGRICULTURAL LANDS IN SOUTH PART OF MARMARA BASIN DEPENDING ON HISTORICAL TRENDS*

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ABSTRACT
Change in amounts and locations of agricultural lands have become a worldwide phenomenon against forcing effects of global urbanization trends. Such trends seemed strongly related with growing population in various regions of the world particularly in developing countries. In present study it was aimed to visualize future situation of agricultural lands in terms of area (ha, %), position and landscape metrics. Freely available Landsat imageries from Thematic Mapper (TM), Enhanced Thematic Mapper Plus (ETM+), and Operational Land Imager of 1984, 1999 and 2014 production season were used to delineate land cover land use (LCLU) maps. Moreover, census data of closest years were utilized to evaluate the population impacts on LCLU formations as main trigger in the area. Markov chains were used to predict pixel demands of future classes whereby the analysis results were modified in simulation step dependently to population relations. The LCLU maps for 2029 and 2044 were simulated through cellular automata based model coupling artificial neural network algorithm and roulette wheel selection mechanism, and results are presented.

Keywords: Agricultural lands, Future Status, Historical Trends, Simulation, South Marmara.

*The paper based on a part of Melis Inalpulat's PhD Thesis.

INTRODUCTION

Alternations in terrain properties against human activities is a common trend that begins from the Neolithic period, Ramankutty and Foley (1999) designated that such activities were accelerated especially in the last three centuries whereby many natural ecosystems were converted to agricultural lands due to significant increase in per capita consumption of resources. The situation usually leads to severe changes on earth surface. One of the most important measures of the consequences of such processes is known to be determination of Land Cover and Land Use (LCLU) changes considering different LCLU types in a certain area. Among various LCLU classes, agricultural land use is reported to be most affected class from human-induced pressures (Plieninger et. al., 2016). Agricultural lands can be defined as complement of production areas for producing plants or animal sourced food, fiber, energy and other raw materials (FAO, 2016). Therefore, identification of changes in magnitudes and locations of agricultural lands is significant for future land use planning, which will also help to guarantee food security in return. Evaluation of transition probabilities between each class by utilizing the historical conditions and periodical changes in LCLU status may facilitate to forecast future conditions for avoiding undesirable levels of vegetative losses, degradation in cultivated areas, maintaining the productivity in all types of agricultural activities, and ensuring the sustainability of environmental system functions (Inalpulat and Genc, 2021). Even though generating these forecasts have great necessity for all scales, since the differencies in LCLU patterns have significant impacts on different components of environment, such as hydrological networks, water quality, budget and distribution, and pollution, conduction of basin scaled studies are highlighted (Miller and Hess, 2017; Aduah, Jewitt ve Toucher, 2018). In this context, determination of recent status and historical changes in LCLU at wider areas have enabled by remote sensing technologies, which provides rapid, reliable and relatively economic results while reducing labor costs in such areas.

Present study focused on the historical ($LCLU_{1984}$, $LCLU_{1999}$, and $LCLU_{2014}$), and simulated LCLU changes ($LCLU_{2029}$ and $LCLU_{2044}$) particularly in occurred agricultural lands in terms of topographic properties and landscape metrics in South part of Marmara Basin, Turkey, using Landsat imageries and ancillary data of population, terrain and location properties.

MATERIAL AND METHOD

The study area is one of the twenty five hydrological basins located in our country, the South part of Marmara Basin, covering a survey area of approximately 635 thousand ha. There are six districts belonging to two different cities located in the area, namely, Biga, Can, Lapseki, Yenice and Provincial centre of Canakkale city, and Gonen district of Balıkesir city (Figure 1).

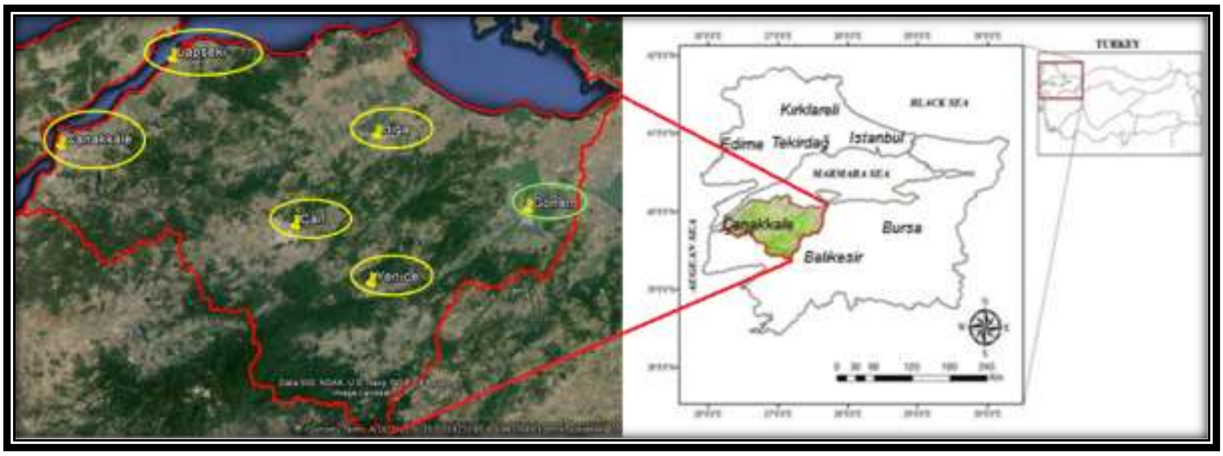

Figure 1. Location and coverage of study area

The census data of available years (1985, 2000, and 2014) were used to evaluate the links between population and settled areas for forecasting future demands for settled areas. Predicting of future settled area demands have provided the losses against urbanization process. The population projections were conducted with cohort component method using open source DemProj software (Spectrum, 2018), whereby the district level inputs were composed using reports of Turkish Statistical Institute (TSI, 2015) and Hacettepe University Institute of Population Studies (HUIPS, 2018).

Landsat imageries of 07 September, 1984, 08 August 1999 and 21 May, 2014 that obtained from Thematic Mapper (TM) with path and row number 181/32 of Enhanced Thematic Mapper Plus (ETM+), and Operational Land Imager (OLI) sensors, respectively, were used to delineate LCLU statuses. The imageries were freely downloaded from United States Geological Survey (USGS) website (USGS, 2015). The visible, NIR and SWIR bands of each image were stacked to generate 6-band images before classification process (Figure 2). Maximum likelyhood algorithym was adopted for supervised classification. The area was separated into five main LCLU classes including forest (F), other vegetation (O), agriculture (A), water surface (W) and residential area bare soil (R-B). Accuracies were controlled through Google Earth application. All preprocessing, classification and accuracy assessment steps were conducted in Erdas Imagine software (Hexagon Spatial, 2014).

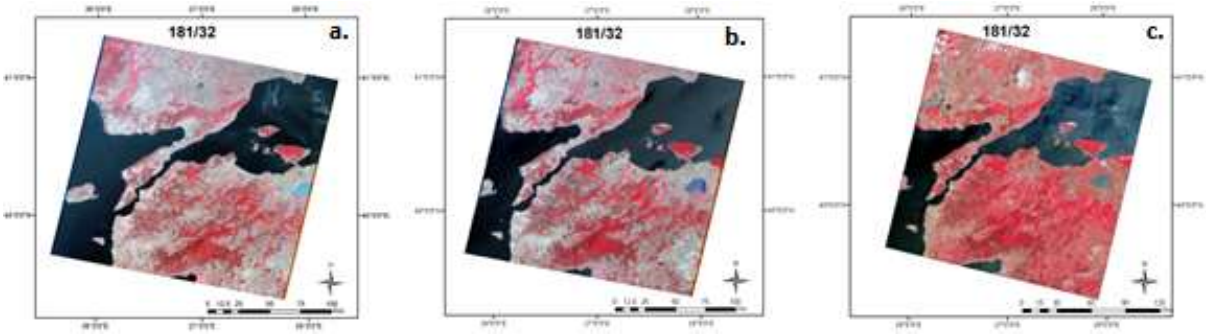

Figure 2. Landsat 181/32 scenes of a. Landsat TM, b. Landsat ETM+, c. Landsat OLI

The LCLU simulation process followed in the study has two basic steps. The first one includes predicting future pixel numbers for each class depending on markov chains (MC), which latter revised considering population relations. The MC is a widely used prediction model for calculation of conversions considering a certain number of possible situations. The equations are given below (Eq. 1 a-c) (Sang, Shang ve Yang, 2011). The distribution of predicted pixel demands into study area depending on historical LCLU type topographic properties of elevation (Figure 3a), slope (Figure 3b), and aspect (Figure 3c), which were obtained from ALOS digital elevation model (DEM) (JAXA, 2020), and location-related properties of proximity to settlements (Figure 4a) and proximity to roads (Figure 4b) data, composed the second step. Verification of simulations were tested by simulating actual pixel numbers of $LCLU_{2014}$ using $LCLU_{1999}$ together with same ancillary data used in future simulation process, and the coherency between actual $LCLU_{2014}$ and simulated $LCLU_{2014}$ assumed to be valid for $LCLU_{2029}$ and $LCLU_{2044}$. Simulation procedures were conducted with ANN based CA model for future land use simulation (GeoSOS, 2020).

$$S\,(t+1) = P_{ij} \times S(t) \quad \text{(Eq. 1 a)}$$

$$P_{ij} = \begin{bmatrix} P_{11} & P_{12} & \dots & P_{1n} \\ P_{21} & P_{22} & \dots & P_{2n} \\ \dots & \dots & \dots & \dots \\ P_{n1} & P_{n2} & \dots & P_{nn} \end{bmatrix} \quad \text{(Eq. 1 b)}$$

$$\left(0 \le P_{ij} < 1 \text{ ve } \sum_{j=1}^{N} P_{ij} = 1, (i, j = 1,2, \dots, n)\right) \quad \text{(Eq. 1 c)}$$

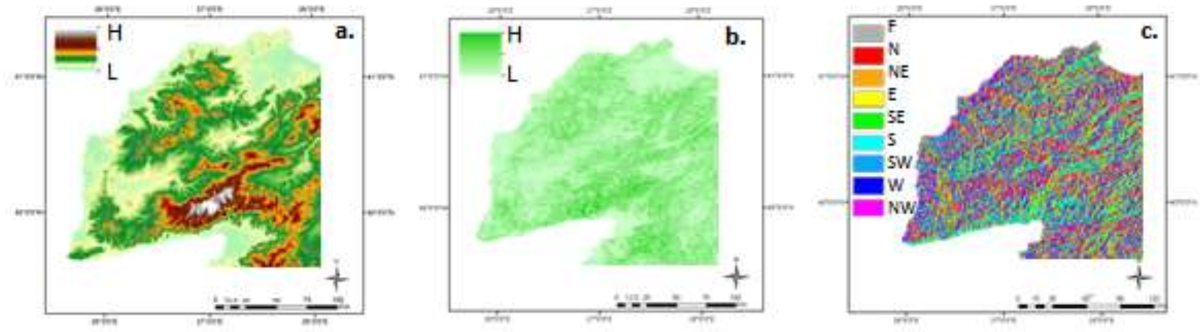

Figure 3. ALOS a. elevation, b. slope, and c. aspect data

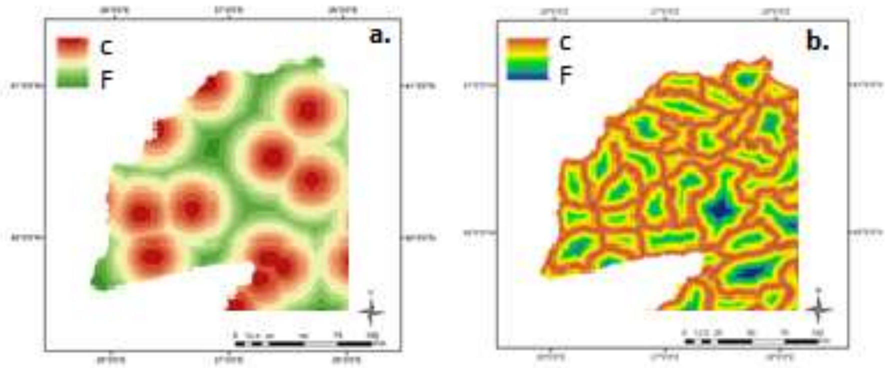

Figure 4. Proximity to a. settlements, and b. roads

The changes in agricultural lands per capita (ALPC, ha person^{-1}), topographic changes including the distributions of agricultural lands according to different elevation and slope intervals, and landscape metrics number of patches (NP), patch density (PD), largest patch index (LPI), and mean patch size (MPS) parameters for $LCLU_{1984}$, $LCLU_{1999}$, $LCLU_{2014}$, $LCLU_{2029}$ and $LCLU_{2044}$ were interpreted in the study.

RESULTS AND DISCUSSION

The $LCLU_{1984}$, $LCLU_{1999}$, and $LCLU_{2014}$ maps of the study area are given in Figure 5 a-c, the magnitudes of different LCLU classes are presented in Table 1, and error matrices of accuracy assessments are summarized in Table 2. As it can be seen from the table, O class including gazing lands, shrubs, brushes and other types of natural vegetation was decreased drastically while the areas of F, A, W and R-B were increased. Deeply analysis of transition amounts have revealed that A and F class have mostly converted from O class whereas W and R-B classes were mainly gained from A class areas, even though total areas of A class seemed to be increased. On the other hand, the similarities between very young forest trees and O class have resulted in misclassifications between F and O classes. However, with the increase in the maturity of younger forest cover, the confusion between these classes reduced within time. Similarly, due to different acquisition months, the patterns of agricultural production were differed and resulted in misclassifications between harvested crops and O class due to almost-identical spectral signatures between these classes. Moreover, the increase in irrigation practices around the area have accelerated the changes of production type from unirrigated crops to various irrigated agricultural plants, and resulted in less misclassification in further years (Inalpulat and Genc, 2021). Therefore, there were both actual and overestimated increases in F and A classes especially between 1984 and 1999 due to above mentioned misclassification situations. All overall accuracies (%) and kappa statistics were shown that classifications were reliable enough since the results were over the threshold value of 0.75 (Lillesand and Kiefer, 2000).

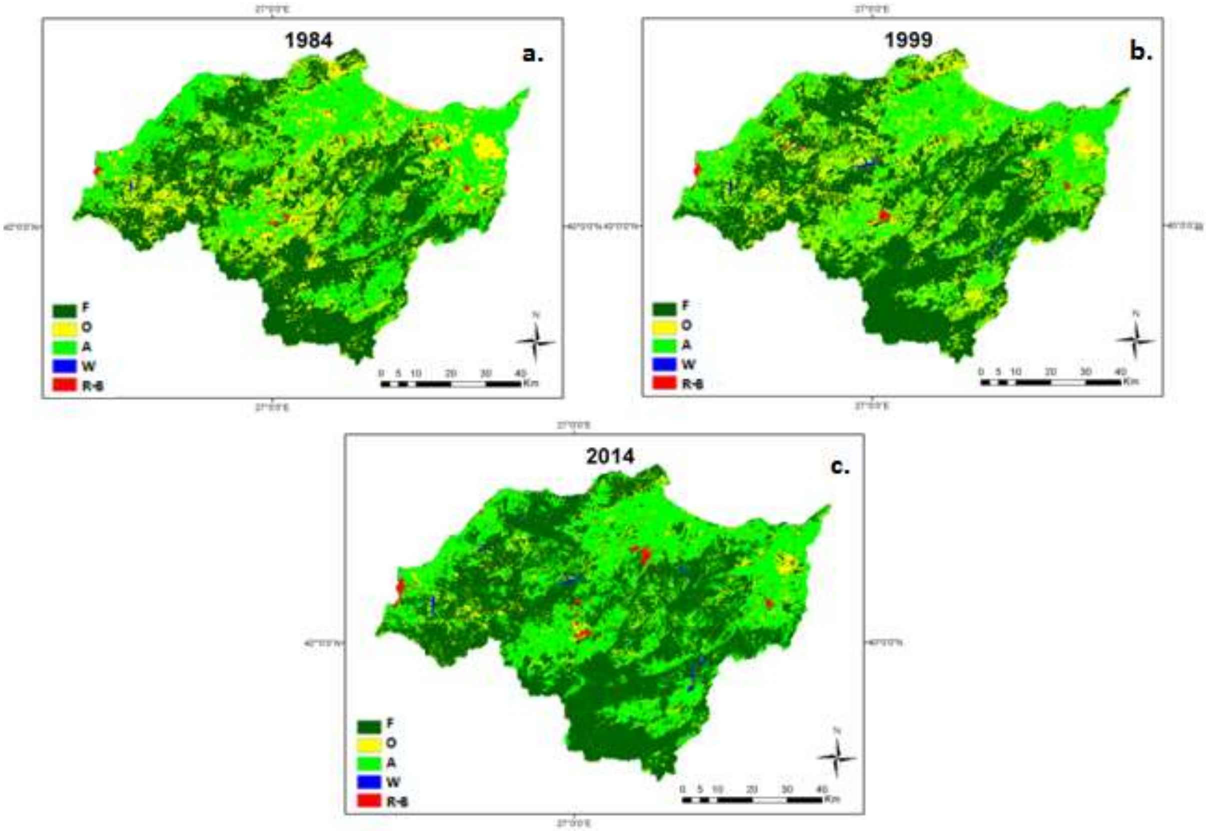

Figure 5. a. $LCLU_{1984}$, $LCLU_{1999}$, and $LCLU_{2014}$

Table 1. Areas of different LULC classes in different years

Class	Area (ha)		
	1984	1999	2014
F	209028	216134	224580
O	129745	101224	65580
A	288573	307165	332877
W	353	2378	2741
R-B	6532	7330	8453
Total	634231	634231	634231

Table 2. Summaries of accuracy reports and error matrices

1984											
C/R	F	O	A	W	R-B	R T	C T	C C	P A %	U A %	K
F	228	6	9	0	0	261	243	228	87,36	93,83	0,9131
O	24	180	18	0	0	195	222	180	92,231	81,06	0,7585
A	9	9	234	0	3	267	255	234	87,64	91,76	0,8829
W	0	0	0	90	0	90	90	90	100,00	100,00	1,000
R-B	0	0	6	0	84	87	90	84	96,55	93,37	0,9262
TOTAL	261	195	267	90	87	900	900	825	90,67		0,8779
1999											
C/R	F	O	A	W	R-B	R T	C T	C C	P A %	U A %	K
F	246	6	0	0	0	261	252	246	91,35	98,96	0,9841
O	9	180	9	0	3	222	201	180	85,71	85,71	0,8244
A	6	36	222	0	0	234	264	222	92,50	85,06	0,7962
W	0	0	0	90	0	93	90	90	100,00	100,00	1,000
R-B	0	0	3	3	87	90	93	87	96,77	96,77	0,9640
TOTAL	261	222	234	93	90	900	900	825	91,67		0,8913
2014											
C/R	F	O	A	W	R-B	R T	C T	C C	P A %	U A %	K
F	267	15	6	0	0	285	288	267	93,68	92,71	0,8933
O	9	150	6	0	3	174	168	150	86,21	89,29	0,8672
A	9	6	240	0	6	255	261	240	94,12	91,95	0,8877
W	0	0	0	90	0	90	90	90	100,00	100,00	1,0000
R-B	0	3	3	0	87	96	93	87	90,63	93,55	0,9276
TOTAL	285	174	255	90	96	900	900	834	92,67		0,9034

R T: Reference Totals, C T: Classified Totals, C C: Correctly Classified, P A %: Producer's Accuracy, O A %: User's Accuracy.

For the simulation step, the relations between census and R-B class were evaluated for all districts located in the area whereby the R^2 values were over 0.99, and the markov chain results were then revised depending on population-sourced potential future urban area demands. Subsequently the predefined simulation steps were applied. The $LCLU_{2029}$ and $LCLU_{2044}$ simulations are given in Figure 6 a, and b. The predicted areas for $LCLU_{2029}$ and $LCLU_{2044}$ are served in Table 3. According to the results, there was a significant reduction in agricultural lands between 2014 and 2029 where as a slight increase was expected between 2029 and 2044. Meanwhile, F and R-B class areas were predicted to be increased unlikely to decrease in O class area. The area of W class assumed to be unchanged since it is mostly dependent to the political decisions on constructions of new water collection units, and thus, not quite possible to foreseen currently. The verification process has revealed that the overall accuracy was75.46% and the kappa value was 0.7527.

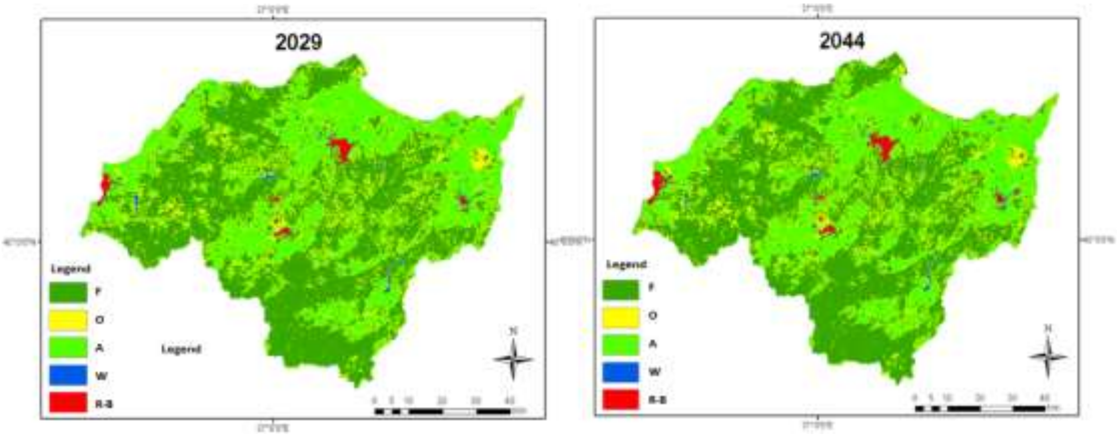

Figure 6. Simulations of a. $LCLU_{2029}$ and $LCLU_{2044}$

Table 3. Areas of $LCLU_{2029}$ and $LCLU_{2044}$

Class	Area (ha)	
	2029	2044
F	339322	341414
O	58703	44459
A	223746	224556
W	2555	2555
R-B	9906	11247
Total	634231	634231

Subsequent to the simulation process, the ALPC, distribution of A class within different elevation, slope and aspect intervals, and landscape metrics (NP, PD, LPI, and MPS) statuses of historical and simulated LCLU maps are given in Figure 7, Figure 8, Figure 9, and Figure 10 a-d, respectively. Depending on the findings from ALPC, it can be said that the ALPC was gradually decreased from 0.88 to 0.78 between 1984 and 2014 although the A class area was found to be increased. Thence, decrease was caused from significant growth of population while it is expected to decrease to 0.36 until 2044, but still considerably higher than mean values calculated for Turkey, Europe, and World (Figure 7). The ALPC serves as an indicator of food security (Devereux ve Maxwell, 2001), further situations should be evaluated with higher resolution scales for composing more precise forecasts since the magnitudes of A areas estimated to decrease while the population showed significant growth. Evaluation of elevation intervals of A class areas designated that, the percentages of the agricultural lands located less than 500 m elevation were slightly decreased whereas there were progressive increases in the areas above 500 m elevation. This was mainly sourced from urbanization process since the urban areas were mainly located in almost flat areas close to sea level. In addition, the percentage of A class located under 6% slope value were gradually decreased whereas there were obvious increases in A class located in 6-18% slope interval. This situation is reported to be a common trend especially in developing countries (Mugagga et. al., 2012). However, the agricultural lands above 18% seemed to be sourced from misclassified pixels, thus were not reflecting the actual changes. Finally, the alternations in landscape metrics were interpreted. The NP values were seemed to increase in the first period, and then decreased until 2014.

However, NP_{2014} was higher than NP_{1984}. Depending on the $LCLU_{2029}$ and $LCLU_{2044}$, it was seen that NP value have a decreasing tendency, designated that agricultural lands expected to have more compact structure. The PD values showed the same trends with NP values, whereas PD values were started from1.82 and decreased to 1.36, with a fluctuation between 1984 and 2014 years. Conversely, LPI decreased from 13.53 to 13.18 between 1984 and 1999, while it was expected to reach to 16.82 until 2044 by a progressive increase. Finally, the change pattern of MPS was similar with LPI values. The initial MPS (MPS_{1984}) was calculated as 19.07 while final simulation year's MPS value (MPS_{2044}) was estimated to be 22.43, supporting the idea of future agricultural lands will be less fragmented.

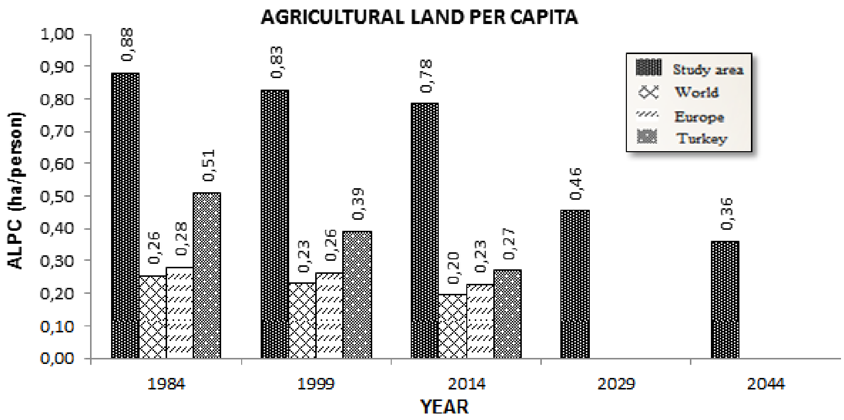

Figure 7. Temporal change in ALPCs of historical and simulated years

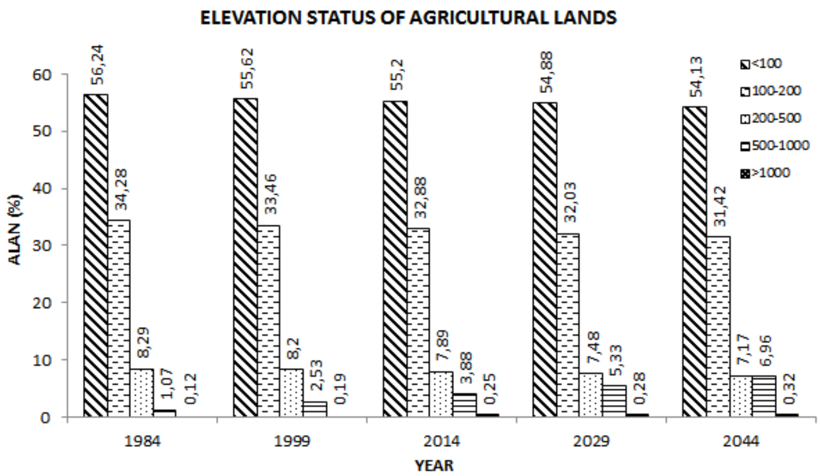

Figure 8. Temporal changes in elevation statuses of A class between 1984 and 2044

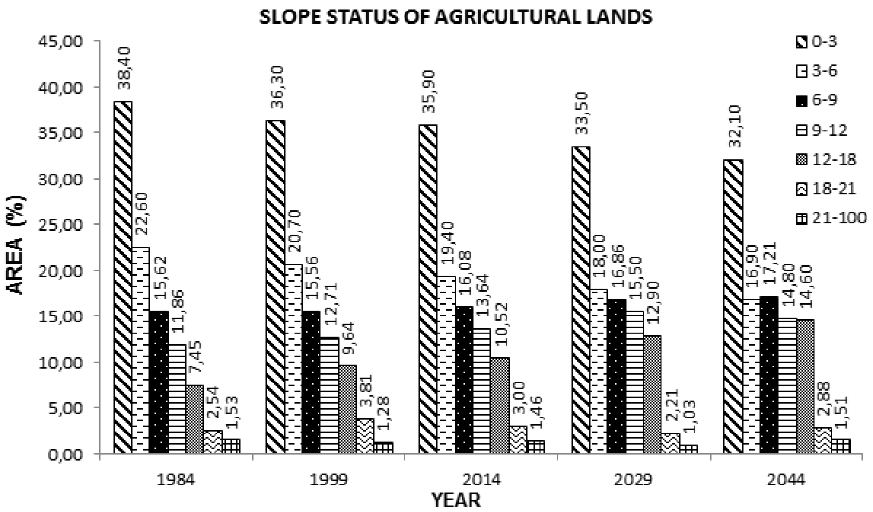

Figure 9. Temporal change in slope statuses of A class from 1984 to 2044

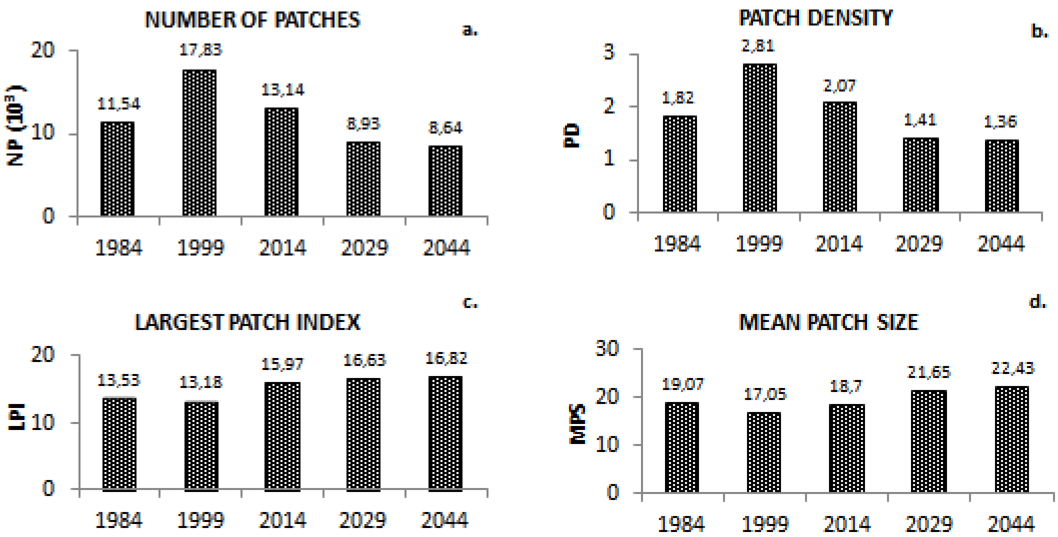

Figure 10. Landscape metrics of a. NP, b. PD, c. LPI, and d. MPS

CONCLUSIONS

The findings of the study revealed that even though agricultural lands showed an increment trend in the past, the situation was occurred due differences in acquisition dates of imageries which leads to some misclassifications between harvested crops and other vegetation types, therefore, overestimation of increased amounts of A class. The actual increases were mostly sourced from increments in agricultural practices which provided both increase of agricultural lands and lass misclassifies sourced from changed production pattern. On the other hand, simulation results designated that the A class areas seemed to be decrease significantly against significantly growing population in the area. Moreover, A class areas area estimated to locate on the areas with more elevation and slope values. On the other hand, the changes in landscape metrics demonstrated that, although the magnitude of A class is predicted to be decreased in the next 30 years, the agricultural lands expected to be less fragmented with larger patches, which results in more compact, and thus, having capability of higher yields and economic incomes obtained from the same unit area due to relative decrements in time, transportation, and labor forces.

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IDENTIFICATION OF RESIDENTIAL DEVELOPMENT IMPACTS ON AGRICULTURAL LANDS USING LANDSAT IMAGERIES: CASE STUDY OF BURSA, NILUFER (1990-2020)

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ABSTRACT

Bursa province of Turkey have been exposed to controlled and uncontrolled urbanization process especially in last three decades due to industrial initiatives, particularly. Nilufer district, which is one of the central residential sites, was foreseen to be significantly effected against increasing artificial surface construction processes. Therefore, the main objective of the study was to analyze the land cover land use (LCLU) changes within ten-year periods between 1990 and 2020, and agricultural land status in response to increased urbanization were highlighted. Images from Landsat series were utilized, and the study area was classified through two different classification techniques of segmentation and random forest considering six main LCLU classes namely, forest (F), agriculture (A), residential (R), bare soil (B), water surface (B) and other (O). According to segmentation results, A class were decreased by 9.40%, 11.03%, 10.41% within 1st, 2nd and 3rd periods, respectively whereas R areas were increased by 58.97%, 75.62% and 29.37% with the same periodical order. On the other hand, according to the random forest classification, the decrease in A areas were calculated as 12.77%, 19.34% in 1st and 2nd period while there was a small increase of 1.10% in 3rd period. Meanwhile, the increases in R areas were found to be 49.96%, 92.19%, and 97.77%, respectively. Results of accuracy assessment revealed that segmentation gave slightly more accurate classifications in the selected area of interest. However, the LCLU maps obtained from both classification techniques showed that the increase in R areas is highly complex and and the gains of R areas were not restricted with only A areas. Especially conversions from F and O classes concluded to be investigated via high resolution imageries for more precise results.

Keywords: Agricultural Land, Bursa, Landsat, Nilufer, Residential Development.

INTRODUCTION

It is known that changes in land use land cover (LULC) occur in accordance with environmental conditions. It is known that the most common LULC change is related to urbanization on a global scale (Weinzettel et al., 2013). Evaluation of information and changes for LULC is a basic need to understand prominent facts in terms of land use (Inalpulat and Genc, 2021). Image interpretation is possible with remote sensing data in terms of examining regional changes in the land and quantitative planning of land use (Liu, 2021). It is preferable to reveal the occurrences of LULC change with remote sensing data (Pijl et al., 2021). Remote sensing data provides quality information production in many fields because it is in different resolution conditions and especially because it allows free use. Landsat satellite images are served for many studies within various resolution concepts (Hua and Ping, 2018; Naikoo et al., 2020; Rodriguez-Galiano et al., 2012; Shawul and Chakma, 2019). Especially over the years, Landsat satellite data is more preferred by experimental research of subjects such as areal changes in agricultural areas, crop patterns, yield (Özelkan et al., 2018) and the relationship between agriculture and urbanization can be understood more clearly. It is known that the change in urban areas is related to agricultural areas and this relationship progresses uncontrollably and irregularly, thus affecting the planning of LULC. Various studies have revealed that the physical change and development structure of residential areas creates pressure on agricultural areas due to multiple reasons (Akın and Erdoğan, 2020; Fazal, 2000; Kumar and Agrawal, 2019; Özelkan et al., 2018; Sharma and Joshi, 2016; Shi et al., 2016). Due to the rapid urbanization in the central areas close to the industrial areas, it is seen that the change in agricultural areas and urban areas is the main subject of many studies. The increasing level of knowledge in the literature shows that the integration of agriculture and remote sensing is considered together and that agricultural practices have increased since 2013 (Weiss et al., 2020).

Different classification techniques are used for different study areas when making LULC maps (Hütt et al., 2016; Lin et al., 2015; Qu et al., 2021). In vegetation mapping, Object Based Classification (OBC) (Johnsson, 1994; Kindu et al., 2013; Petropoulos et al., 2013; Yu et al., 2006; Zhou et al., 2008) and Random Forest (RF) (Chan and Paelinckx, 2008; Ghimire et al., 2010; Pal, 2005) classification techniques have increased in use. OBC is a technique in which segmentation is characterized by sequential and classification processes (Lourenço et al., 2021). It provides classification by structural recognition of lower and upper spectral classes, keeping it in a dependent relationship with the information learned (Vieira et al., 2012) and information extraction, spectral features as well as geometric and structural features (shape, texture, context) (Aggarwal et al., 2016). This optimal and in itself efficient classification approach can also improve the overall accuracy of the image (Aggarwal et al., 2016). RF classification is a classification technique used to pixels into certain classes (Kumar and Agrawal, 2019). It involves selecting random features and assigning them to classes with preloaded information of training data (Pal, 2005). The requirements of learning are compared with the determination of many necessary parameters within its own network structure and the high accuracy of extraction in large data sets (Wu et al., 2021). In its design, the changes to be made on the classification should be created in a way that will affect the final output (Magidi et al., 2021).

For the sustainability of agricultural areas, it must ensure their integration with the environment in an intense balance (Tarolli and Straffelini, 2020) and the changes to be determined can be achieved by means of satellite images obtained at different times (Bayar, 2018). Remote sensing, which is a fast and economical method in determining the change in LULC from the local to the global level, is used extensively to determine the relationship between urban and agricultural areas (Genc and Inalpulat, 2016). In determining this change, different models are used to see the change and development in classes (Radhika and Varadar, 2016). Basic information or basic accuracies in the field must be estimated for the classified image that occurs when using models (Kumar and Agrawal, 2019). In this context, understanding, estimating and modeling local, regional and environmental change is considered sustainable in terms of agricultural and urban dynamics (Fazal, 2000).

Bursa, which is the 4th largest city in Turkey, is called the city of industry and agriculture. The contribution of the rapid change in the field of industry in the last thirty years to the national economy has increased by 11.7% in the field of industry and 7.5% in the field of agriculture as the Gross Domestic Product (GDP) volume index (TUIK, 2021). The increase in the GDP ratios in the industrial-centered regions increases the migration to the industrial cities and thus the migration affects the speed of urbanization and agricultural areas. The aims of the study are to reveal the changes by comparing the vegetation mapping to examine the temporal variation of the loss of the city of Bursa in agricultural areas against urban development and growth, and to examine the urban/agricultural dynamics, and to reveal the changes on the basis of area (%, ha) and with Landsat images in 1990, 2000, 2010 and 2020 seasons and It is a comparison of the performance of different classification techniques. In the findings at the end of the study, there was no ABPM mapping comparison, which was formed by using different classification techniques in integration with the remote sensing data related to the city of Bursa in terms of agriculture and settlement areas in different studies, and therefore, this to study is qualified to form a base for future evaluation.

MATERIAL and METHOD

Material

Study Area

The study area, Nilüfer, is surrounded by Osmangazi in the east, Karacabey in the west, Mudanya in the north, Orhaneli in the south, with a total area of 423.181 decares, consisting of 42 neighborhoods and 22 villages (Bursa Governorship, 2019). The total population of the district consists of 484,832 people (TUIK Address Based Population Registration System, 2020). Nilüfer is under the influence of subtropical dry summer climate and Mediterranean climate according to Köppen Climate Classification (GDM, 2020). Considering the general soil quality of the study area on a provincial basis, it has alluvial soil group and agriculture is carried out in different cultural structures such as peach, olive, wheat, corn, sunflower, tomato and pepper (Turan et al., 2010).

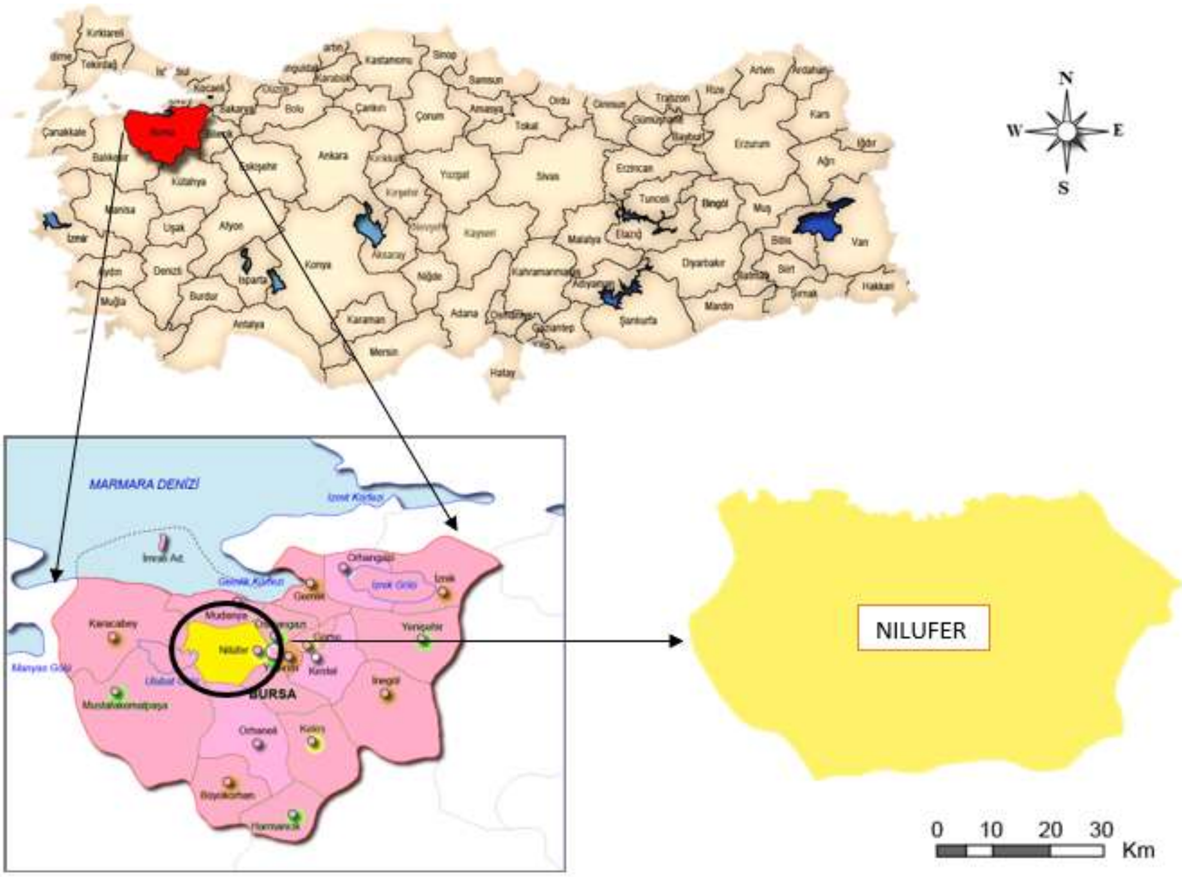

Figure 1. The location of the study area

Data Used

In the study, Landsat 5-TM (1990, 2000, 2010) and Landsat 8-OLI (2020) satellite images were obtained as open access from the United States Geological Survey (USGS) Earth Explorer site (USGS, 2021). Detailed information and features about the satellite images used are given in Table.1.

Table 1. Characteristics of satellites used in the study area.

Landsat 5 - TM	Obtaining Date	: 31 July 1990
	Spatial Resolution	: 30 m
	Spectral Bands	: 7
Landsat 5 - TM	Obtaining Date	: 26 July 2000
	Spatial Resolution	: 30 m
	Spectral Bands	: 7
Landsat 5 - TM	Obtaining Date	: 4 August 2010
	Spatial Resolution	: 30 m
	Spectral Bands	: 7
Landsat 8 - OLI	Obtaining Date	: 1 July 2020
	Spatial Resolution	: 30 m
	Spectral Bands	: 9

Preprocessing

In order to obtain LULC maps, image processing steps were performed using Erdas IMAGINE (2014), ArcGIS 10.5, eCognition Developer 64 and SNAP 7.0 programs. In the study 1, 2, 3, 4, 5, 7 bands for Landsat 5-TM satellite images (1990, 2000, 2010, 2020) to be used and 2, 3, 4, 5, 6, 7 bands for Landsat 8-OLI satellite image (2020) by determining a new image was obtained. The images of each year were determined for the Nilüfer district by considering the political borders of Bursa and were cropped from the image.

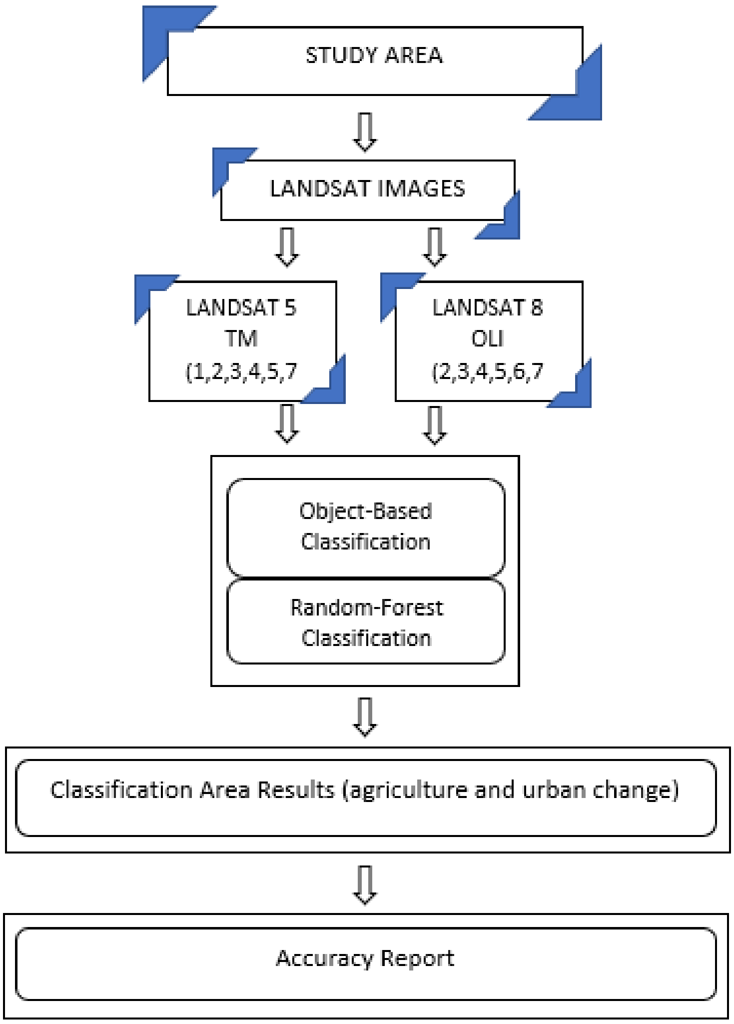

Figure 2. Flowchart

Method

Classification

The study area was classified using Landsat imageries through two different classification techniques of segmentation and random forest considering six main LULC classes namely, forest (F), agriculture (A), residential (R), bare soil (B), water surface (B) and other (O). The choice of classes in the study was determined by the fact that the field conditions were known by the researchers and therefore visual interpretation was made.

Object-Based Classification (OBC)

In the images where the preprocessing was finished, OBC was made with eCognition Developer 64 software. The first step of the classification process is segmentation, which enables the creation of objects similar to real world objects on the image (Bıyıklı, 2019). The process starts with the trial selection of the three main parameters required in segmentation. These are the parameters of scale, shape and density. The scale parameter determines the average size of the objects (Trimble, 2007). Shape and color parameters have an integral effect on image objects. Objects created by image segmentation for OBC with the parameters whose values are provided begin to be assigned to classes (Kindu et al., 2013). In the study, the satellite image was first converted to false colors, facilitating the assignment of classes. Segmentation parameters were determined as scale, density, shape, 40, 0.5, 0.5, respectively. Recognition of objects was created by making homogeneous selections for six classes over the segmentation image formed. After the objects were introduced, the machine learning assisted classification algorithm was applied and the classified images emerged. The homogeneity criterion for the segmentation process is given in Equation.1 (Baatz and Schäpe, 1999).

$$f = \sum_{i=1}^{j=n} Wi\left(n_{Merge}\sigma_{Merge} - \left(n_{Obj1}\,\sigma_{Obj1} + n_{Obj2}\sigma_{Obj2}\right)\right) \qquad \text{(Eq.1)}$$

Starting from the starting point, the individual double objects in the image are converted into a single object at each step. The merge is formed according to the homogeneity criterion in the segmentation (Wang et al., 2004). n is the number of bands, Wi is the weight matrix, nMerge, nObj1 and nObj2 represent the ordered state of the combined objects, and the other equation elements represent the variances of the combined objects (Baatz and Schäpe, 1999).

Random-Forest Classification (RF)

SNAP 7.0 software program was used to work with the RF classification technique. The RF classification method consists of structural classifiers in which independent vectors are randomly distributed and using decision trees to organize the data (Goel and Abhilasha, 2017). Decision trees are formed by branching training tests in random vectors (Breiman, 2001). In its content with the rational learning technique the classes, that make up the trees are determined by assignment. Input data includes pixel values in the content of classes for classification (Phiri and Morgenroth, 2017). In the study, homogeneous selections were made by creating six main vector selections separately on the satellite images. The selected classes were processed on the algorithm suitable for the RF tree structure, and as a result, classified images were obtained. The Gini index, which is the equation used in the classification process for RF, is given in Equation.2.

$$\sum\sum_{j\neq i}(f(C_i\,,T)/|T|)(f(C_j,T)/|T|) \qquad \text{(Eq.2)}$$

The Gini index branches the tree structure by measuring the purity of the features depending on the classes, where T represents the training data set and Ci performs the process of assigning random pixels to the class representative (Pal, 2005).

Accuracy Assesment

For the accuracy assesment determined in the Google Earth Engine system, the class of the sample points was determined with the help of the terrain outputs and used for accuracy assesment. In the study area, 440 homogeneous ground sample points were selected in 1990, 2000, 2010 and 2020. Accuracy assesment results in classified images were made in ArcGIS 10.5 for OBC technique and SNAP 7.0 for RF classification technique. The eCognition program, in which the OBC technique was created, determined the accuracy assessment in itself by class similarity matching, and it was understood that this process had no effect on the overall accuracy. For this reason, it has been determined that the ArcGIS 10.5 program for the OBC technique will reveal the general accuracy with clearer results. By changing the file extensions of the homogeneous ground sample points obtained from the Google Earth Engine program, the classified image opened in the ArcGIS program was overlaid and accuracy assesment was created over the reference numbers. No different program was used, as accuracy assesment was created through the SNAP program on the images created as a result of RF. For this reason, the ground sample points thrown on the classified image showed the accuracy by finding the conjugate points directly in the SNAP program. The equations used to create the error matrix in the study are given in Equation.3 and Equation.4 (Zaidi et al., 2017).

$$Overall\ Accuracy = \frac{Sum\ of\ true\ random\ points}{Cumulative\ sum\ of\ all\ random\ points} \quad \text{(Eq.3)}$$

$$k_c = \frac{Observed - Expected}{1 - Expected} \quad \text{(Eq.4)}$$

RESULTS AND DISCUSSION

The maps showing the change in the classified study area are shown in Figure 3 and Figure 4 for both classification techniques. Accuracy assesment results were homogeneously distributed with 440 ground sample points. It was determined for NTS in 1990, respectively (overall accuracy: 57.78%, kappa: 44.84%), 2000 (69.25%, 60.22%), 2010 (76.92%, 70.43%) and 2020 (82.94%, 78.19%), and in the same order, for RF in 1990 (overall accuracy: 57.63%, kappa: 44.26%), 2000 (63.36%, 52.80%), 2010 (75.29%, 68.29%) and 2020 (77.73%, 71.53%). An example of the error matrices related to classification techniques for 2020 is given in Table 2.

Table 2. Accuracy assesment error matrix for 2020

RANDOM FORESTS (2020)							
CLASS	**Forest**	**Agriculture**	**Other**	**Residential**	**Bare Area**	**Water**	**Total**
Forest	108	12	0	1	0	0	121
Agriculture	2	75	0	26	1	0	104
Other	7	3	30	13	0	0	53
Residential	0	7	6	84	0	0	97
Bare Area	0	15	0	5	2	0	22
Water	0	0	0	0	0	43	43
Total	117	112	36	129	3	43	440
Producer	0,892562	0,721154	0,566038	0,8659794	0,0909091	1	
User	0,923077	0,669643	0,833333	0,6511628	0,6666667	1	
Row	0,275	0,236364	0,120455	0,2204545	0,05	0,097727	
Column	0,265909	0,254545	0,081818	0,2931818	0,0068182	0,097727	
Row-Column Average	0,073125	0,060165	0,009855	0,0646333	0,0003409	0,009551	0,21767
Overall Accuracy	0,777273						
KIA	0,715302						
OBJECT-BASED CLASSIFICATION (2020)							
CLASS	**Forest**	**Agriculture**	**Other**	**Residential**	**Bare Area**	**Water**	**Total**
Forest	114	7	0	0	0	0	121
Agriculture	2	100	0	2	0	0	104
Other	0	2	36	15	0	0	53
Residential	0	20	4	71	2	0	97
Bare Area	6	15	0	0	1	0	22
Water	0	0	0	0	0	43	43
Total	122	144	40	88	3	43	440
Producer	0,942149	0,961538	0,679245	0,7319588	0,0454545	1	
User	0,934426	0,694444	0,9	0,8068182	0,3333333	1	
Row	0,275	0,236364	0,120455	0,2204545	0,05	0,097727	
Column	0,277273	0,327273	0,090909	0,2	0,0068182	0,097727	
Row-Column Average	0,07625	0,077355	0,01095	0,0440909	0,0003409	0,009551	0,218538
Overall Accuracy	0,829545						
KIA	0,781877						

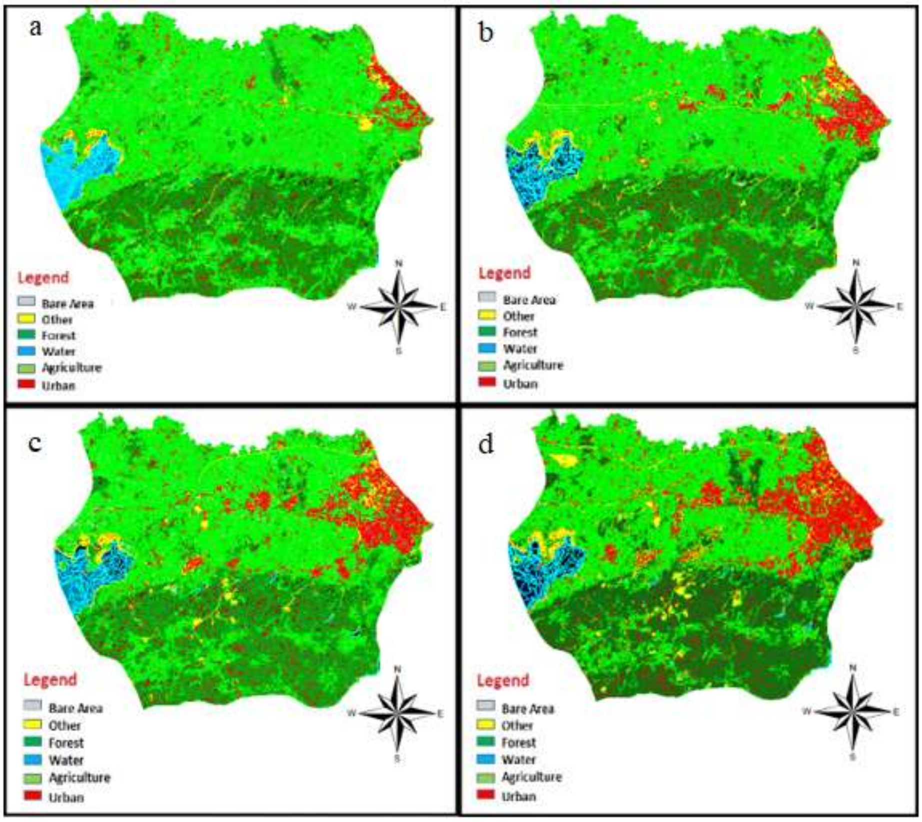

Figure 3. OBC technique created maps a)1990, b)2000, c)2010, d)2020

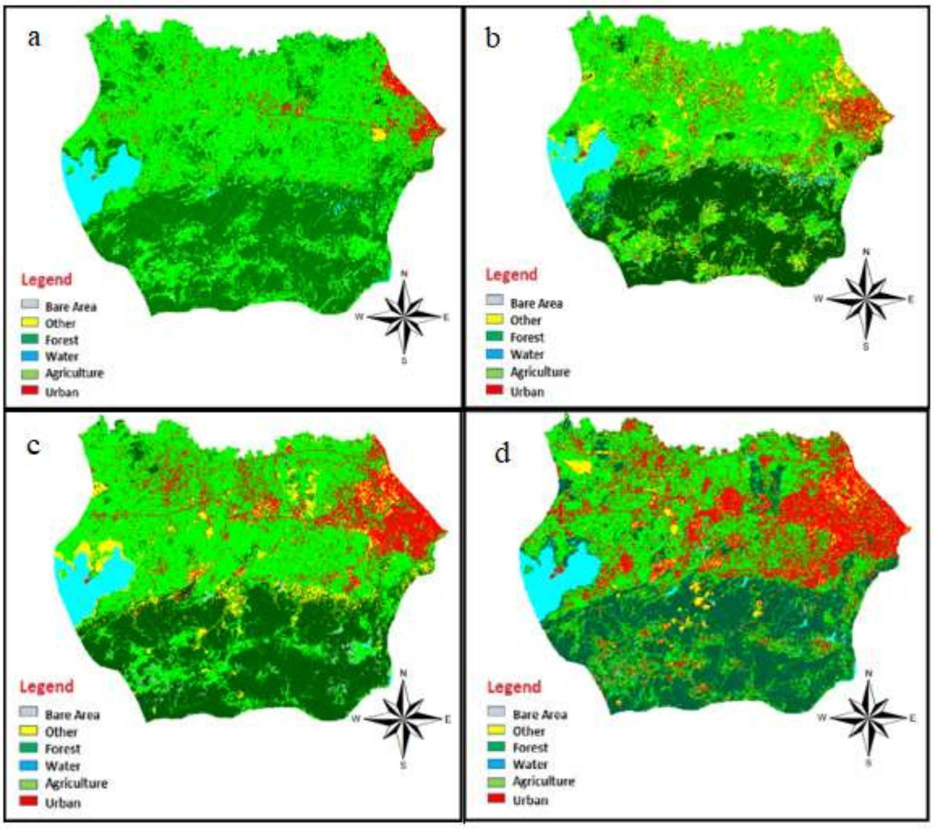

Figure 4. RF technique created maps a)1990, b)2000, c)2010, d)2020

Table 3. OBC area change results (ha, %)

CLASS	OBC-AREA (ha)				OBC-CHANGE (%)		
	1990	2000	2010	2020	1990-2000	2000-2010	2010-2020
Agriculture	33865,2	30679,2	27294,48	24450,48	9,4 ↓	11,03 ↓	10,41 ↓
Residential	1676,61	2665,44	4681,08	6056,01	58,97 ↑	75,62 ↑	29,37 ↑

LULC Classification

The proportional percentage of the results obtained by the areal changes and accuracy assessment on the basis of A and R classes in the study area differs for all years. It is aimed to examine the effect to T areas of the change of Y areas in the selected area (Çolak and Memişoğlu, 2018). Spatial changes in LULC were evaluated in the same periods.

OBC Classification

It showed in the A class with an average accuracy rate of 71.73% with OBC a decrease of 9.40% between 1990-2000, 11.03% 2000-2010, and 10.41% 2010-2020. The cumulative change in the years 1990-2020 emerged as 27.80%. The data obtained in the same period revealed 58.97%, 75.62% and 29.37% increases in the R class. The total change in the R area between 1990 and 2020 was observed as 261.20%.

RF Classification

It showed in the A class with an average accuracy rate of 68.57% with RF a decrease of 12,77% between 1990-2000, 19,34% 2000-2010, and a increase of 1,10 % 2010-2020. The cumulative change between 1990 and 2020 was 28.87%. In the same dates and periods, 49.96%, 92.19% and 97.77% increases were observed in the R class. The total change in the years 1990-2020 was determined as 470.00%.

Table 4. RF area change results(ha%)

CLASS	RF-AREA (ha)				RF-CHANGE (%)		
	1990	2000	2010	2020	1990-2000	2000-2010	2010-2020
Agriculture	28752,3	25078,3	25355,9	20450,8	12,77 ↓	1,1 ↑	19,34 ↓
Residential	2159,1	3237,8	6222,8	12307	49,96 ↑	92,19 ↑	97,77 ↑

When the LULC maps were examined with both classification techniques, it was observed that the OBC technique gave better results compared to the RF technique in terms of area. The accuracy assessment process also provided supporting results. The results will support making more informed decisions in geopolitical areas for industrialization in order to regulate land use and environmental impacts (Habte et al., 2021).

Table 5. Results of accuracy assessment

	1990		2000		2010		2020		AVERAGE
	OVERALL	**KIA**	**OVERALL**	**KIA**	**OVERALL**	**KIA**	**OVERALL**	**KIA**	
RF	57,63	0,443	63,36	0,528	75,29	0,683	77,98	0,718	68,565
OBC	57,79	0,448	69,25	0,602	76,92	0,704	82,95	0,782	71,7275

As a result of the study, it was foreseen that the agricultural structures at the macro and micro level should be examined with various techniques and supported with existing information and data (Turhan and Erdal, 2013). The effect of increasing construction on agricultural lands has been shown by analyzing with numerical data (Çolak and Memişoğlu, 2018). It has been revealed that the areal changes are not only from A fields to R fields. Considering the results of the classification processes, it should be said that A areas firstly transformed into O areas and then grew by changing to R areas with zoning regulations, and especially the central districts in big cities were greatly affected by this construction. When the results are examined, it is seen that the negative change in A areas continues rapidly and the areas affected by service/industry development spread to R areas (Bayar, 2018).

DISCUSSION

In this study, which was carried out with the aim of vegetation comparison mapping of the dynamics of the temporal change of the loss of agricultural areas against urban development and growth of Nilüfer district with satellite images, in 1990, 2000, 2010 and 2020, it was found that the OBC technique gave more accurate results than the RF classification technique with a rate of 71.73%. It has been understood that the increasing artificial surface structuring in the study area has increased primarily towards agricultural areas and therefore the agricultural lands in the district have suffered a serious loss. It has been understood that the spread in urban areas does not progress towards one-way A areas, and it affects other classes as well.

It has been seen that the available information supports the major findings of the study. Findings obtained within the scope of obtaining Landsat satellite images on desired dates and processing them due to their resolution, knowing the distribution of vegetation in the study area and making visual interpretations, comparing and mapping the classification techniques applied to satellite images support the existing information. Considering the literature studies, it is thought that mapping the Nilüfer district with satellite images by applying different classification techniques can form a basis for future studies.

The increase in urbanization as an artificial surface in Nilüfer district has led to losses in agricultural areas. Controlled and uncontrolled spread that comes with urbanization has caused loss in other classified areas besides agricultural areas. An accuracy of 71.73% was achieved with the OBC technique and 68.57% with the RF technique. With the OBC technique, between 1990 and 2020 there was an increase of 261,20% in urban areas and a decrease of 27,80% in agricultural areas; With the RF technique, these rates were determined as 470.00% increase and 28.87% decrease. This research, which is examined on a district basis, can also be applied to other vegetation classes in Bursa and a detailed analysis can be made with different classification techniques.

Research highlights the importance of examining the dynamics of change in LULC. In a study conducted in the state of Kansas, USA, they calculated the growth of urban expansion and the loss rate in agricultural areas over a twenty-two-year period according to the classification of satellite images made in the central region (Zubair et al., 2019) and an urban growth rate of 369% and a decrease in agricultural area at a rate of 20% were calculated and a similar approach was adopted with the findings of our study. Addressing the continuous urbanization-based loss of fertile agricultural lands in Egypt, the study estimated agricultural land consumption through urbanization in the Alexandria and El-Behiera regions and the future pattern of this process (EL-kawy et al., 2019). Although our analyzes are in line with the main dynamics of this study, the modeling approach related to future planning does not match our findings and it is anticipated that this approach will be used for our future studies. The study conducted in the capital region of India, focused on the characteristics of ten and a half years of urbanization and the resulting LULC, and the study was supported by remote sensing vegetation indices (Sharma and Joshi, 2016). Our study is supported by similar findings by examining the urbanization-related features of the LULC change with the help of important information other than the index concept. In a study conducted in China, they produced an empirical model of the reduction in agricultural lands between 2000 and 2014, which occurred for various reasons, including intense urbanization (Deng et al., 2020). The outputs created in the study match the findings of our study by suggesting that the loss of agricultural lands is based on urbanization, but our study highlights that the increase in urbanization does not only affect agricultural areas. Again, a study conducted in China revealed how the sprawl of urbanization affects agricultural losses due to urbanization, using vegetation indices from 2001 to 2013 (Shi et al., 2016). Our analysis provides information that is consistent with the study in China in determining the impact of urbanization on farmland.

This study to address the above existing problems aimed at mapping urban expansion and hence agricultural areas. In this direction, our analysis has shown that there are regional and global migrations in areas that have started industrialization, as indicated in the GDP data, and therefore, the irregular increase in the rate of urbanization increases the pressure on agricultural lands and brings socio-economic implications. It has been determined that the pressure created by urbanization is not only effective on agricultural areas and also interacts with other classes. It has been understood that, with the controlled and uncontrolled structuring in the last forty years, destructions have occurred in the central areas and have affected the change in LULC quantitatively. It shows that the use of remote sensing data and classification techniques can increase the accuracy in future studies in terms of examining the dynamics of LULC.

CONCLUSION

The study was created to present the results of comparing and mapping the land use vegetation classification in Nilüfer district. It has been determined how urban sprawl affects agricultural areas and therefore what are the dynamics of change that occur throughout the district. In 1990, 2000, 2010 and 2020, OBC and RF classification techniques were applied to Landsat 5-TM and Landsat 8-OLI satellite images in ten-year periods. Accuracy assessment was revealed on the classified images with homogeneous ground sample points taken from the Google Earth Pro program.

The findings of the study showed that the OBC technique gave better results than the RF classification technique. Since the recognition of objects is based on the OBC technique, the distinguishability of A and R fields is higher, but since the RF technique creates trees with close random vectors in the tree structure, it has been observed that there is confusion between the classes. In both techniques, it was determined that the increase in urban areas did not provide a unidirectional effect and did not only affect the decrease in agricultural areas. In this context, it has been understood that Bursa has been exposed to controlled and uncontrolled construction in the last forty years. In Nilüfer, which is the subject of the study, it is predicted that there will be greater losses in the coming years in areas with agricultural, with the increase of urban structures as artificial surfaces. Bursa province where Nilüfer is located, it should be analyzed in detail with higher resolution satellite images and different imaging techniques in different land cover classes, in areas such as districts and villages, including the area change between A-R class. Understanding the change trend of Nilüfer district LULC for future studies will form the infrastructure of the studies to be carried out for Bursa in general. In this sense, the use of machine learning methods will facilitate easier and faster understanding of LULC changes and making predictions for the future.

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VILLAGE-LEVEL CHANGE DETECTION AND FUTURE PREDICTION OF OLIVE GROVES IN EZINE, CANAKKALE USING LANDSAT IMAGERIES

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ABSTRACT

Olive (*Olea europeae* L.) is one of the most economically important agricultural products in Turkey, not only in terms of nutrition but also for other sectors such as, cosmetics. It is grown within in whole Aegean-Mediterranean coast, South-East Anatolian region and some parts of Black Sea region, the highest yield per tree (kg) is known to be obtained around production areas of Canakkale and Balıkesir provinces. The publicly available agricultural production statistics demonstrated that olive grown areas are increased, and this situation declared to be sourced from financial supports. However, it was reported that the increment was insufficient since expected level of increase could not be reached yet. On the other hand, such statistical data is at district level and also becomes available after the harvest season. Thence, present study aimed to quantify the changes in olive areas between 2009 and 2019 at village level, and to predict 2029 year's status in pilot area of Ezine District, located in Central Aegean coast of Canakkale Province. Landsat imageries acquired from TM and OLI sensors in dry season of selected years were used as main data source, local production data was used to verify remotely sensed results and actual status whereas ALOS digital elevation model, markov chain and future land use simulation model were used to predict future olive grove areas and their distributions.

Keywords: Change detection, Ezine, Landsat, Olive, Future Prediction.

INTRODUCTION

Olive (*Olea europeae* L.) is known to be an important product for nutrition, as well as other sectors such as; cosmetics and pharmacy sectors. The majority of olive production constitutes in Mediterranean countries, which produce over 95% of total amount. Even though olive production is conducting in Aegean, Mediterranean, and some parts of Black Sea coasts together with South-East Anatolia of Turkey, the highest yield in terms of kilograms per tree is usually obtained from Canakkale and Balikesir production areas (Savran, 2017). However, the terrestrial average of the yield is declared to be very low in comparison with other Mediterranean countries. For instance, the average yield per tree is reported to be approximately 12 kg tree^{-1} whereas it reaches to 50 kg tree^{-1} in Italy while the differences can be sourced from natural (elevation, slope, soil, water or climate) or human induced factors including cultivation activities (Efe et. al., 2013; Ozturk et. al., 2021).

The data including district based magnitudes of olive grow areas and yields of annual olive cultivation are publicly available in databases of national institutions such as Turkish Statistical Institute (TSI). These agricultural production statistic data have revealed that there were increments in the produced amounts, which were expected to be occurred due to financial supports, but still highly away from the desired levels.

Main purpose of the study was to determine the decadal change in olive grove areas (ha, %), and predict the magnitudes and locations of future olive grove areas in Ezine district of Canakkale province, Turkey, using remote sensing technologies. Landsat TM and OLI imageries of 2009 and 2019 dry season were used to create LCLU maps, local village level production data was used to verify the study findings and actual status within the first ten villages where the olive groves mainly located. Finally, ALOS digital elevation model, markov chain and simulation model were used to prediction and simulation of future olive grove areas and locations.

MATERIAL AND METHOD

The study was conducted in Ezine district of Canakkale province (Figure 1). Landsat TM image of 26 July, 2009 and Landsat OLI image of 07 August, 2019 were used as the main data source for designating locations of olive groves in selected years. The images are publicly available in United States Geological Survey website (USGS, 2020). Six bands of each image that obtained from both TM and OLI sensors covering visible, near infrared and short wave infrared channels were utilized in present study (Figure 2 a, b). These six-band images were subset depending on the Ezine district boundaries. Images were classified via supervised classification maximum likelyhood algorithm. The considered classes were Olive (O), agriculture (A), forest (F), other vegetation (OV), impervious surfaces (I) and water surface (W).

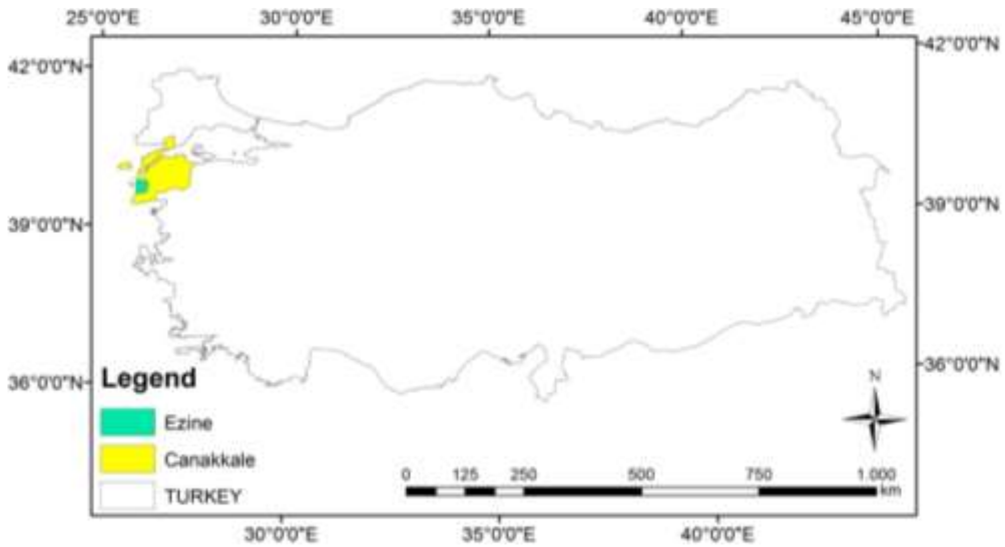

Figure 1. Ezine district location in Canakkale province, Turkey

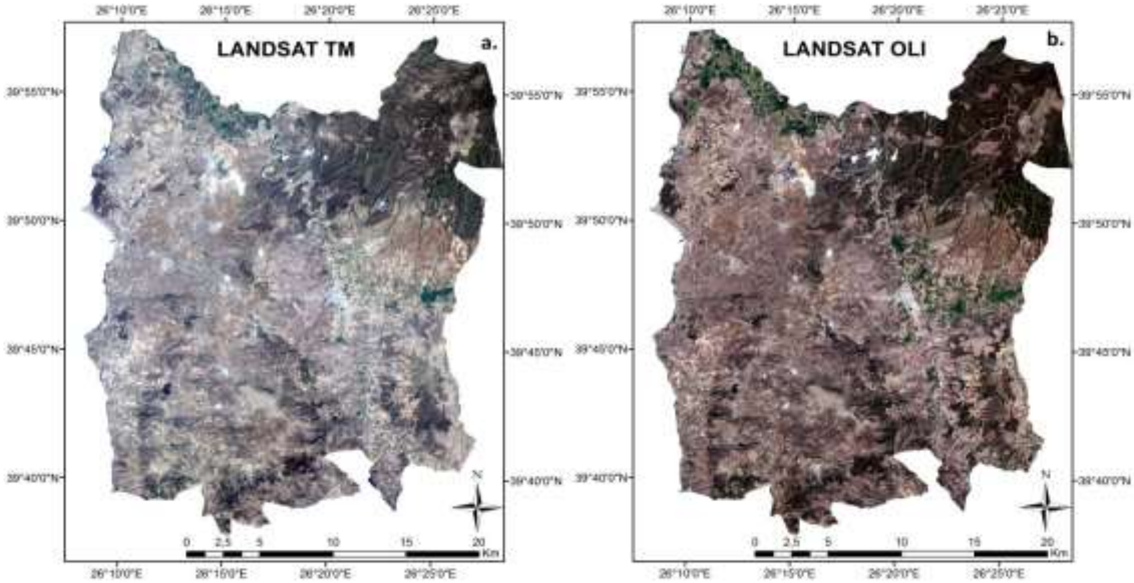

Figure 2. Six-band imageries of a. 2009 Landsat TM, and b. 2019 Landsat OLI

The accuracies of LCLU maps of 2009 and 2019 years (hereafter, $LCLU_{2009}$ and $LCLU_{2019}$, respectively) were determined according to the procedure presented by Congalton and Green (2009). The accuracies of 150 equalized randomized points were controlled through Google Earth (hereafter, GE) application for validation process (Figure 3).

Figure 3. A view from accuracy assessment procedure for in GE

Subsequently, the $LCLU_{2009}$ and $LCLU_{2019}$ were subset into village-level depending on the boundaries of villages located in the district, after the accuracies area validated. The local inventory records of olive production areas were compared with the remotely sensed findings of the study for the villages where the majority of olive grove areas are located.

Finally, future olive grove areas were predicted by markov chain model depending on the changes between 2009 and 2019 years. The predicted amounts for future olive grove areas in terms of number of pixels were distributed using an ANN based cellular automata model for future land use simulation were used (Geosos, 2020). The digital elevation model of ALOS (JAXA, 2020) with 30 m spatial resolution were utilized for determination process of elevation (Figure 4a), slope (Figure 4b) and aspect (Figure 4c) statuses of olive areas for simulation step. $LCLU_{2019}$ was predicted by using $LCLU_{2009}$ as underlie for determining the validation of future simulations by comparing actual $LCLU_{2019}$ and simulated $LCLU_{2019}$.

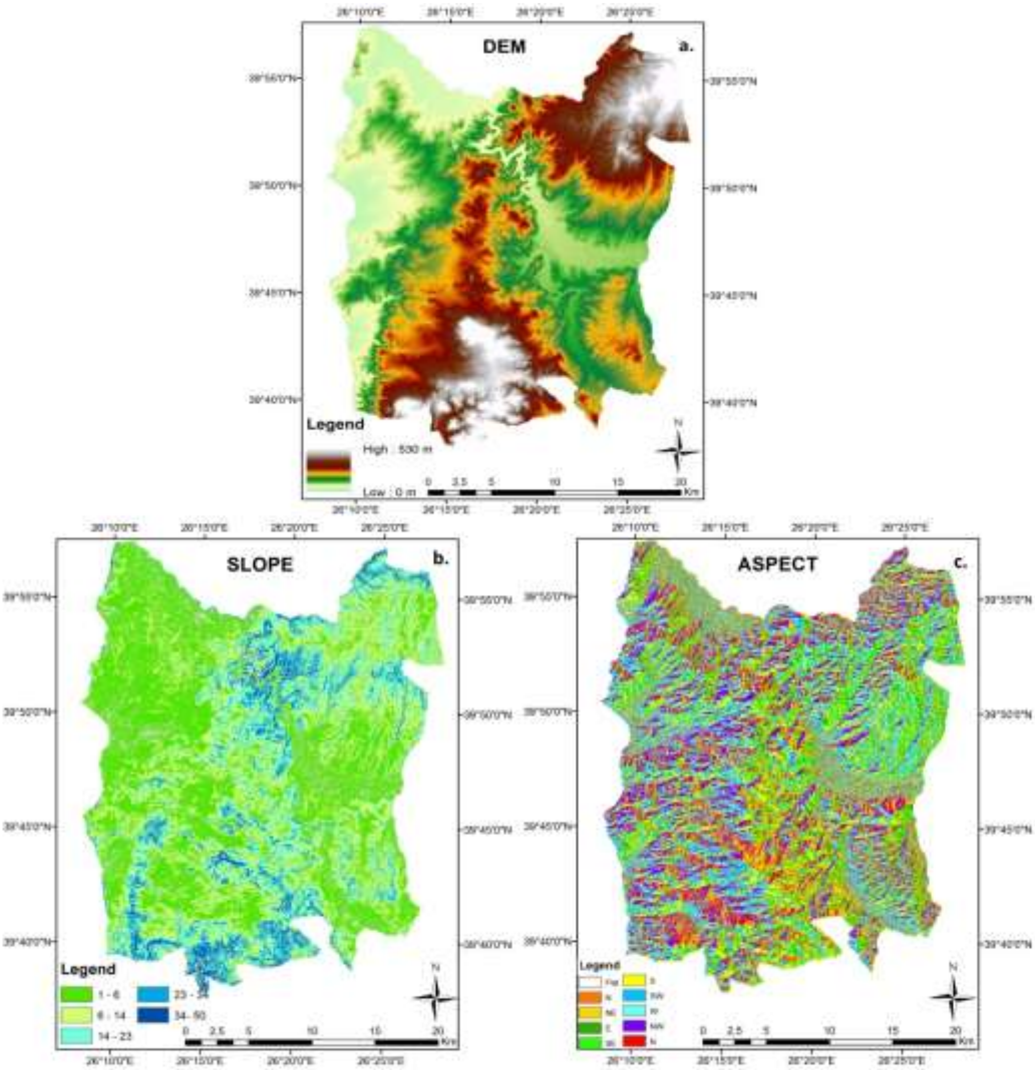

Figure 4. a. DEM, b. slope, and c. aspect maps of the study area

RESULTS AND DISCUSSION

The $LCLU_{2009}$ and $LCLU_{2019}$ that obtained through supervised classification process can be seen on Figure 5a and Figure 5b, respectively. The results for the change analysis for all LCLU classes are detailed in Table 1. The findings of LCLU maps have revealed that olive areas are increased within last decade. As it can be seen, an increase of approximately 1670 ha has occurred between 2009 and 2019 years which corresponds to 2.2%. This finding was consistent with the local statistical data. As well as olive class, the areas of O and OV classes were also increased slightly in the area. On the contrary, the areas of A, F and W classes were decreased. Even though the area of OV class was found to be increased, the gains from O class were found to be mostly came from A and OV classes. On the other hand, visual interpretation of LCLU maps were highlighted that the increases in OV have significantly gain from F class on the North-Eastern part of the study area. Change analysis was pointed out that the most conspicuous alternation in the area was decrease in F and increase in O areas within last decade.

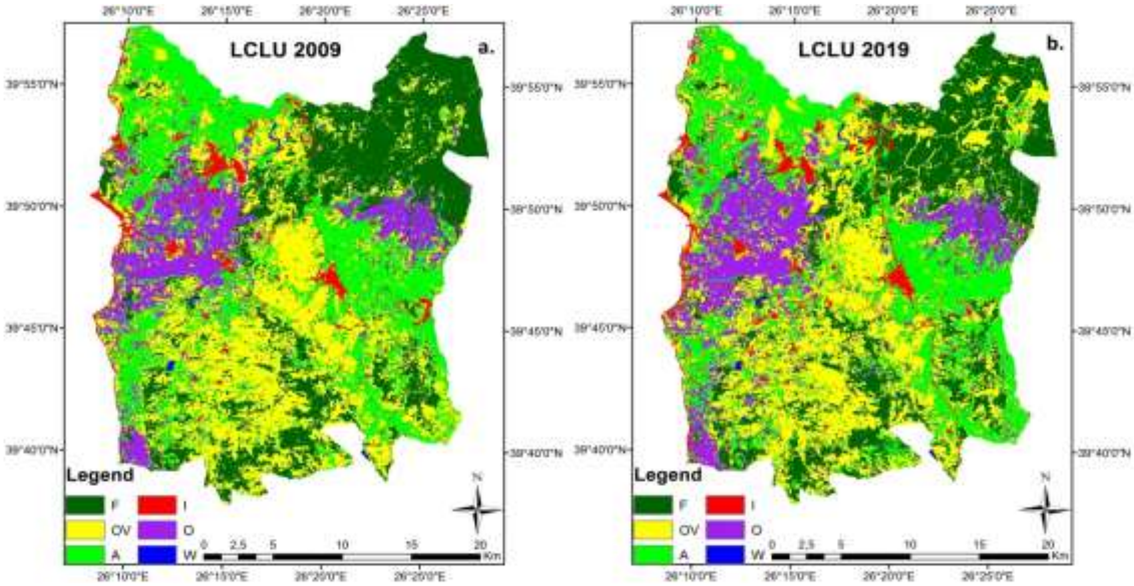

Figure 5. The maps of a. $LCLU_{2009}$, and b. $LCLU_{2019}$

Table 1. Areas (%) and changes in LCLU classes

Classes	Year				Change	
	2009		2019		%	Direction (+/-)
	Area (ha)	Area (%)	Area (ha)	Area (%)		
O	9252	12.4	10921	14.7	2.2	+
A	19325	26.0	18841	25.3	0.7	-
F	19958	26.8	18210	24.5	2.4	-
OV	22923	30.8	23081	31.0	0.2	+
I	2736	3.7	3191	4.3	0.6	+
W	173	0.2	123	0.2	0.1	-
TOTAL	74367	100.0	74367	100		

According to the results of accuracy assessments, the overall accuracy of the $LULC_{2009}$ was 88.67% with a kappa value of 0.8640 while the overall accuracy of $LULC_{2019}$ was found as 89.33% with a kappa of 0.8720. According to the findings, the producer's and user's accuracies for O class were 95.45% and 84.00% in 2009. Both producer's and user's accuracies of $LULC_{2019}$ were lower than $LULC_{2009}$, whereby they were obtained as 95.24% and 80.00%, respectively. Therefore, the kappa value of $LULC_{2019}$ O class (0.7674) was lower than $LULC_{2009}$ (0.8125). Accuracy results have demonstrated that the classifications were reliable since all accuracies and kappa statistics were over 0.75 as reported in the literature (Lillesand and Kiefer, 1999).

Table 2. Summary of accuracy assessment results

Class	Year					
	2009			2019		
	Producer's Accuracy (%)	User's Accuracy (%)	Kappa	Producer's Accuracy (%)	User's Accuracy (%)	Kappa
O	95.45	84.00	0.8125	95.24	80.00	0.7674
A	78.75	88.00	0.8475	75.86	88.00	0.8512
F	88.89	96.00	0.9512	92.00	92.00	0.9040
OV	86.96	80.00	0.7638	88.77	84.00	0.8065
O	100.00	84.00	0.8140	95.83	92.00	0.9048
W	100.00	100.00	1.0000	100.00	100.00	1.0000
Overall Accuracy	88.67%			89.33%		
Overall Kappa	0.8640			0.8720		

Considering the major olive grove areas, it was seen that the local data and remotely sensed results of the study showed strong relations (Figure 6). The R^2 value was found to be 0.9929 for 2009 year whereas it was calculated as 0.9954 in 2019 year for the first ten olive grove located villages in the area. In addition, the village based findings were coherent with precious studies conducted in some parts of the area of interest (Inalpulat et al., 2012; Inalpulat and Genc, 2019).

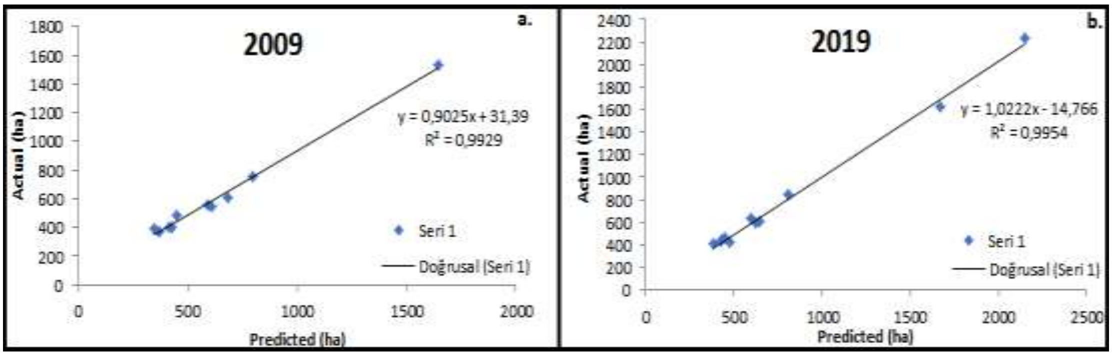

Figure 6. The relations between predicted and actual olive grove areas in ten major olive grove located villages a. 2009 year, b. 2019 year

Depending on the above mentioned findings, it can be said that determination of olive grove areas in similar climate regions with similar topographic properties seemed feasible with moderate spatial resolution Landsat imageries.

Moreover, the simulation process was resulted in increase of OV and I classes together with O. On the other hand, the areas of F and W tent to decrease, as well as it happened in the past, within last decade. The coverage of olive areas was predicted to be increased from 14.7% to 17.3% until the end of 2029 production year. The simulation map of the study area for 2029 is given in Figure 7 and the predicted areas for each considered class are given in Table 7. On the other hand, validation results of simulation $LULC_{2019}$ was quite low with 68.81% overall accuracy and 0.6652 kappa value, indicating that reliability of simulation result should be increased. This situation is expected to be sourced from using the driving factors only limited with topographic data. Moreover, among such data, using aspect data considered to be another restrictive factor for obtaining more accurate simulations. Therefore, depending on the findings of the study, it should be noted that, in addition to elevation and slope data, using soil-related data instead of aspect is expected to be improve the simulation accuracy in similar areas.

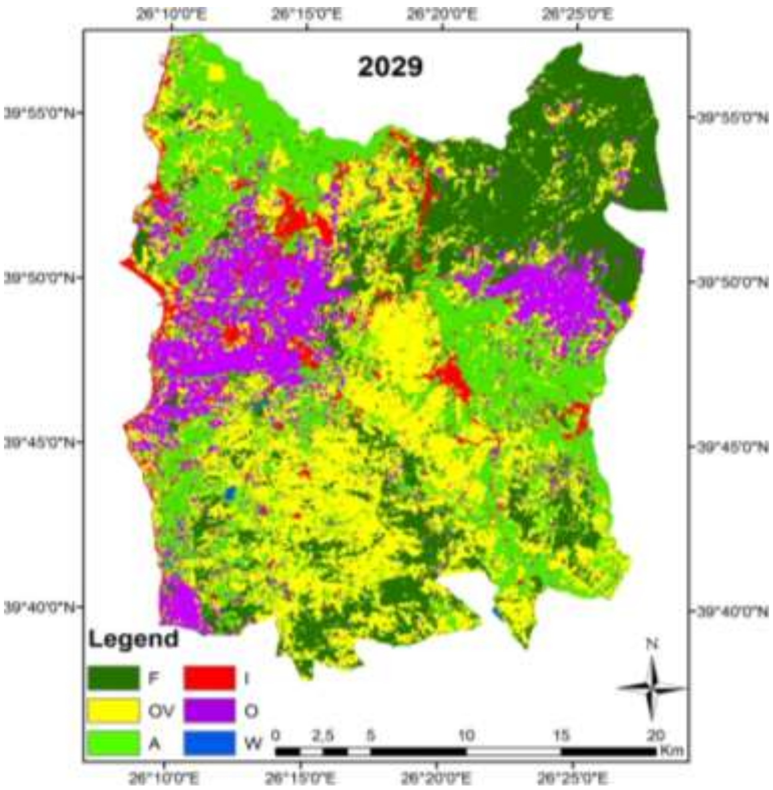

Figure 7. Simulation map of $LULC_{2029}$

Table 3. Predicted areas for $LULC_{2029}$ (ha, %)

Classes	Year	
	2029	
	Area (ha)	Area (%)
O	12835	17.3
A	18602	25.0
F	16707	22.5
OV	23168	31.2
I	2982	4.0
W	73	0.1
TOTAL	74367	100.0

CONCLUSIONS

In present study, Landsat based LCLU maps were produced for 2009 and 2019 years to discriminate olive groves from other main LCLU types in study area of Ezine. The findings demonstrated that olive groves were increased by 2.2% within last decade. The overall accuracies, individual and overall kappa values designated that the classifications were highly accurate. In addition, the first ten villages that have majority of olive groves were selected to compare amounts of remotely sensed and actual olive grove areas whereby they present strong correlations with R^2 0.99. Simulations were conducted through an open source simulation model using ancillary data related with topography. Validation of the simulation was tested by simulating 2019 year's actual pixel demands with $LULC_{2009}$ and the same ancillary data, and as well as all simulation studies, it was assumed that the result is valid for $LULC_{2029}$ since there is no other data representing the predicted situation. The relatively low simulation accuracy of 69% predicted to be mainly appeared due to use of only topographic restrictions without considering soil or other conditions. Moreover, a secondary reason of this situation could be sourced from utilization of aspect data, which makes the pixel distribution process highly complicated. Thence, ongoing study is focused on better simulation of future olive grove areas whereas yields will also be predicted depending on different elevation and slope interval zones.

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DETERMINATION OF PHENOLOGICAL STAGES AND THE CHANGE OF YIELD POTENTIAL ACCORDING TO HARVEST IN BLUEBERRIES GROWN IN OUTDOORS IN POTS AND RAISED BED

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ABSTRACT
In the study, three years old 5 different northern highbush blueberry cultivars ('Bluecrop', 'Brigitta', 'Denise Blue', 'Patriot' and 'Blugold') were grown in pots with acidic peatmoss and raised beds under open field conditions. Several phenological stages, total and individual weekly yielding and harvest duration of the cultivars were determined. Flower buds burst during last week of February for all growing conditions while raised bed grown cultivars bloomed earlier than pot ones. Fruit set of raised bed grown blueberries was also earlier than pot plants like veraison. Pot grown 'Denise Blue' bushes gave the highest yield (955.14 g/plant) and raised bed grown 'Bluegold' was the lowest yielded (178.87 g/plant) cultivars. Individually the most yielded cultivar is 'Denise Blue' and pot grown plants are the best. Harvest begins during last week of June in raised bed grown blueberries and pot grown blueberries harvested later but pot grown blueberries had longer harvest periods than raised bad ones. Yield was also varied according to growing conditions, cultivars and harvest period. 'Bluecrop', 'Bluegold' and 'Brigitta' grown in raised bad 'Bluecrop', 'Denise Blue' and 'Bluegold' grown in pots starts earlier harvest than other cultivars. 'Patriot' is the latest matured cultivar for both growing conditions.

Key Words : *Vaccinium corymbosum*, pot, raised bed, yield, maturation

INTRODUCTION

The homeland of blueberries is the American continent, and it is a berry fruit that can grow in temperate climates. Blueberry is in the heather family (*Ericaceae*), which also includes species such as rhododendron, azalea, heather, cranberry, Caucasian whortleberry and bilberry. Blueberry is a deciduous shrub, can be grown in acidic soils, with a very high yield and very beneficial for health. Blueberry grows well in light-textured, sandy-loamy or loamy-millady soils containing at least 3% organic matter. Soils where blueberry will be grown are strongly acidic and pH is between 4.2-5.5. In Turkey, four different *Vaccinium* species (*V.vitis-idea, V. myrtillus, V. uliginosum and V. arctostaphyllos*) grows wildly in the forest and/or plateaus of the Balck Sea (Artvin, Rize, Trabzon, Ordu, Giresun, Gümüşhane, Samsun, Sinop, Kastamonu, Zonguldak, Bolu, Bartın and Düzce), the Marmara (Kocaeli, Sakarya, Istanbul, Kırklareli, Bursa and Balıkesir) and some special part of Eastern Anatolian Region (Erzurum-Şenkaya and Ardahan). Natural acidic soils been in these areas is suitable for the natural growth of some species in the genus of *Vaccinium* (Çelik, 2012c). However wild *Vaccinium* species in these regions are of no commercial importance. *Vaccinium* species grown commercially in the world are blueberries, cranberry and lingonberry. The most widely grows Vaccinium species is blueberry and it was bred in the United States and Canada in the 1900s. Blueberry varieties cultivated in the world are included in the highbush (*Vaccinium corymbosum*), lowbush (*Vaccinium angustifolium*) and rabbiteye (*Vaccinium ashei*) species. Blueberry, which is in the *Vaccinium* genus and called blueberry because of its blue colored fruits, was first introduced to Turkey by Prof. Dr. Hüseyin ÇELİK and it was brought as "maviyemiş" to our Turkish and Black Sea Region agriculture as an alternative berry fruit (Çelik, 2007). According to the statistics of 2019, world blueberry production is 823300 tons, America comes first with 308760 tons, followed by Canada with 176130 tons, Peru with 142430 tons, Spain with 53380 tons, Mexico with 49000 tons and Poland with 34770 tons (FAO, 2020). In Turkey, there are 5-6 thousand tons estimated blueberry production and planting area is over 10000 decares. Growing in pots has accelerated in recent years and reached 2000 decares in Mediterranean, Aegean and Marmara regions.

Weather and climatic conditions significantly affect blueberry productivity. Drought, extreme cold or extreme heat during rest and flowering cause poor fruit set, decrease in berry size and decrease in yield. These conditions can also negatively affect flower bud formation (Pavlovski, 2010). Blueberries need very special soils. Plants grow well in sandy fertile soils with good drainage, light, high organic matter content and pH value in the range of 4.5-5.5 (Jaworski, 2015; Strik et al, 2008). Iancu et al. (2010), determine the effect of manure + peat moss and/or pine barks add in raised bed on the yield of newly bred 7 blueberry cultivars ('Simultan', 'Delicia', 'Lax', 'Compact', 'Augusta', 'Azur' and 'Blueray') in Romania. It was determined that the use of manure and peatmoss during planting increased the TSS and they revealed that the blueberry varieties could react positively or negatively to the mixtures in the growing places where they were planted. Strik et al. (2012) stating that the planting system has no effect on fruit firmness, and they found that fruits fed with a high percentage of fish emulsion were much harder. The researchers determined that the fruit firmness of the blueberries planted in the plain was lower than the ones in the raised beds. On the other hand, it was revealed that the yields per plant of blueberries planted in the plain were lower than those of the raised beds and fruit firmness decreased in sawdust + compost, sawdust and mulch applications, respectively. Butkus et al. (1987) investigated the performance of 6 highbush blueberry cultivars in Vilnius, Russia, and they determined their phenology and growth characteristics. Yu et al. (2006) tried to adapt some rabbiteye blueberry varieties to Southern China, and they investigated the growth, development, yield and phenology of 12 cultivars. It was determined that all cultivars were adapted to the region, but 'Baldwin', 'Brightwell', 'Gardenblue', 'Powderblue' and 'Tifblue' varieties came to the fore. Çelik (2009b) made an adaptation trial for the first time in the Eastern Black Sea Region between 2002-2005 with the highbush blueberry varieties cultivated in the world. For this purpose, 'Berkeley', 'Ivanhoe', 'Jersey', 'Northland' and 'Rekord' northern highbush cultivars were tested in İkizdere, Rize, for their growth, development, phenological and morphological characteristics, yield and some fruit characteristics. The researcher determined that the cultivar 'Ivanhoe' was the most productive variety (2567.80 g/plant), and the cultivar 'Berkeley' was the lowest yielding variety with 455.21 g/plant.

This study was carried out to compare the growth, development, yield and quality of blueberries grown in outdoors in raised beds and pots. Blueberries can grow in strongly acid soils and can spread in limited areas in the Black Sea Region and Marmara Region. In this study, the possibilities of growing them in pots or raised tubes were investigated in places with suitable ecology.

MATERIAL AND METHOD

The experiment, which determined the yield and some phenological stages of blueberries grown in pots and raised beds, was carried out in Samsun Atakum district between 2016-2018. The trial area is at an altitude of 195 m and at the GPS point 41°21'52 N and 36°11'29 E. In the experiment, Lithuanian acidic peatmoss (Lithuanian Peat Moss), pH: 4.5-4.8, salt ratio < 0.2, white sod peat containing trace elements and iron, NPK-free and offered for sale as pallets, were used in raised tubes and pots. Raised beds, on the other hand, are 60 cm wide and 40 cm high, using a mixture of forest soil, farmyard manure, pine sawdust and acidic peat (1:1:1:1 (v/v)). The raised bed was sulphurated with elemental (granular) sulfur at 150 grams per square meter between 2011 and 2014, the soil surface was covered with weed mat after sulphuration and the soil acidity was reduced by ensuring that the sulfur was dissolved with routine irrigations throughout the year. Thus, the soil pH value was tried to be reduced to 5.5, which would be suitable for blueberries for 3 years. The blueberry bushes of the cultivars used in the experiment were planted on the raised beds in the autumn of 2014 and they were 3 years old at the beginning of this trial. Therefore, the observations and measurements made in the current trial were made on 4-year-old bushes. In the experiment, 80 liter pots were used for growing in pots. The pots were filled with acid peatmoss and blueberry bushes were planted in the autumn of 2014. Starting one month after planting, slowly soluble fertilizer (Osmocote Pro, 17-11-10 + 2Mg0 + Te, 8-9 months) (1 time and 100 g/plant), ammonium sulfate and composite fertilizer (10:10:10) (50 grams per plant, 3 times) were applied to both pots and raised bed plants. Blueberry plants grown in pots or raised bed were irrigated with a drip irrigation system. After the first week of May, the top of the blueberry plants, both in the pots and in the raised beds, was shaded from 3 m with the green shade material, which gives 55% shade. A total of 150 plants (5 varieties x 3 replications x 5 plants x 2 growing areas = 150 plants) were included in the experiment, which was established with 3 replications according to the randomized blocks trial design, with 5 plants in each replication. The data obtained from the experiment were analyzed in the SPSS statistical analysis program. Differences between growing medium were separated by Duncan Muliple Range Test at 5% or 1% level.

RESULTS AND DISCUSSION

While the burst in the flower buds of blueberry varieties planted in pots started on February 24 in 3 varieties ('Bluecrop', 'Denise Blue' and 'Bluegold'), 'Patriot' started to burst the flower buds on February 27 and 'Brigitta' on March 2. While the bursting of the flower buds of the blueberry cultivars grown in the raised beds took place in almost the same period, it was determined that the bursting in the flower buds of the 'Brigitta' cultivar took place earlier than the ones in the pots and on 27 February together with the 'Patriot' (Tables 1 and 2). In blueberries, the flower buds are activated first and the leaf buds last later. Therefore, the leaf buds of blueberries grown in pots last on March 14 ('Bluecrop'), March 28 ('Bluegold', Denise Blue' and 'Brigitta') and April 1 ('Patriot'), while the leaf buds of varieties grown on raised beds are more likely to bloom. occurred late and on March 9 ('Bluecrop'), March 13 ('Bluegold'), March 14 ('Denise Blue'), and March 15 ('Patriot' and 'Brigitta') and later (Tables 1 and 2). Flowering in pot-grown blueberry varieties started with 'Bluecrop', 'Bluegold' and 'Denise Blue' in the first week of April and continued with 'Patriot' and 'Brigitta' varieties in the second week (Table 1). On the other hand, the flowering of blueberries grown in the raised beds was earlier and took place on March 17 in 'Patriot', on March 28 in 'Bluecrop' and 'Denise Blue' varieties, and on April 5 in 'Brigitta' and 'Bluegold' varieties (Table 2). In the pot-grown blueberry cultivars, the earliest fertilization occurred on June 12 in 'Bluecrop' and 'Bluegold' cultivars, while it was followed by 'Denise Blue' (June 16) and 'Brigitta' (June 19), while the first coloration in 'Patriot' cultivar took place on July 3 (Table 1). On the other hand, the first colorations of the same blueberry cultivars grown in the raised tube started much earlier than those in pots, and on May 29 in the cultivars 'Bluegold', on June 2 in the cultivars 'Bluecrop', 'Denise Blue' and 'Patriot' and on June 7 in the 'Brigitta' cultivars. also started to color (Table 2). As can be seen in Figures 1 and 2, the beginning of the harvest and the harvest period of the blueberries grown in pots and raised beds were also different according to the varieties. For blueberries grown in pots, the first harvest started in late June with 'Bluecrop', 'Bluegold' and 'Denise Blue' cultivars, and in the second week of July for 'Brigitta' and 'Patriot' cultivars (Figure 1). Harvest in 'Bluecrop' and 'Bluegold', which are grown in pots, lasted until the end of August, while it ended earlier in 'Denise Blue', 'Patriot' and 'Brigitta' cultivars (Figure 2). In the blueberry varieties grown in the raised beds, the harvest period started much earlier than the ones in the pots and was completed in the first weeks of August. While the first harvest of blueberries grown on raised beds started with the varieties 'Bluecrop', 'Bluegold', 'Brigitta' and 'Denise Blue', 'Patriot' started to be harvested later, but the harvest period of this variety was also the shortest (Figure 2).

Table 1. Phenological stages of flower, leaf bud and berry development of northern highbush blueberry cultivars grown in pot

	Flower Bud Burst	**Full Bloom**	**Leaf Bud Burst**	**Fruit set**	**Veraison**
Bluecrop	24 February	10 April	14 March	18 April	12 June
Denise Blue	24 February	12 April	28 March	24 April	16 June
Bluegold	24 February	10 April	28 March	18 April	12 June
Patriot	27 February	19 April	01 April	28 April	03 July
Brigitta	02 March	19 April	28 March	28 April	19 June

Table 2. Phenological stages of flower, leaf bud and berry development of northern highbush blueberry cultivars grown in raised bed.

	Flower Bud Burst	**Full Bloom**	**Leaf Bud Burst**	**Fruit set**	**Veraison**
Bluecrop	24 February	03 April	09 March	12 April	02 June
Denise Blue	24 February	03 April	14 March	12 April	02 June
Bluegold	24 February	12 April	13 March	21 April	29 May
Patriot	27 February	05 April	15 March	18 May	02 July
Brigitta	27 February	12 April	15 March	21 April	07 July

It has been determined that there are differences between leaf and flower bud phenologies of tall blueberry cultivars grown in pots and raised beds, while bursting in flower buds occurs earlier than in leaf buds (last week of February), there is a 3-4 day difference in flower bud bursting times between cultivars. It was determined that the flower buds of the blueberry varieties grown in pots and raised beds were bursting at almost the same periods and in late February (Tables 1 and 2). As a matter of fact, Retemales and Hancock (2012) state that flower buds bursting earlier than leaf buds in blueberries, which may be affected by variety, chilling time and environmental factors. It has been revealed that the bursting in the leaf buds, which start to wake up 7-12 days after the bursting in the flower buds, also differs according to the varieties, and that the bursting in the leaf buds of the blueberries grown in pots is earlier than those in the raised beds. Similarly, the dates of full flowering, fruit set, veraison and blue color in fruits occurred earlier in blueberry cultivars grown in the raised beds than those in pots. It is estimated that this situation is due to the increased shade conditions after the trees and buildings facing north and surrounding the blueberries in the pots have leafed out. As a matter of fact, the conditions for shadowing around the garden, where the blueberries grown on the raised beds are located, are almost non-existent, and the garden partially faces south. As a matter of fact, phenological stages in blueberries can vary depending on variety, altitude, lighting, direction and temperature (Retamales and Hancock, 2012; Çelik, 2012 a and c). In addition, Darnell and Davies (1990), who stated that the duration of illumination affects the bursting of flower buds in blueberries, determined that the bursting rate of flower buds may increase by 50% when the illumination decreases from 10 hours to 8 hours. The researcher stated that the day length does not only affect the formation of flower buds, it also affects the uniform flowering, fruit development and maturation.

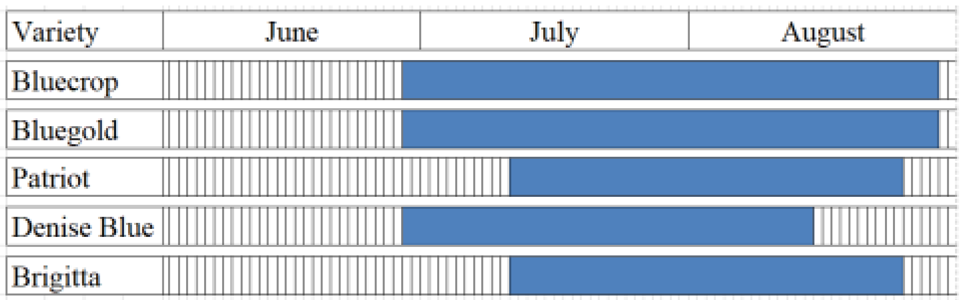

Figure 1. Distribution of ripening and harvest period by months in highbush blueberry varieties grown in pots

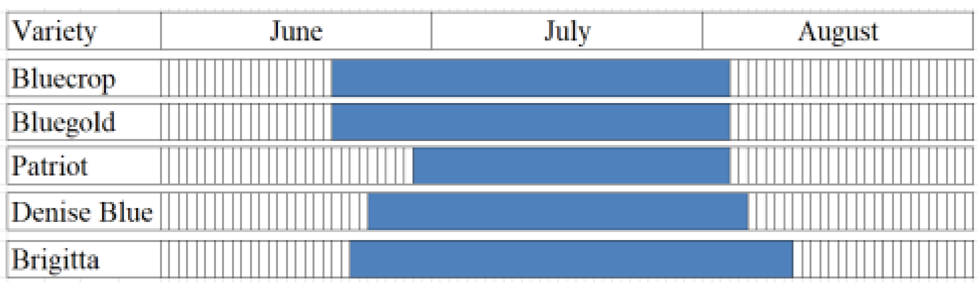

Figure 2. Distribution of ripening and harvest period by months in ighbush blueberry varieties grown in raised beds

It is desirable that the harvest period is long in blueberries. Earliness is more important for the southern regions, and it is known that early flowering varieties have a short ripening period. On the other hand, fruit maturity progress is very slow in late varieties. Ratemales and Hancock (2018), stating that there is a close relationship between flowering and fruit ripeness, are 'Duke', 'Huron' and 'Spartan' from the northern highbush blueberry cultivars and 'Santa Fe' from the southern highbush cultivars, which bloom on the same dates as the average cultivars. Early maturing cultivars such as ‘Star' were also developed., Eck et al. (1990) state that harvesting is done once a week or less and they stating that blueberries start to collect when they turn 100% blue. Blueberries do not mature at the same time, even if they grow on the same shoot in a plant. Maturation takes place between three and four weeks (Greeff, 2003). The red-blue stage is an extremely important stage with rapid improvement in quality characteristics. The fact that fruits are collected at this stage or at previous maturity (color) stages will cause all the positive organoleptic characteristics related to quality not to be formed in the fruit after harvest and will cause deprivation of these substances, which will prolong the storage life of the fruits to be stored especially after harvest (Peano et al., 2014). On the other hand, the fact that ripe fruits are easily damaged, lose their firmness quickly and become more susceptible to fungal diseases will also reduce their storage potential (Peano et al., 2014; Ballinger et al., 1978; Boyette et al., 1993).

The variation of the yield value of blueberry cultivars grown in pots and raised tubes in all harvest periods according to growing locations and cultivars is given in Table 3. As can be seen in Table 3, it has been determined that there are statistically significant differences in yield values obtained in all harvests according to the place of growth and varieties. In the first harvest, the 'Bluecrop' variety grown in pots had the highest yield value with 403.76 (g/plant), while the 'Bluegold' variety grown in pots took the second place with 317.59 (g/plant). The cultivar 'Patriot' grown on the raised bed was found to have the least yield with 32.64 (g/plant). In the second harvest, the 'Patriot' variety grown in pots had the highest yield value of 440.30 (g/plant), while the 'Bluegold' variety grown in the raised bed had the lowest yield value with 46.20 (g/plant). In the third harvest, the 'Denise Blue' variety grown in pots had the highest yield value with 251.46 (g/plant), while the 'Bluegold' variety grown on the raised bed had the lowest third harvest yield value with 53.44 (g/plant). In the fourth harvest, the 'Denise Blue' variety grown on a raised bed ranks first with 131.17 (g/plant), followed by the 'Patriot' variety grown in pots with a value of 112.70 (g/plant). 'Bluegold' cultivar grown on raised bed had the lowest fourth harvest yield value with 11.30 (g/plant), followed by 'Bluecrop' 16.46 (g/plant) cultivar and 'Bluecrop' 19.60 (g/plant) cultivars grown in pots. In the fifth harvest, while the 'Patriot' cultivar grown in pots produced the fifth highest yield value with 75.14 (g/plant), no product could be obtained from 'Bluecrop' and 'Bluegold' cultivars grown in pots. In addition, it was determined that the 'Bluegold' cultivar grown on the raised bed could not get any product in the last harvest (Table 3). It was determined that the yields of blueberry cultivars changed according to whether they were early, mid-season or late, and the yields were higher in the first harvests in the early ones and in the second or third harvests in the late ones (Table 3). On the other hand, the place of cultivation also has an importance on productivity in blueberries. As a matter of fact, the difference in productivity values of blueberry varieties according to their growing places is clearly seen in Table 5. It has been determined that the yield of blueberries grown in pots and outdoors is much higher than those grown in raised beds, the amount of fruit obtained in the second harvest of blueberries grown in pots almost doubled, the highest yield was obtained in the second harvest and then this amount decreased. As a matter of fact, in the second harvest, it was determined that the highest fruit was obtained from blueberries grown in pots, with 371.37 g per plant. It was determined that the total yield values also changed according to the growing place and cultivars, there were significant differences between the cultivars, and the total yield changed significantly according to the growing place. As a matter of fact, the 'Denise Blue' variety grown in pots gave 955.14 g per plant. While the 'Patriot' variety grown in pots is in the first place with total yield, it is 937.41 g per plant. 'Bluecrop' (921.84 g/plant) and 'Bluegold' (918.08 g/plant) varieties take the third and fourth place with fruit. On the other hand, it was determined that the 'Bluegold' variety grown on the raised bed had the least total yield with 178.87 (g/plant).

Table 3. Variation of fruit yield values according to harvest x variety interactions in some blueberry cultivars (g/plant)

Medium	**Variety**	**Yield (g/bush)** 1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	**Total Yield (g/bush)**
Pot	Bluecrop	403.76 a	369.20 abc	129.28 abc	19.60 b	0.00 d	921.84 a
	Bluegold	317.59 a	332.07 abc	227.17 ab	41.25 b	0.00 d	918.08 a
	Patriot	58.15 cd	440.30 a	181.78 abc	112.70 a	75.14 a	937.41 a
	Denise Blue	142.42 bc	431.31 ab	251.46 a	76.56 ab	53.38 ab	955.14 a
	Brigitta	225.44 b	283.94 c	63.08 bc	19.46 b	31.21bcd	624.01 b
Raised bed	Bluecrop	87.55 c	90.13 de	69.12 bc	16.46 b	13.36 cd	276.62 c
	Bluegold	63.37 cd	46.20 e	53.44 c	11.30 b	4.54 d	178.87 c
	Patriot	32.64 d	107.37 de	67.40 bc	32.67 b	0.00 d	243.42 c
	Denise Blue	122.89 cd	180.68 d	165.95 abc	131.17 a	48.33 abc	649.15 b
	Brigitta	43.28 cd	107.87 de	95.09 abc	45.04 b	7.85 d	299.14 c
	Significancy	*0.00*	*0.00*	*0.00*	*0.002*	*0.001*	*0.00*

There are no statistical differences between the data has the same letter given in the column

Table 4. Variation of fruit yield values in some blueberry cultivars according to harvest (g/plant)

Variety	Yield (g/bush) 1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	Total Yield (g/bush)
Bluecrop	245.65 a	229.67 bc	99.20 ab	18.03 c	6.68 bc	599.23 b
Bluegold	190.48 ab	189.14 c	140.31ab	26.28 bc	2.27 c	548.47 b
Patriot	45.39 b	273.84 ab	124.59 ab	72.69 ab	37.57 ab	590.41 b
Denise Blue	132.36 ab	306.00 a	208.71 a	103.87 a	50.85 a	802.14 a
Brigitta	134.36 ab	195.91 c	79.09 b	32.25 bc	19.53 abc	461.57 b
Significancy	*0.08*	*0.654*	*0.099*	*0.004*	*0.018*	*0.500*

There are no statistical differences between the data has the same letter given in the column

Accordingly, it was determined that the total yield values of the blueberry varieties grown in pots were 2-3 times higher than those of the raised bed (Figure 3). On the other hand, the total yield also changed according to the cultivars and the 'Denise Blue' cultivar was 802.14 g per plant. 'Bluecrop' (599.23 g/plant) and 'Patriot' (590.41 g/plant) followed it with total yield. 'Brigitta' was the least productive variety. In the experiment, it was determined that the total yield values per plant also changed according to the growing environments, and it was also determined that the blueberries (871.29 g/plant) grown in pots gave much higher yields than those grown in raised beds (329.43 g/plant).

Table 5. Variation of fruit yield values at the beginning of harvest in some blueberry varieties according to growing place (g/plant)

Variety	Yield (g/plant)					Total Yield (g/bush)
	1st Harvest	2nd Harvest	3rd Harvest	4th Harvest	5th Harvest	
Pot	229.47 a	371.37 a	170.56 a	53.91	31.94 a	871.29 a
Raised Bed	69.94 b	106.45 b	90.20 b	47.33	14.81 b	329.43 b
Significancy	*0.00*	*0.00*	*0.024*	*NS*	*0.131*	*0.000*

There are no statistical differences between the data has the same letter given in the column
NS: Non significant

The total yield was also changed to medium x vareity interactions and cultivars and growing media. As seen in Table 3, 4 and 5; ‘Denise Blue’ cultivar grown in pots gave the highest total yield (955.14 g/plant) however four cultivars instead of ‘Brigitta’ been in the same significant group with ‘Denise Blue’ (Figure 3 and 4). On the other hand, raised bed grown blueberry bushes gave the lower total yield than pot ones (Table 4 and 5).

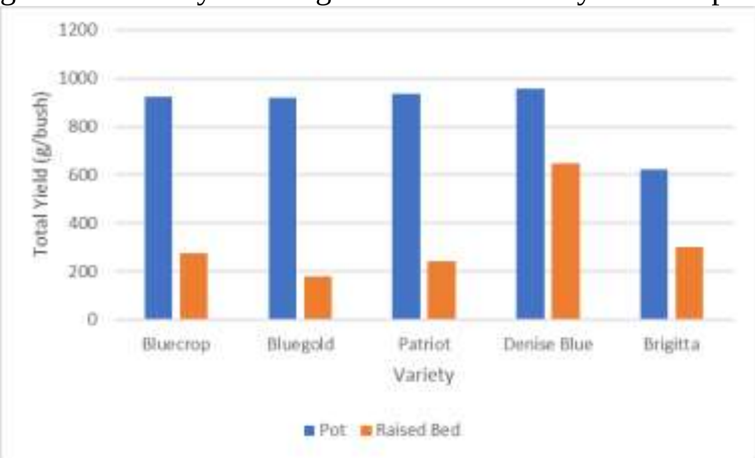

Figure 3. Variation of total yield values in blueberry cultivars grown in pot and raised bed (g/plant)

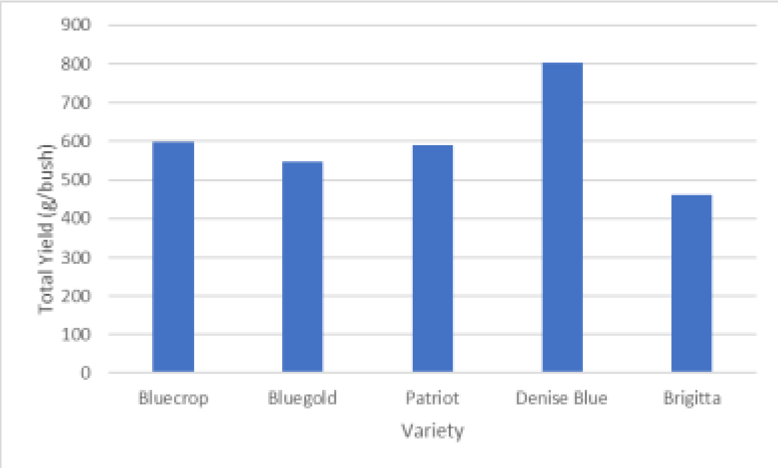

Figure 4. Variation of total yield according to varieties (g/plant) in blueberry cultivars grown in pots and raised tubs

The most important criterion examined in the adaptation abilities of blueberry cultivars is the yield value per plant (Çelik, 2012a, b and c.; Ratemales and Hancock, 2018). In the experiment, it was determined that the total yield value per plant of the northern highbush blueberry cultivars grown in pots and raised beds differs significantly according to the growing media and cultivars. Accordingly, the 'Denise Blue' cultivar grown in pots and outdoors was in the first place with a yield of 955.14 g per plant, while the 'Patriot' cultivar grown in pots took the second place with a fruit yield of 937.41 g per plant, and 'Bluecrop' (921.84 g/plant) and 'Bluegold' (918.08 g/plant) varieties. In addition, it was determined that the 'Bluegold' variety grown on the raised bed had the least total yield with a yield of 178.87 g per plant. On the other hand, it was determined that the total yield in the pot (871.29 g/plant) was higher than the total yield in the raised bed (329.43 g/plant). Considering the blueberry cultivars in terms of total yield, the cultivar 'Denise Blue' had the most (802.14 g/plant) and the 'Brigitta' cultivar had the least (461.57 g/plant) total yield; It was determined that 'Bluegold' (548.47 g/plant) and 'Patriot' varieties (590.41 g/plant) followed this. Çelik (2009b) stated that the yield of blueberries may vary according to the cultivars. He was determined that the cultivar with the highest yield (2567.80 g/plant) and 'Berkeley' cultivar with the lowest yield (455.21 g/plant) grown in soil conditions. Heiberg and Stabhaug (2006) investigated the effects of different substances in pot growing places on the development of tall blueberries and determined that the 'Bluecrop' variety yielded 326 g of fruit per plant, according to three-year averages. The same investigators revealed that 'Bluecrop' and 'Nui' blueberry cultivars showed different responses to the components in the growing places and these cultivars developed better in pots. As a matter of fact, the total yield per plant in blueberries can be affected by factors such as the variety, age of the plant, growing places, the ecology of the grown region, the number of shoots, the number of flower buds in the shoot and the berry weight. On the other hand, **Darnell (2006),** who states that ripening as well as fruit color and yield are dependent on the duration of light, found that fruit set ratio and fruit size increase in hot conditions (16-20 °C), and that the fruit development period decreases in highbush blueberries of northern origin. Researcher stating that the opposite result occurs in cool conditions (8-24°C), the researcher also revealed that high night temperatures (21°C) reduce fruit size. Meanwhile, Austin and Bondari (1990), who determined that the soil pH value also affects the yield and quality of blueberries, determined that the total yield per plant and the uniformity at fruit maturity depend on the soil pH. On the other hand, Shoemaker (1978), who stated that the roots of the blueberry plant do not go very deep and spread in the organic matter on the surface, determined that the plant growth and yield value were directly affected by the soil organic matter. Gough (1994) also revealed that the yield of blueberries planted directly on the soil surface is lower than those mulched with sawdust. The data obtained from our study are in parallel with the literature (Heiberg and Stabhaug, 2006; Çelik, 2008; Çelik, 2009a and b; Retamales and Hancock, 2012).

CONCLUSIONS

The homeland of blueberry is America, and it was started to be cultivated in the early 1900s by using individuals selected from nature. Blueberry, which is examined among berry fruits, is a fruit that is very beneficial for health and has a high yield per unit area, which is cultivated with varieties that fall into the *Vaccinium* genus in the heather family and are classified into three different types commercially. The cultivars of highbush, lowbush and rabbiteye blueberry have plants that can grow in strongly acidic and organic matter-rich soils, deciduous and has a bushy form. Although it has a history of one hundred and twenty years, a production of 823000 tons can be made according to the data of 2019 in highbushl blueberries, which can be grown in very limited regions and areas due to its ability to grow in strongly acid soils. Although blueberry consumption has doubled in recent years, the increase in world blueberry production is very limited.

It has been determined that there are differences in terms of phenological stages, total yield and distribution of yield according to harvest between northern origin highbush blueberry varieties grown in outdoor in pots and raised beds under Samsun conditions. The main objective of this study is to determine the differences in the variation of total yield and yield according to harvest in cultivation of blueberries with low pH demand in open field pots and specially prepared raised beds. As a result, it was determined that there were significant differences in terms of yield, quality and fruit content, as well as phenological and biological characteristics, in 5 northern origin highbush blueberry cultivars grown in pots and raised beds.

According to the results obtained from the experiment, the flower buds of blueberry cultivars grown in raised beds were burst at the same time as those grown in pots and at the end of February, while the leaf buds of blueberry cultivars grown in pots were awakened later than those grown in raised bed. On the other hand, the flowering of blueberries grown in the raised bed started earlier than the plants in the pots. It has been determined that veraison starts much earlier in blueberry varieties grown in raised beds than in pots. The first harvest and the length of the harvest period in blueberries grown in pots and raised beds were different according to the varieties. The first harvest of blueberry cultivars grown in the raised bed was much earlier than those in pots, starting in the second week of June and continued until the first weeks of August. The blueberry varieties grown in pots started to be harvested at the end of June and the harvest continued until the end of August and the harvest periods were longer than those in the raised bed. Similarly, it was also determined that the dates of full flowering, fruit set, veraison and blue coloring in the fruits occur earlier in the blueberry varieties grown in the raised beds than in the pots.

According to these results, it has been revealed that blueberries can be grown in pots using peat with adjusted acidity in areas where soil pH is limiting. It has been determined that high pH conditions in the soil, due to infiltration, may negatively affect the growth, development and yield values of plants by negatively affecting the pH value in the raised beds. It is necessary to carry out new studies on the possibilities of covering the blueberries grown in pots in order to get the harvest time earlier, the color and shade ratios of the shading material and the laying and / or removal periods of the covers in order to extend the harvest period to a longer period.

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EFFECTS OF NITROGEN FERTILIZATION ON LIGNOTUBER DEVELOMENT AND SPROUTING OF Arbutus unedo L.

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Abstract: Strawberry tree (*Arbutus unedo* L.) is an important species for nature. It is resistant to fire and drought. It is a species which can survive with increasing temperatures of global climate change. Arbutus unedo is one of the important fruits and is valuable for health. Lignotubers develop over the years in the underground part of *Arbutus* stem. Lignotuber sizes vary between 41cm to 3068 cm^2 size that they are the source of new meristems. After forest fire, plant re-grow from meristem of lignotubers. In this study, plant growth and lignotuber formation of arbutus plants was evaluated under plastic tunnel condition. After plantation 40, 80, 120, 160 and 240 ppm N fertilization was applied to the seedlings. Granular ammonium sulfate (21N–0P– 0K–24S) was solved in water and given to each plant. Fertilization was made monthly for two growing seasons before irrigation. Sucker and sprouts number and length was recorded. The experiment was conducted in randomized plot design with three replicates, 3 plants per replicates. All the data were evaluated using the analysis of variance (ANOVA) with statistical program Minitab 17 and the groups that showed variance were determined with Tukey test with a significance value $P \leq 0.05$. The results were given as average of two growing season. The number of new sprouts and suckers were increased with N fertilization. 120 and 160 ppm N application was the most effected nitrogen doses in the study. Lignotuber size was increased by doses of N fertilization but decreased at 200 ppm and higher doses. All doses positively affected plant development according to control plants. The results obtained from the studies will be of great benefit in terms of breeding studies.

Keywords: *Arbutus unedo* L., nitrogen fertilizer, lignotuber, germination

INTRODUCTION

Arbutus unedo L.(strawberry tree) is one of the important natural plants of the Mediterranean region (Torres et al., 2002; Takrouni et al., 2012). It is one of the important alternative fruits for the future and produces minor fruits that used in pharmacy sector and valuable for various traditional food products (Pawlowska et al., 2006; Alexandre et al., 2020). It has a wide continental distribution area that includes the western coast of North America and Central America, Eastern Europe, the Mediterranean Region, North Africa and parts of the Middle East. *Arbutus unedo L.* is one of the good drought tolerant maquis plants. It survives easily in hot climate.

Strawberry tree plant forms lignotuber under the ground parts that accumulated nutrients. After a forest fire or when the plant is damaged, the plant regrows again from the meristem in the lignotuber. All this makes it a good planting material for the Mediterranean region (Ogaya et al., 2003). The number of studies focusing on the genetic diversity of natural populations of *Arbutus unedo* L. in different countries is increasing (Ogaya et al., 2003; Kostantinidis et al., 2006; Sulusoglu et al., 2011). In subsequent breeding work, researchers should pay attention to the capacity to form lignotubers and the energy re-sprout from lignotubers. The high sprouting capacity of plants will not only reduce the cost of afforestation but may also increase the success of regeneration efforts in planted gardens. Rooting rate of Arbutus unedo L. cuttings are moderately low and changeable with the genotype and maturity (Pignatti and Crobeddu, 2005; Sulusoglu, 2012). Among mineral nutrients, nitrogen (N) is one of the most limiting nutrients for crop production (Taiz and Zeniger, 2010) and had positive effects on growing. The present study, was deals with the effect of nitrogen fertilization on growth capacity of *Arbutus unedo* L.

MATERIAL AND METHODS

This study was organized at Kocaeli University Faculty of Agriculture in 2012-2013. *Arbutus unedo* L. seedlings grown with seeds collected from the Black Seaside of Kandıra-Kocaeli, were used in the experiment. Seeds were sown in February and 3-month-old seedlings were transferred to plastic bags in June. *Arbutus unedo* L. seedlings were planted to soil in the high plastic tunnel in September. In this plantation, seedlings with the same growth strength were used as much as possible. Plants were treated with six N rates of ammonium sulfate (40, 80, 120, 160, 200 and 240 ppm) used as a nitrogen source in the fertilization. Granular ammonium sulfate (21N–0P– 0K–24S) was solved in water and given to each plant.

Fertilization was made monthly before irrigation and repeated during the two growing seasons. Plants were irrigated from early March to late October each year. Irrigation was scheduled based on weather and daily estimates of crop evapotranspiration.

At the end of the experiment (18 months later), lignotuber size (cm), sucker number and length (cm), sprouts number and length (cm) was recorded. The experiment was conducted in randomized plot design with three replicates, three plants per replicates. All the data were evaluated using the analysis of variance (ANOVA) with statistical program Minitab 17 and the groups that showed variance were determined with Tukey test with a significance value $P \leq 0.05$. The results were given as average of two growing season.

RESULTS AND DISCUSSION

Strawberry tree can tolerated differences in production and nutrient concentration and proved to be unaffected by N-NH+ 4 concentrations. *Arbutus* could regulate the N-NH+ 4 absorptions dependently and to require a greater N fertilizer (Martin et al., 2006). Ammonium sulfate $(NH_4)_2SO_4$) was used as nitrogen source in the study. This fertilizer contains 21% nitrogen as ammonium cation formula and sulfate anion form 24 sulfur. Sucker formation and sprouts formation were increased with N fertilization doses. 120 ppm nitrogen fertilization (6,9 cm) has an important effect on lignotuber diameter, but this was not different form 40, 80 and 160 ppm N doses effects. 120 ppm nitrogen doses also have a major impacts on sprouting from meristems on lignotubers. 17,7 sprouts per plant was occurred from under-ground dormant buds by stimulative effects of fertilization (Table 1).

Table 1. Effect of nitrogen fertilization on *Arbutus unedo* L. plant growth

N doses (ppm)	Lignotuber size (cm)	Sucker/plant	Sucker lenght (cm)	Sprouting/plant	Sprouts lenght (cm)
0	4,0 bc	0,0 b	0,0 b	1,0 c	24,7
40	5,3 ab	0,0 b	0,0 b	5,7 bc	12,8
80	5,5 ab	6,0 ab	31,2 a	9,0 b	13,5
120	6,9 a	5,0 ab	23,3 ab	17,7 a	13,0
160	5,3 ab	6,7 a	14,8 ab	7,3 bc	10,8
200	4,2 bc	8,3 a	26,5 ab	7,3 bc	20,9
240	2,5 c	3,0 ab	15,4 ab	1,7 c	8,3

Studies signed that when above-ground part of plant is removed by fire or other disturbance, resprouting is occurred. Sucker formation was close to each other in plants where different nitrogen fertilizer doses were applied (Figure 1). The highest number of suckers were observed at 200 ppm N but differs only statistically from the 40 ppm dose. Suckers from plants treated with 200 ppm nitrogen were the longest shoots (26.5 cm) in the trial.

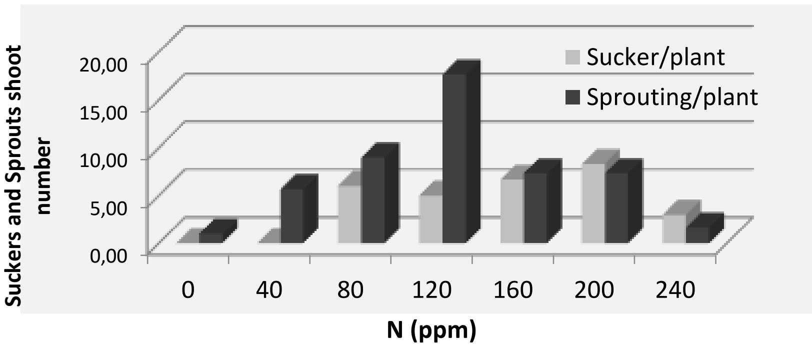

Figure 1. Effect of N doses on sucker formation and sprouting from lignotubers

Other study results showed that N applications increased soil nitrate concentrations in the soil, thus plant available N in all treatments (Salter and Bonnet, 2000). Sucker number was increased following N applications. This result is in a harmony with our results. However, at different doses of N application, the number of suckers/plants was similar in all treatments, but plants were in a very early growing stage in this experiment. Next growing seasons increasing of lignotuber size, the results of N fertilization will be more visible. Younger trees are able to produce sprouts because they have dormant buds around stem trunk, but as a tree matures these buds are lost (Tredici, 2001). In our study, plants were incredibly young and sprout formation was strong.

This close number of suckers as a result of flush of small suckers in high doses of N. The last fertilization was applied in early autumn and have a pronounced effect on number of new suckers. Root suckers are often produced in a disturbance situation such a forest fire kills the above ground parts of the plant (Frey et al., 2003), while in this study N fertilization fastened the growth.

CONCLUSION

Nitrogen fertilization affected plant growth of *Arbutus unedo* L. Lignotubers grew better with fertilization. This will help the plants to be ready in adverse conditions such as forest fire, It will also increase the tolerance to drought conditions. The results of these studies show that nitrogen fertilization will improve plant growth after above-ground cutting of plants. This information will also assist in the management of forested areas after a fire.

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CHERRY LAUREL (P. LAUROCERASUS L.) FLOWERING, POLLINATION AND FRUIT SET

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ABSTRACT:

Cherry laurel (*Prunus laurocerasus* L.) is an evergreen shrub or small tree that can grow up to 6 m high. It is native to the regions bordering the Black Sea in most part of Europe and in Southwestern Asia. It is naturally grown in the northern part of Turkey and very common in the Kocaeli. There are many cultivars as sources of germplasm, provide rich parental material for breeding studies. It is consumed as fresh fruit in Turkey. The fruit is traditionally known for its rich medicinal properties against diabetes and kidney stones. It is an integral part of the life of the people of the Black Sea region where it is known as amazing fruit for their life circle. Cherry laurel gardens have been recently planted. In this study flower characteristics was investigated, and pollination requirements was discussed. Fruit set was evaluated for cherry laurel cultivars. All observation was made on three replicates. One tree was taken as a replicate and 15 flower stalk per tree. Fruit color, size and quality was changed with pollen source. Fruit set was increased with different pollen resources compare with open pollination. There was not fruit set on self-pollinated flowers. Results of the study gave about pollination biology of cherry laurel that very useful for plantation of modern orchards. We propose that, in future, it can be grown in small-scale conventional farms.

Keywords: Cherry laurel, flowering date, pollination, fruit set, breeding

INTRODUCTION

Cherry laurel (*Prunus laurocerasus* L.) is an evergreen fruit trees, may be in the form of low, compact spreading shrub or small tree. It is native to the regions bordering the Black Sea in Southwestern Asia and Southeastern Europe. There are many cultivars with differing characteristics in Turkey and provide rich parental material as a germplasm for crossing.

Normally cherry laurel was grown like a border tree, there are not closed orchards and productivity is very low because of insufficient cultural treatments. The major utilization of the crop is as fresh fruits. The tree is highly resistant to drought, arduous soil conditions, as well as biotic and abiotic stress conditions (Sulusoglu, 2011; Macit and Demirsoy, 2012). This fruit will be one of the most important species with the effect of climate change for the near future. Such minor ; fruits are predicted to become more popular in the future, mainly due to their resistance to strenuous conditions.

Plant breeding has become an important subject and numerous studies were contributed commercially to be used in artificial pollination (Sütyemez 2011; Nikolic et al. 2012).. In almost many fruit species, pollination and fertilization play the main role in fruit set and fruit quality. Studies are focused on cherry laurel fruit set is insufficient. In this paper, we discussed the effect of pollen source on fruit set and quality.

MATERIAL AND METHODS

The study was conducted in 2017-2018 years with the four genotypes selected as superior types according to the results of previous studies (Sulusoglu 2011.). These genotypes were planted in the garden of Kocaeli University Agriculture Faculity. Trees were the same age, size and in fruiting status. Irrigation was applied all over the year and growing condition is protectable as much as possible.

Flowering dates (first flowering, full bloom flowering and end of flowering time) were determined for each genotypes. Un-opened flowers were collected in white balloon stage of the trees and carried to the laboratory immediately. Anthers isolated from flower buds and placed on a black paper under incandescent lamp on a table overnight. Pollen were placed in a small glass bottles, lids were closed and wrapped with stretch film and were preserved at the refrigerator temperature (4 °C) until use. Bottles were prepared separately for cross-pollination treatments of each type. Pollen germination rates (%) were determined with *in vitro* germination of pollen in the agar-plate tests and the germination rate was determined after 48 hours incubation period (Sulusoglu and Cavusoglu, 2014). The germinated pollens were counted and results were determined as germinated pollen percentage.

Artificial pollination was carried out in order to determine the pollinator effect on fruit set. Flowers were emasculated at the white balloon stage and counted before cluster were isolated with parchment paper bags to prevent pollen contamination. Cross and self-pollination treatments were performed by applying pollens to the receptive stigmas of the flowers with a brush. Cross pollination was done with other genotypes and their own pollen for each genotype. castrated and bagged, only marked at the same time as artificial pollination. After self-pollination and cross-pollination, the cluster was again insulated with parchment paper bag and the bags were removed after completion of flowering.

Fruit set records were taken at the end of the ripening period, that is, in July. The percentage of fruit set was calculated. Fruit weight was determined for 30 fruits in each application. The experiment was set up in a completely randomized plot design. For each combination of pollination treatments, ten flower clusters, at the equal size and at the same phenological period, were chosen on different side of trees as a replication and three replications were used. Totally 10 flowers shoots were used in each application. Statistical analyzes of fruit weight and fruit set values were performed separately for each genotype. The data were analyzed using the analysis of variance (ANOVA) and the differences among mean values was determined using Tukey test at $P<0.05$. Data containing percentage values were angle transformed before the statistical analysis.

RESULTS AND DISCUSSION

Cherry laurel flower buds were appeared in early spring and opened from end of March to first week of May, depends on genotypes. The full flowering phenophase occurred in 4 to 10 days after beginning of flowering and average duration of flowering phenophase was 12 to 17 days. In this work, the first flowers opened in March on the GT10. The flowering period for all genotypes was completed between 9-18 April (Table 1).

Flowering time is an important criterion for the perfect completion of pollination period. The phenological calendar of genotypes showed that the flowering periods of mother and pollinator types overlapped in the study. The results agreed with the previous findings (Sulusoglu and Cavusoglu, 2014).

Table 1. Flowering dates of cherry laurel genotypes

Genotypes	First flowering	Full flowering	End of flowering
GT10	March 26	April 4	April 14
GT31	April 2	April 10	April 18
GT32	April 1	April 7	April 11
GT5	March 29	April 4	April 9

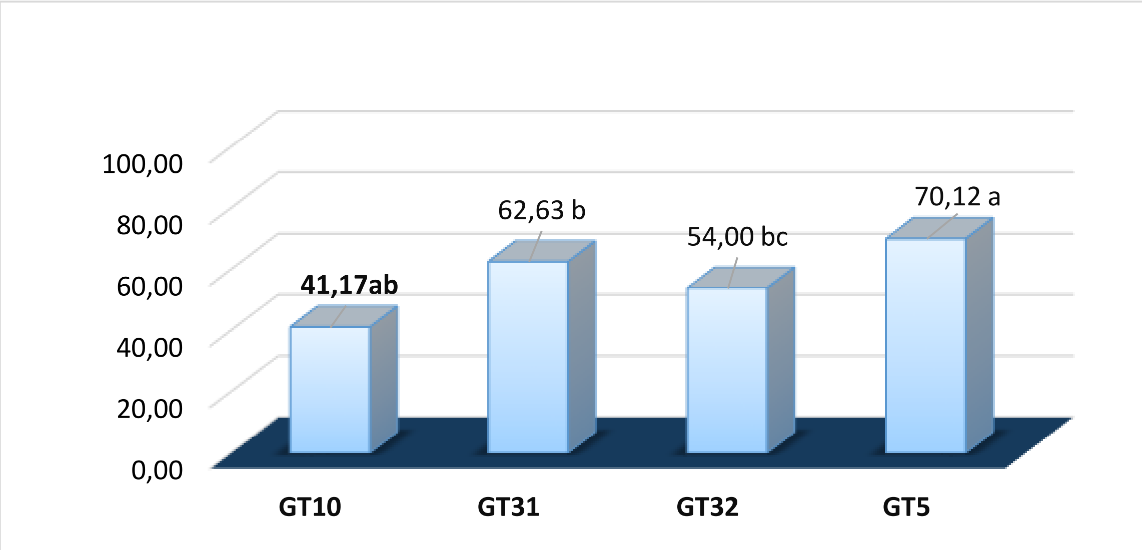

Figure 1. Pollen germination rate (%) of cherry laurel genotypes

At the end of the 48 hours incubation period, the highest pollen germination was obtained from GT5 pollens (70.12%) and according to the mean germination values, this genotypes pollen showed statistically higher germination rates than GT31 and GT32 (Figure 1). Pollen germination rates of fruit varieties vary as indicated in the literature (Gaaliche et al. 2013; Çavuşoğlu and Sulusoğlu 2013), and the results presented here support previous studies.

Fertilization success highly depends on male-female interaction so that pollen performance may have a significant role on pollination (Sütyemez, 2011; Nikolic et al., 2012). Fruit set was recorded after June fruit drop for all genotypes. Result showed that fruit set was strongly changeable depends on pollinator genotypes or self-pollination (Figure 2). Such a case has been reported in plum cultivars and initial fruit set was determined relatively high (Nikolic et al., 2012). Fruit set was relatively high in cross-pollinated combinations (Figure 2). Unpublished observations showed us that, fruit drop was very severe when genotypes self-pollinated and self-pollination resulted with a few fruits set in types 10, 31 and 32 while there was no fruit set in Type 5 at the harvest time. Open pollination gave the similar fruit set percentages between types while cross-pollination had a positive effect in fruit set in all types. Fruit drop was very severe after pollination and decreased considerably over time towards the harvest time. (Fig. 2). Generally, fruit set was lower on Type 32 in all cross-pollinated combinations. As has been frequently observed in the other species (Botu et al., 2002; Koskela et al., 2010, Sütyemez, 2011), there was a variation of the number of set fruits with different pollinizers that fruit set ratios of species could change from 0.0% to 70% and our results agreed with these studies. Cross pollination increased fruit set in all treatments under controlled conditions and fruit set rates were lower in open pollination than in cross pollination. The results were agreed with the results of Nikolic et al. (2012). To obtain adequate yields in cherries 25 to 30% of flowers should set fruits (Bekefi, 2004, Davarynejada et al., 2014,). Our final fruit set rates were sufficient for an effective yield.

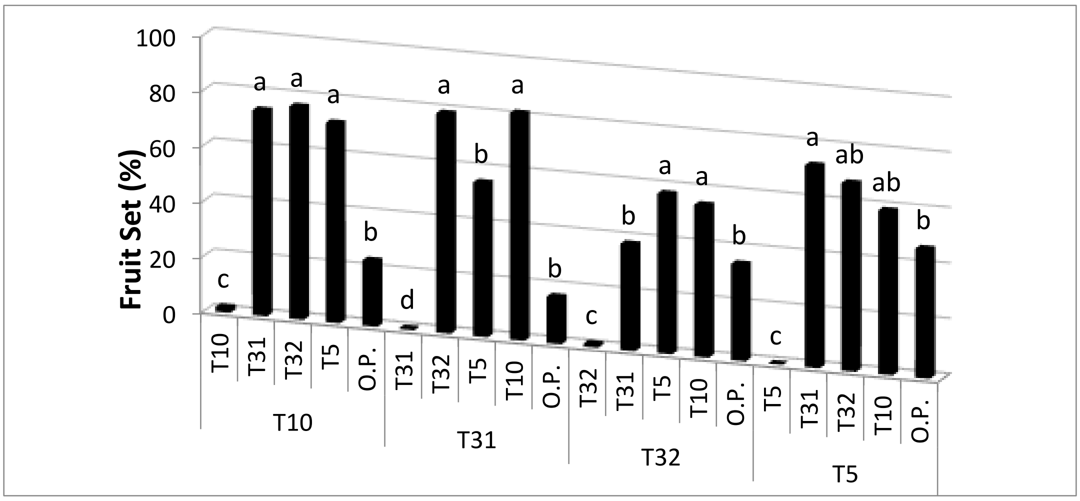

Figure 2. Fruit set of cherry laurel genotypes

Fruit weight was decreased on self-pollinated treatments, and resulted with small, undeveloped amorphous fruits such as lost the roundness and in poor coloration (Figure 3).

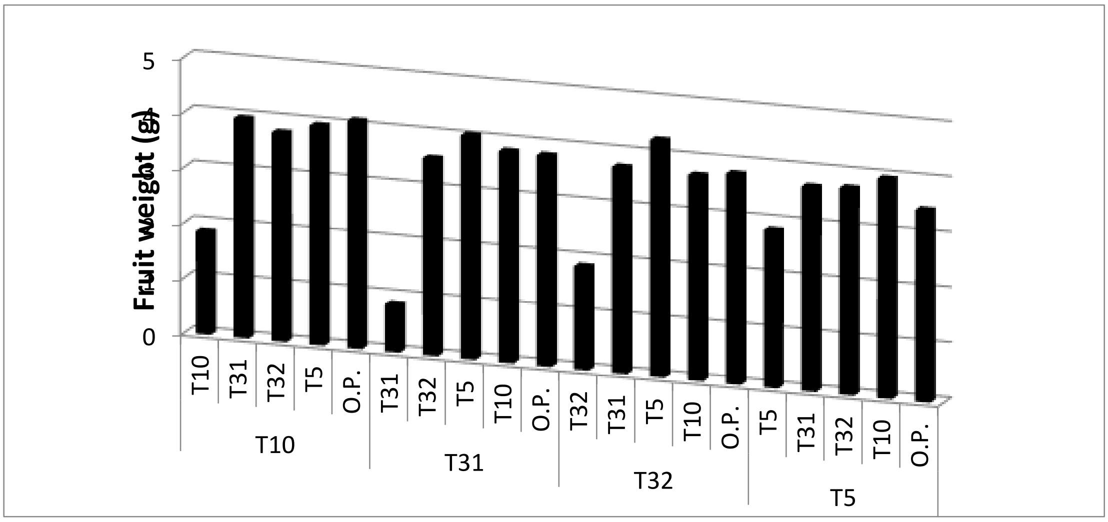

Figure 3. Fruit weight of cherry laurel genotypes

CONCLUSIONS

The types investigated in this study had satisfactory *in vitro* pollen viability. Fruit set was the number of satisfactory standards and differ depending on the pollinators pollen source.

This is an important study about on cross-pollination of the cherry laurel (*Prunus laurocerasus* L.) and results draw under point of cherry laurel needs a pollinizer for high fruit set. Pollinator genotypes also very important for the quality of fruits. This study represents important implications for the plantation establishment of this fruit. Further research will be based on utilization of new pollinizers and orchard establishment. We propose that, in future, it can be grown in small-scale conventional farms, and this research will support the breeding studies in future. Improved and sustainable production of cherry laurel in commercial orchards would be possible by planting suitable combination. Results of the study important for the future breeding studies.

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CHANGES IN SOME BIOCHEMICAL PARAMETERS OF SWEET RED PEPPERS AT DIFFERENT STORAGE TEMPERATURES

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ABSTRACT
Nowadays, a healthy life and nutrition are among the most important problems of humanity. Therefore, the demands of consumers to products with high quality and nutritional value are becoming the focus of recent studies. In line with the demands of the consumers, issues of reducing the biochemical compositions changes of crops are increasingly important. For this reason, sweet red peppers (capia type) stored at different storage temperature (4 ºC, 8 ºC and 20 ºC) for 21 days. Peppers were analyzed for ascorbic acid content, total phenolic content, antioxidant activity, electrolyte leakage, chilling injury, soluble solids content, titratable acidity, visual quality and weight loss at the harvest time and 7 days intervals. Storage period and storage temperatures affected significantly the visual quality of peppers during storage. Loss in AsA of peppers was accelerated with storage temperature increase. The total phenolic and AsA decreased regularly throughout storage. However, the decrease of these compounds was delayed in peppers stored at 4 and 8 ºC. The storage time and temperatures significantly affected the weight loss of peppers. Weight loss increased with increasing time and temperature as expected; but these increases was limited at 4 ºC. The chilling injury was not recorded peppers stored at 8 ºC and 20 ºC during storage. Chilling injury was recorded on 21th days of storage at 4 ºC.

Keywords: Antioxidant activity, Ascorbic acid, Chilling injury, FRAP, Red pepper

INTRODUCTION

Peppers with the scientific name of *Capsicum annuum* L. belongs to the *Solanaceae* family. Peppers are grown in almost every region of the world and it is consumed dried or fresh (Arslan et al., 2020). Morever, peppers are much appreciated by consumers worldwide as a result of phytochemical compounds with antioxidant properties, mainly comprising carotenoids, flavonoids, phenolic compounds, anthocyanins and vitamin and minerals (Kılıç and Calam, 2020; Rodríguez-Rodríguez et al., 2020). These components in peppers are important for human health (Sathasivam and Ki, 2018).

After harvest, fresh peppers can quickly decay due to high water content (Soysal et al., 2009). The biochemical quality of pepper is greatly affected by several pre-and postharvest factors. It is known that storage temperature plays a key role of metabolism and is the most important factor affecting postharvest quality or shelf life quality of fresh products (Hong et al., 2013). The main aims of postharvest storage are to slow down the metabolic activity of fresh products (Zhou et al., 2020) and preserve the quality. And storage temperature is direct effect on fresh products quality and metabolism.

Phytochemical compounds such as anthocyanin, vitamin C, carotenoids are generally very susceptible to degradation and unstable (Hellström et al., 2013). And chemical structure and concentration of these compounds is affected by several factors like variety, climate, ripening stage and storage temperature (Kirigia et al., 2018). Improper storage temperatures and conditions affect their concentrations of these compounds in products. Spinardi and Ferrante (2012) reported that the unfavorable storage temperatures caused the degradation of these compounds in vegetables. So storage temperature is very important factor for delay the quality changes and degradations.

The main aim of this work was to evaluate the changes in biochemical compositions of red pepper during storage at low temperatures (4 ºC and 8 ºC) and room temperatures (20 ºC). In addition, some other quality parameters were also measured during storage.

MATERIAL AND METHOD

Sweet red peppers cv. Diyar F1 (capia type pepper) were hand harvested (Antalya-Turkey, at 2020 year), in the full red stage, in the early morning hours. Capia type pepper is intensively consumed and cultivated in Turkey. After harvest, peppers immediately transferred to the laboratory (Postharvest Physiology). Damaged peppers were removed as well as un-uniform size, color and shape ones to obtain uniform sample. After homogenization and visual examination, the peppers were grouped into three sets. All groups were packaged into the plastic boxes. First group were stored at 4 ºC, second were stored at 8 ºC and third group was stored at 20 ºC for 21 days. The following analyses were measured as three replicates using randomly selected pepper samples.

Peppers were analyzed for weight loss (%), soluble solids content (%), titratable acidity (g/100 ml), visual quality (5: very good, 4: good, 3: moderate, 2: poor, 1: very poor), ascorbic acid content (mg/l00g), total phenolic content (mg GAE/100g), antioxidant activity (g/kg), electrolyte leakage (%), chilling injury (CI) index and physiological disorders at the harvest time and 7 days intervals.

Weight loss: Peppers were weighed initially and weekly during storage. Results were expressed as %.

Soluble solids content (SSC) and titratable acidity (TA): SSC were measured by Atago PAL 1 digital refractometer and expresses as %. For TA, pepper juice (10 mL) was titrated using sodium hydroxide (0.1 N) up to pH 8.1. The results were expressed as g citric acid /100mL.

Total phenolic content (TPC): TPC of peppers was determined using the Folin-Ciocalteu method as described by Thaipong et al. (2006). The standard curve was developed using gallic acid standart (Merck). The absorbance was read by a spectrophotometer (Varian Cary Bio 100, Australia) at 725 nm. Results were calculated as mg of gallic acid equivalent (GAE) per 100 g^{-1} FW.

Antioxidant activity (AA): The ferric reducing antioxidant power (FRAP) assay were used for evaluation of antioxidant capacity in peppers (Thaipong et al., 2006)**.** The calibration curve was developed using Trolox standard (Sigma, Aldrich). The absorbance was read by a spectrophotometer (Varian Cary Bio 100, Australia) at 593 nm. Results were calculated as Trolox equivalents (TE) in g/kg FW.

Ascorbic acid (AsA): AA of peppers was performed by HPLC method recommended by Watada (1982). The AsA content was expressed as mg in 100 g (FW).

Visual quality and chilling injury (CI) index: Visual quality of peppers were evaluated according to the 5-1 scale (5: very good, 4: good, 3: moderate, 2: poor, 1: very poor). CI index was measured by using the 0-4 scale (0: no sign of CI, 1: mild, 2: moderate, 3: severe 4: very severe) (Kasım et al., 2018).

Electrolyte leakage (EL): EL of peppers was analyzed to using method as described by Kasım and Kasım (2014).

Statistical Analysis

The experiment was set up according to the factorial randomized design with 3 replications. All analyses were performed with SPSS software package v.18.0 for Windows by General Linear Model (GLM) univariate test.

RESULTS AND DISCUSSION

Weight Loss and Visual Quality

Prevention of the water loss is one of the most important factors in the storage of fruits and vegetables (Erdoğmuş, 2015). The weight loss of peppers increased with increasing storage period and storage temperature as expected; 4 ºC limited these increases compared to other storage temperatures. The increases in the weight losses during the storage were statistically significant ($p<0.05$) (Table 1). Peppers at 20 ºC showed the highest weight loss than the others. Weight loss of peppers at 20 ºC reached unmarketable level days of 14. However, others (4 ºC – 4.69 % and 8 ºC – 4.52 %) did not reach unmarketable even after 21 days of storage (Figure 1). The loss of weight increased with increasing storage temperature. Similar results were obtained with loquat (Ding et al., 1998).

Storage period and storage temperatures affected significantly the visual quality of peppers during storage (Table 1). As can be seen in Figure 1, the visual quality of peppers decreased regardless of storage temperature during storage. At the end of the storage, the highest score (3.83) were obtained from peppers stored at 8 ºC while the lowest value (2.33) were obtained from peppers stored at 20 ºC.

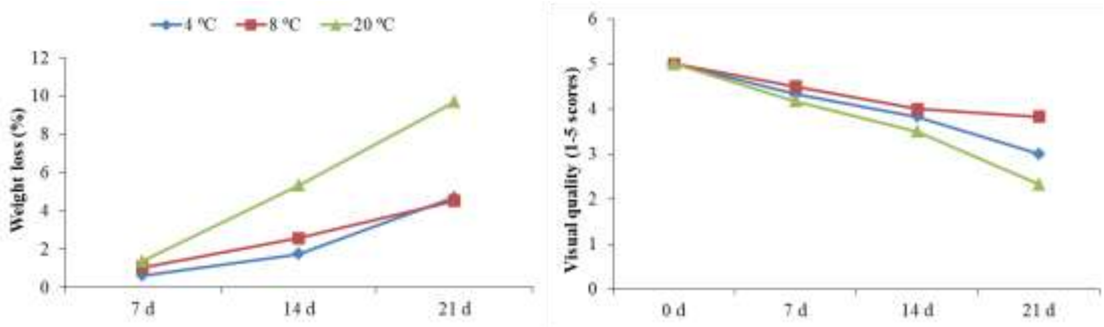

Figure 1. The effect of different storage temperatures on weight loss and visual quality of peppers. d: days.

Soluble Solids Content (SSC) and Titratable Acidity (TA)

Storage period and storage temperatures affected significantly the SSC of peppers during storage (Table 1). Changes in SSC during storage are presented in Figure 2. SSC, which represents soluble sugars in horticultural crop, generally increased throughout the storage period. At the end of the storage, SSC of peppers varied between 6.02 % (4 ºC) and 6.32 % (8 ºC), while the initial value was 5.63 %.

The effect of storage temperatures on TA of peppers was statistically significant ($p<0.05$) but effects of storage period were not significant (Table 1). The TA of peppers generally increased with increasing storage period in all sample groups, but was delayed at 4 ºC. TA reduced as storage temperature increased but there were no significant differences among the storage temperatures. TA was declined more slowly in pepper stored at 4 °C than 20 °C. Similar results were reported by Pailly et al. (2004).

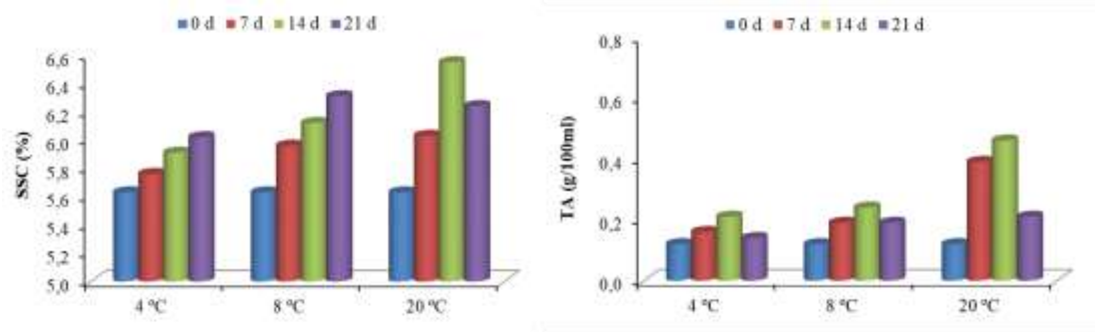

Figure 2. The effect of different storage temperatures on SSC and TA of peppers. d: days.

Ascorbic Acid Content (AsA)

Temperature is one of the most important factors to maintain vitamin C of horticultural crops during storage. The effect of storage temperatures and storage periods on AsA of peppers was statistically significant ($p<0.05$) (Table 1). The AsA of peppers significantly tended to decrease throughout the storage regardless of storage temperatures. The AsA content of peppers changed from 43.97 mg/100g (20 ºC) to 74.65 mg/100g (8ºC) at the end of the storage, while initial value was 132.02 g/100g. The best preservation of AsA was obtained from 8 ºC after 21 day of storage. AsA significantly decreased in 20 ºC compared to other groups. In our study, loss in AsA of peppers was accelerated with storage temperature increase. Similar findings were obtained with citrus and spinach (Lee and Kader, 2000).

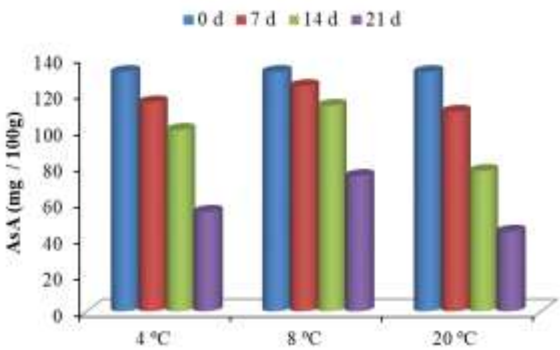

Figure 3. The effect of different storage temperatures on AsA content of peppers. d: days.

Total phenolic content (TPC) and Antioxidant activity (AA)

As shown in Figure 4, total phenolic content of peppers were reduced with extending storage period all storage temperatures. No statistical effects due to the storage temperatures on TPC of peppers were found, but storage periods significantly affected TPC of peppers (Table 1). Stored at 8 ºC prevented the degradation of phenolic compounds during storage. Our results were agreement with Ding et al. (1998).

There is a highly correlated relationship between total antioxidant activity and total phenolic in fruits and vegetables. In accordance to TPC, the antioxidant activity decreased with extending storage period. At the end of the storage, the highest (34.67 g/kg) AA value was found in peppers stored at 8 ºC while stored at 20 ºC gave the lowest value (20.36 g/kg) (Figure 4). The effects of storage temperature and storage period on AA values were significant ($p < 0.05$) (Table 1).

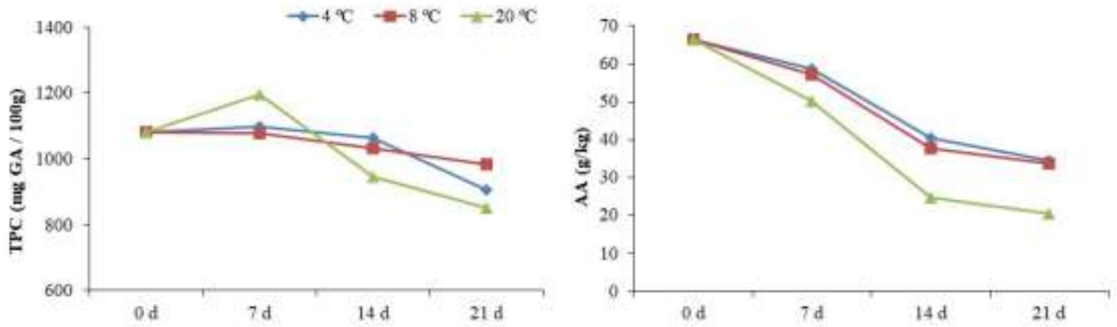

Figure 4. The effect of different storage temperatures on TPC and AA content of peppers. d: days.

Electrolyte Leakage (EL) and Chilling Injury (CI)

The EL values of peppers progressively increased with increased storage period. At the end of the storage period, maximum EL (43.01 %) values was obtained from the 4 ºC, while 8 ºC samples showed minimum values (27.03 %). The effects of storage temperature and storage period on EL values of peppers were significant ($p < 0.05$) (Table 1).

The chilling injury was not recorded peppers stored at 8 ºC and 20 ºC during storage. Chilling injury was recorded on 21th days of storage at 4 ºC. Storage period affected significantly ($p<0.05$) the CI of peppers during storage (Table 1).

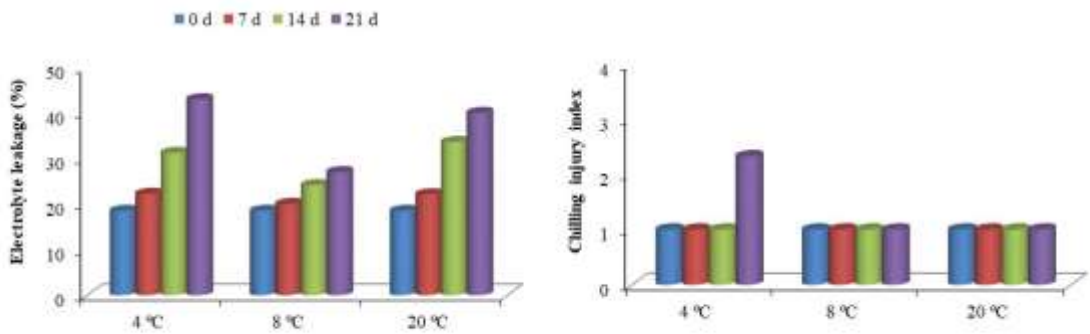

Figure 5. The effect of different storage temperatures on electrolyte leakage and chilling injury index of peppers. d: days.

Table 1. ANOVA for storage temperatures, storage period and their interactions

	ST	SP	ST×SP
Weight loss	**	*	**
Visual quality	*	*	ns
SSC	*	**	ns
TA	ns	*	ns
AsA	**	*	*
TPC	ns	*	ns
AA	*	**	ns
EL	**	*	ns
CI	ns	*	ns

ST: Storage temperatures, SP: Storage periods, SSC: Soluble solids content, TA: Titratable acidity, AsA: Ascorbic acid, TPC: Total phenolic content, AA: Antioxidant activity, EL: Electrolyte leakage, CI: Chilling injury, ns: represents non-significance at $p < 0.05$, **: represents significance at the 0.01 level, *: represents significance at the 0.05 level.

CONCLUSIONS

This study investigated changes in biochemical parameters of sweet red peppers stored at 4°C, 8°C and 20°C. And study demonstrated the importance of storage temperature for the preserve of phenolics, ascorbic acid and antioxidant activity of peppers during storage. Moreover 8°C delayed the visual quality loss and chilling injury compared to other temperatures and retarded changes in biochemical compositions of peppers. Stored at room temperatures has accelerated the quality loss of peppers.

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DETERMINATION OF FORAGE QUALITY OF DIFFERENT COOL SEASON TURFGRASS SPECIES AND VARIETIES

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ABSTRACT

This research was planned to determine the forage quality of the varieties of different grass species used to create green areas. The research was carried out in Erzurum in 2018 and 2019 according to the randomized completely blocks experimental design with 3 replications. The hay taken from the green fields of 14 varieties of *Lolium perenne*, *Festuca arundinacea*, *Poa pratensis*, *Festuca rubra rubra*, *Festuca rubra trichophylla*, *Festuca rubra commutata* and *Agrostis tenuis* were dried and the dry matter ratio, crude protein ratio, ADF (Acid detergent fiber) and NDF (Neutral detergent fiber) and RFV (Relative Feed Value) values were determined. The obtained data were subjected to variance analysis with the help of SPSS package program, and the differences between the averages were revealed by Duncan multiple comparison test.

According to the results obtained, it was determined that there were great differences in the forage quality parameters of the hay according to the varieties. The highest crude protein (19,17%) Miracle, the lowest ADF (23,17%) Olimpus and the lowest NDF ratio (38,89%) were determined in Allstater varieties according to the two-year averages. The relative feed values of the cultivars also varied between 91,8 and 216,6.

Keywords: Turfgrass, Species, Hay, Forage quality

INTRODUCTION

The indispensable element of green areas is the grass family plants. Grasses are the most preferred species for creating green areas as well as being used in many fields such as human nutrition, meadows and pastures, hay production, silage production, soil conservation, industry and art. It is very important to choose plant species and varieties suitable for the purpose of use in creating green areas. In this respect, the variety to be selected should be suitable for the purpose and climate of the place where it will be used. In this regard, choosing clean seeds of varieties that are resistant to cold, heat and drought, have strong resistance to diseases, pests and weeds, have a uniform appearance, and have a homogeneous structure are among the issues to be considered. There are 5 species of grasses commonly used to create green areas in cool season regions around the world. The most prominent of these is the perennial ryegrass (*Lolium perenne* L.). The biggest disadvantage of turfgrass areas is that it is not very resistant to low temperatures, has no spreading feature and has a short lifespan. Another type of red fescue (*Festuca rubra* L.) that has been used frequently to create green areas in recent years. There are three different subspecies of red fescue in the market: *rubra*, *commutata* and *trichophylla*. However, turfgrass quality deteriorates under high temperature and lighting conditions (Oral and Açıkgöz, 2015). Kentucky bluegrass (*Poa pratensis* L.) is a very valuable green field plant that creates a very dense and finely structured green area. The leaves are hairless, blue-green in color. It is very difficult to plant due to the slow germination and spreading speed. In addition, seed production is difficult and seeds are expensive. Tall fescue (*Festuca arundinacea* Schreb.) is a plant that has been used in the establishment of green areas in recent years. The most important advantages of this plant are its durability and low maintenance. One of the most valuable plants in green areas is the delicate common bent (*Agrostis tenuis* L.). It spreads with rhizomes and stolons and creates a dense grass texture. Since it is based on short form, it is one of the indispensable types of quality mixtures. With its thin and soft texture, it provides beautiful textured surfaces. It is preferred in the establishment of special areas such as golf courses. However, since it is a plant of cool and rainy climates, it cannot adapt to the arid-terrestrial regions of our country, so its import has been restricted (Avcıoğlu, 2014).

Scientific studies on green areas and turfgrass types are quite new in Turkey. Conscious efforts to create green areas started in the 90s (Avcıoğlu, 2014). Studies in this area show that mostly cool season grass species and varieties are used (Varoğlu, 2015; Hosaflıoğlu and Yılmaz, 2017; Charif et al., 2019; Alkan, 2019). The Eastern Anatolia Region of the country has different ecological characteristics from other regions. High altitude, cold and snowy winter period, cool and short summer period are its characteristic features. The most important source of livelihood in the region is animal husbandry. The people of the region are engaged in pasture-based livestock activities in large and productive pastures. However, the winter period in the region is quite long and cold. In this period when the pastures are covered with snow, it is imperative that the animals be fed in the shelters. Therefore, there is a great need for roughage in the Eastern Anatolia Region in the winter period. Studies show that there is a shortage of roughage in the region, and the need for quality roughage of animals cannot be adequately met (Acar et al., 2020, Tan and Yolcu, 2021). For this reason, it is of great importance to evaluate alternative feed sources in the region.

Biomass produced from green areas in the Eastern Anatolia Region can be a valuable alternative feed source. It is possible to use this herb, which is produced in every form, to be used in the nutrition of animals in the winter period. However, there are not enough studies on this subject. Bilgili and Açıkgöz (2007) reported that the total grass production of turf plants formed with different plant mixtures is 1020-1276 g m^2 in spring, 502-544 g m^2 in summer, 198-245 in autumn and 73-83 g m^2 in the winter period. As can be seen, the biomass produced from these areas is too high to be underestimated. On the other hand, Biber (2013) determined that the use of green areas (park, roadside, sports field) affects the quality of the grass produced. The researcher found that the hay produced from green areas have 15-29% crude protein, 47-62% NDF and 22-29% ADF ratio. However, the most effective factor on the quality of hay produced from green areas is undoubtedly the grass species and varieties used. Therefore, there is a need for more information on the evaluation and nutritional value of the material produced by different species and cultivars. This study was carried out to determine the hay quality of grasses produced from different grass species and varieties used in green areas.

MATERIALS AND METHODS

The research was carried out in the wet trial areas of Atatürk University Plant Production, Application and Research Center Directorate in 2018 and 2019. In the field study, five different turfgrass species (*Lolium perenne, Festuca arundinacea, Poa pratensis, Festuca rubra, Agrostis tenuis*) and 14 varieties belonging to these species were used (Table 1).

Table 1. Grass species and varieties used to create green areas in the research

Species	Varieties
Lolium perenne L.	Allstarter
Lolium perenne L.	Jackento
Lolium perenne L.	Cutter2
Lolium perenne L.	Neruda
Poa pratensis L.	Miracle
Poa pratensis L.	Volt
Poa pratensis L.	Evora
Festuca rubra L. var. *trichophylla*	Pinafore
Festuca rubra L. var. *rubra*	Sergei
Festuca rubra L. var. *commutata*	Longfellow
Festuca arundinacea Schreb.	Olimpus
Festuca arundinacea Schreb.	Brigantine
Festuca arundinacea Schreb.	Master Piace
Agrostis tenuis L.	Denso

The study was set up in a randomized complete blocks design with three replications. Sowing was done manually in the pre-prepared seed bed in April 2018. The area of each parcel is planned as 2 m^2 (2 m×1 m). In sowing, 30 g of perennial ryegrass, 25 g of red fescue, 22 g of tall fescue, 15 g of Kentucky bluegrass and 10 g of common bentgrass were used per square meter (Avcıoğlu, 2014; Açıkgöz and Oral, 2015). After the seeds were spread homogeneously on the plots, a 1-2 cm thick pre-prepared mortar (2/3 sifted soil, 1/3 burnt and sieved barn manure) was laid on it and pressed with a roller. During planting, standard fertilization was made at 5 kg N $da^{-!}$ and 5 kg P_2O_5 da^{-1}, and then nitrogen was given to the plots at 4 g m^2 every month (Avcıoğlu, 2014; Açıkgöz and Oral, 2015; Bilgili et al., 2017). Irrigation operations were started immediately after planting in the form of sprinkling, and it was carried out every 2-3 days depending on the precipitation. Weeds seen in the plots were removed by manually removing them before they were allowed to grow. The forms are made from a height of 4-5 cm with a lawnmower when the plants reach 7-8 cm in height (Arslan, 2010).

The samples taken in the second form of each year were first dried in the open air, then in a drying oven set at 65 °C and ground. DMR (dry matter rate), CPR (crude protein rate), ADF (acid detergent fiber rate), NDF (neutral detergent fiber rate) ratios and RFV (relative feed value) were determined in the milled samples. Crude protein ratios were performed by Mikro Kjeldahl method according to Kacar and İnal (2013), and ADF and NDF analyzes were performed using ANKOM Fiber Analyzer by Van Soest et al. (1991) was made according to the principles stated. By using the ADF and NDF parameters, the digestible dry matter (DDM), dry matter intake (DMI) and relative feed value (RFV) of the cultivars were calculated using the following equations (Oddy et al., 1983; Sheaffer et al., 1995; Fonnesbeck et al., 1984).

DDM (%) = 88,9 - (0,779 x % ADF)

DDI (%) = 120 / NDF

RFV = (% SKM x % SPG) / 1,29

The obtained data were subjected to variance analysis according to the randomized complete blocks arrangement with the help of the SAS package program. The differences between the means found to be significant were compared and grouped according to the Duncan Multiple Comparison Test (Yıldız and Bircan, 1991).

RESULTS AND DISCUSSIONS

Dry matter ratio was significantly affected by both the year and the variety, and the year x variety interaction was found to be significant (Table 2). According to the two-year average, the dry matter ratios of the cultivars ranged from 28,24% to 38,25%. The highest values were determined in Longfellow and Brigantine cultivars. Dry matter ratio showed a significant change according to years, the value of the first year (37,70%) was higher than the other year (30,80%).

Table 2. Dry matter rate of turfgrass varieties used for green fields (%)[1]

Varieties	Dry Matter Rate (%)		
	2018	2019	Mean
Allstarter	$37,26^{AB}$	$30,32^{BC}$	$33,80^{A-E}$
Jackento	$42,90^{A}$	$30,80^{ABC}$	$36,82^{ABC}$
Cutter2	$39,70^{A}$	$31,20^{ABC}$	$35,44^{A-D}$
Neruda	$41,30^{A}$	$32,40^{ABC}$	$36,83^{ABC}$
Miracle	$35,40^{ABC}$	$29,14^{CD}$	$32,27^{A-E}$
Volt	$44,00^{A}$	$28,30^{CD}$	$36,12^{ABC}$
Evora	$44,00^{A}$	$28,22^{CD}$	$36,11^{ABC}$
Olimpus	$36,60^{AB}$	$32,43^{ABC}$	$34,51^{A-D}$
Brigantine	$43,90^{A}$	$31,70^{ABC}$	$37,80^{AB}$
Master piace	$33,80^{ABC}$	$30,13^{BC}$	$32,00^{B-E}$
Pinafore	$27,80^{BC}$	$34,11^{AB}$	30.94^{CDE}
Sergei	$25,10^{C}$	$31,40^{ABC}$	$28,24^{E}$
Longfellow	$41,40^{A}$	$35,11^{A}$	$38,25^{A}$
Denso	$34,60^{ABC}$	$25,48^{D}$	$30,02^{DE}$
Mean	37,70	30,80	34,22
F values			
Year	-	-	51,182**
Variety	3,037**	3,266**	3,03**
Year x Variety	-		3,468**

[1] Means marked with different letters are statistically different at 0,01 probability level

Crude protein content of hay cut from green fields in the study did not change depending on the years, but showed significant differences depending on the varieties (Table 3). In the two-year average, the highest crude protein rate (19,172%) was determined in Miracle variety, followed by Denso, Cutter2 and Jackento varieties. The Olimpus cultivar of *Festuca arundinacea* had the lowest (13,650%) crude protein content. The year x variety interaction was found to be important in the research, and the Cutter2 variety had the highest crude protein ratio with a value of 21,000% in 2018. *Lolium* varieties are already plants with abundant leaves and high crude protein as fodder. On the other hand, *Festuca arundinacea* cultivars are coarse in structure and low protein ratios are an expected result.

ADF ratios of the grasses of the cultivars were between 28,310% and 28,693%, depending on the years; It varied between 23,175% and 35,227% depending on the cultivars (Table 4). While the effect of years was found to be insignificant, the difference between cultivars was determined to be very significant ($P<0,01$). According to the two-year average, Evora had the highest (35.227%) and Olimpus had the lowest (23.170%) ADF. While the Olimpus cultivar had a low ADF ratio in the first year, it had more ADF from the second year. In some cultivars, the low rate of ADF in the first year may be due to the long duration of the seedling period.

Table 3. Crude protein ratios of turfgrass varieties used for green fields (%)[1]

Varieties	Crude Protein Rate (%)		
	2018	2019	Mean
Allstarter	15,670[b–e]	15,707[ab]	15,632[bcd]
Jackento	17,330[abc]	18,547[ab]	17,832[ab]
Cutter2	21,000[a]	16,770[ab]	18,903[ab]
Neruda	20,330[a]	15,090[ab]	17,530[abc]
Miracle	19,670[ab]	18,990[a]	19,172[a]
Volt	11,000[f]	17,450[ab]	14,202[cd]
Evora	11,330[ef]	17,453[ab]	14,362[cd]
Olimpus	13,670[c–f]	13,470[b]	13,650[d]
Brigantine	18,000[abc]	15,387[ab]	16,587[a–d]
Master piace	18,670[ab]	15,520[ab]	17,227[ab]
Pinafore	12,670[def]	15,673[ab]	14,210[cd]
Sergei	18,330[ab]	15,590[ab]	16,982[a–d]
Longfellow	15,330[b–f]	17,260[ab]	16,440[a–d]
Denso	17,000[a–d]	18,630[ab]	17,833[ab]
Mean	16,399	16,538[ab]	16,469
F values			
Year	-	-	0,060
Variety	5,63**	1,08	2,99**
Year x Variety	-	-	2,89**

[1] Means marked with different letters are statistically different at 0,01 probability level.

Table 4. ADF ratios of turfgrass varieties used for green areas (%)[1]

Varieties	ADF (%)		
	2018	2019	Mean
Allstarter	25,230[cde]	26,013[a]	25,622[cd]
Jackento	30,157[bc]	29,487[a]	29,822[abc]
Cutter2	30,370[bc]	26,247[a]	28,308[bcd]
Neruda	21,380[de]	28,517[a]	24,948[cd]
Miracle	27,213[cde]	26,817[a]	27,015[bcd]
Volt	28,327[bcd]	30,383[a]	29,355[a–d]
Evora	40,580[a]	29,873[a]	35,227[a]
Olimpus	20,200[e]	26,140[a]	23,170[d]
Brigantine	30,970[bc]	28,640[a]	29,805[abc]
Master piace	30,240[bc]	29,460[a]	29,850[abc]
Pinafore	26,810[cde]	29,677[a]	28,243[bcd]
Sergei	27,023[cde]	31,593[a]	29,308[a–d]
Longfellow	22,000[de]	28,203[a]	25,102[cd]
Denso	35,833[ab]	30,650[a]	33,242[ab]
Mean	28,310	28,693	28,501
F values			
Year	-	-	0,143
Variety	5,504**	0,356*	2,942**
Year x Variety	-	-	1,720*

[1] Means marked with different letters are statistically different, *: 0,05, **: 0,01.

The NDF contents of the grasses produced by different types of grass fields did not change according to the years, but there were significant differences between the varieties (Table 5). Evora and Olimpus had high NDF contents (48,997% and 48,648%). On the other hand, Allstarter took the last place with 38,895% NDF rate. Year x variety interaction was found to be significant in NDF ratio of grass, Evora in the first year and Olimpus in the second year had the highest values. The lowest value was determined in the Allstarter variant in the first year. The NDF contents of the cultivars have emerged as a result of their genetic characteristics and developmental status.

Table 5. NDF ratios of turfgrass varieties used for green areas (%)[1]

Varieties	NDF (%)		
	2018	2019	Mean
Allstarter	36,620^{d}	41,170 ^{ab}	38,895^{e}
Jackento	43,030^{bcd}	43,787 ^{ab}	43,408 $^{a–e}$
Cutter2	45,167^{bcd}	41,930 ^{ab}	43,548 $^{a–e}$
Neruda	39,983^{bcd}	41,920 ^{ab}	40,952^{cde}
Miracle	39,080^{cd}	43,377 ^{ab}	41,228$^{b–e}$
Volt	38,097^{cd}	43,813 ^{ab}	40,955^{cde}
Evora	56,180^{a}	41,813 ^{ab}	48,997^{a}
Olimpus	47,243^{abc}	50,053^{a}	48,648^{a}
Brigantine	47,993^{abc}	44,630 ^{ab}	46,312$^{a–d}$
Master piace	45,637^{bcd}	42,483 ^{ab}	44,060$^{a–e}$
Pinafore	45,767^{bcd}	43,833 ^{ab}	44,800$^{a–d}$
Sergei	50,233^{ab}	45,477 ^{ab}	47,855^{ab}
Longfellow	48,203^{abc}	46,820 ^{ab}	47,512^{abc}
Denso	41,450^{bcd}	37,853 ^{ab}	39,652^{de}
Mean	44,620	43,497	44,058
F values			
Year	-	-	1,076
Variety	3,087**	1,179*	2,84**
Year x Variety	-	-	1,621*

[1] Means marked with different letters are statistically different, *: 0,05, **: 0,01.

The relative feed value of the hay obtained from the green areas did not change according to the years, but significant differences were found depending on the varieties (P<0,01; Table 6). RFV was found between 168,16 and 121,70 according to the cultivars. *Lolium perenne*'s Allstarter pair had the highest relative feed value, while Evora from *Poa pratensis* had the highest value (Table 6).

Table 6. Relative feed values of turfgrass varieties used for green areas[1]

Varieties	Relative Feed Value		
	2018	2019	Mean
Allstarter	179,10[a]	157,24	168,16[a]
Jackento	151,41[abc]	142,67	147,04[a–d]
Cutter2	135,40[a–d]	152,14	147,80[a–d]
Neruda	171,90[ab]	148,85	160.37[ab]
Miracle	162,74[abc]	146,10	154,42[abc]
Volt	165,61[abc]	139,38	152,50[abc]
Evora	94,88[d]	148,45	121,70[d]
Olimpus	144,12[abc]	127,90	136,00[bcd]
Brigantine	127,14[bcd]	139,25	133,20[bcd]
Master piace	133,23[bcd]	144,43	138,83[bcd]
Pinafore	138,30[a–d]	139,80	139,04[bcd]
Sergei	125,94[cd]	132,39	129,16[cd]
Longfellow	139,70[abc]	134,30	137,00[bcd]
Denso	136,74[a–d]	163,23	150,00[a–d]
Mean	143,30	143,93	143.94
F values			
Year	-	-	0,023
Variety	2,64*	0,80	2,108*
Year x Variety	-	-	1,651

[1] Means marked with different letters are statistically different, *: 0,05

CONCLUSIONS

The results obtained from the research; shows that the materials harvested from the green areas can be used for animal feeding. These hay have high crude protein content and low fiber content (ADF and NDF). Accordingly, the relative feed values were found to be high. The feed values of turfgrass show significant changes depending on the years and especially the varieties. Biber (2013) also determined that the content of turfgrass in lawns varies depending on the material used and the way it is used. As a matter of fact, many studies on forage crops reveal that feed quality characteristics change depending on years and varieties (Tan, 208). However, studies on the storage method (silage or drying with different additives) and animal nutrition related to the use of these materials are required.

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EVALUATION OF ANTIOXIDANT CAPACITY AND FLAVONOID CONTENT IN ANNUAL AND PERENNIAL WILD *HELIANTHUS* SPECIES

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ABSTRACT

The genus *Helianthus,* belonging to the *Asteraceae* family, is a very diverse genus that consists of 53 species including 14 annuals and 39 perennials. The large number of wild sunflower species constitutes a genetic pool for crop improvement providing breeders with potentially useful agricultural traits, such as increased disease resistance, high oil content, better abiotic stress tolerance, early maturity, enhanced antioxidant potential. These advantageous agronomic characteristics could be introduced into elite parental lines by interspecific crosses. The main goal of the present investigation was to examine the antioxidant capacity of different sunflower species grown in the Institute of Plant Physiology and Genetics (Bulgarian Academy of Sciences, Sofia, Bulgaria) and Trakya University (Edirne, Turkey) using Ferric reducing antioxidant power (FRAP) and 2,2-diphenylpicrylhydrazyl (DPPH) assays, and leaf flavonoid content. We analyzed the annuals (2n=2x=34) *Helianthus petiolaris ssp. petiolaris, H. praecox ssp. runyonii, H. praecox ssp. praecox, H. praecox ssp. hirtus,* the diploid perennials (2n=2x=34) *H. divaricatus, H. salicifolius* (2 populations), *H. pauciflorus, H. smithii, H. grosseserratus*, and hexaploid (2n=6x=102) *H. tuberosus.* Altogether, this work provides experimental evidences for the highly variable antioxidant activity of the studied sunflower accessions, which could be used as a basis for development of novel cultivars with desirable antioxidant *properties*.

Key words: antioxidant capacity, flavonoids, *Helianthus annuus***,** sunflower, wild accessions

INTRODUCTION

The antioxidants play an important role in living organisms since they prevent excessive free radical formation in cells. The overproduction of free radicals beyond those needed for the effectiveness of the antioxidant defense may cause disruption of biologically imported molecules and in consequence the onset of various diseases. Reactive oxygen species (ROS) that are produced in living organisms generate oxidative stress by oxidizing the bio-molecules, such as nucleic acids, proteins and lipids (Harborne and Williams, 2000; Heim et al., 2002; Shahidi and Ambigaipalan, 2015; Colin, 2019). Since antioxidants are effective elements in preventing oxidative stress, attention has been focused on the importance of natural antioxidants and their utilization in food. The consumption of antioxidants in food and dietary supplements has been linked to a reduced risk of these diseases (Zhang and Tsao, 2016; Onoja et al., 2020).

Cultivated sunflower (*Helianthus annuus* L.), an oleaginous plant grown worldwide is the fourth largest source of edible oil after soybean, rapeseed and peanut (Seiler et al., 2017). Due to presence of phenolic, flavonoid, biologically active compounds, polyunsaturated fatty acids and vitamins sunflower seeds contain valuable antioxidant, antimicrobial, anti-inflammatory, antihypertensive, wound healing and cardiovascular benefits (Karamać et al., 2012; Sharif et al., 2018). Globally, the crop wild relatives (CWRs) such as wild *Helianthus* species are sources of beneficial genetic diversity in sunflower ranging from disease resistance to drought tolerance and yield-related traits and therefore, they have been employed in crop improvement process by crossing cultivated varieties with wild species (Dempewolf et al. 2017; Seiler et al., 2017).

The aim of the present study was to evaluate antioxidant capacity and total flavonoid content of annual and perennial wild *Helianthus* species as a potential source of natural antioxidants - a trait of interest for sunflower research and breeding.

MATERIALS AND METHODS

The sunflower collection at the Institute of Plant Physiology and Genetics (IPPG), Sofia, Bulgaria (42˚50´ N, 23˚ 00´ E, 595 m above the see level) contains annual and perennial wild sunflower genotypes represented by a large number of different populations as well as lines in an advanced selection cycle. The wild annual and perennial populations were grown from the seeds originally obtained from the Trakya University, Edirne, Turkey. Of all genotypes in the collection, morpho-biochemical traits of eleven representatives were analyzed (Table 1).

Table 1. Investigated annual and perennial wild *Helianthus* species

Species	Accession number
Annual (2n=2x=34)	
Helianthus annuus cv 1114	
Helianthus praecox ssp. runyonii	N 187
Helianthus praecox ssp. *praecox*	N 186
Helianthus praecox ssp. *hirtus*	N185
Helianthus petiolaris ssp. *petiolaris*	N 179
Diploid perennial species (2n=2x=34)	
Helianthus divaricatus	N 62
Helianthus pauciflorus	N 153
Helianthus salicifolius	N 203
Helianthus salicifolius	N 205
Helianthus smithii	N 216
Helianthus grosseserratus	N 85
Hexaploi perennial species (2n=6x=102)	
Helianthus tuberosus	N 234

The cultivated sunflower *H. annuus* cultivar 1114 developed at IPPG was used as a control. To evaluate and compare the biochemical parameters of the individual wild species, the following characteristics were assessed: antiradical activity by 2,2’-diphenyl-1-

picrylhydrazyl (DPPH) Radical Scavenging assay, antioxidant capacity by Ferric Reducing Antioxidant Power (FRAP) assay, and total flavonoid content by the aluminium colorimetric assay. Data were subjected to one-way ANOVA analyses of variance for comparison of means, and significant differences were calculated according to Fisher LSD-test ($p \leq 0.05$) using a statistical software package.

RESULTS AND DISCUSSION

DPPH

The recognized high antioxidant potential of sunflowers makes it a promising source of antioxidants (De Leonardis et al., 2005; Vassilevska-Ivanova et al. 2014; Zoumpulakis et al., 2017). The antioxidant activity of sunflower leaves extracts was determined as the ability to scavenge free radicals ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$) and to reduce ferric ions (FRAP). The results of these estimations are presented in Fig. 1 (a, b). The lowest level of DPPH among annuals was observed in extract of *H. petiolaris* ssp. *petiolaris* (acc. N 179). The three analyzed subspecies of *H. praecox* (acc. N 175, 186, and 187) revealed lower values of DPPH than those of cultivated sunflower *H. annuus*. The *H. praecox* subsp. *hirtus* and *H. praecox* ssp. *praecox* showed almost identical values of DPPH. Among the studied perennial species, the hexaploid *H. tuberosus* (acc. N 234) had significantly higher DPPH value as compared with cultivated sunflower *H. annuus* and other species evaluated in the current study (Fig. 1b).

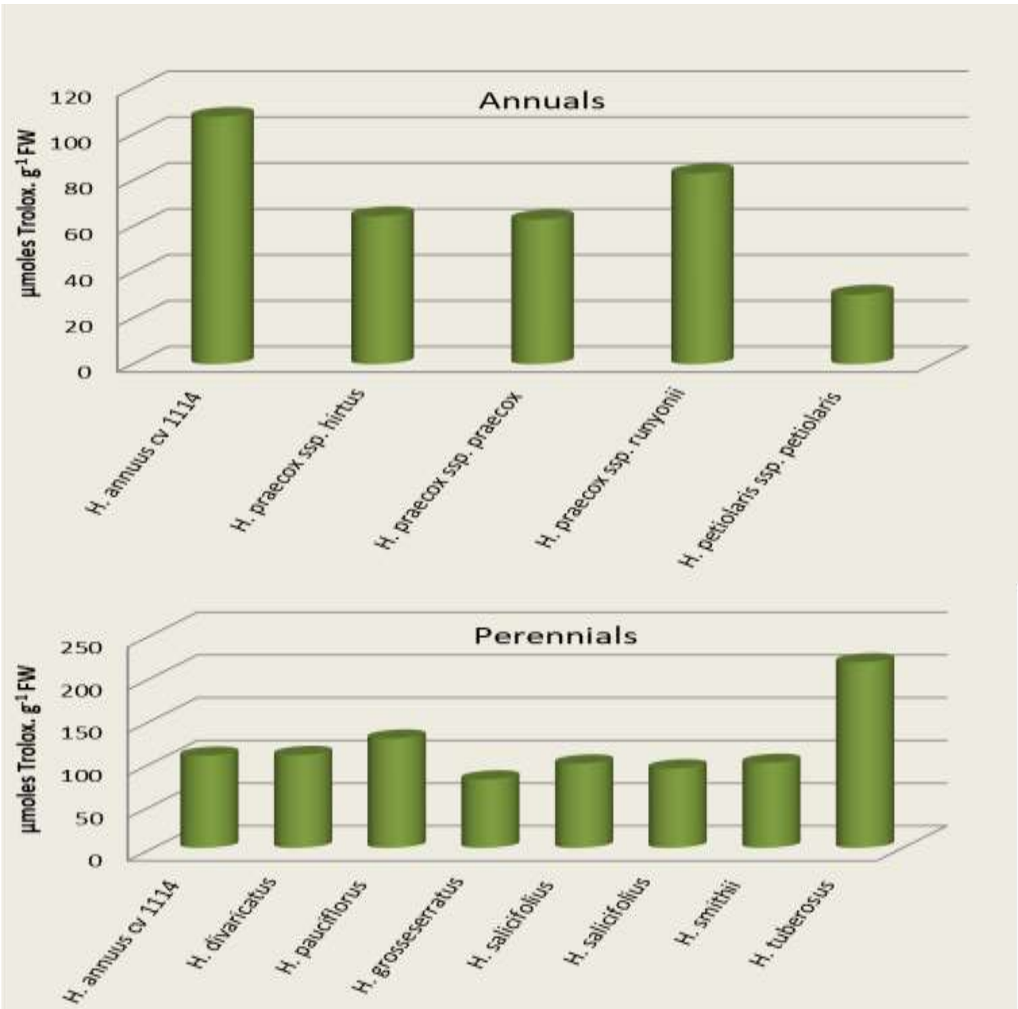

Fig. 1. The antioxidant capacity (DPPH) of different annual and perennial *Helianthus* species.

FRAP

Ferric Reducing Antioxidant Power (FRAP) values of the studied plants varied similarly to the way it varied in the DPPH evaluation. According to the FRAP method used, *H. praecox* ssp. *hirtus* showed the highest value of antioxidant capacity as compared with investigated annual species but lower than *H. annuus* (Fig. 2a). For perennial species (Fig. 2b), the highest antioxidant properties were observed in the extracts from hexaploid species *H. tuberosus* (acc. N 234). The current results are in agreement with the finding of Liava et al. (2021), who reported that, the three main bioactive constituents in the tubers and aerial parts (flavonoids, phenolic acids, and sesquiterpenoids) of *H. tuberosus* effectively participate in biological processes. These functional and bioactive ingredients exhibit antioxidant, anti-inflammatory, antitumor, and antibacterial activities beneficial to human and animal health (Kays and Nottingham, 2007). In addition, it was found that wild *H. tuberosus* (Jerusalem artichoke) is a plant with strong stress resistance to abiotic stress (drought, salinity, waterlogging). Producing hybrid plants from crosses between cultivated sunflower *Helianthus annuus* and *H. tuberosus* is an advantage, since it will allow for selection within hybrid progeny for specific characters (high antioxidant capacity or stress resistance).

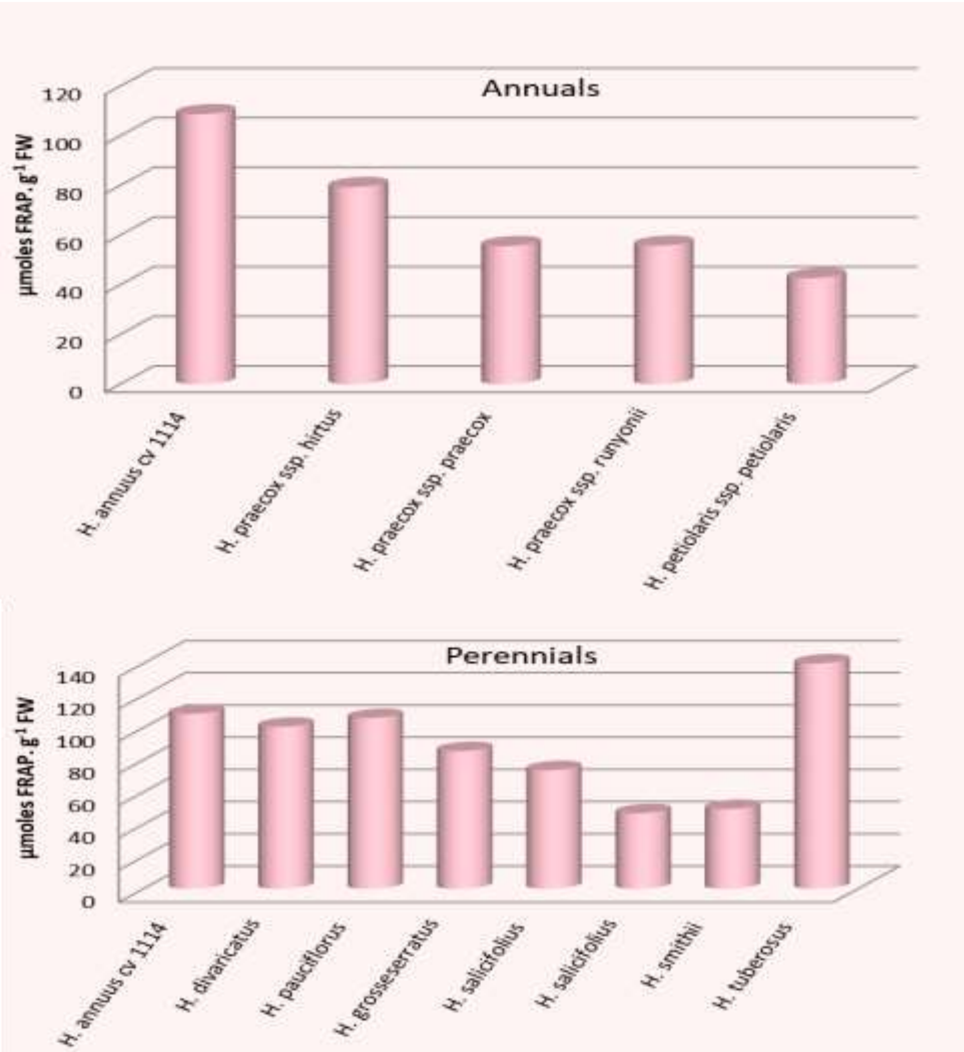

Fig. 2. Free radical scavenging activity (FRAP) in fresh leaves of wild annual and perennial *Helianthus* species

FLAVONOID CONTENT

Flavonoids are phenolic substances, which exhibit a wide range of biological benefits, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory. Flavonoids are the important metabolites found in the sunflower family (Guo et al., 2017), which belong to the group of natural antioxidants such as tocopherols and phenolic acids, peptides (reduced glutathione), carotenoids etc. In the current study, among annual species, the lowest level of flavonoids was found in *H. petiolaris ssp. petiolaris* (acc. N 179). The all three *H. praecox* species (ssp. *hirtus*, *praecox* and *runyonii*) exhibited higher flavonoid content than *H. petiolaris* but much lower that the cultivated *H. annuus* cv 1114 (Fig. 3a). For perennial species tested in this study (Fig. 3b), *H. tuberosus* (acc. N 234) showed the highest mean of total flavonoids followed by *H. smithii* (acc. N 216) and one accession of *H. salicifolius* (acc. N 216). According to our data, the hexaploid perennial species *H. tuberosus* (2n=2n=102) seems to be a potential source of bioactive compounds, due to its high flavonoid content and antioxidant activity.

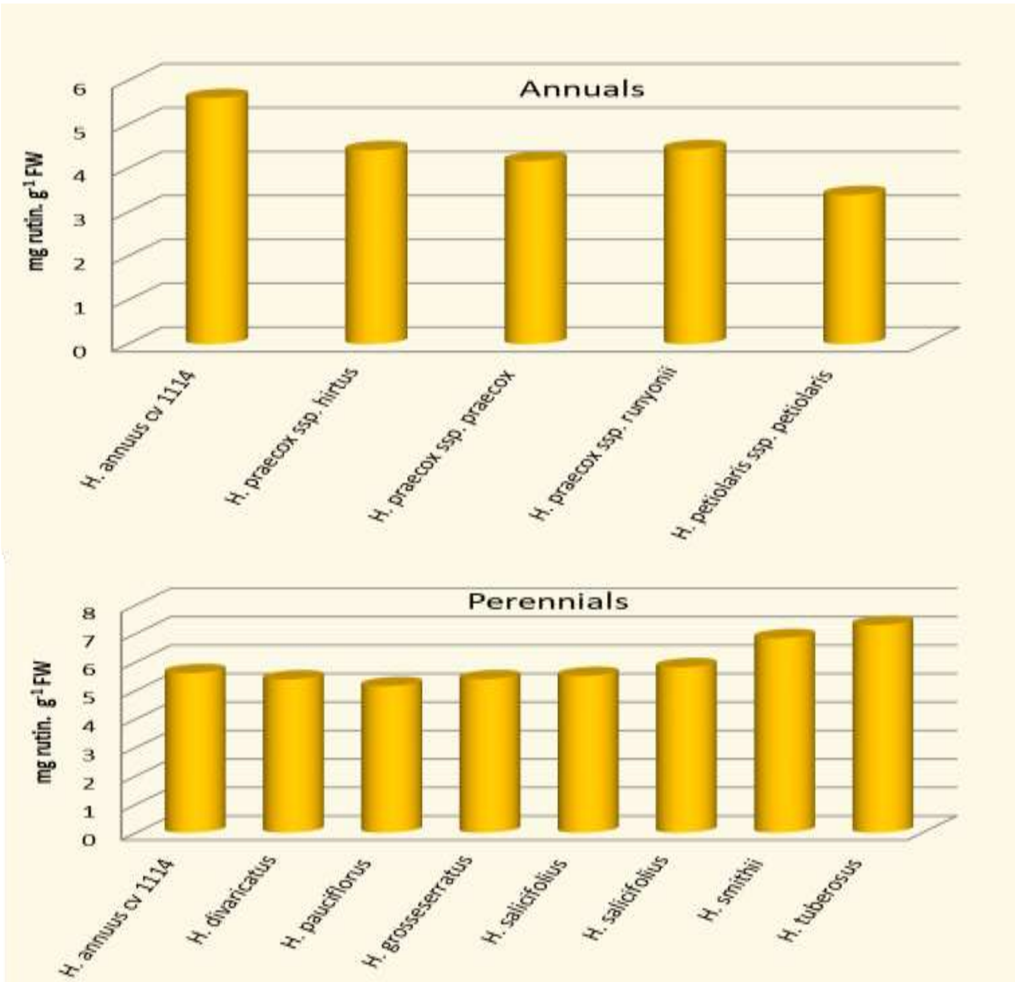

Fig. 3. The total flavonoid content of different annual and perennial wild Helianthus species

CONCLUSION

Based on the above results, it could be concluded that antioxidant capacity and total flavonoid content of studied sunflower genotypes varied considerably both within populations and between individual wild species. Further detailed screening of the wild species as a potential good source of natural antioxidants as the trait of interest for sunflower breeding will be carried out.

ACKNOWLEDGEMENT

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DETERMINATION OF ADULT LONGEVITY OF *COTESIA GLOMERATA L.* (*HYMENOPTERA: BRACONIDAE*) ON DIFFERENT DIETS

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ABSTRACT

The purpose of the study was to determine the differences between adult longevities of *Cotesia glomerata* (*Hymenoptera: Braconidae*), larva parasitoid of *Pieris brassicae* (*Lepidoptera: Pieridae*), on different diets. With this purpose, *C. glomerata* adults gathered from *P. brassicae* larvae from cabbage fields in 2019 and 2020, were fed with honey water (15%), molasses water (15%), sugar water (15%), mixture diet (15% honey + 15% molasses + 15% sugar + 55% distilled water) and distilled water. In addition, adult longevity was determined with adults without any food as control. Number of days alive was recorded for each adult with daily checks. At the end of the study, data were compared with statistical analysis. According to the results, there was no statistical difference between the longevities from honey water (3,27 days), molasses water (3,32 days), sugar water (2,84 days) and mixture diet (3,53 days), while longevity from distilled water and control were lower than the other diets. At the same year, longevities of males and females were also higher from honey water, molasses water, sugar water and mixture diet than others. When longevities on different diets were compared, female longevities were higher than male longevities in molasses water, sugar water and mixture diet. Longevities on honey water, distilled water and control did not significantly differ between sexes. On the second year of the study, highest longevity was determined from sugar water (2,56 days), while honey water (2,08 days), molasses water (2,00 days) and mixture diet longevities did not differ from each other significantly. Longevities from distilled water (1,36 days) and control (1,33 days) were significantly lower than the other diets. When the effect of diets on longevity was compared based on the sexes, similar to the general longevities, longevity from sugar water were higher on both sexes. On sugar water and mixture diet, female longevity was found to be longer than males, but there was no significant difference from other diets and control. As the result of the study, sugar water can be considered as an alternative to honey and molasses, as there is not significant differences between longevities from these diets, and sugar is cheaper in general.

Keywords: *Cotesia glomerata*, Adult Longevity, Diet, Honey, Molasses, Sugar

INTRODUCTION

Cabbage (*Brassica oleracea*) is an important cultivated plant with over 70 million tones annual production in the world (FAO, 2019). Like all other cultivated plants, cabbage also has agricultural pests that cause yield and quality loss. One of the most important of these pests is cabbage butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae). Larvae of the pest feed on the leaves of cabbage in groups and sometimes bore into the head, which causes important quality and economical value loss (Younas et al. 2004; Jainulabdeen and Prasad 2004; Hasan 2008; Bhandari et al. 2009, Hasan and Ansari, 2011). Farmers generally prefer synthetic insecticides for the management of this pest, but the negative effects of intensive insecticide usage has become an important problem in agricultural production in recent years (Tiryaki and Temur, 2010). Because of these problems, other control methods are becoming more common in practice. Biological control, one of these methods, is an important tool in pest control because of its ability to target specific pests and low impact on the environment.

Cotesia glomerata L. (Hymenroptera: Braconidae) is a gregarious larval endoparasitoid of *P. brassicae* (Dorn and Beckage 2007; Gu et al. 2003; Laing and Levin 1982). The process of parasitization of *P. brassicae* by *C. gelomerata* starts with the female parasitoids searching the host plants of the pest (Sato & Ohsaki 1987; Ohsaki & Sato 1999). When a female locates the host plant, it searches the plant leaves by trying to detect the chemicals in *P. brassicae* larva's saliva (Sato, 1976; Horikoshi *et al.*, 1997). If the female detects these chemicals, it starts a more detailed search to find the larvae and oviposits her eggs into the detected *P. brassicae* larva. In today's broad cabbage fields, the first step is not important anymore, because the parasitoids are already around the host plants.

Many parasitoid adults need nutrition to live longer and successfully reproduce (Godfray, 1994). Most of the parasitoid adults feed on carbohydrate sources like floral nectar or honeydew as the main energy source (Rivero and Casas, 1999; Wackers, 1999; Jervis & Kidd, 1986; Heimpel et al., 1997). In addition, adult longevity and fecundity decreases with the lack of food (Jacob & Evans, 2000; Schmale et al., 2001; Siekmann et al., 2001).

This study was conducted with the purpose of the determination of different carbohydrate sources on the longevity of *C. glomerata* adults gathered from the cabbage fields of Çanakkale in 2019 and 2020.

MATERIAL AND METHOD

The Trial Setup

The trials were conducted with *C. glomerata* adults gathered from *P. brassicae* larva from the cabbage fields in Çanakkale in 2019 and 2020 (Figure 1). With the purpose of the determination of the effects of different carbohydrate sources on the longevity of *C. glomerata* adults, they were fed with 5 different diets. The diets were honey water (15%), molasses water (15%), sugar water (15%), mixture diet (15% honey + 15% molasses + 15% sugar + 55% distilled water) and distilled water. In addition, adult longevity was determined with adults without any food as control. Adults were placed in glass tubes (10x1 cm) and the diets were applied inside the tubes as a thin line by a needle, then, the tubes were placed into a climate chamber with 25±1°C temperature, %60-70 r.h. and 16:8 Light: Dark photoperiod conditions (Figure 2). The number of adults used in the study is shown in table 1 and table 2.

Adults were checked daily until the date of their death and this date was recorded for each adult individually. Longevity of adults were calculated from these data.

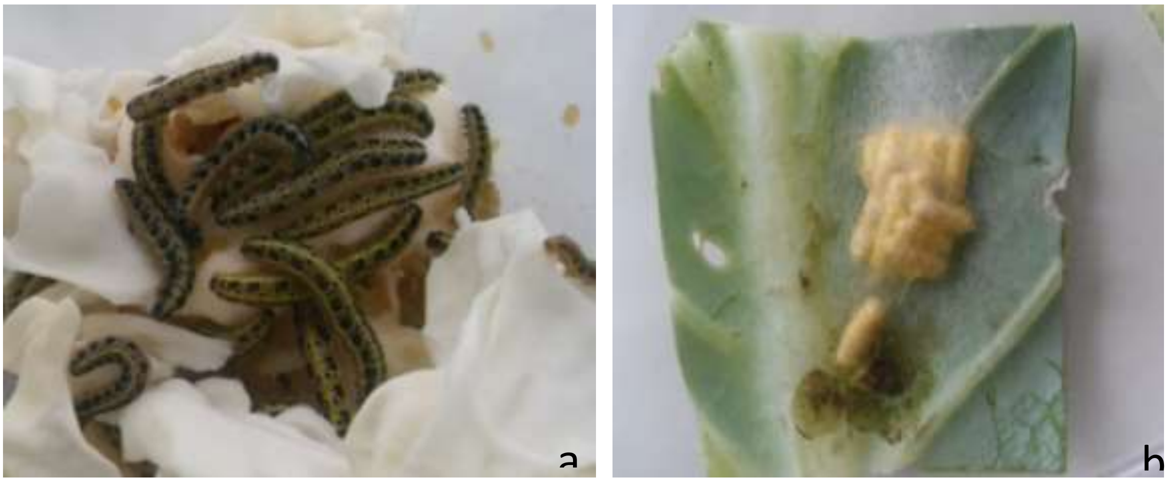

Figure 1. *Pieris brassicae* larvae gathered from cabbage fields (a), *Cotesia glomerata* pupa on cabbage leaf part (b)

Figure 2. The trial tubes (a), climate chamber (b)

Table 1. Number of adult *Cotesia glomerata* used in the trials (M: Male, F: Female) in 2019

	Honey Water		**Molasses Water**		**Sugar Water**		**Mixture Diet**		**Distilled Water**		**Control**	
	M	F	M	F	M	F	M	F	M	F	M	F
	53	94	82	83	64	76	50	80	45	68	63	95
Total	148		165		140		130		113		158	

Table 2. Number of adult *Cotesia glomerata* used in the trials (M: Male, F: Female) in 2020

	Honey Water		**Molasses Water**		**Sugar Water**		**Mixture Diet**		**Distilled Water**		**Control**	
	M	F	M	F	M	F	M	F	M	F	M	F
	3	41	9	42	16	48	15	47	26	51	9	36
Total	44		51		64		62		77		45	

Statistical Analysis

The data was statistically analyzed to determine the differences between the longevities from different diets by ANOVA with Minitab 17 statistical software. In the case of statistically important differences, Tukey multiple comparison test was used to determine which means are statistically different from the other means.

RESULTS AND DISCUSSION

The Effect of Different Diets on the Longevity of *Cotesia glomerata* Adults

At the end of the study, the longevities of *C. glomerata* fed with different diets were statistically different in 2019 (F=29,98, df=5, p=0,000) and in 2020 (F=29,98, df=5, p=0,000) (Table 3). In 2019, the highest longevity was from molasses water with 3,32 days but the difference with other carbohydrate diets was not significant, while the adults fed with distilled water and from control lived considerably shorter than the others.

Table 3. Mean adult longevities of *Cotesia glomerata* from different diets in 2019 and 2020 (Mean±Standart Error) (Days)

	Honey Water	**Molasses Water**	**Sugar Water**	**Mixture Diet**	**Distilled Water**	**Control**
2019	**3,27±0,14 n=147 A**	3,32±0,13 n=165 **A**	2,84±0,12 n=140 **A**	3,53±0,24 n=126 **A**	1,78±0,07 n=113 **B**	1,35±0,04 n=158 **B**
2020	**2,08±0,06 n=47 B**	2,00±0,07 n=51 **B**	2,56±0,12 n=64 **A**	2,10±0,08 n=62 **B**	1,36±0,03 n=77 **C**	1,33±0,03 n=45 **C**

Note: Means with different letters on the same row are not statistically different (p>0,05).

In 2020, the highest longevity was from adults fed on sugar water with 2,56 days, which was followed by the other carbohydrate diets. Similar to the previous year, longevities on distilled water and control were significantly lower than the other diets. Similar to our results, Wacker (2001) has reported that *C. glomerata* longevity was highest from the nectars containing carbohydrates like sucrose, glucose and fructose. In addition, according to Hausmann et al. (2005), feeding starving females with diets containing sucrose, glucose and fructose caused the females to start flying again.

In both years, longevity of adults fed with only distilled water was very low and not significantly different from unfed adults. Lee and Heimpel (2008) also found the same results and have reported that the in the absence of carbohydrates, water did not prolong the longevity of *C. glomerata* adults.

In the first year, there was not a significant difference between different carbohydrate diets, while sugar water diet, which contains sucrose, had significantly higher longevity than the other carbohydrate diets. According to Wackers (2001), the highest longevities of *C. glomerata* adults were fed on sucrose, glucose and fructose. While there was no significant difference in that study between different carbohydrates, sucrose was the carbohydrate with the highest longevity.

The Effect of Different Diets on the Longevity of *Cotesia glomerata* Adults in Genders

In 2019, longevity of *C. glomerata* significantly differed based on gender on molasses water (F=4,24, df=1, p=0,041), sugar water (F=6,52, df=1, p=0,012) and mixture water (F=7,60, df=1, p=0,007), where females lived longer than males. On honey water (F=1,78, df=1, p=0,185), distilled water (F=0,05, df=1, p=0,818) and control (F=0,02, df=1, p=0,891), there was not any significant difference between the longevities of males and females.

In males, there was not a statistically significant difference between the longevities on honey water, molasses water, sugar water and mixture diet, while control had significantly lower longevity than the other diets. Longevity on distilled water was not statistically different than the other diets except honey water (F=4,69, df=5, p=0,047).

In females, longevities between honey water, molasses water, sugar water and mixture water was not significant, while distilled water and control both had significantly lower longevity than the others (F=12,87, df=5, p=0,047).

In 2020, longevity of *C. glomerata* from different diets in male and females were significantly different in sugar water (F=4,11, df=1, p=0,047) and mixture diet (F=5,56, df=1, p=0,021), which the females lived longer than the males. However, the difference between longevities was not significant in honey water (F=0,29, df=1, p=0,059), molasses water (F=0,23, df=1, p=0,063), distilled water (F=0,13, df=1, p=0,072) and control (F=1,63, df=1, p=0,209) (Table 4).

When longevities were compared in males, there was not any significant difference between honey water, molasses water and sugar water, while longevities on mixture diet, distilled water and control were significantly lower than the other group (F=8,67, df=5, p=0,000).

Table 4. Mean adult longevities of *Cotesia glomerata* from different diets in genders in 2019 (Mean±Standart Error) (Days)

2019	**Honey Water**	**Molasses Water**	**Sugar Water**	**Mixture Diet**	**Distilled Water**	**Control**
Male	3,30±0,29 n=53 **a A**	2,91±0,12 n=82 **b AB**	2,59±0,14 n=64 **b ABC**	2,83±0,28 n=46 **b ABC**	1,69±0,10 n=45 **a BC**	1,33±0,06 n=63 **a C**
Female	3,24±0,14 n=94 **a A**	3,71±0,23 n=83 **a A**	3,05±0,18 n=76 **a A**	3,94±0,34 n=80 **a A**	1,84±0,10 n=68 **a B**	1,37±0,05 n=95 **a B**

Note: Means with different small letters on the same column are not statistically significant (p>0,05). Means with different capital letters on the same row are not statistically significant (p>0,05).

In females, longevity on sugar water was significantly higher than all other diets and control. There was not any significant difference between honey water, molasses water and mixture diet, while longevities on distilled water and control were both lower than the other diets (F=27,01, df=5, p=0,000).

Table 5. Mean adult longevities of *Cotesia glomerata* from different diets in genders in 2020 (Mean±Standart Error) (Days)

2020	**Honey Water**	**Molasses Water**	**Sugar Water**	**Mixture Diet**	**Distilled Water**	**Control**
Ma1e	3,83±0,48 n=6 **a AB**	3,78±0,46 n=9 **a AB**	4,69±0,68 n=16 **b A**	3,07±0,27 n=15 **b B**	1,96±0,16 n=26 **a C**	1,89±0,11 n=9 **a C**
Female	4,59±0,29 n=41 **a B**	4,36±0,36 n=42 **a B**	8,35±0,87 n=48 **a A**	5,34±0,51 n=47 **a B**	1,90±0,08 n=51 **a C**	1,81±0,10 n=36 **a C**

Note: Means with different small letters on the same column are not statistically significant (p>0,05). Means with different capital letters on the same row are not statistically significant (p>0,05).

CONCLUSIONS

At the end of the study, it is apparent from the results that, *C. glomerata* adults live longer with a carbohydrate source provided. Generally, there was not a significant difference between the longevities from different carbohydrate containing diets, while distilled water and control had significantly lower longevity than the other diets. With gender in mind, longevity of *C. glomerata* females were generally longer than males on carbohydrate diets except in 2020 with honey water and molasses water.

Longevity on sugar water was not significantly lower than the other carbohydrate diets, as a matter of fact it was higher than the other diets in 2020. Thus, we concluded that sugar water is a possible diet to prolong the longevity of *C. glomerata* in mass production studies considering the lower cost of sugar. However, further and detailed studies with multiple generations may be beneficial to definitively conclude this subject.

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THE EFFECTS OF ADDITION OF ORGANICACIDS AND SUCROSE TO THE DRINKING WATER OF BROILERS INTHE PRE-SLAUGHTER FEED WITHDRAWAL PERIOD ON PERFORMANCE AND NUMBER OF MICROORGANISMS

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ABSTARCT

Pre-slaughter starvation ensures that the digestive tract is cleaned, minimizing contamination during processing and reducing the amount of undigested feed. As the crop empties during feed withdrawal, the development of lactic acid bacteria decreases and the pH of the crop rises, and this condition 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 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 at the right amount in order to reach the ideal pH level of 4-4.5. In this study, the effects of adding organic acids and sucrose to broilers will be emphasized. It is recommended to add sucrose in order to prevent the loss of live weight during fasting and the reduction of darkness due to loss of hemoglobin in liver color. In this study the effects of adding organic acids and sucrose to broilers in combination will be discussed.

Keywords: Organic acids, sucrose, poultry, pre-slaughter feed withdrawal, performance, microorganism contamination

INTRODUCTION

For the supply of quality and healthy products to the consumer, a pre-slaughter feed withdrawal period is applied to broilers in order to prevent microbial contamination. Thus, the digestive tract is cleaned of residues and microbial contamination originating from crops and intestines is prevented (Northcutt, 2010). 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). As the crop empties during feed withdrawal, the development of lactic acid bacteria decreases and the pH of the crop rises, and this increases pathogens such as Salmonella. Most pathogenic bacteria are Gram-negative and sensitive to acidic environments with a bacteriostatic effect. At pHs below five, 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 4-4.5 pH.

ADDITIONAL ORGANIC ACIDS IN POULTRY FEED

In many studies, it has been reported that the addition of organic acids reduces the pH of the digestive system and decreases the effectiveness of the pathogenic bacterial population (Byrd et al., 2001; Biomin, 2020; Harris et al., 2019; Bryd et al. 2001). The adding citric acid (especially 4.5% and 6% levels) to drinking water for 8 hours in pre-slaughter feed withdrawal lowers the pH of the gizzard and cecum content, and reduces the pathogen load in the broiler carcass by reducing fecal contamination (Alzawqari et al., 2013).

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 generally used in combinations of two or more, rather than being used alone (Dhama et al. , 2014; Krisham and Narang, 2014). However, it should be noted that some acidifying products can make water less palatable. On the other hand, prolongation of the fasting period (12 hours) increases the amount of blood loss, reduces carcass defects and improves meat quality (Halva and Aksit, 2019). Blood flowing well during slaughter reduces redness and spot hemorrhages on the wing tip and thigh due to the decrease in hemoglobin in the muscle tissue (Helva and Aksit, 2019). Decreased water loss causes a decrease in the loss of nutrients dissolved in the sarcoplasm and increases the shelf life of meat (Baracho et al., 2006). 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. It has been reported that it reduces the pathogen load in the broiler carcass (Alzawqari et al., 2013). Many studies have shown that the addition of organic acids to broiler rations can positively affect growth, nutrient utilization and the microbial population in the digestive tract (Abdel-Fattah et al., 2008; Ao et al., 2009; Hassan et al., 2010; Mohamed et al. , 2014; Hassan et al., 2015).

ADDITIONAL SUCROSE IN POULTRY FEED

Studies on sucrose additions are a new approach and the reports presented are still limited. The word sugar is the general term used in carbohydrate chemistry for all nutritive mono and disaccharides such as glucose, fructose, galactose, maltose and sucrose. Regardless of whether the source is sugar cane or beet, the term sugar is used both in technology and in food terminology. However, commercially, a distinction is made between cane and beet sugar (Schiweck et al., 2007).

There are studies reporting that the addition of sucrose before feed withdrawal reduces bacterial contamination. For example, Hinton et al. (2002) examined the crops of broiler chickens that were given sucrose-supplemented cocktail supplementation during the 12-hour period during pre-slaughter feed withdrawal to broilers previously administered orally with

Salmonelletyphimurium, and it was reported that S. typhimurium and Campylobacter activity were significantly reduced. Kop-Bozbay and Ocak (2015) obtained 75% carcass yield from the chickens that were given additional sucrose during the pre-slaughter feed withdrawal period, and 69.8% in the control group.

Karacay et al. (2007) conducted a study to determine the effect of adding sucrose to drinking water on liver, breast and thigh meat color during the fasting period of broiler chickens. In their study, they reported that control group animals had a lower carcass percentage, higher abdominal fat and liver ratio and lighter thigh meat color than the fasted animals.

CONCLUSION

When the studies were examined, it was observed that the addition of organic acids during the pre-slaughter feed withdrawal period increased the acidity of the digestive system pH, decreased the bacterial load, increased the level of beneficial microorganisms, and had positive effects on some performance parameters (carcass weight, meat and edible internal organ colors). The combination of sucrose addition and acidification may produce beneficial results in order to prevent the reduction of red color due to loss of meat quality and body weight and decrease in hemoglobin in the liver. With this application, the risk of microbial contamination in the carcass can be reduced. The addition of sucrose to compensate for the loss of hemoglobin during feed withdrawal may contribute to both preventing color loss and reducing weight loss.

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USE OF ORGANIC ACIDS AS FEED ADDITIVES IN POULTRY FEED

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ABSTARCT

Organic acids, which are formed by the oxidation of an aldehyde group, are either free or in the form of salts or esters in the structure of plants. They are also called carboxylic acids because of the presence of a carboxyl group in their structure. Microorganisms that make up the natural microflora of the digestive system produce organic acids such as lactic acid, acetic acid, and propionic acid. Organic acids used as feed additives create an acid environment by lowering the pH in the digestive tract. Thus, the balance of microflora in the digestive tract is turned in favor of beneficial microorganisms and the reproduction of pathogenic microorganisms is prevented. In addition, organic acid additions to the feed have a positive effect on possible feed deterioration. As such, organic acid supplements are used intensively in the field of animal nutrition, and this study will focus on the additions made in poultry nutrition.

Keywords: Organic acids, poultry, feed additives, nutrition, performance,

INTRODUCTION

Due to the negative effects of antibiotics, their use as feed additives has been prohibited, and the trend towards feed additives such as probiotics, prebiotics, organic acids and enzymes has increased. With the use of organic acids as feed additives, the balance of microflora in the digestive tract is turned in favor of beneficial microorganisms and the reproduction of pathogenic microorganisms is prevented (Cakmakci and Karahan, 1999; Sanli and Kaya, 1991). Organic acids such as lactic acid, fumaric acid, propionic acid, citric acid, formic acid (Alp et al., 1999), acetic acid (Cakmakci and Karahan, 1999) have wide usage possibilities in animal nutrition. Organic acids used as feed additives are digestible. They create an acid environment by lowering the pH in the digestive tract. The resulting acid environment prevents the development of pathogenic microorganisms (Canibe et al., 2001; Cakmakci and Karahan, 1999; Sanli and Kaya, 1991), and increases the enzyme activity (Alp et al., 1999; Kahraman et al., 1999). In addition, the digestibility and usefulness of minerals such as iron (Porres et al., 2001), calcium, phosphorus, magnesium, zinc, protein and amino acids increase depending on the acid environment and the increase in enzyme activity (Canibe et al., 2001; Omogbenigun et al., 2003).

ORGANIC ACIDS

Organic acids formed by the oxidation of an aldehyde group include lactic acid, formic acid, oxalic acid, malonic acid, malic acid, acetic acid, succinic acid, aspartic acid, citric acid, pyruvic acid, fumaric acid and their salts (Aripinar and Sulu, 2005; Nir and Senkoylu, 2000). Organic acids are used in the nutrition of all farm animals in the European Union countries in order to increase the acidity of the feed and prevent the deterioration of the feed, to maintain the balance between pathogens and beneficial microorganisms in the digestive system, to improve the digestion and absorption of the nutrients taken, to stimulate growth and to protect the health (Çelik, 2007; Garipoğlu, 2005; Arıpınar and Sulu, 2005).

ORGANIC ACIDS IN POULTRY FEED

Generally, this practice is done in the form of acidification of drinking water. In many studies, it has been reported that the addition of organic acids reduces the pH of the digestive system and decreases the effectiveness of the pathogenic bacterial population (Bryd et al. 2001; Harris et al., 2019). Lactic or formic acid (0.5%) reduces Salmonella in the crop (Bryd et al. 2001) It has been reported that the addition of citric acid (especially 4.5% and 6% levels) to drinking water for 8 hours in pre-slaughter feed withdrawal reduces the pH of the gizzard and cecum content and reduces fecal contamination, thereby reducing the pathogen load in broiler carcass (Alzawqari et al., 2013).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.

CONCLUSION

The positive effects of the addition of organic acids in both ruminants and poultry have been demonstrated in many studies. In particular, it prevents spoilage by increasing the acidity of the feeds, decreases the pH of the digestive tract and has a negative effect on pathogens, and may positively affect growth and performance parameters. It will minimize the risk of bacterial contamination, since positive effects can be observed in terms of acidification of the crop and reduction of bacterial load, especially by adding it to drinking water in poultry. Due to these positive effects, it is seen that the addition of organic acids as feed additives is important in terms of performance, carcass quality and reducing the number of pathogenic microorganisms infecting the carcass.

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STUDY OF THE BIODIVERSITY AND THE STATE OF NATURAL PASTURES IN STRANDZHA MOUNTAIN AND OPPORTUNITIES FOR THEIR USE

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ABSTRACT

The productivity and quality of the production of the natural pasture depends primarily on the use and manifestation of climatic factors. Observations have been made on 4 natural pasturelands in the border region of Southeastern Bulgaria (Strandzha Mountain), during the period 2013-2015.The botanical composition, productivity and chemical composition of the grass cover in the natural pastures were studied. It has been found that the variation in yields from natural pastures depends not only on the weather conditions and soil fertility, but also on the botanical composition and method of use. The non-systematic use of meadows and pastures has strongly influenced the species composition, weeds are increasing at the expense of legumes. To increase the productivity and nutritional value of the fodder obtained from the natural pastures in Strandzha mountain, it is necessary to introduce hay mowing periods with grazing periods.

Key words: Strandzha mountain, natural pastures, productivity, climatic factors.

INTRODUCTION

Pastures are natural grasslands that form part of the country's vegetation cover and are of major economic and ecological importance. They are a major source of grass protein fodder and the annual amount of raw protein derived from them is about 200 000 t. Most of the pastures are mainly located on low productive and degraded lands with the risk of water erosion. From the natural pastures in Bulgaria 700-1200 kg/ha of hay are obtained (Pavlov, 2007).

Southeast Bulgaria is one of the driest regions in the country. Rainfall is insufficient and is predominantly during the autumn-winter period (Palova, Stoeva, 2006). As a result of the drought in the spring and summer, grass vegetation ceases its growth and dries (Stoeva K., H. Yancheva, P. Todorova, 2002).

The grassland of the Strandzha region amounts to about 1500000 decares of natural meadows and pastures.(Ministry of Agriculture, Foods and Forestry of the Republic of Bulgaria). Most of the Strandzha areas are characterized as low mountainous, hilly areas. The northern area is mostly hilly and suffers more from drought. The southern zone is more mountainous, rainfall is more evenly distributed over seasons, and relative humidity is lower.

The most widespread soils in the area are cinnamon forest. (Atlas of Soils in Bulgaria). They occupy the sites with an altitude of 10 to 500-600 m. Most of the soils have humus content below 2% and high acidity. A considerable part of them are subjected to water erosion I and II degree. The hilly places of Strandzha are formed from dry xerophyte meadows. (Yancheva, H. 2007).According to Stoeva et al. (2005) the pastures in Strandzha are coach grass-ryegrass (Cynodon dactylon L. - Lolium perenne L.)

The purpose of this study was to investigate grass associations in Strandzha region, botanical composition and grassland status. To investigate the productivity and chemical composition of grass cover in natural pastures. To identify the need for surface improvement of natural grassland.

MATERIAL AND METHODS

The survey was conducted by organizing tours to monitor designated sites during the period 2013-2015.

Observations have been made on 4 natural pasturelands in the border region of Southeastern Bulgaria (Strandzha Mountain) situated on the territory of Sredets Municipality and Malko Tarnovo Municipality. The studied natural pastures are of the clover-ryegrass type. They are situated at different locations, different altitudes and exposition of the slope and are selected to cover the most typical and used natural pasturelands at the foothills of the region.

The selected sites for observation and analysis are as follows:

Site 1, (Variants 1) -240 m.Altitude with a slope 8^0 with east exposure.
Site 2, (Variants 2) -350 m. Altitude with a slope 6^0 with south exposure.
Site 3, (Variants 3) -380 m. Altitude with a slope 12^0 with northwest exposure.
Site 4, (Variants 4) -280 m. Altitude with slope 4^0 with southeast exposure.

At the designated sites three mows have been made - I-25.05., II- 30.07., III- 25.09. The mowing has been carried out manually in maturity phase, by mowing down, and the fresh mass obtained has been weighed directly on site.

During the study period, the climatic indicators and their impact on the productivity of natural grassland have been monitored.

The soil is leached cinnamon forest, poorly stocked with nutrients, humus-poor (0.65%) and acidic reaction - the pH is 4.06 in KCl.

RESULTS AND DISCUSSION

Climate conditions in the habitat area are one of the most important factors for developing the growth and productive potential of any vegetation, including natural pastures.

Weather conditions, especially rainfall, are one of the factors that have the greatest impact on the productivity of natural meadows and pastures.

The observation period 2013-2015 (Figure 1) is characterized by different rainfall quantities with a maximum in June and October, and minimum in August. The rainfall quantities in 2013 and 2015 are lower than the established rate for the area - 650 mm and they are in the range 507 - 552 mm. Apparently 2014 is very wet with an annual sum of 1138 mm, which exceeds almost twice the average rainfall. The first and third years of monitoring are quite critical with rainfall during the summer months well below the multiannual period. The average annual air temperature (Figure 2) is a relatively constant value and varies between 13.3 -13.5^{0}C during the years of monitoring.

The average air temperature during the study period was 0.6^{0}C higher than the regional average of 12.8^{0}C.Generally speaking, during the years of monitoring a tendency for increasing the annual temperature values has been formed.

The climatic situation in Strandzha region and during the three years of monitoring shows an inconsistency in the provision of moisture, which is manifested both with long droughts as well as more heavy rainfall. These anomalies, combined with the presence of soil poor in nutrients are the reason for the low productivity of the pastures in Strandzha mountain.

On (Figure 3), the botanical composition of the natural grassland in Strandzha is shown. The percentage ratio of cereals, legumes and weeds is given. It is obvious that pastures have a higher content of cereal components, the increase being at the expense of lower participation of legumes and weeds. Option 3 is an exception, where the weeds are more than 50%.

On (Table 1) the results of the measurements made are shown, before the first mow, the height of the cereal species is in the order of 60 - 85 cm, and of the legumes species between 32.4 - 50 cm. Before the second and third mow the grass height is almost 50% lower in cereal and legumes species.

The density of the grassland, expressed in pcs/m^2 is shown on (Figure 4). The number of plants per m^2 is the smallest in site № 3, -1402 pcs/m^2, and the highest in site №1, -1730 pcs/m^2. The largest number of cereals is in site №1, and the legumes in site №2.

The highest yield of green mass (Table 2) in the first mow is recorded in the grass of site №4, 1860 kg/dka respectively. Site №3 has the lowest productivity, 940 kg/dka green mass, respectively. This is also the case with the second and third mows. Dry mass production is also reported - (Table 2). The results are as follows: the highest yield has been obtained from the grass of site No.4, 869 kg/dka., and site №3 has the lowest productivity, 432 kg/dka dry mass at the first mow. For the second and third mow the ratio is the same.

This lower productivity of site №3 can be explained by the fact that this site is situated on a slope in the municipality of Malko Tarnovo, at a higher altitude, with northwest exposure, the presence of diverse grasses is quite large here.

Chemical composition of the dry matter – it is determined by analyzing average plant samples taken at the harvest of each young sprout - for crude protein, raw fibers, raw fats, mineral substances and nitrogen-free extracts (NFE). The data are averaged and shown in (Table 3). The results show that for the dry matter no significant differences are observed. The protein level is between 8 and 12%.

CONCLUSIONS

The productivity and quality of the production of the natural pastures depends primarily on the manners of use and manifestation of climatic factors.

The natural pastures in the Strandzha region can be successfully transformed into high-productive grasslands by applying the appropriate agricultural technology.

To increase the productivity and nutritional value of the fodder, obtained from the natural pastures in Strandzha, it is necessary to introduce hay mowing periods with grazing periods.

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Table 1. Height of the sward from Strandzha, on average for the period 2013-2015 years.

Variants	Height of (cm)					
	Grasses			Legumes		
	I	II mows	III	I	II mows	III
Variants 1	85.0	30.8	23.8	38.5	20.8	16.8
Variants 2	72.5	29,1	26.2	42.3	19.1	15.2
Variants 3	68.5	26.2	17.2	32.4	17.2	12.2
Variants 4	60.0	42.5	32.5	50.0	28.5	22.5

Table 2. Yield of greenanddry mass the sward from Strandzha, on average for the period 2013-2015 years.

Variants	Yield of green mass					
	I mow		II mow		III mow	
	kg/dka	%	kg/dka	%	kg/dka	%
Variants 1	1120	100.0	710	100.0	690	100.0
Variants 2	1560	139.3	890	125.3	720	104.3
Variants 3	940	96.4	520	73.2	480	69.6
Variants 4	1860	166.0	990	139.4	780	113.0

Variants	Yield of dry mass					
	I mow		II mow		III mow	
	kg/dka	%	kg/dka	%	kg/dka	%
Variants 1	570	100.0	300	100.0	276	100.0
Variants 2	748	131.2	340	113.3	290	105.0
Variants 3	432	75.8	205	68.3	190	68.8
Variants 4	869	152.5	400	133.3	310	112.3

Table 3. Chemical composition of the nature sward from Strandzha. on average for the period 2013-2015 years.

Indicators	Variants 1	Variants 2	Variants 3	Variants 4
Water, %	74.81	74.92	74.85	74.83
Dry matter,%	25.19	25.08	25.15	25.17
Crude protein,%	8.81	12.1	8.05	11.8
Crude fat,%	1.61	2.78	1.42	2.55
Crude fiber,%	23.09	22.62	25.57	24.54
Mineral traces,%	6.54	7.94	6.08	7.32
NFE,%	59.87	54.56	58.88	53.79

Figure 1. Average monthly precipitation for the period 2013-2015 years.

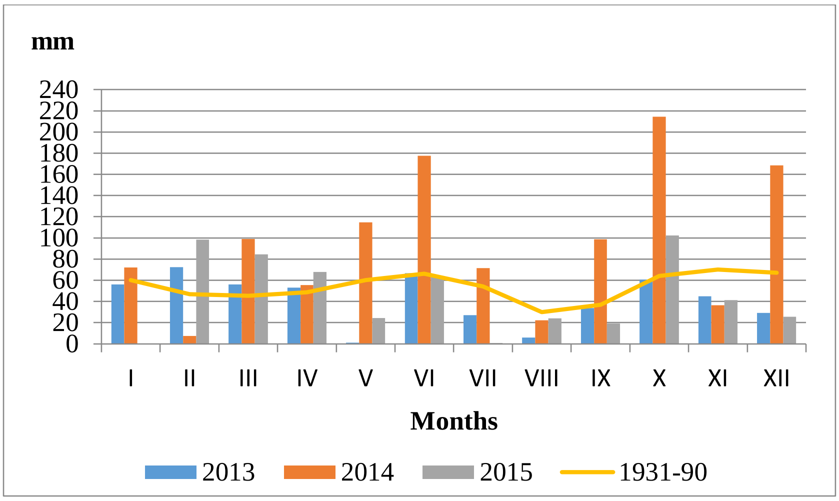


Figure 2. Average monthly temperatures for the period 2013-2015 years.

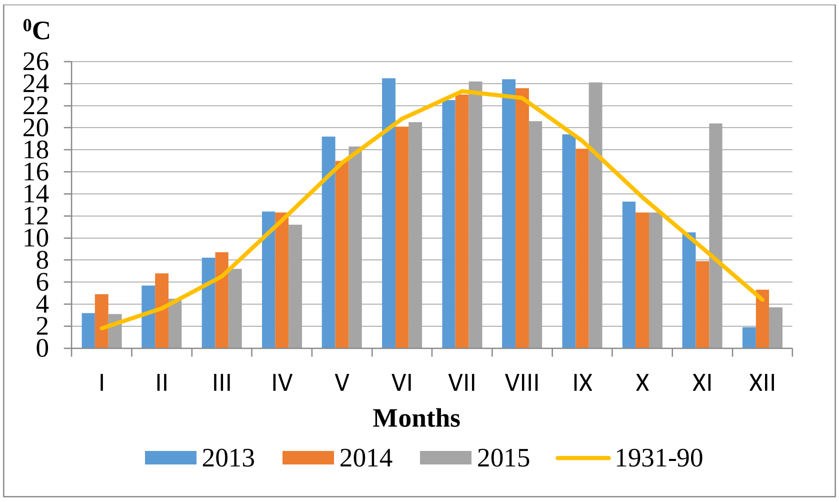


Figure 3. Botanical composition of the swards, % from Strandzha

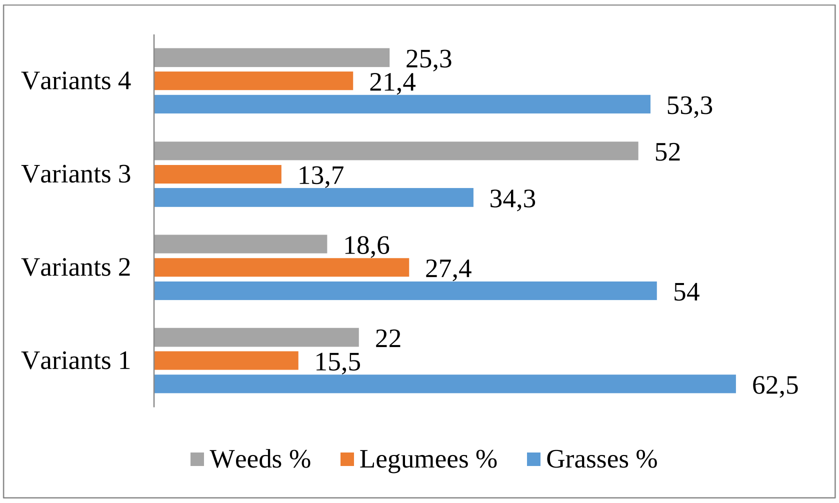
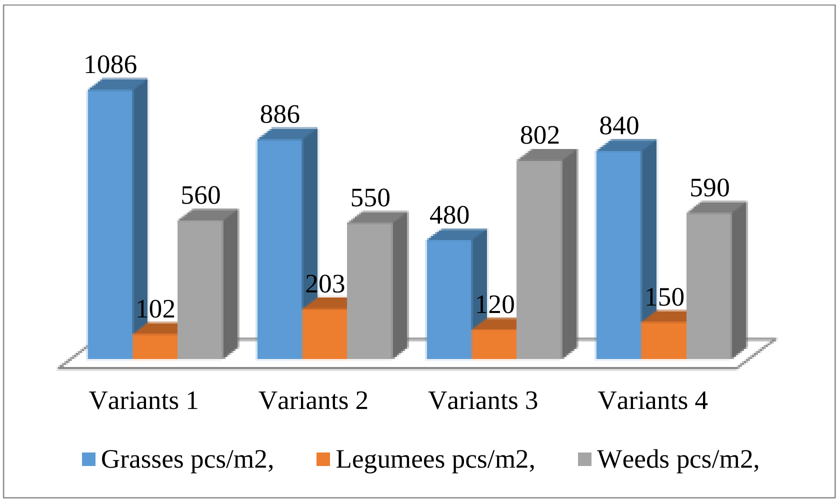


Figure 4. Density of the swards (nb/m^2) from Strandzha



EFFECTS OF ENCAPSULATION ON BIOAVAILABILITY OF FEED ADDITIVES

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ABSTRACT

Encapsulation is a technology based on keeping an active substance in capsules by being coated with one or more coating materials. Encapsulation is a system used to increase the stability and bioavailability of bioactive components, to preserve their structure and to release them in a controlled way to target tissues. Although it is used in areas such as food, medicine, pharmacy, veterinary medicine, industry, encapsulation is also possible in feed additives. Vitamins, minerals, enzymes, proteins, organic acids, probiotics, prebiotics, essential oils, sweeteners, preservatives, colours, flavors, fatty acids (ω-3, konjugated linoleic acid), carotenoids (β-carotene and lycopene) and antioxidants (tocopherol, flavonoids and polyphenols) are the active ingredients mostly used in the encapsulation process. In recent years, it has been suggested that the encapsulation method can provide significant advantages for more effective use of feed additives used in the nutrition of farm animals. In this review, the advantages of encapsulation technology applied to additives in order to reduce the negative effects of environmental factors on the quantity and quality of active substances or to increase their stability, and the effects of these products on farm animals are discussed.

Key words: Encapsulation, coating, animal feeding, feed additives, bioavailability.

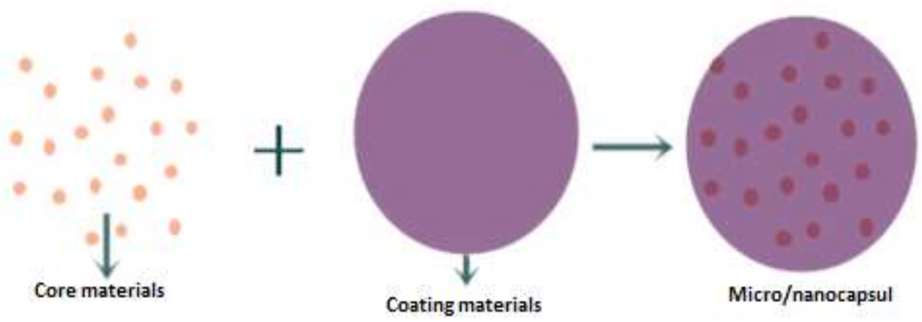
INTRODUCTION

Antibiotics have been frequently preferred for many years for the purpose of preventing and treating diseases as well as for the purpose of increasing feed efficiency and growth rate in animal nutrition. Since the use of products such as antibiotics in farm animals has been banned as a feed additive since 2006, due to the increasing concerns about the formation of resistant bacteria (cross-resistance) that may pose a carcinogenic and mutagenic risk to human health and leaving residues in animal products. After the prohibition of growth factor antibiotics, researchers focused their studies on powders and extracts of medicinal and aromatic plants, organic acids, prebiotics and probiotics and similar natural products (Karasu and Öztürk, 2014; Kim et. al., 2019). One of the newest technologies, the technique of using the aforementioned feed additives seperately or by microencapsulating their mixtures in the feeding field, has also been added to the ongoing researches in order to ensure efficiency in production after the prohibiton of the use of antibiotics (Lippens et al., 2006; Bakry et al., 2015).

ENCAPSULATION

Encapsulation is defined as incarceration of a substance or mixture into capsules by covering it with another substance or system (Madene et al., 2006). Encapsulation can also be expressed as packaging of solid, liquid or gaseous components, enzymes, cells and other substances, microorganisms with a protein or carbohydrate-based coating material (Gökmen et al., 2012). Encapsulation technology, today, is an application that is used in many differents fields such as pharmacology, chemistry, cosmetics, medicine, biotechnology and food and offers wide opportunities to improve the functional properties of the product it is applied to (Gouin, 2004; Poshadri and Aparna, 2010). In encapsulation processes, which are used to give many beneficial properties to the capsules, mostly fats and oils, flavor components, vitamins, minerals, color components (β-carotene, lycopene), fatty acids (ω-3, conjugated linoleic acid), antioxidants (tocopherol, flavonoids, polyphenols) and enzymes are encapsulated with a protective material, thus providing many benefits (Gökmen et al., 2012; Güngör et al., 2012). The encapsulation process is given in Figure 1.

Figure 1. Encapsulation Process



The encapsulation process is applied to solidify liquids to ensure easy transport in areas of use, to prevent evaporation loss of volatile substances, to hide undesirable taste and odor, to protect from atmospheric conditions, to increase stability, to extend the effect period and to perform controlled release and similar activities (Kaş, 2002). In the field of nutrition and food, encapsulation is used to protect the bioactive component in the microcapsule from the negative effects of the current environment (such as humidity, temperature, light, air), to prevent its loss by evaporation, to increase its stability during processing, transportation and storage of foods. In addition, it is used for purposes such as facilitating food processing, protecting living cells such as probiotics, preventing the effects of bad aromas, preventing oxidation, preventing reaction with other different substances and increasing the bioavailability of active ingredients (Desai and Park, 2005).

EFFECTS ON ENCAPSULATION ON BIOAVAILABILITY OF FEED ADDITIVES

It has been stated that efficient and satisfactory results are obtained in encapsulation technology in increasing the stability and bioavailability of various feed additives (Çoruhli, 2013). It is desired that the active substance reaches the small instestine without being affected by the environmental conditions and the acidic conditions of the stomach environment and is absorbed from there to obtain products with high bioavailability. For this reason, by applying encapsulation technology in recent years, it is provided that it is not affected by environmental conditions, staple at low pH, partially resistant in stomach conditions, and swells and disperses at high pH such as the small intestine (Anbinder et al., 2011; Çoruhli, 2013; Mohammedi et al., 2016). In addition, the relation of the active ingredient encapsulated with the system is limited by microencapsulation applications. If it is necessary to limit the relation of the active substance with its environment, it is easier to separate it from the environment by the agency of the microcapsules obtained by encapsulating this substance.

The capsules used in this method have the ability to preserve their structure and become functional in environments where the necessary conditions are met, when desired (Kınık et al., 2003). As a matter of fact, in a study on broiler chickens, it was reported that the encapsulated form of garlic and *Phyllanthus niruri L.* mixture, in which the capsule forms are coated with arabic gum and whey, between powder and capsule form, had more positive effects on live weight gain and feed evaluation than the powder form, but did not affect the intestinal microflora (Natsir et al., 2013). In a study in which turmeric extract, chitosan and sodium tripolyphosphate were used as encapsulation materials and added to the diets of broiler chickens, it was concluded that it could be used as an alternative feed additive instead of antibiotics and improved nutrient digestibility by lowering meat cholesterol (Sundari et al., 2014). Haafez et al. (2015), stated that encapsulated phytogenic feed additives added to broiler rations positively affect the rate of feed conversion compared to powder form. In a study in which powder and encapsulated forms of various essential oils and organic acids were added to the diets of broiler chickens, it was reported that the encapsulated form had a positive effect on CAA compared to the powder form (Lippens et al., 2006).

A study investigating the effects of adding sodium alginate-chitosan encapsulated essential oils and Lactobacillus plantarum TN8 strain to the diet on total cholesterol (HDL, LDL) and growth performance; the results showed that the groups supplemented with encapsulated Lactobacillus plantarum TN8 or essential oils showed higher growth than the control group, the weekly feed consumption and feed conversion rates were positively affected, and the cholesterol ratio (HDL, LDL) was significantly reduced as well as the content of triglycerides (Bouchaala et al., 2016). In a study on broiler chicks, the effects of microencapsulated thyme and cinnamon essential oils on performance, some blood parameters and carcass characteristics in chicks were investigated. In the results obtained in general; it has been stated that microencapsulated essential oils reduce cholesterol concentration, increase the enzyme activity of glutathion peroxides, and have positive effects on broiler chicks in terms of decreasing liver and abdominal fat weight (Rozmehr et al., 2018).

CONCLUSION AND RECOMMENDATIONS

Encapsulation technology has been used in many fields for many years. In the field of animal nutrition, it is used to encapsulate feed additives such as lipids, vitamins, peptides, fatty acids, antioxidants, minerals and probiotics, which have an important place for the metabolic requirements of farm animals in recent years. It is preferred for many purposes such as preventing oxidation, preserving aroma, increasing stability, prolonging the duration of action, controlled release to target tissues and increasing bioavailability. Although most of the products produced with encapsulation technology are at laboratory stage, many of the studies carried out at the laboratory scale have the potential to turn into commercial products in the coming years. As a result, encapsulation is becoming an increasingly important issue in the fields of food, pharmacy, medicine, veterinary medicine, biotechnology, textile, industry, agriculture and animal nutrition. In this context, application areas can be created with purpose-oriented and demand-oriented studies and projects in bioactive components and feed additives, thus, many laboratory-scale researches can be applied on an industrial scale.

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EFFECTS OF BENTONITE ON PERFORMANCE, AFLATOXICOSIS, IMMUNE SYSTEM, LIVER AND KIDNEY PATHOLOGY, INTESTINAL MICROORGANISMS AND HISTOMORPHOLOGY OF BROILERS

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ABSTRACT

Aflatoxins, which are secondary metabolites of fungi, easily develop in agricultural products such as grains, oilseeds, feed raw materials and mixed feeds. The formation of aflatoxins is largely dependent on environmental temperature, relative humidity, insect infestation and mechanical losses during harvest, and when consumed in high concentrations, they have serious toxic effects in humans and animals. Aflatoxins, which occupy an important place among mycotoxins, pose a serious threat in terms of their prevalence in animal feeds, animal health and productivity with various forms of toxicity, profitability of livestock enterprises, food safety, human and environmental health. Removal of aflatoxins, which can easily multiply in many feeds and cause serious health problems, performance and yield losses when consumed for a long time, is an important problem and effective, cheap and practical decontamination methods are needed. Protecting the feed from moisture, physical damage and insects during the period from harvest to storage and delivery to the animal are some of the precautions to be taken against mold fungi and toxins. In order to reduce the absorption of aflatoxins from the digestive system, some non–nutritive compounds and adsorbents are used in the diet. In this study, the use of toxin–binding bentonites, known as aluminosilicates, as a mold inhibitor in broiler mixed feeds, their effects on performance, immune system, aflatoxicosis, liver and kidney pathology, the number of some intestinal microorganisms and intestinal histomorphology were investigated.

Key words: Bentonite, toxin binder, feed additives, aflatoxicosis, broiler.

INTRODUCTION

The poultry sector is important for our country in terms of both providing the production of white meat, which is a very valuable and economical protein source, and the employment and economic values it creates. Feed raw materials constitute the most important input of the sector. The feed industry in our country, as a very important part of the poultry industry, has developed at a level parallel to the industry and continues its development to increase the productivity of the industry. Yet, the feed industry has not completely solved its problems despite its development. One of the most important problems is the supply of quality and cheap raw materials. All of the raw material needs of the feed industry cannot be met by domestic resources in our country and they are imported. Sometimes for economic reasons, sometimes because of necessity, low quality feed raw materials are used in the rations and economic losses occur. Mycotoxins cause significant losses in the quality and quantity of foods and feeds that can develop very easily in the environments where humans live and in nature, and adversely affect the health of humans and animals (EFSA, 2004; Pozzo et al., 2013). It is reported that approximately 40% of the world's mixed feeds are under the threat of mycotoxins (Şanlı, 2001). In recent years, taking into account the viral diseases that cause pandemics beyond bacterial infections, it has been proven once again how essential it is to protect human and animal health and to support the immune system. Thus, the fight against mycotoxins, which collapses the immune system and makes it vulnerable to diseases, has become one of the top priorities of the scientific community. Bentonite sources (Na-Ca Bentonite) included in the aluminosilicate group are thought to be an effective solution against mycotoxicosis and aflatoxicosis events.

MYCOTOXINS IN FEED AND THEIR EFFECTS

Consumption of various mycotoxins individually or in combination may cause different toxicity lesions in animals. Adverse effects of mycotoxins can result in acute, overt disease or chronic, insidious conditions. Toxicity may vary depending on the toxin(s) present, dosage, duration of exposure, and a variety of other factors, including animal species, age, sex, nutritional status, and concomitant disease. Intestinal microflora may also modulate mycotoxin toxicity. Aflatoxin B1 is the most potent known carcinogen, possibly inhibiting fetal and infant physical and cognitive development, immunosuppressive in livestock and humans, and can be fatal to both in acute poisoning cases (FAO and WHO, 2019). Even if the presence of mold-causing fungus is removed from moldy products, invisible mycotoxin varieties can remain undegraded for years. Therefore, some feeds and foods that are not noticeably moldy can pose a very serious health risk to humans and animals because they are contaminated with mycotoxins (Qu et al., 2018; Mondal et al., 2018; Chen et al., 2019a; FAO and WHO, 2019). Moreover, since the majority of mycotoxins, especially aflatoxin varieties, are resistant to heat treatments and chemical reactions, they maintain their toxic effects during the preparation of compound feeds and foods. Aflatoxins, which can easily multiply in many feeds, cause very important health problems in living things when consumed for a long time. This condition, called acute aflatoxicosis, causes depression, anorexia, anemia, runny nose, bleeding, weakness, respiratory distress, feathering disorder, bloody diarrhea and high mortality rate in poultry (Eraslan et al., 2005; Farag et al., 2017; Mondal et al., 2018). On the contrary, in animals exposed to low levels but long-term aflatoxin consumption, chronic aflatoxicosis is seen, the immune system is the first to be affected, and performance, feed consumption, feed utilization, egg production, and reproductive ability are reduced (Fowler et al., 2015; Yenice et al., 2015; Chen et al., 2016; Shannon et al., 2017; Mondal et al., 2018; Prasai et al., 2018; FAO and WHO, 2019).

BENTONITE AS TOXIN BINDER

Protecting the feed from moisture, physical damage and insects from harvest, storage and feeding to the animal is one of the measures to be taken against mold fungi and toxins. 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 adsorbents are used in the detoxification of feeds contaminated with mycotoxins. Mycotoxins in feedstuffs are tried to be detoxified or inactivated by many methods such as physical decomposition, thermal inactivation, radiation microbial degradation. Another strategy is to bind aflatoxin molecules with a chemical compound, preventing their absorption from the digestive systems of animals and excreting them with feces. 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). Clays containing predominantly montmorillonite, formed as a result of chemical weathering of volcanic ash, tuff and lavas rich in aluminum and magnesium, are defined as 'bentonite'. General chemical formula; Bentonite, which is (Na,Ca) (Al,Mg) 6(Si4O10) 3(OH)6 nH2O (Tecer, 2020), contains various minerals such as quartz, cristobalite, feldspars, zeolites. It is widely used in industry, agriculture, mining and engineering geology. It is stated that the organic molecules of bentonite act either by adsorption on the outer surface or by binding with cations in the interlaminar spaces in the inner parts. 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 quater neramonium 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-Wahhabet al., 2002).

With the addition of bentonite to 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., 2019b), heart, liver, kidney weight and health (Eraslan et al., 2003; 2005; Farag et al., 2017) found to have 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., 2019d). Recent studies have shown that montmorillonite can improve the intestinal antioxidant capacity, intestinal immunological barrier functions of chickens during the laying period and improve the laying performance of chickens by improving intestinal health status (Chen et al., 2019b; 2019c; 2019d). There are literature reports (Clark et al., 1998; Chattopadhyay and Puls, 1999; Li et al., 2003; Abduljauwad, 2019; Abduljauwad et al., 2020; Mishra et al., 2020) that bentonite clay has the ability to absorb and adhere to toxins, poisons and viruses, and that chemically modified nano bentonites can prevent HIV (Aids) and influenza viruses by adsorbing pathogenic viruses. Moreover, it has been found that COVID-19 can be retained by clay particles of kaolinite or preferably bentonite, minimizing pathogenic properties (Mishra et al., 2020).

Some antioxidants such as SOD and CAT in the body can eliminate excess reactive oxygen species in the body, making an important contribution to maintaining intestinal barrier integrity by preventing infections caused by pathogens (Kelly et al., 2004). Increased T-SOD and CAT activities observed in the small intestinal mucosa of poultry (Chen et al., 2019d) lead to an improvement in antioxidant capacity (Zhu et al., 2017; Qu et al., 2018; Chen et al., 2019b). It has been shown that montmorillonite can improve the performance of animals by improving intestinal antioxidant and barrier functions by reducing the concentration of MDA, which is used as one of the most important terminal products of peroxidation in monitoring the lipid oxidation state in the body, in the duodenum and jejunum (Chen et al., 2019c; 2019d).

The gut immunological barrier is formed primarily by gut-associated lymphoid tissues and immune cells, and gut homeostasis is maintained by the production of immunoglobulins, cytokines, interferons, and the like (Lotz et al., 2007). Chen et al., (2019c) and (2019d) reported that dietary montmorillonite in laying hens contributed to an improvement in the IgA concentration of the duodenal mucosa. IgA plays a crucial role in intestinal mucosal defense, which is the first line of defense in preventing damage to the intestinal epithelium from endotoxins and pathogenic microorganisms and maintaining mucosal homeostasis in the intestinal tract (Mantis et al., 2011). It has been reported that clays can prevent intestinal mucosal damage, protect intestinal epithelial cells, increase the number of intraepithelial lymphocytes and goblet cells (Ivkovic et al., 2004; Wu et al., 2013), and contribute to the increase in immunoglobulin production in the intestinal mucosa. It has been suggested that montmorillonite may be beneficial in improving the intestinal mucosal immunity of laying hens or reducing inflammatory responses, maintaining the intestinal physical barrier (Xing et al., 2015; Chen et al., 2019c).

Total villus height and crypt depth measurements are often used to assess intestinal integrity. It has been shown that chickens fed diets containing bentonite develop higher villus height and villus height-crypt depth ratio in the jejunum (Chen et al., 2019c; 2019d). This may contribute to the integrity of the epithelial cells and the tight junctions in the intestines of laying hens. It is known that montmorillonite, a mucus stabilizer, acts effectively by binding to mucus to protect and repair the intestinal mucosa (Albengres et al., 1985). Montmorillonite can benefit the temporary colonization of beneficial bacteria in the intestine and form a complex by adhering to pathogens such as E. coli and Salmonella and can be excreted through the intestine (Abudabos et al., 2019; Chen et al., 2019c).

CONCLUSION AND RECOMMENDATIONS

Mycotoxins contaminate agricultural systems globally. When consumed in high concentrations with feed derived from plant material, they exhibit severe toxic effects in animals, may reduce their productivity, and may accumulate in edible tissues and animal products, causing human exposure and health effects. Besides Aflatoxin M1 (carcinogenic) in milk and ochratoxin in meat, milk and eggs, other well-known mycotoxins (Aflatoxin B1, zearalenone, fumonisins) seriously affect animal health and productivity. 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. Because it is very difficult to remove mycotoxins from contaminated feed, preventing their accumulation in agricultural products is the most effective strategy to combat the problem. Preventive measures range from crop rotation and resistant cultivation to vaccination with microbial antagonists and storage management. Continuing monitoring is necessary to deal with epidemics and the risks from low-level exposure, and effective detoxification strategies are needed.

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INVESTIGATION OF SAME AGRICULTURAL CHARACTERS OF CROP HYBRID CORN VARIETIES

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ABSTRACT
Since the corn plant is used in many ways and so that it is an open pollinated plant, it is widely cultivated in each region. Therefore, the research of yield characters of 17 hybrid corn varieties (Tavascan, Motri, Calgary, Sancia, P.573, P.32T83, Hydro, Performer, Capuzi, 72MAY80, Simon, Macha, PL712, Torro, Bolsan, KB5562 and KB3961) were aimed to the in the second crop growing season in Kahramanmaraş conditions. In the study, the tassel emergence period, plant height, stem diameter, 3rd leaf angle, number of leaves in the plant and grain yield values were investigated. The research was conducted in random blocks in the trial design in 3 replications in 2016. It was recorded that the tasselling period, plant height, stem diameter, 3. leaf angle, number of leaves in plant, grain yield is among 49-55 days, 164.1-233.1 cm, 24.6-28.3 mm, 21.5-38.1º, 12.2-15.4 number, 410.3-1069.9 kg da-1 respectively.

Keywords: Corn, variety, grain yield, second crop.

INTRODUCTION

Agricultural practices differ according to regional conditions. These differences are due to climate, soil and water sources. For this reason, agricultural practices are planned according to regional conditions. Adaptation is made by trying plant or animal assets suitable for the region, or agricultural production is expanded by continuing the improvement work in that direction. The new varieties due to the versatile use of corn plant are introduced to the market by commercial companies every year. Therefore, new varieties should be tested and their compatibility should be investigated. Corn can be cultivated in the northern hemisphere countries in the summer season. Since the temperature demand of the corn plant is high, In some regions, climatic conditions allow corn to be grown as a single product, while in some regions it allows it to be grown as a second product after the main product. In regions with the ecology of growing two crops per year, silage maize can be produced three times during the maize growing season. Varieties of the corn plant that can be grown between 80 and 135 days have been improved. Due to the fact that it has different maturation groups, it has found a wide spread area in the world and in Turkey. Corn cultivation area for 2019 in Turkey is 6,388.287 decares, production is 6 000 000 tons (Anon, 2020). Second crop corn is planted place in June or July. In the comparison of the 30 June and 15 July sowing times for silage sowing in the Aegean region, it was stated that early sowing increased the yield and there were significant differences between the varieties (Geren ve ark., 2003). The desired yield will be achieved in the second product by processing the field without delay after the harvest of the pre-plant as main product, preparing a good seed bed, ensuring full plant emergence in the unit area and keeping the organic matter and nutrient element in the soil at a sufficient level for healthy plant growth (Altuntaş ve Sade, 2007). In addition, in order to reach the desired level in corn production, it is necessary to using quality seeds, to importance of fertilizer application, and solving of irrigation problem (Şahin 2001). When the areas with irrigation facilities are evaluated for second crop corn, it contributes to animal production since the stubble left in the field is used for grazing (Sarikurt ve Bengisu 2020). Due to the increase in maize cultivation areas every year, the constant introduction of new hybrid corn varieties to the market by commercial firms and the interest in new varieties, 17 different hybrid maize varieties were tested in Kahramanmaraş-Turkey conditions.

MATERIAL AND METHOD

The research was carried out in the second crop growing season, using 17 hybrid maize cultivars in a randomized block design in Kahramanmaraş conditions. Material as, Tavascan, Motri, Calgary, Sancia, P.573, P.32T83, Hydro, Performer, Capuzi, 72MAY80, Simon, Macha, PL712, Torro, Bolsan, KB5562 ve KB3961 like hybrid corn varieties were used. Corn varieties were planted on July 1, 2016 with 70 cm row spacing and 20 cm spacing. Net 6 kg da-1 phosphate and 25 kg da-1 nitrogen fertilizer were given to the plants. Although the plants reached harvest maturity in October, it was done by hand on November 9 due to the rains. The tassel emergence time, plant height, stem diameter, leaf angle (3rd leaf), number of leaves per plant and grain yield values of the cultivars were investigated.

It has been recorded that in the soil sample taken at 0-30 cm depth of the experimental area, the organic matter rate is 1.52, the pH is 7.55, the lime rate is 15.71, the amount of suitable potassium is 74.72 kg da-1, the amount of phosphorus is 5.44 kg da-1, and it had a sandy clay loamy texture (Anonim 2016a). It was reported that in the period of growth of the corn crop (July, August and September) relative humidity of 36-42%, the minimum average temperature of 14-24 ° C, the maximum average temperature of 23-38 ° C, the average temperature of 24-30 ° C varies, no precipitation in July and August, only 23.7 mm of precipitation fell in September (Anonim, 2016b). For this reason, the plants were irrigated 8 times with 10-day intervals using the flood irrigation method. In the statistical analysis of the research, variance analysis was performed according to the anova procedure using the SAS package program for varities evarage. Means were compared according to Duncan's ($P<0.05$) multiple test.

RESULTS AND DISCUSSION

In the research conducted with 17 hybrid corn varieties in the second crop plant growing season in Kahramanmaraş conditions, it was noted that there were statistically significant (1%) differences among varieties in terms of the tassel emergence time, plant height, stem diameter, leaf angle, number of leaves per plant and grain yield characteristics. The values of the examined characteristics of the cultivars and their formed groups were given in Table 1.

Tassel Emergence Time (day)

In the study conducted with 17 hybrid corn varieties grown as a second crop in Kahramanmaraş conditions, the average tassel emergence time was recorded as 52.3 days. It was observed that 17 hybrid corn varieties formed four groups that were statistically different from each other in terms of tassel emergence time. It was recorded that the longest tassel period was 55.0 days for Calgary, Hydro, Simon and KB5562 cultivars, in the same group, and they were statistically significantly different from the others. Tavascan and KB 3961 hybrid maize cultivars were recorded that tassel emergence were achieved as 53 days and diferent form another group. Motri, Sancia, P.32T83, 72MAY80, Marca, PL712, Torro, Bolsan hybrid corn cultivars showed tassel emergence in 52 days, and it was determined that they were in a separate group in the others. It was determined that the earliest tassel emergence was observed in P.573, Performer and Capuzi cultivars with 49.0 days and was in a group that was statistically significantly different from the others (Table 1).

The tassel emergence time is a feature that determines the earliness or lateness of the corn plant. The variety with early tassels will reach harvest maturity earlier. Yield will be lower than late varieties due to the early arrival of harvest maturity in the corn plant and the short transport time of carbohydrates to the storage organs (Bonelli et al., 2016). In the study conducted with 17 hybrid corn varieties grown as a second crop in Kahramanmaraş conditions, the tassel emergence time varied between 49.0-55.0 days. The tassel emergence time in the corn plant were found 47-53 days on 16 hybrid corn varieties in Kahramanmaraş conditions by Cesurer and Ünlü (2001), 51.0-58.0 days in different row spacing and nitrogen dose applications by Alıcı (2005), 51.3 -55.3 days in Hatay conditions by Gözübenli et al. (2007), 40-52 days in preliminary plant and fertilizer dose application by İdikut et al. (2009), 47.8-50.5 days with different nitrogen dose applications in Çukurova conditions by Türkay et al. (2007), 51-54 days with preliminary plant and nitrogen dose applications İdikut and Kara (2011), 46.00 - 57.00 days on 15 hybrid corn varieties by İdikut and Kara (2013), 57.7-63.5 days in the main product in Diyarbakır conditions by Kahraman (2016), 50-66 days in popcorn in Çanakkale conditions by Idikut et al. (2012), 61.5 - 68.0 days in Samsun conditions by Özata et al. (2013), 60.7-72.3 days in Tokat-Kozova conditions by Çakar (2015), 50.0-56.3 days in Çukurova conditions by Saygı (2016).It is also seen from the previous research results that the tassel emergence time in corn varieties varies according to the variety, planting times, application factors, region and climate factors.

Plant height (cm)

It was determined that the average plant height of the second crop varieties in Kahramanmaraş, where the study was carried out, was 189.1 cm. The lowest plant height of 164.1 cm was found in Performer cultivar. Performer hybrid maize cultivar showed statistically significant difference from cultivars except P.32T83, Capuzi, P.573, Hydro, Sancia hybrid maize cultivars with plant heights of 167.6, 175.7, 176.9, 180.8, 181.1 cm, respectively. Among the cultivars, the highest plant height was found in Macha cultivar with 233.1 cm and it was recorded that it created statistically significant difference from other cultivars. Simon and KB3961 hybrid maize cultivars had plant heights of 204.5, 204.3 cm, respectively, it was determined that there were significant differences in plant height from other cultivars, except for Bolson (202.0 cm), 72 MAY80 (190.5 cm), KB5562 (198.5 cm), Tavascan (189.8 cm), Torro (188.1 cm), Motri (187.5 cm) hybrid maize cultivars. It was observed that Calgary and PL712 hybrid corn varieties had plant heights of 185.1 and 184.2 cm, respectively, did not make a statistical difference among themselves in terms of plant height, and were in the same transition group. (Table 1).

It was determined that plant heights of 17 hybrid corn varieties used in the study ranged from 164.1 to 233.1 cm. Ahmed et al., (2020) emphasized that the plant height of corn was 107.20 cm with the use of bacteria, phosphate-dissolving bacteria and synthetic fertilizers and 98.50 in the control, and it may vary according to environmental factors. In second crop conditions, plant height of maize plant was 137.9-197.8 cm in different row spacing and nitrogen dose applications of Alıcı (2005) in Kahramanmaraş conditions, 253.53 - 289.30 cm on 12 cultivars in findings of Sarikurt and Bengisu (2005) in Diyarbakir conditions, 168.2 - 206.8 cm in findings of Öktem and Öktem (2006), 207.0-246.7 cm in researches of Gözübenli et al. (2007), 195.6-224.7 cm in findings of Türkay et al. (2007) in Çukurova conditions, 182-

213 cm in researches of İdikut and Kara (2011) Kahramanmaraş conditions, 153 - 196 cm in the main product study of Cesurer (1994) in Kahramanmaraş Conditions, 172 - 220 cm in findings of İdikut and Kara (2013), 255.8 - 335.8 cm in findings of Özata et al. (2013), 256.25 - 296.50 cm in the first year, 245.5 - 297.75 cm in the second year researches of Coşkun et al. (2014), 252-280 cm in findings of Kuşvuran and Nazlı (2014), 269.2 - 315.0 cm in studies of Özata and Öz (2014) in Samsun conditions, 170-232 cm in finding of Çakar (2015), 202.66-247.18 cm in researches of Khan et al.(2016) and 267.6-301.8 cm in finding of Saygı (2016). Kahraman (2016) noted that the plant height of 16 cultivars was between 247.8-289.5 cm in the second crop and 233.9-277.3 cm in the first crop in Diyarbakir conditions, and the plant height of the cultivars changed according to different planting times in the same place. From the results obtained by previous researchers, it is seen that plant height is highly affected by environmental factors. Knowing the plant height in corn varieties is important for determining the plant density, choosing the silage variety, determining the planting time, and choosing the variety according to the relative humidity level and wind speed of the region.

Stem diameter (mm)

It was determined that the average stem diameter of hybrid corn cultivars was 26.4 mm. The lowest stem diameter value of 24.6 mm was observed in Performer and Sancia hybrid maize cultivars. Performer and Sanciya hybrid maize cultivars made a statistically significant difference in terms of stem diameter from other, except for Calgary, Simon P.573, (25.3, 25.2, 25.6 mm) hybrid maize cultivars. It was seen that Tavascan and Bolsan cultivars did not make a statistical difference with stem diameters of 25.8 and 26.2 mm, respectively, and formed the same group. The highest stem diameter value was in Macha variety with 28.3 mm, followed by Hydro variety with 28.2 mm, and it was seen that there was no statistical difference between them and they were in the same group. Macha and Hydro hybrid maize cultivars had statistically significant differences in terms of stem diameter from others, except for 72MAY80 (27.9 mm), Torro (27.6 mm) hybrid maize cultivars. It was determined that the stalk diameter of P.32T83, PL 712, KB3961 hybrid maize cultivars was 26.6, 26.7, 26.6 mm, respectively, and they did not make a statistical difference among themselves and formed the same group. It was determined that hybrid corn cultivars Motri with 26.5 mm and Capuzi with 26.5 mm stem diameter did not make a statistical difference each other, and formed the same group. It was determined that KB5562 hybrid corn variety was statistically in the transition group with a stem diameter of 26.9 mm (Table 1.)

It was noted that the stalk diameter of 17 hybrid maize cultivars grown as a second crop ranged from 24.6 to 28.3 mm. In the first crop conditions, the stalk diameter of the corn plant was stated that between 21 - 24 mm by İdikut and Kara (2013), 22.3-26.4 mm by Han (2016), 17.0-24.2 mm by Saygı (2016). And in the second crop conditions, it was measured as 11.3-19.6 mm by Alıcı (2005), 33.40 - 36.80 mm by Sarikurt and Bengisu (2005), 19.3-24.5 mm by Öktem and Öktem (2006), 22.3-26.0 mm by Gözübenli et al. (2007), 16 -18 mm by İdikut et al. (2009). Ahmed et al., (2020) stated that the internode diameter of the corn plant was increased with the use of growth-promoting bacteria, phosphate-dissolving bacteria and synthetic fertilizers, and it was affected by environmental factors. Stem diameter was been an important feature in terms of lying feature in the corn plant, holding on to the soil, and transporting the nutrient element (Incognito et al. 2002).

Third Leaf Angle (°)

It was determined that the average of the third leaf angle of the second crop corn varieties was 27.6 ° in Kahramanmaraş conditions. The lowest leaf angle value among the cultivars was seen in KB 5562 with 21.5 °. It was determined that there was no statistical difference between KB 5562 and KB 3961 (leaf angle of 22.5°) varieties, and they were in the same group. The highest leaf angle value among the cultivars was found in Sancia cultivar with 38.1^{o}, and it was recorded that there was a statistically significant difference from other cultivars. It was determined that Tavascan (30.9^{o}) and Calgary (30.8^{o}) varieties did not make a statistical difference among themselves and were in the same group. 72MAY80 (29.1^{o}) hybrid maize cultivars differed statistically in terms of leaf angle from other cultivars, except PL712, Macha, Performer, Motri, Capuzi hybrid maize cultivars. P.573, P.32T83 and Torro hybrid maize cultivars were included in the statistically related transition groups in terms of leaf angle. It was determined that the leaf angle of Hydro and Bolsan hybrid corn cultivars were 24.7^{o} and 24.5^{o}, respectively, statistically in the same group and significantly different from other cultivars, except Simon (25.6^{o}) hybrid corn cultivar (Table 1).

It was noted that the third leaf angle values of the hybrid cultivars used in the study varied between 21.5 and 38.1^{o}. Leaf angle is related to the fact that the leaf is upright and leaning. If the leaf is flat, the sun falls directly on the leaf blade, if it is upright, the sun falls on the front and back surfaces of the leaf. In ecologies where light is insufficient, it is important in terms of light balance point of C4 plants. The uprightness of the leaves in the corn plant has been increased yield due to increasing contributes to photosynthesis and tolerance to plant density (Li et al., 2011). The leaf angle was varied between 32-38.3^{o} in second crop (Çokkızgın, 2002), 41.1-44.5° in main crop corn (Topal 2016), 12.7-55.7˚(Lu et al. 2018). Knowing the leaf angle is important for adjusting the row spacing (Maddonni et al. 2001).

Number of Leaves Per Plant (piece/plant)

It was noted that the number of leaves of hybrid corn cultivars ranged from 12.2 to 15.4 per plant, and the everage of leaves number per plant was 13.2 piece. Among the cultivars, the lowest number of leaves per plant was seen in Hydro cultivar with 12.2. Hydro hybrid maize cultivars differed statistically from the other cultivars in terms of the number of leaves per plant, except for the Motri, Calgary, Sancia, P.573, P.32T83, Performer, Capuzi, Torro, Bolsan and KB3961 hybrid maize cultivars. The number of leaves in the highest plant was found in the PL712 variety with 15.4, and it was determined that there was a statistically significant difference between the PL712 variety and the other varieties, except for the Macha (14.4 units/plant) variety. It was determined that the number of leaves per plant in Bolsan, 72MAY80, KB 5562, Tavascan and Simon hybrid corn cultivars was 13.4, 13.5, 13.7, 14.0 and 14.1, respectively, and it was statistically in the transition group (Table 1).

It was determined that the number of leaves of the second crop hybrid corn cultivars varied between 12.2 and 15.4 pieces/plant. Leaves make the biggest contribution to yield. It has been noted that the yield loss in reducing the number of plants per unit area is higher than the damage to the leaves of the plant at different periods (Haag et al., 2017). It was emphasized by Ahmed et al., (2020) that the number of leaves may change depending on environmental factors, the number of leaves in the corn plant was 16 with the use of bacteria, phosphate-dissolving bacteria and synthetic fertilizers that promote plant growth, and 11 in the control. The number of leaves of the corn plant was stated 7.9- 11.1 units/plant in findings of Sönmez et al. (2013), 7.60 - 16.60 units/plant in study of Öner (2011), 12.80 - 13.67 units/plant in research of Aygün (2012), 13.6-15.7 units/plant in finding of Kahraman (2016), 13.3 - 14.8 number/plant in study of Topal (2016), for the first product, 12.49 - 18.49 units/plant in finding of Taş (2010), 13.4-15.7 units/plant in research of Kahraman (2016), for the second crop. Although the genetic influence of cultivar on the number of leaves in the plant is higher, it is affected by soil and climatic factors.

Grain Yield (kg/da)

It was recorded that the grain yield values of 17 hybrid corn varieties grown as a second crop in Kahramanmaraş conditions varied between 410.3-1069.9 kg da^{-1}, and the average grain yield was 822.2 kg da^{-1}. It was determined that P.573 hybrid corn cultivar had the lowest grain yield with 410.3 kg da^{-1} among the cultivars. P.573 hybrid maize cultivars was differ statistically from other cultivars in terms of grain yield, with the exception of Tavascan, Capuzi, Simon, Macha, Bolsan and Calgary (799.1, 829.9, 821.6, 815.8, 749.0 and 667.6 kg da^{-1}) hybrid corn cultivars. It was determined that the grain yield of PL712, Hydro, Sancia, KB3961, P.32T83, Motri and Torro hybrid maize cultivars were 917.7, 902.8, 894.0, 887.2, 860.1, 851.9, 850.7 kg da^{-1}, respectively, in the statistically related transition groups. 72MAY80 hybrid corn variety was statistically different from the other varieties, except Bolsan with 696.0 kg da^{-1} grain yield. The highest grain yield value was seen in Performer hybrid corn variety with 1069.9 kg da^{-1} and it was recorded that it was statistically different from other varieties in terms of grain yield. KB5562 (954.0 kg da^{-1}) cultivar followed the Performer hybrid corn variety in the second place in grain yield and it was found to be in a different group (Table 1).

It was recorded that the grain yield values of 17 hybrid corn varieties grown as a second crop in Kahramanmaraş conditions varied between 410.3-1069.9 kg da^{-1}. Milander et al (2017) stated that the variety, ripening time and plant density were effective on the grain yield of maize plant. It was explained by Ahmed et al., (2020) that plant growth-promoting bacteria, phosphate-dissolving bacteria significantly increase grain yield in corn plant and the yield is affected by environmental factors. In the previous main crop corn studies, grain yield of per decare was found 758 - 1209 kg by Cesurer (1994), 276-440 kg in 10 hybrid corn varieties by Dok (2005) in Şanlıurfa conditions, 822.33- 1213.67 kg in green manure and farm manure application by Gürses (2010) in Çukurova conditions, 696 - 1290 kg by İdikut and Kara (2013), 909.4-1,224 kg by Özata et al. (2013) in Samsun conditions, 655 - 975 kg da by Han (2016) in Giresun conditions. And also in second crop corn, grain yield was between 572.7 - 849.0 kg in Şanlıurfa and Diyarbakır conditions in Çölkesen ve ark. (1997) finding, 341 - 797 kg in the study of Budak (2001), 643.1 - 1248.8 kg in researhces of Cesurer and Ünlü (2001) in Kahramanmaraş conditions, 472.1 - 991.6 kg in finding of Alıcı (2005) at Kahramanmaraş conditions, 682.8 - 966.8 kg in 10 hybrid corn cultivars in study of Dok (2005)in Şanlıurfa conditions, 1052.4 - 1249.3 kg in finding of Türkay et al. (2007) in Çukurova conditions, 622 - 794 kg da in pre-plant and fertilizer dose in researches of İdikut et al. (2009) in Kahramanmaraş conditions, 875 kg in Ödemiş location, 816 kg/da in Bornova location on four maize genotypes in findings of Budak et al. (2014), 879 - 1050 kg in findings of İdikut and Kara (2011), 511.9-818.9 kg in studies of Qi et al.(2020) in Northwest-China, 1137.67 -1489.67 kg on 12 corn varieties in researches of Sarikurt and Bengisu(2020) in Diyarbakır conditions.

It is understood from the findings of previous researchers that the grain yield of the corn plant is affected by many yield components, as well as the variety character, planting time, application factor, soil factor, nutrient element, and the climatic conditions of the region. In hybrid corn varieties, private companies are constantly introducing new varieties that give high yields according to the regions. In Kahramanmaraş conditions, there is almost no precipitation in the second crop growing season, and the lack of relative humidity required by the corn plant causes the yield of the grain product to decrease.

Table 1. Average and groups of tassel emergence time, plant height, stem diameter, leaf angle, number of leaves per plant and grain yield of second crop hybrid corn cultivars

Çeşitler	Tassel emergence time (day)	Plant height(cm)	Stem diameter (mm)	Leaf angle (°)	Number of leaves per plant (piece)	Grain yield (kg de^{-1})
Tavascan	53.0 b	189.8 bcde	25.8 def	30.9 b	14.0 bc	799.1 ef
Motri	52.0 c	187.5 bcde	26.5 de	27.7 cdef	13.3 bcdef	851.9 cde
Calgary	55.0 a	185.1 cdef	25.3 fg	30.8 b	12.9 cdef	667.6 fg
Sancia	52.0 c	181.1 defg	24.6 g	38.1 a	12.3 ef	894.0 bcd
P.573	49.0 d	176.9 efg	25.2 fg	26.2 fgh	12.4 ef	410.3 f
P.32T83	52.0 c	167.7 fg	26.6 cde	26.9 efg	12.9 cdef	860.1 cde
Hydro	55.0 a	180.8 defg	28.2 a	24.7 ı	12.2 f	902.8 bcd
Performer	49.0 d	164.1 g	24.6 g	28.4 cde	12.7 def	1069.9 a
Capuzi	49.0 d	175.7 efg	26.5 de	27.6 cdef	12.3 ef	829.9 cdef
72May80	52.0 c	190.5 bcde	27.9 ab	29.1 c	13.5 bcde	696.0 g
Simon	55.0 a	204.5 b	25.6 efg	25.6 ghı	14.1 bc	821.6 def
Macha	52.0 c	233.1 a	28.3 a	28.5 cd	14.4 ab	815.8 def
PL712	52.0 c	184.2 cdef	26.7 cde	28.7 cd	15.4 a	917.7 bc
Torro	52.0 c	188.1 bcde	27.6 abc	27.2 def	13.2 bcdef	850.7 cde
Bolsan	52.0 c	202.0 bc	26.2 def	24.5 ı	13.4 bcdef	749.0 fg
KB 5562	55.0 a	198.5 bcd	26.9 bcd	21.5 j	13.7 bcd	954.0 b
KB 3961	53.0 b	204.3 b	26.6 cde	22.5 j	12.7 def	887.2 bcde
	52.3	**189.1**	**26.4**	**27.6**	**13.2**	**822.2**

CONCLUSIONS

In the research conducted using 17 commercial hybrid corn varieties in the second crop season in Kahramanmaraş conditions, it was noted that there was no problem in obtaining grain corn in terms of completing physiological maturity. Performer, KB 5562, PL 712, Hydro cultivars were considered as recommended cultivars due to their grain yield above 900 kg da^{-1} as a second crop. It was emphasized that adaptation studies should be continued with new varieties every year.

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NUTRITIONAL VALUES OF PLANT RESIDUES OF BEAN VARIETIES GROWN AS SECOND CROPS

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ABSTRACT

Bean plant is produced for fresh, canned and dried grain. The green pods of 11 bean varieties, which were grown in Kahramanmaraş conditions as second crops, were harvested due to the cold weather in November. After the fresh pods are taken, the nutritional values of the plant residues were examined by taking into account the contribution they will make to the feed or soil. In the plant residues of bean varieties used in the research, the dry weight of plant residue, ratio of dry matter, Protein, Ca (Calcium), Mg (Magnesium), K (Potassium), P (Phosphorus), ADF (Fiber Insoluble in Acid Detergent), ADP (Protein Insoluble in Acid Detergent), NDF (Insoluble Fiber in Neutral Detergent) ratios were investigated. At the end of the research, it was noted that the average values were varies plant residues weight from 1202.5 to 217.3 kg/de, dry matter ratio from 89.54 to 88.92%, protein from 17.09 to 8.49%, Ca from 1.91 to 1.42%, Mg from 0.67 to 0.51%, K from 1.23 to 0.55%, P from 0.30 to 0.21%, ADF from 76.70 to 38.03%, ADP from 1.46 to 0.9%, NDF from 66.74 to 46.39%. It was thought that it would be a very good pre-plant for the plant to be planted in winter, as it provides organic matter to the soil and fixes nitrogen to the soil with the help of nodosity formed in the plant roots. In addition to being used as fodder.

Key Word: Bean, second crops, residues of plant, chemical content.

INTRODUCTION

It is known that the homeland of the bean, which is cultivated in many different parts of the world, is South America, it was brought to Europe at the beginning of the 16th century, and the bean cultivation has been carried out in Turkey for about 250 years (Nemli 2013). The bean plant, which is in the legumes group, is important in terms of meeting the nutritional needs of people. It is an important field plant that is consumed abundantly in Turkey and in the world due to reasons such as the high nutritional value of the bean plant, its trade as fresh, canned and dried, and the indispensable food of the kitchens. In terms of sustainable agriculture and environmentalism, the contribution of legumes to today's agriculture is great. Leguminous plants form nodosites in their roots during nutrient uptake from the soil and they attach the free nitrogen of the air to their roots thanks to the rhizobium bacteria thus, they both meet their own nitrogen needs and leave some nitrogen to the next plant (Voisin et al., 2014). About two-thirds of the nitrogen usually fixed by a legume crop becomes available to the plant in the growing season after a legume rotation (Deakin and Broughton, 2009). For this reason, legumes are seen as a good crop rotation plant. Rotation of the bean plant with other field crops reduces fertilizer and energy intake in arable land therefore, it plays an important role in reducing greenhouse gas emissions (Deakin and Broughton 2009; Stagnari et al. 2017). Leguminous plants reduce the use of commercial nitrogen fertilizers, enrich the soil in terms of organic matter, aerate the soil and increase the water holding capacity of the soil, as well as provide an economic advantage by providing two crops in a year for the plants entering the crop rotation. Legumes also play an important role in animal nutrition due to the low content of cellulose in the stem and straw of edible grain legumes. The use of plant residues as feed is another plus. Bean plant stem, leaves, bean pods are important for small-scale animal enterprises and the yield and nutritional value of the component of plant residues should be taken into account when selecting genotypes (Asfaw and Blair, 2014; Beebe et al., 2013; Blümmel et al., 2012; Mekbib, 2002; Tullu et al., 2001; Dejene et al. 2018). It is the anatomical structure and chemical composition of the plants that reveal the difference between the plants. For this reason, there may be differences in nutritional value between graminae or legumes, between cool and warm season plants and genotypes within the same species. The bean plant, which is grown for its grain, is grown together with many graminae plant species and used as silage in animal feding and it is known that it is used as an alternative roughage source that stands out with its straw. Studies on the chemical content of plant residues of the bean plant are very few. In cereal and legume plants, it should be evaluated as feed by considering the nutritional value of plant residues without sacrificing grain yield (Kafilzadeh and Maleki, 2012; Nigam and Blummel, 2010; Prasad et al., 2010; Singh et al., 2003). Knowing the contribution and nutritional value of the plant parts remaining from the commercially used production materials of the cultivated products to the soil will increase the knowledge of the producer about the usage areas. (de Tonissi e Buschinelli de Goes et al., 2013). Since the bean plant is a temperate climate plant that does not tolerate cold, it needs an optimum soil temperature of 18 °C and a growth temperature of 20-25 °C for germination. Plant growth decreased at temperatures below 15 °C and inhibition of fertilization at temperatures above 32 °C cause yield loss (Porch and Jadn, 2001). In places with Mediterranean climate, the bean plant is generally grown as the first crop in the spring. In this study, bean varieties were grown as a second crop in autumn in Kahramanmaraş region. In this study, it was aimed as a pre-plant of bean to contribute to be planted in winterand to investigate its adaptability as a grain product and the pods of bean varieties were taken and the nutritional values of the remaining plant parts were examined.

MATERIAL AND METHOD

Research material used in the experiment was obtained from research institutes and commercial companies in Turkey. Karacaşehir 98, Bermaz, Önceler 98, Akman 98, Topçu, Noyanbey 98, Göksun, Göynük 98, Aras 98, Yunus 90, Alberto bean varieties were used in the study. The research was carried out under the conditions of Kahramanmaraş, which has the effect of Mediterranean climate. Kahramanmaraş province, which is 568 m above sea level, has a monthly minimum (23.2, 21.0, 14.6, 9.2 C^0), maximum (36.8, 34.7, 26.4, 17.6 C^0), average temperature (29.1, 27.2, 19.8, 2.7 C^0), average relative humidity (48.3, 38.4, 51.5, 66.7) and precipitation (4, 8, 45, 70 mm) values were recorded for the months of August, September, October and November, when the research was carried out in 2018 (Anon, 2018a). Soil properties, Saturation, pH, salt (%), calcalereous (%), organic matter (%), potassium (mg/kg), phosphorus (mg/kg) values are 85.80, 7.28,0.30, 1.00, 2.08, 266.8, 10.46 respectively for 0-30 cm depth. (Anon, 2018b). It has been determined that the soil structure of the experimental area has a slightly alkaline and clayey texture, medium level of organic matter, slightly salty and calcareous, and sufficient level of phosphorus and potassium. Wheat plant was grown as a preliminary plant in the experimental area. After the wheat harvest, the field was plowed with a plow. Before planting, the trial field was plowed with second-class agricultural tools, and then the trial field was made ready for planting by pulling the cultivator. The research was sown on August 7, 2018 at 50 cm inter- row and 10 cm row spacing, It was sown by hand in 3 replications in 4 rows on 5 meter long plots. Before planting, 6 kg/da of phosphorus and 2-3 kg/da of nitrogen fertilizer were applied. After the planting was completed, the drip irrigation system was installed and irrigation was done. When the plants reached a height of 15 cm, 3-7 kg/da of nitrogen fertilizer was applied. Hand hoeing was done 3 times for weed control. During the growing period of the bean plant, the drip irrigation system was operated for approximately 7 hours and irrigated for a total of 12 times due to the almost non-existent rainfall in August and September. Due to insufficient climatic condition for physiological maturity of bean varieties for dry grain harvest, the middle two rows of each plot were manually removed from the soil on 6-10 November, and observations, measurements and analyzes were made on the plant parts. Except for the pods of the harvested bean varieties, the plant parts were kept at 105 °C for 72 hours and dried in the oven. Established samples were then ground into flour. Laboratory analysis of 48 samples taken as flour was made using the WINISI package program on the FOSS 6500 NIR system device. In the study, dry weight of plant parts, amount of dry matter, Protein, Ca (Calcium), Mg (Magnesium), K (Potassium), P (Phosphorus), ADF (Fiber Insoluble in Acid Detergent), ADP (Protein Insoluble in Acid Detergent), NDF (Fiber Insoluble in Neutral Detergent) ratios were investigated. In the statistical analysis of the results obtained in the study, variety averages were compared using the SAS package program, variance analysis was performed according to the anova procedure, and the averages were compared according to Duncan's ($P<0.05$) multiple test.

RESULTS AND DISCUSSION

Dried weight, dry matter ratio, Protein, Ca (Calcium), Mg (Magnesium), K (Potassium), P (Phosphorus), ADF (Fiber Insoluble in Acid Detergent), ADP (Protein Insoluble in Acid Detergent), NDF (Insoluble in Neutral Detergent) average values of eleven bean varieties grown as a second crop in Kahramanmaraş conditions are given in Tables 1 and 2.

Dried Plant Parts (kg da^{-1})

In the research carried out with different bean varieties, it was noted that the dried weight of the remaining plant parts after the green pods of the bean plant varied between 217.0-1202.5 kg da^{-1}. It was determined that there were statistically significant differences between the varieties in terms of the dried weight of the plant parts. Önceler 98 variety with 1202.5 kg da^{-1} plant component weight received the highest value and except for Göynük 98 and Yunus 90 cultivars, it was determined that there was a statistically significant difference from other cultivars. It was determined that the lowest plant parts were recorded in Bermaz variety with 217.3 kg da^{-1}, and there was no statistically significant difference between Bermaz variety and Akman 98, Topçu, Noyanbey 98, Göksun and Alberto varieties. It was determined that there was no statistical difference between Karaçaşehir 98 and Aras 98 varieties, but they were in the same transition group (Table 1). Kılınç and Uslu (2021) found fodder yield as 129.7-978.0 kg da^{-1} in 70 bean genotypes in Kahramanmaraş-Afşin conditions, Budak (2017) found the fodder yield as 644.7-741.3 kg da^{-1} in his studies conducted on vetch plant in Iğdır conditions, Dejen et al. (2018) recorded that the weight of bean stem and bean pod in four different locations in Ethiopia varied between 74-254 kg da^{-1}. The presence of legumes in feed sources increases animal nutrition and unit area yield.

Table 1. Dried weight, dry matter ratio, Protein, Ca (Calcium), Mg (Magnesium) mean values of bean varieties and formed groups.

Variety	Dry weight of plant parts kg da^{-1} **	Dry matter (%) *	Protein (%) **	Calsium (%) *	Magnesium (%) *
Karacaşehir 98	623.7 bc	88.92 b	17.09 a	1.86 a	0.59 abc
Bermaz	217.3 d	89.35 ab	10.56 cd	1.50 b	0.52 bc
Önceler 98	1202.5 a	89.39 ab	12.89 bc	1.67 ab	0.56 abc
Akman 98	546.3 cd	88.98 b	14.64 ab	1.86 a	0.62 abc
Topçu	534.0 cd	88.97 b	10.80 bcd	1.91 a	0.67 a
Noyanbey 98	530.0 cd	88.97 b	14.35 abc	1.88 a	0.64 ab
Göksun	509.9 cd	89.32 ab	8.49 d	1.49 b	0.53 bc
Göynük 98	868.7 abc	89.26 ab	13.00 bc	1.74 ab	0.56 abc
Aras 98	711.0 bc	89.44 a	10.59 cd	1.42 b	0.51 c
Yunus 90	960.3 ab	89.54 a	13.58 abc	1.69 ab	0.53 bc
Alberto	474.2 cd	88.93 b	10.95 bcd	1.61 ab	0.52 bc

Dry Matter Ratio (%)

Bean varieties formed 2 groups in terms of dry matter and dry matter values varied between 88.92-89.54%. The highest dry matter value was in Yunus 90 variety with 89.54%, followed by Aras 98 variety with 89.44%, and Yunus 90 and Aras 98 variety were recorded in the same group. In terms of dry matter content, the varieties Önceler 98, Bermaz, Göksun, Göynük 98 had values of 89.39%, 89.35%, 89.32%, and 89.26%, respectively and were in the same transition group. Bermaz, Göksun, Göynük 98 varieties were not statistically different from Yunus 90 and Aras 98 varieties. The lowest dry matter content was obtained from Karacaşehir 98 variety with 88.92%. Karacaşehir 98 variety made a statistically significant difference with Yunus 90 and Aras 98 varieties, but it was determined that there was no statistically significant difference between other varieties (Table 1). Kavak (2019) stated that after the harvest of some astragalus taxa in the Southeastern Anatolia Region, the rate of dry matter was found to be between 88.92-91.04%. Kaplan et al. (2014) found the dry matter ratio between 77.73-82.39% in different forage pea genotypes. Zulkadir and İdikut (2021) stated that the dry matter content of quinoa plant parts in Kahramanmaraş conditions varied between 91.08 and 87.94%. It is also understood from the findings of previous researchers that the amount of dry matter varies according to the plant species, the time of intake of dry matter and climatic conditions.

Protein Ratio (%)

It was determined that the protein values in the plant parts of the bean varieties used in the study varied between 8.49 and 17.09%. The highest protein value was obtained from Karacaşehir 98 variety with 17.09%. In terms of protein values, Akman 98, Noyanbey 98, Yunus 90 variety are in the transition group with 14.64%, 14.35% and 13.58% values, respectively, and it was stated that these varieties did not statistically differ significantly from Karacaşehir 98 variety. The lowest protein value was obtained from Göksun variety with 8.49% and Göksun variety showed statistically significant differences from other varieties except Bermaz, Topçu, Alberto varieties. It was noted that Önceler 98 and Göynük cultivars were in the same transition group (Table 1). Kılınç and Uslu (2021), in their study in Kahramanmaraş Afşin region, found the protein ratio in the plant parts after the bean harvest in the range of 5.65-14.64%. Cebeci et al. (2017) found the protein ratio in the stem of bean variety between 10.0-13.7% in different row spacing studing in Çanakkale conditions. Zulkadir and İdikut

(2021) determined that the protein ratio in quinoa plant parts was between 4.40% and 7.37% in Kahramanmaraş conditions. It is seen from previous studies that there are differences according to plant species, climate, soil structure and application factors.

Calcium Ratio (%)

It was determined that in bean cultivars planted as a second crop, the cultivars formed 2 groups in terms of Ca ratio and varied between 1.42% and 1.91%. It was recorded that the highest Ca value was obtained from Topçu variety with 1.91%, followed by Noyanbey 98 with 1.88%, Akman 98 and Karacaşehir 98 varieties with 1.86% and included in the same group with an insignificant difference. In terms of Ca values, Göynük 98, Yunus 90, Önceler 98 and Alberto cultivars had values of 1.74%, 1.69%, 1.67% and 1.61%, respectively, and it was stated that they were in the same transition group. The lowest value in terms of Ca value was obtained from Aras 98 variety with 1.42%. Aras 98 was followed by Göksun with a value of 1.49%, Bermaz with a value of 1.5%, Alberto with a value of 1.61%, Önceler 98 with a value of 1.67%, Yunus 90 with a value of 1.69%, and Göynük 98 with a value of 1.74% and it was determined that there was no statistically significant difference between them (Table 1). Özbahçe (2008) determined that the amount of Ca as a result of Manganese application to bean varieties in Konya ecological conditions changed between 1.32% and 1.94%. In other plant species made in previous years, Çaçan et al. (2012) found the Ca ratio in green grass values between 1.09% and 1.61% in alfalfa species collected from natural pastures in Diyarbakır ecological conditions. Kavak (2019) reported that the Ca ratio of some astragalus taxons in the Southeastern Anatolia region was between 0.96-2.14% and Zulkadir and İdikut (2021) reported that the Ca ratio of quinoa plant parts varied between 0.93-3.33% in Kahramanmaraş conditions. The literature results indicate that the amount of applied nutrients, plant taxons and ecological conditions cause changes in the Ca ratio.

Magnesium Ratio (%)

It was determined that the Mg values examined in the plant parts of the bean cultivar ranged between 0.51-0.67%. The highest Mg value was obtained from Topçu variety with a value of 0.67%. In terms of Mg values, it was determined that Noyanbey 98, Akman 98, Karacaşehir 98, Göynük 98, Önceler 98 cultivars had 0.64%, 0.62%, 0.59%, 0.56% values, respectively, in the transition group and did not statistically differ from Topçu cultivar. Yunus 90 and Göksun varieties were in the same transition group as Alberto and Bermaz varieties. It was stated that the lowest value in terms of Mg value was obtained from Aras 98 variety with 0.51% (Table 1). Özbahçe (2008) stated that the Mg ratio in the application of Manganese to bean varieties in Konya ecological conditions varied between 0.191-0.266%.In previous studies on other plant species, Çaçan et al. (2012) reported that they found the Mg ratio in green grass values of clover species collected from natural pastures in Diyarbakır ecological conditions to be between 0.22-0.31%.Kavak (2019) reported that after some astragalus harvests in the Southeastern Anatolia region, the Mg content varied between 0.30-0.53%, and Zulkadir and İdikut (2021) reported that the Mg ratio in the quinoa plant parts in Kahramanmaraş conditions changed between 0.20-1.24%. As understood from previous studies, the amount of applied nutrients, plant taxons, ecological conditions, harvest time cause changes in Mg ratios.

Potassium Ratio (%)

It was determined that the K values of the plant parts of the bean cultivars tried as the second crop ranged between 0.55- 1.23%. The highest K value of 1.23% was obtained from Karacaşehir 98 variety. In terms of K values, it was noted that there was a statistically significant difference between Karacaşehir 98 cultivars, Önceler 98, Topçu and Göynük 98 cultivars, but there was no statistically significant difference between other cultivars. While Topçu cultivar, which has the lowest K value of 0.55%, did not make a statistical difference between Noyanbey 98, Akman 98, goynuk 98 and Alberto cultivars, it was determined that there was a significant statistical difference between other cultivars (Table 2). Özbahçe (2008) determined that the potassium ratio varies between 0.73-3.82% as a result of manganese application to bean varieties in Konya ecological conditions. In other plant species, Çaçan et al. (2012) reported that they found the K ratio between 1.76% and 2.54% in green grass values of alfalfa species collected from natural pastures in Diyarbakır ecological conditions. Kavak (2019) determined the K ratio between 0.57-2.23% after some astragalus harvest in the Southeastern Anatolia region, and Zulkadir and İdikut (2021) recorded that the K ratio in quinoa plant parts changed between 0.73-2.24% in Kahramanmaraş conditions. As can be seen from the researches, it has been determined that the K ratio varies according to the time of the samples, the plant species and the applied plant nutrient.

Table 2. Average values and groups of bean varieties of K (Potassium), P (Phosphorus), ADF (Fiber Insoluble in Acid Detergent), ADP (Protein Insoluble in Acid Detergent), NDF (Fiber Insoluble in Neutral Detergent)

Variety	**Potassium (%) ***	**Phosphorus (%) ***	**ADF (%) ****	**ADP (%) ***	**NDF ** (%)**
Karacaşehir 98	1.23 a	0.30 a	38.03 d	1.09 bcd	46.39 c
Bermaz	0.94 abc	0.24 ab	51.78 ab	1.17 a-d	61.69 ab
Önceler 98	0.76 cd	0.25 ab	45.78 bcd	1.06 cd	54.52 bc
Akman 98	0.93 a-d	0.26 ab	42.49 cd	1.15 bcd	52.06 bc
Topçu	0.55 d	0.21 b	51.29 ab	1.40 ab	60.64 ab
Noyanbey 98	0.89 a-d	0.29 a	47.00 bc	1.46 a	55.40 bc
Göksun	1.11 abc	0.21 b	76.70 a	1.27 abc	66.74 a
Göynük 98	0.83 bcd	0.24 ab	44.16 bcd	1.07 cd	52.70 bc
Aras 98	1.12 abc	0.22 b	47.39 bc	1.14 bcd	58.30 ab
Yunus 90	1.19 ab	0.26 ab	40.47 cd	0.9 d	46.55 c
Alberto	0.86 a-d	0.22 b	51.36 ab	1.26 abc	61.77 ab

Phosphorus Ratio (%)

It was determined that the P value in plant parts of bean cultivars varied between 0.21-0.30% and formed 2 different groups. Karacaşehir 98 cultivar had the highest P value with 0.30%, followed by Noyanbey 98 cultivar with 0.29% value and was recorded in the same group. It was determined that the lowest P value was obtained from Topçu and Göksun cultivars with 0.21%, followed by Alberto and Aras 98 cultivars with 0.22% value. While Topçu cultivar made a statistically significant difference with Karacaşehir 98 and Noyanbey 98 cultivars, it was determined that there was no statistically significant difference between other cultivars. Bermaz, Önceler 98, Akman 98, Göynük 98, Yunus 90 cultivars did not differ statistically among themselves in terms of P values, and they were in the same transition group (Table 2). Özbahçe (2008) determined that the P content of Manganese application to bean varieties varies between 0.27-0.45% in Konya ecological conditions. In other plant species, Çaçan et al. (2012) found the P ratio between 0.29-0.35% in green grass values of alfalfa species collected from natural pastures in Diyarbakır ecological conditions. Kavak (2019) found the P ratio between 0.25 and 0.45% after the astragalus harvest in the Southeastern Anatolia region, while Zulkadir and İdikut (2021) reported that the P ratio in quinoa plant parts in Kahramanmaraş conditions changed between 0.22-0.30%. As understood from previous studies, it is understood that P content varies according to plant species, application factors, ecological conditions, soil structure and the structure of taxons.

ADF (% Acid Detergent Insoluble Fiber)

It was determined that the ADF values of the plant parts of the bean varieties used in the study varied between 38.03-56.70%. The highest ADF value was obtained from Göksun variety with 56.70%. It was stated that Bermaz, Alberto and Topçu cultivars were in the same transition group with the values of 51.78%, 51.36% and 51.29%, respectively, and did not statistically differ significantly from the Göksun cultivar. The lowest value in terms of ADF was determined in Karacaşehir 98 variety with 38.03%. It was determined that Karacaşehir 98 cultivar was followed by Yunus 90 with 40.47% value, Akman 98 with 42.49%, Göynük 98 with 44.16%, and Önceler with 45.78% value and there was no statistically significant difference. However, statistical differences were found between other cultivars and Karacaşehir 98 cultivar. Aras 98 and Noyanbey 98 varieties were recorded in the same transition group with the values of 47.39% and 47.00%, respectively (Table 2). ADF values are important in determining forage quality standards. It has been reported that the best quality class is <31% in ADF values. It has been reported that the first class has the values between 31-35% and the 5th class has the lowest roughage value with a value of >45% (Güney, 2016). It can be seen from Table 2 that the ADF values of some cultivars were moderate in the research conducted. Kılınc and Uslu (2021) reported that after the dry bean harvest in Kahramanmaraş Afşin region, the rate of ADF varied between 28.00-58.00%. Dejene et al. (2018) reported that ADF values ranged between 502-585 g kg^{-1} in four different places in Ethiopia. Since ADF values are used as an important criterion in determining the quality of the feed, in previous studies on other plants, Koivisto et al. (2003) found in a study conducted in England that the ADF ratio of soybeans harvested in early and late seasons varied between 31.5 and 57.8%. Cebeci et al. (2017) found the ADF ratio in the range of 24.1-21.6% in the different row spacing of gum bean weed yield application in Çanakkale conditions. Gebreyowhnas and Gebremeskel (2014) found the ADF ratio in cowpea to be between 47.0-57.2% in Ethiopian conditions. In the study of Çaçan et al. (2012) determined the ADF ratios of alfalfa species collected from natural pastures in Diyarbakır ecological conditions between 30.1-37.36%. Temel et al.(2015) reported that ADF values changed between 28.94-35.71% after the common vetch harvest in Iğdır ecological conditions. It has been determined that the literature values vary according to the plant species, harvest time, ecological conditions and the structure of the taxon.

ADP (% Acid Detergent Insoluble Protein)

It was determined that the ADP values of the plant parts of the bean cultivars tried as the second crop ranged between 0.9-1.46%. The highest ADP value of 1.46% was obtained from Noyanbey 98 variety. In terms of ADP values, Topçu, Göksun, Alberto and Bermaz cultivars had 1.40, 1.27%, 1.26%, 1.17% values, respectively, in the transition group and did not differ significantly from Noyanbey 98 cultivar. In terms of ADP values, it was determined that the Yunus variety had the lowest value with 0.9%. Akman 98, Aras 98, Karacaşehir 98, Göynük 98, Önceler 98 varieties have values of 1.15%, 1.14%, 1.09%, 1.07, 1.06, respectively, and are in the same transition group. It was observed that there was no statistically significant difference between the Yunus cultivar (Table 2). Kılınç and Uslu (2021) reported that the NDF ratio in plant residues of 70 different bean varieties in Kahramanmaraş-Afşin conditions varied between 28.00-58.00%. In other plant species made in previous years; In meadow pasture taxa in the Southeastern Anatolia Region, drying of the plants from which fresh grass samples were taken during the flowering period and ADP values in Yalçınkaya(2019) 0.38-1.28%, Çakış (2019) 0.7-1.12%, Başbağ (2018) Diyarbakir ecological conditions, in the weed analysis of grass forage forage plants in the flowering period. determined that the values varied between 0.08-0.63%. In the literature results, it is seen that the ADP values vary according to the plant species, the harvest period of the plant, ecological conditions and soil structure.

NDF (% Neutral Detergent Insoluble Fiber)

It was determined that the NDF values of the plant parts of the bean cultivars grown as a second crop ranged between 46.39% and 66.74%. The highest NDF value was obtained from Göksun variety with a value of 66.74%. In terms of NDF value, it was determined that Bermaz, Alberto, Topçu and Aras 98 varieties had 61.99%, 61.77%, 60.64%, 58.30% values, respectively, in the same transition group and did not statistically differ significantly from Göksun variety. In terms of NDF values, Noyanbey 98, Önceler 98, Göynük 98 and Akman 98 varieties have 55.40%, 54.52%, 52.70%, 52.06% values, respectively, and they are in the same transition group and these cultivars made a statistically significant difference from the Göksun cultivar, but it was noted that there was no statistically significant difference between them and the other cultivars. It was recorded that the lowest value in terms of NDF value was determined in Karacaşehir 98 variety with value of 46.39%, followed by Yunus 90 variety with value of 46.55% in second place. While Karacaşehir 98 and Yunus 90 cultivars did not make a statistically significant difference with Akman 98, Göynük 98, Önceler 98 and Noyanbey 98 cultivars, it was determined that there were statistically significant differences between them and other cultivars (Table 2). Güney (2016) stated that according to the roughage quality standards, the best quality class is <40% in NDF values, the first class has the lowest roughage value between 40-46% and the fifth class with >65%. It is seen from Table 2 that the plant residues of some of the bean varieties used in the research are at a very good level in terms of NDF values. Dejene et al. (2018) found the NDF values of bean genotypes in four different places in Ethiopia between 648-739 g kg^{-1}. Kılınç and Uslu (2021) reported that the NDF ratio in plant residues of 70 different bean varieties in Kahramanmaraş-Afşin conditions varied between 48.15-80.00%. Since NDF values are used as an important criterion in determining the quality of the feed, Koivisto et al. (2003) found, in a study conducted in England, the NDF ratio in early and late harvested soybeans varied between 43.5-72.8%, Cebeci et al. (2017) reported that the NDF ratio in the different row spacing application of gum bean in Çanakkale conditions was between 48.9-37.8%, Gebreyowhnas and Gebremeskel (2014) found the NDF rate of 56.3-60.7% in cowpea plants in Ethiopian conditions, Çakış (2019) The NDF rate of 33.68%-51.23% in dried grass samples taken during flowering period in meadow pasture taxon in the Southeastern Anatolia region, and Çaçan et al. (2012) determined that the NDF rate in alfalfa species collected from natural pastures under the ecological conditions of Diyarbakir varies between 38.9-49.8%. As it is understood from previous studies, it is understood that there are differences according to the plant species, the development level of the material taken and the time it was taken, the climate and soil structure and the structure of the taxon.

CONCLUSIONS

In order to evaluate the vacant area after the wheat harvest, 11 bean varieties were cultivated as the second product in the Mediterranean climate region of Kahramanmaraş, and the nutrient content of the remaining plant parts after the green bean pods were harvested. Excluding the green pods of the bean plant, 8-17% protein value in the dried plant parts, showing higher protein value than wheat straw, and medium roughage properties of ADF, ADP and NDF values will contribute to the increase of feed resources in regions with Mediterranean climate. Considering that it contributes to the soil in terms of organic agriculture and leaves nitrogen for the plant to be planted afterwards, it is predicted that green bean cultivation can be cultivated in summer as the pre-plant of winter plants. Considering the limited literature on the chemical content of plant residues of the bean plant, it is thought that it will guide the next studies and contribute to science.

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ARONIA BERRY TEA AS ANTIOXIDANT FUNCTIONAL BIOACTIVE PHENOLIC SOURCE

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Tea is popular beverage and currently, herbal infusions based on dried fruit products have gained in popularity because of their fragrance, fruity flavor, lower amounts of caffeine, and low astringent and bitter taste. Chemical composition and biological activity of berries and their products have been widely reportED BUT THERE ARE LIMITED WORKS DEALING WITH BERRY FRUIT TEAS. THE genus Aronia (Rosaceae family) includes two species of shrubs, native to eastern North America and Eastern Canada: *Aronia melanocarpa* (Michx.) Ell., known as black chokeberry and *Aronia arbutifolia* (L.) Pers. (red chokeberry). The aronia berries contain high levels of flavonoids, mostly proanthocyanidins and anthocyanins, and in vitro and in vivo studies indicate that the berries may have potential health benefits, e.g. hepatoprotective effects, cardioprotective effects, antidiabetes effect and anticancer effects on selected CA cells. In our current research, aronia based new products including aronia berry teas (as decoction and infusion types), aronia powder and aronia ice-cream were developed. Aronia berry (black chokeberry) fruit teas was found as valuable source of flavonoids and anthocyanins compared to the most of commonly consumed berry teas. This application was performed by using a certain combination of teaware, steeping process, water temperature, water to aronia berry tea ratio. The total phenolics and the anthocyanin level of infusion was determined as 101.02 ± 0.55 mg GAE/100 ml (*n*=3) and 9.05 ± 0.05 mg/100 ml (*n*=3), respectively. For aronia (chokeberry) powder production, aronia berries were subjected to freze drying (FD) and spray drying process (B-290, Buchi Labour Technik,AG,Flawil, Switzerland) based on our determined conditions; the content of total polyphenols in aronia powder product was 444.72 ± 4.33 mg GAE/100 ml (*n*=3) whereas the anthocyanin level of powder was 151.30 ± 1.53 mg/100 ml (*n*=3). Aronia berry based new nutritive food products could be utilized in functional food industry as valuable antioxidant sources and could be evaluated as innovative foods.

Key words: Aronia berry, black chokeberry, *Aronia melanocarpa* (Michx.), aronia tea, powder

Introduction

Black chokeberry or aronia (Aronia melanocarpa) is a shrub of the Rosaceae family that is a berry plant native to North America and was transferred to Europe about a century ago (Chrubasik et.al.,2010). Edible parts of black chokeberry are mainly small cherry-like berry parts. The genus Aronia (Rosaceae family) includes two species of shrubs, native to eastern North America and Eastern Canada: Aronia melanocarpa (Michx.) Ell., known as black chokeberry and *Aronia arbutifolia* (L.) Pers. (red\ chokeberry). The aronia berries contain high levels of flavonoids, mostly proanthocyanidins and anthocyanins, and in vitro and in vivo studies indicate that the berries may have potential health benefits, e.g.
hepatoprotective effects, cardioprotective effects, antidiabetes effect and anticancer effects on selected CA cells. The consumption of low levels of antioxidants in the form of fruit and vegetables has been shown to more than double the incidence of certain cancers. (Tokusoglu and Boz, 2019; Tokusoglu 2019; Sidor et.al. 2019; Gavaric et.al.2019; Toli et.al 2015; Savikin et.al.2014).

Unprocessed fresh black chokeberry fruits are generally not consumed routinely owing to their so astringent taste; aronia berries widely consumed as juices, syrups, jams, fruit teas and dietary supplements (Tokusoglu,2019; Tokusoglu and Boz,2019). Chemical composition and biological activity of berries and their products have been widely reported but there are limited works dealing with berry fruit teas. Powder forms of berries and industrial ice-cream form of berries are also utilized as functional food products for nutrition. Tea is popular beverage and currently, herbal
infusions based on dried fruit products have gained in popularity because of their fragrance, fruity flavor,
lower amounts of caffeine, and low astringent and bitter taste. In this proceeding content, aronia based product aronia berry teas was manufactured as functional drink and its and bioactive phenolics were determined by HPLC-DAD and LC-ESIQTOFF-Mass Spectrometry.

Material and Methods

Aronia berry [Aronia melanocarpa (Michx.) (black chokeberry) was harvested at Yalova Research\ Institute, Yalova, Turkey. In our current research, aronia based new products including aronia berry teas (as decoction and infusion types), was developed by Dokuz Eylul University Technology Development Zone Depark Technopark Spil Innova LLC, Izmir Project.

For aronia (chokeberry) powder production, aronia berries were subjected to freeze drying (FD) and spray drying process (B-290, Buchi Labour Technik, AG, Flawil, Switzerland) based on our determined conditions

In manufacturing, decoction method was applied by boiling of aronia berry material in a nonaluminum pot during 8 min until up to two-thirds of the water was evaporated and was strained by home-made tea strain apparatus.

Phenolics were extracted according to Tokusoglu (2019). The obtained extract was used for determination of total phenolic content (TPC), for antioxidant capacity assay by DPPH method and chromatographic analyses including HLPC-DAD and Q-TOFF-MS.

In Q-TOFF-MS Analyses, 30 °C of column temperature, 2 µL of injection volume, flow rate 0.5 ml/min was performed. Gradient elution was as 0–0.5 min, 5% B; 0,5–2 min, 25% B; 2–4 min, 50% B; 4–6 min, 75% B; 6–10 min, 95% B; for column conditioning için 10-16 min, 5%B. For MS analyses, dryer gas flow rate 140 L/min; nebulizer gas pressure

as 35 psi, dryer gas temperature as 290 °C; sheath gas temperature as 400°C; sheath gaz flow as 12 L/min, Agilent Dual Jet Stream elektrosprey ionization (Dual AJS ESI) intermediate surface unit 6550 iFunnel was utilized.

Results and Discussion

After harvesting, the content of total polyphenols of fresh aronia berry was 1012.67 ±34.62 mg GAE/100 ml (n=3) and the monomeric anthocyanin level was 425.65 ±3.65 mg/100 ml (n=3). The content of total polyphenols in aronia powder product was 444.72 ± 4.33 mg GAE/100 ml (n=3) whereas the anthocyanin level of powder was 151.30 ±1.53 mg/100 ml (n=3).

Total phenolics was found as 87.72 ± 0.83 mg GAE/100 ml (n=3) whereas total anthocyanin content was measured according to European Pharmacopoeia 6.0 method with slight modifications. In the study, aronia tea infusion was also carried out. Infusion means achieving a desired taste and aroma results of aronia berry by dissolving a certain proportion of the tea materials into water. This application was performed by using a certain combination of teaware, steeping process, water temperature, water to aronia berry tea ratio. The total phenolics and the anthocyanin level of infusion was determined as 101.02 ±0.55 mg GAE/100 ml (n=3) and 9.05 ±0.05 mg/100 ml (n=3), respectively.

It has been determined phenolic acids, gallic acid, epigallocatechin, catechin, chlorogenic acid, hydroxycinnamic acid, neochlorogenic acid, vanillic acid, siringic acid, caftaric acid, p-coumaric acid, ferulic acid, t-sinapic acid as flavanols (catechins); rutin (quercetin-3- glycoside), kaempferol-3-rutinoside, quercitrin (quercetin-O-glycoside), quercetin, astragalin (kaempferol-O-glycoside), kaempferol as flavonols and their glycosides. It has been identified that chlorogenic acid (ChA) was found major phenolic matter in 3/5/10/15 min brewed aronia teas that were determined as 90.28 mg/100g, 95.63 mg/100g, 188.42mg/100 g, and 195.33 mg/100g, respectively and it has been provided strong antioxidative property ($p< 0.05$). Quercetin (QE) was found major flavonol in 3/5/10/15 min brewed aronia teas that were determined as 307.20 mg/100g; 328.96 mg/100g; 380.56 mg/100g; and 387.25 mg/100g, respectively ($p< 0.05$).

Aronia berry (black chokeberry) fruit teas was found as valuable source of flavonoids and anthocyanins compared to the most of commonly consumed berry teas. Aronia berry based new nutritive tea products could be utilized in functional food industry as valuable antioxidant sources and could be evaluated as innovative teas.

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USE OF GARDEN RADISH IN JOHNSONGRASS CONTROL IN COTTON

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** This article is dedicated to his cherished memory

ABSTRACT

Cotton, an industrial crop, have many problems in producing stage, which include weeds. Johnsongrass (*Sorghum halepense* (L.) Pers.) is among important weeds in cotton as well as many other crops worldwide. Chemical control of johnsongrass and the other weeds in cotton have been the dominant method in traditional cotton and GM cotton. High reliance to herbicides resulted in problems such as herbicide resistant weeds, side effects on non-target organisms, carryover, pollution in environment so on. Farmers have mostly ignored non-chemical methods although alternative methods had been available before chemical methods were invented. Allelopathy is a phenomenon has got attention more since the second half of the previous century. But use of allelopathy in weed control has not been adapted in practice. Garden radish (*Raphanus sativus* L.) has been proved with its allelopathic effect on many weed species including johnsongrass in vitro or in situ in some instances but not implementation of weed control in crop field conditions. In this study, garden radish was applied as cover crop, intermediate cropping (half of the radishes harvested) rotational crop, or organic amendments (whole radish or vegetative parts) comparing to herbicide application (haloxyfop, group A/1) in cotton. All applications controlled johnsongrass, but it was less in amendment applications. Cover crop gave the best cotton yield. Effect of rotational radish regarding to herbicide application was comparable. It is concluded that garden radish can be used in johnsongrass control in cotton as rotational crop as the best economically sound and good technically applicable method. Use of garden radish and other Brassicas needs to be improved. Furthermore, combination of methods should be kept in agenda.

Keywords: Cover crop, intermediate cropping, rotational crop, organic amendments, herbicide application, haloxyfop, ACCase inhibiting herbicide

Introduction

Allelopathy is biochemical interactions among plants including microorganisms as well as biochemical effects of plants on the other organisms or some components of environment (Rice, 1984; Dakshini et al., 1999; Uludag et al., 2006). Allelopathy has role on weed interference and plant invasions as well as crop competitive ability, plant use for medicine, and weed and the other pest control (Uludag et al., 2006; Farooq et al., 2011; Jabran, 2017; Lydon and Duke, 2018; Macias et al., 2019; Zhang et al., 2021). There have been few studies on use of allelopathy in field conditions to control weeds despite huge number of papers dealing with allelopathy has been published (Weston, 1996; Jabran et al., 2015; Rusen et al., 2015; Uludag et al., 2017; Macias et al., 2019; Zhang et al., 2021).

Impact of weeds has been increasing in modern agriculture due to priorities of crop breeding and husbandry techniques as well as misuse of inputs and mis implementations. Widely relying on chemical weed control, with other word herbicides, has created new problems or increased their incidences such as herbicide resistance weeds, pesticide residues in soil and water, non-target effects on all creatures including direct effects on human, which imposes finding out new techniques or improving current ones (Travios et al., 2019; Akın and Uludag, 2021; Heap, 2021). Use of allelopathy is one of innovative options in weed control (Farooq et al., 2011; Jabran, 2017).

Johnsongrass (*Sorghum halepense* (L.) Pers), a perennial plant with Mediterranean origin, is among the prevalent weeds worldwide (Mill, 1985; Holm et al., 1991; McWhorter, 1993; Uludag and Üremiş, 2000; Gozcu and Uludag, 2006; Uludag et al., 2016; Peerzada et al., 2017; Arslan, 2018; Peterson et al., 2020; Klein and Smith 2021). It causes very significant crop losses resulted in economic impact in many crops including cotton (Bridges and Chandler, 1987; Keeley and Thullen, 1989; Wood et al, 2002; Güneş et al., 2008; Klein and Smith, 2021). Season-long competition of one johnsongrass plant in a square-meter can cause around 30% yield loss (Uludag et al, 2007). Different control methods can be used in cotton to control johnsongrass but still herbicides have kept importance (Uludag et al., 1998; Yazlık and Uremis, 2015; Tursun et al., 2016; Ferrell et al., 2020).

The role of Brassica crops on weed control have been studied (Petersen, 2001; Arslan et al., 2005; Uremis et al., 2005; Uremis et al., 2009; Uludag and Uremis, 2016; Jabran, 2017; Rehman et al., 2019; Ozdemir and Uremis, 2019; Kural and Özkan, 2020). Garden radish (*Raphanus sativus* L.) has had special attention with its allelopathic effect on weeds while it was not affecting many crop species (Uygur and Koseli, 1998; Uygur et al., 1990; Dogan, 2004, Arslan et al., 2005; Uremis et al., 2005; Uludag et al., 2006; Rasul and Ali, 2020). Effect of garden radish on johnsongrass and crop species have been reviewed (Uludag et al., 2006). Garden radish has been used to control johnsongrass in cotton in very small extent in the east Mediterranean part of Turkey since 1980's. But it has been just a local knowledge using radish kind of cover crop/rotational crop despite studies has been done so far. The aim of current paper is to reveal effective, reliable, and economic method to use garden radish in cotton fields to control johnsongrass.

Materials and Methods

Experiments were conducted in Agricultural Research Institute fields in the Türkoğlu District of the Kahramanmaraş province in three consecutive years from 2002. Seeds of garden radish was obtained from local populations. In the experiments, cotton cv Maraş-92 and herbicide haloxyfop ethoxyethyl ester were used. Seven treatments including no radish check and herbicide check were established (Table 1).

Table 1. Treatments of the experiments to determine best method(s) for garden radish use to control johnsongrass

Treatments	Abbrevation	Features
No Treatment Check	NTC	No herbicide or garden radish applied
Organic Amendment (Vegetative Parts)	OAV	0.20 kg/m^2 vegetative part of garden radish was added
Organic Amendment (Whole Radish)	OAW	0.15 kg/m^2 of root and 0.15 kg/m^2 vegetative part of garden radish added
Rotational Crop	RC	3/4th of garden radish harvested and remaining was incorporated
Intermediate Implementation	INT	Half of the garden radish harvested and remaining was incorporated
Cover Crop	CC	All garden radish plants kept and incorparated
Haloxyfop Check	HH	Until herbicide applied plot was kept as NTC

Three experiments were set in three consecutive cotton growing seasons (Appendix 1). Experiments were named 2002, 2003, and 2004 after corresponding cotton growing seasons. All experiments were four replications. Garden radish was grown using techniques that have applied by local farmers. Before sowing and two-three stages of radish, 20-20-0 and ammonium nitrate fertilizers were applied in the rates of 60 kg/ha pure phosphorus and 200 kg/ha pure nitrogen. Radish were irrigated five times.

In addition to method determination experiment another experiment were set to assess side effect of garden radish on cotton in 2003 and 2004 using the same procedures and implementation details (Table 2). Two different treatments were established, one was no garden radish CC and the other was no-garden radish. All plots were kept weed free during throughout cotton growing season by hand hoeing and/or hand picking.

All data, namely the number of johnsongrass shoot and seed cotton yield obtained from experiments were underwent to ANOVA and Duncan multiple comparison test using MSTAT-C software. Furthermore, a correlation analysis was run with R software.

An economic analysis was done to estimate income increase due to applications comparing to NTC using the last two years' results of the study and 2004 monetary values. The garden radish yield is considered 40 t/ha with gross income 4000 TRY/ha, total growing cost 1868 TRY/ha, total carrying cost for organic amendments 500 TRY/ha. For cotton, herbicide application cost was 87 TRY/ha and cotton price 0.732 TRY/kg. The conversion rate was 1.7 TRY/Euro.

Results

The first year of experiment the infestation level of johnsongrass was not high enough, then herbicide was not applied. Johnsongrass at cotton harvest time in 2002 was not significantly affected by garden radish treatments. But, the following two years garden radish treatments were significantly affected johnsongrass densities (Table 2). In the early stages of cotton crop before any intervention such as no hoeing, tillage or herbicide applying, cover crop application caused the least shoot number, 1.25 and 0.86 in both 2003 and 2004, respectively while the shoot number was 4.42 and 3.23, respectively in no-garden radish check, which corresponds over 70% pressure (Figure 1). The effect of herbicide assessed around 20 days after its application after first irrigation was highest on johnsongrass (81% in 2003 and 2004) followed by cover crop garden radish where the pressure rate on johnsongrass the same as before any intervention (Figure 2). Garden radish as rotational crop was not effective on johnsongrass as much as cover crop application although the effect intermediate application was in between rotation and cover crop applications (Figure 1 and 2). In addition, the effect of whole plant organic matter on johnsongrass was comparable with rotational crop choice (Table 2). At harvest the effect of applications was still going on, especially in 2003 (Figure 3). It was concluded before that garden radish gave acceptable suppresses on johnsongrass with 70% applied as CC and 50% as INT (Uygur et al., 1991), which are parallel to current results. Haloxyfop reduced johnsongrass shoot number 82% which is similar to earlier studies and the effect of herbicide lasted in closer level (Mueller et al., 1989, Uludag et al., 1998).

Table 2. The effect of garden radish applications on johnsongrass

Treatments	The Number of Johnsongrass in Assessment Times and Years (shoot/ m^2)												
	2002	2003						2004					
	At Harvest	Before Hoeing		After First Irrigation		At Harvest		Before Hoeing		After First Irrigation		At Harvest	
NTC	0.51	4.42	a	4.64	a	1.68	a	3.23	a	2.76	a	0.66	a
OAV	0.61	3.36	b	3.73	b	1.25	b	2.48	b	2.25	b	0.63	ab
OAW	0.41	3.01	bc	3.26	c	0.87	c	2.03	bc	1.44	c	0.53	bc
RC	0.46	2.76	cd	2.75	d	0.60	d	1.67	cd	1.26	c	0.47	c
INT	0.42	2.46	d	2.64	d	0.53	d	1.20	de	0.92	d	0.33	d
CC	0.41	1.25	e	1.51	e	0.52	d	0.86	e	0.69	de	0.26	d
HH	N/A	N/A		0.87	f	0.41	d	N/A		0.51	e	0.23	d
CV (%)	39.9	10.1		11.4		17.5		17.3		14.6		21.6	

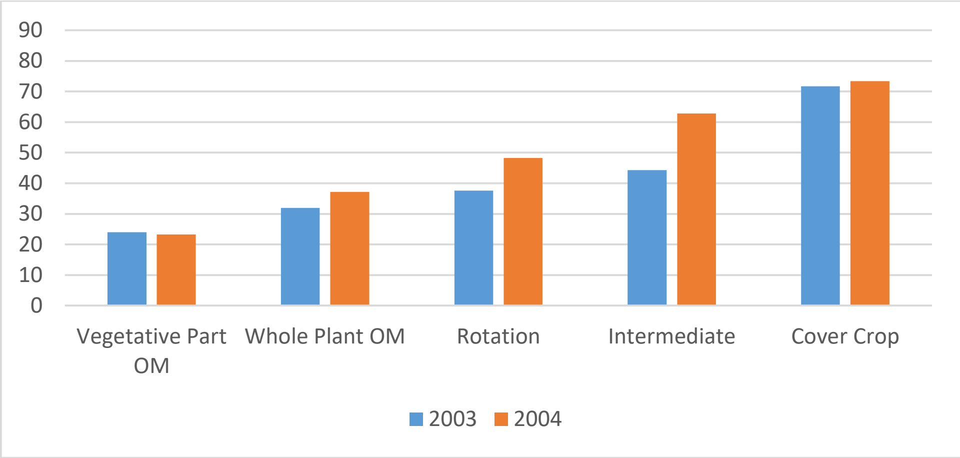

Figure 1. The effect of garden radish applications on johnsongrass (%) before herbicide application

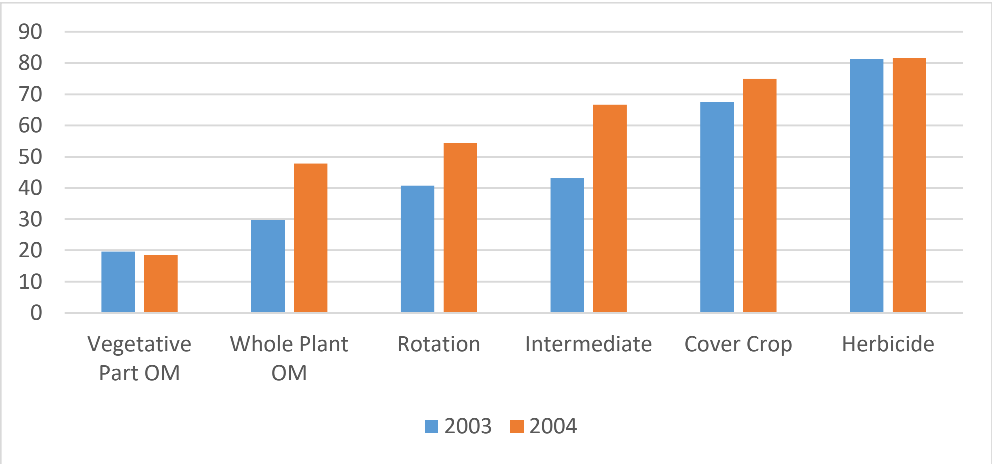

Figure 2. The effect of garden radish applications on johnsongrass (%) at assessment of herbicide application

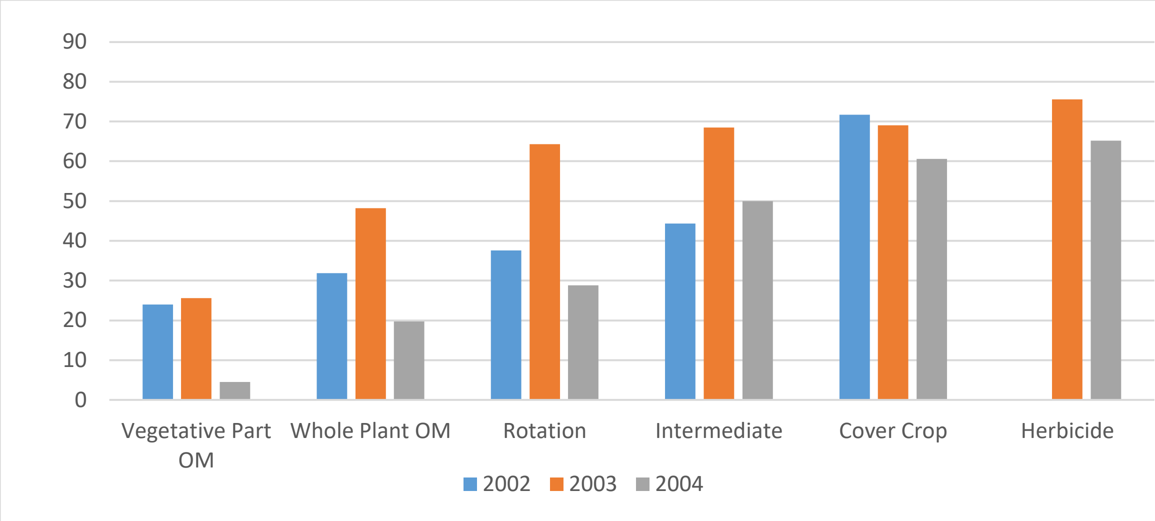

Figure 3. The effect of garden radish applications on johnsongrass (%) at harvest

Seed cotton yield was not affected by treatments in 2002 on the contrary to 2003 and 2004 (Table 3). The highest seed cotton yield was obtained from CC in 2003, and CC and INT 2004. The effect of garden radish applications on johnsongrass were significantly correlated with seed cotton yield in 2003 and 2004 (output is not shown). The correlation between seed cotton yield and johnsongrass control was very low in 2004 when herbicide application was included in the analyses. It can be interpreted that early season effect of johnsongrass on cotton is higher because the effect of radish on johnsongrass was apparent in early season. The lower influence of herbicide application on cotton yield via controlling johnsongrass can be attributed to critical period of weed control (CPWC) for cotton which had been found that starting at 2nd-3rd week after cotton emergence (Tursun et al., 2015; Tursun et al., 2016) because time that herbicide was applied in current experiment might be let johnsongrass compete with cotton in early times of CPWC.

Table 3. The effect of garden radish on cotton yield (kg/ha)

Treatments	2002	2003	2004
NTC	3108	3298 c	2152 d
OAV	2828	3368 bc	2365 c
OAW	3100	3540 bc	2628 b
RC	2933	3635 abc	2640 b
INT	3165	3765 ab	2885 a
CC	2838	3983 a	2850 a
HH	N/A	3775 ab	2235 cd
CV (%)	8.55	6.91	6.04

The side effect assessment experiment showed there was no side effect of garden radish grown as CC on seed cotton yield (Table 4). Studies in field conditions showed that radish does not have side effect on following crops such as maize (Lawley et al., 2011; Sandler et al., 2015) as well as cotton (Uygur et al., 1991)

Table 4. The effect of garden radish on cotton yield to assess side effect

Treatments	2003	2004
With Garden radish	376	234
No Garden radish	369	242
CV	2.78	3.14

Despite the highest johnsongrass control and cotton yield providing method was CC, it cannot be used in practice because of its high cost (Table 5). This makes RC a best option technically and economically.

Table 5. Economic analyses to find out additional income due to garden radish use to johnsongrass control in cotton

Treatments	Garden Radish				Cotton			Overall Profit Increase TRY / ha	Overall Profit Increase Euro / ha
	Incorporated to soil (t/ha)	Income TRY / ha	Cost TRY / ha	Profit TRY/ha	Yield Increase kg/ha	Income due to Increase TRY/ha	Profit TRY / ha		
HH	0	0	0,0	0,0	360	205	118	118	69
OAV	2	0	100	-100	140	102	102	2	1
OAW	3	0	150	-150	360	264	264	114	67
RC	10	3000	1868	1132	410	300	300	1432	842
INT	20	2000	1868	132	600	439	439	571	336
CC	40	0	1868	-1868	690	505	505	-1818	-1069

The results showed that garden radish can control johnsongrass effectively without a side effect on cotton crop. The best results were obtained from no harvest CC option, but it was not economically bearable. Economically the soundest method was rotational crop option with ¾ of crop harvested. Even half is harvested gave comparable johnsongrass control and cotton yield with economically acceptable. These results suggest that there are needs to improve use of garden radish as rotational crop or organic amendment that can be applied easily and economically. Instead of one weed species, several species together should be studied. Following crops' safety needs to be studied. Especially summer Brassicas can be studied as companion crops. Combination of allelopathic crops with other techniques including chemical weed control can be studies as well. It should be kept in mind the necessity of alternative techniques to control weeds and other pests because of combating to climate change, herbicide resistance and environmental safety and some more modern problems.

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Appendix

Appendix 1. Details of experiments

Process	Details	2002	2003	2004
Plots established	28 plots at size 39 m2 (3.9*10) including 6 rows.	15 September 2001	05 September 2002	22 September 2003
Johnsongrass propagules added	To be able to have homogenous plots, 20 rhizomes parts and 50 g seeds of johnsongrass were added to each plot although area was naturally infested by johnsongrass	15 September 2001	05 September 2002	22 September 2003
Garden radish sown to RC, INT, CC plots	Sowing rate was 10 kg/ha	15 September 2001	05 September 2002	22 September 2003
Garden radish sown outside of experiment	To be used in OAV and OAW	15 September 2001	05 September 2002	22 September 2003
Garden radish harvested with a subsoiler	RC and INT plots established via removing harvested percentage	20 December 2001	14 December 2002	27 December 2003
Garden radish added to OAV and OAW		20 December 2001	14 December 2002	27 December 2003
Garden radish shredded and incorporated	RC, INT, CC, OAV, and OAW plots using rototiller and disk harrow	20 December 2001	14 December 2002	27 December 2003
Seed bed preparation for cotton	No soil herbicide applied	20 April 2002	12 April 2003	22 April 2004
Cotton seed sown	Fertilizer applied also as 60 kg/ha Phosphorus and 200 kg/ha Nitrogen	04 May 2002	04 May 2003	06 May 2004
First assessment	In the center four rows. Johnsongrass shoots counted	N/A	08 June 2003	25 May 2004

First tilling and hoeing	All experiment	27 May 2002	08 June 2003	25 May 2004
Haloxyfop application	Only HH plot at 125 g/ha with backpack sprayer mounted flat fan nozzle	N/A	08 June 2003	25 May 2004
The first irrigation	All experiment	20 June 2002	26 June 2003	17 June 2004
Second assessmen Including herbicide efficacy assessing	In the center four rows. Johnsongrass shoots counte and removed with hand hoe	N/A	26 June 2003	17 June 2004
The last irrigation	All field irrigated seven times	18 Septembe 2002	10 Septembe 2003	22 September 2004
The third assessment and Harvest	In the center four rows. Seed cotton data were obtained	10 October 2002	10 October 2003	15 October 2004

NEGATIVE EFFECTS OF GLOBAL WARMING AND CLIMATE CHANGE ON PLANT HEALTH

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ABSTRACT

There are so many signs about negative effects of global warming and climate change over the world. Each living organism has different experiment under this new conditions. Many events such as drought, drying up of lakes, extreme temperature, floods, mucilage tell us to new living conditions although we are not used to, but from now on we have to get used to. The death of so many flamingos in Tuz Gölü (Salt Lake) in Konya province, the excessive flood and landslide in Black See, the drought in so any regions of Turkey especially South and Central Anatolia even East Anatolia Region due to the scarcity of water the loss of quality and yield in agriculture are clear examples of negative effect of global warming or climate change. Moreover, there are similar events in some Europe countries which tell us that floods, droughts and famines that in Africa and Asia would also be happened in other continents from now on. Therefore, all over the world is under the threat and risk of global warming. In addition to drought and global warming, phytosanitary problems such as; combating against new pests and diseases, will increase the negative effects of global warming and climate change even more dramatic. In this article we would like to take attention and increase awareness of public to emphasis negative effect of climate change not only would badly effect human and animal effect, but also plant health would be a problem in the future.

Key Words: Global warming, pest management, early warning system, plant health,

INTRODUCTION

There has been yield and quality loss in so many cultivated plants such as cereal, fresh fruits, vegetables in 2021, due to unexpected drought (the lack of irrigation, rain and water). Only the yield loss in wheat production estimated at least two million tons, it means over 5 billion Turkish Lira. In addition, the yield loss in citrus, fruits, vegetables and other crops, it could give us an idea to estimate how much we are under the risk of global warming (Gürkan et al., 2017a). It could be tolerated for one year, however if this challenge continues for so many years, it would be main cause of so many economic and social trouble of many countries. It also may change the map of agriculture in the next. While some countries will find new opportunities to produce and cultivate new productions even it was impossible before, some countries will not able to produce their main productions, although they could easily produce beforehand (Giorgi and Lionello, 2008).

Similar to human being, plants and animals' reactions to new unexpected conditions, which are much more different than before will change. While global warming is a big challenge and hard experiment for some organisms, it is a big opportunity to live, multiply and widespread for the others. Only increase of a degree temperature or a little bit of humidity change is enough to multiply in a short period for microorganisms such as viruses, bacteria and fungi including insects and weeds. It means that the list of main pest organisms will be changed and increased soon. In this case some new pest organisms will be challenge even they aren't a problem until now, or maybe some of them will be main pest even we have no any idea about them (Bajwa et al., 2020). In addition to drought and other problems, phytosanitary problems will increase the negative effects of global warming and climate change even more dramatic. By using excessive and overuse pesticide will cause residue and resistance problems as well (Turgut, 2021). Therefore, long lasting new strategies, comprehensive projects, and sustainable agricultural plans including innovative studies should be carried out to tackle global warming and climate change. Otherwise, food scarcity could be a main social problem for so many countries (Mendelsohn, 2009; Gürkan et al., 2017b).

There are so many signs about negative effects of global warming and climate change over the world. Each living organism has different experiment under this new conditions. Many events such as drought, drying up of lakes, extreme temperature, floods, mucilage tell us to new living conditions although we are not used to, but from now on we have to get used to.

It could be given an example from Turkey to understand how global warming affects our climate. There has been the death of so many flamingos in Tuz Gölü (Salt Lake) in Konya province, the excessive flood and landslide in Black Sea. There was drought in so many regions of Turkey especially in South and Central Anatolia even East Anatolia Region due to the scarcity of water. As results, it is observed that the loss of quality and yield in agriculture due to negative effect of global warming or climate change (Bayraç and Doğan, 2016).

Moreover, there are similar events in some other countries which tell us that floods, droughts and famines that in Africa and Asia would also be in the other continents from now on. Therefore, all over the world is under the threat of global warming (Rojas-Downing, 2017).

Konya has the second largest number of sinkholes in the World other than Florida. However, reliance on groundwater has seen, underground water levels drop by more than two meters in the past five years, contributing to an increase in massive sinkholes across the province, worrying farmers as they spread closer to residential areas (Figure 1).

Figure 1. A large sinkhole consumes a road in the village of Ekmekçi, Konya, Turkey on June 03. 2021

THE EFFECTS OF THE DROUGH TO PLANT PRODUCTION AND PLANT HEATH

To determine the effect of drought, extreme temperature, global warming on plant health should consider carefully to reduce its effects on future human life. It should be calculated well how the increase of 1°C degree of temperature, or 2-3% change of humidity for pest organisms to guess the real effects on our living organisms in our world. Furthermore, it should be determined that how microorganisms show reaction against these warming and it could be possibly to easy managing of the main pests, diseases and weeds to diminish the effects of the crops which are our main food sources for human in the world (Malhi et al., 2021).

There has been yield and quality loss in so many cultivated plants such as cereal, fresh fruits, vegetables in 2021, due to unexpected drought (the lack of irrigation, rain and water). Only the yield loss in wheat production estimated at least two million tons, it means over 5 billion Turkish Lira. In addition, the yield loss in citrus, fruits, vegetables and other crops, couldgive us an idea to estimate how much we are under the risk of global warming (Bayraç and Doğan, 2016). This situation could be tolerated for one year, however if this problem continues for so many years, it would be main cause of so many economic and social trouble of many countries. The most important point is that this situation could change the map of agriculture in the world. It could be seen that in Figure 1, 2 and 3 how Turkey will affect from drought. While some countries will find new opportunities to produce and cultivate newproductions even it was impossible before, some countries will not able to produce their maincrops, although they could easily produce beforehand.

Figure 2. Drought map of Turkey in recent years

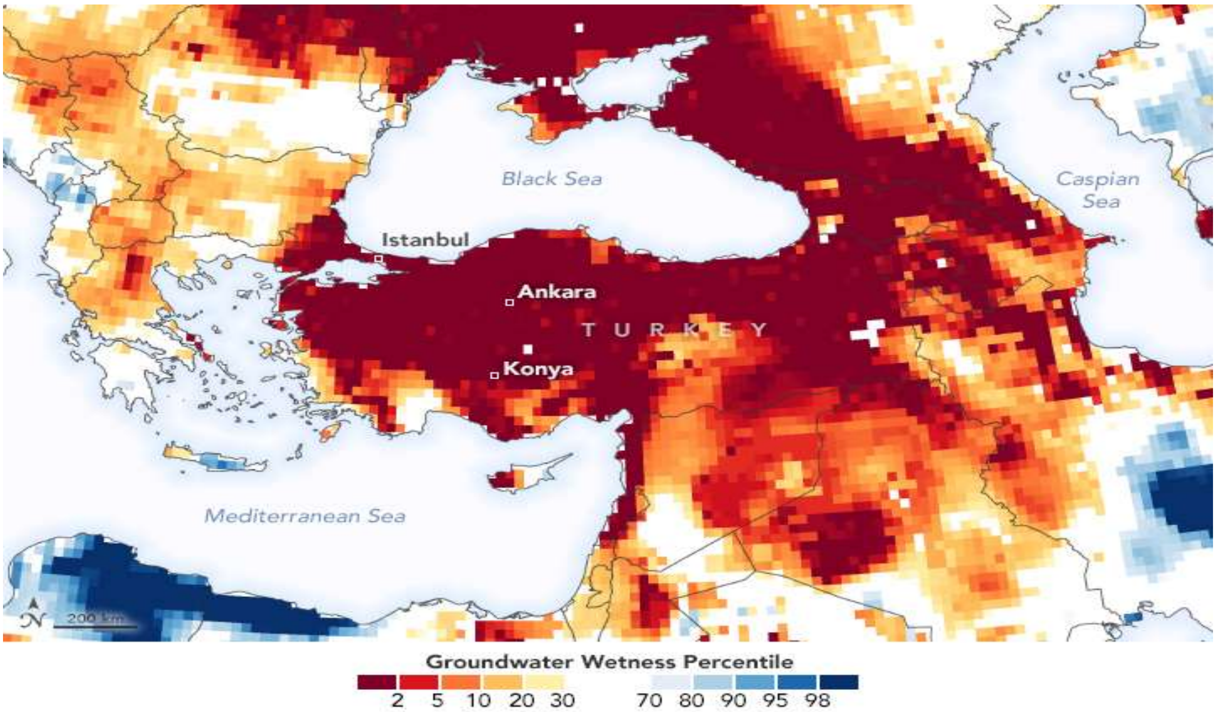

Figure 3. Drought map of Turkey in 2020 (NASA Report, 2021)

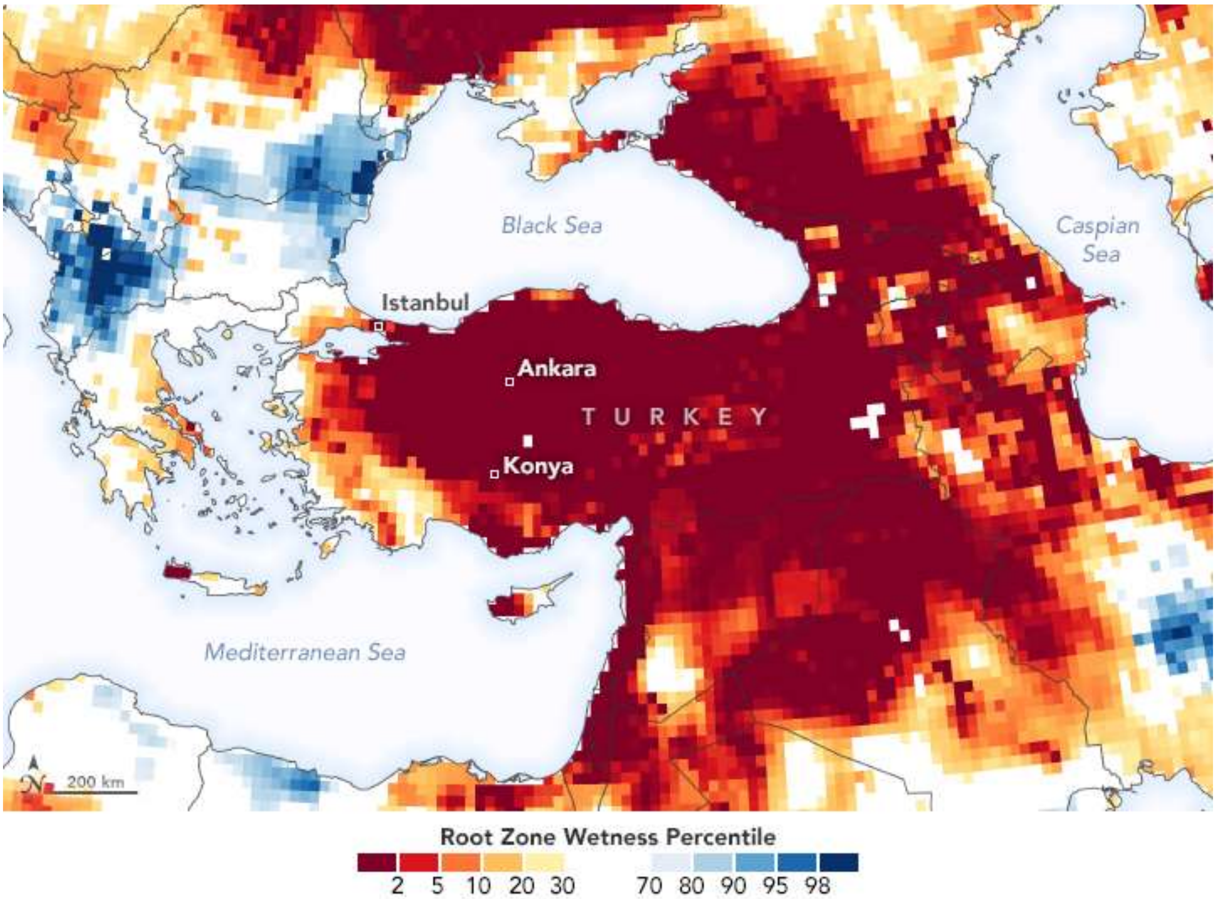

Figure 4. Drought map of Turkey in 2020 (NASA Report, 2021)

Similar to human being, plants and animals' reactions to new challenging, unexpected conditions, which are much more different than before will change after living these recent disasters. While global warming is a big challenge and hard experiment for some organisms, it is a big opportunity to live, multiply and widespread for the others (Ray, 2019; FAO, 2020).

Only increase of a degree temperature or a little bit of humidity change is enough to multiply in a shortperiod for microorganisms such as viruses, bacteria and fungi including insects and weeds. It means that the list of main pest organisms will be changed and increased soon. In this case, some new pest organisms will be challenge even they aren't problem until now, or maybe some of them will be main pest even we have no any idea about them (Misiou and Koutsoumanis, 2021).

In addition to drought and other problems, phytosanitary problems such as; combating against new diseases and pests, will increase the negative effects of global warming and climate change even more dramatic. By using excessive and overdose pesticide will cause residue and resistance problems. Yield and quality loss of production will cause food supply shortage.

SOME SUGGESTIONS TO REDUCE EFFECTS OF THE GLOBAL WARMING

To reduce the serious effects on agricultural productions, there are some suggestions:

- A scientific council could be organized under the responsibility of Ministry of Agriculture and Forestry to take urgent measures intime for each new development.
- An action plan should be published to manage and control outbreak of new pest organisms.
- New strategies, new studies should be carried out in order to establish a sustainable plant health system against global warming and climate change.
- Monitoring, surveys, control programs, technical instructions all should be updated according to new conditions.
- Technical staff should be educated, researchers and lecturer should investigate and share new developments in case of new pestorganism.
- An effective and quick information system should be established among all stakeholders, and awareness of producers about new diseases and pests should be increased.
- By developing new early warning systems via artificial intelligence, it should be ensured that diseases and pests are controlled atthe right time before their widespread.
- With a comprehensive and sustainable action plan, the negative effects of climate change and global warming can be minimized. Thus, by correctly planning the pest management in wide areas, the successful control could be made and resistance and the residue problem of pesticide could be managed with the right method.
- A national Plant Health Comity has been established for coordinating all plant health studies betweendirectorates, universities, unions and other stakeholders for the most important main pest organisms monitoring teams and groups were established.
- Communication and information line established between groups to follow up all development and give answers or orders in time.
- An action plan for main pests has been prepared. We are trying to found new strategies, to establish a sustainable plant health system against global warming and climate

change.

The ministry of Agriculture in Turkey has been trying to develop a new early warning systems via artificial intelligence, which it has alreadyexperienced for Sunny pest since 2015. They were following international development to take action for any spread of pest organisms. They are ready for all stakeholders to collaborate and study for establishing a better control and managementsystem.

DISCUSSION

In order to overcome global warming, climate change including drought and irregular or unexpected extreme rain and flooding conditions, new strategies, new studies should be carried out for each main problem and for each region or country. Projects about breeding including new techniques biotechnological and molecular studies for resistant varieties against pests and diseases, identification of new pest organisms, survey programs, control methods, irrigation, soil treatment, mechanization including artificial intelligence tools and machines should be carried out.

Technical instructions of pests, diseases and weeds should be updated according to new conditions. Technical staff should be educated, researchers and lecturer should investigate and share new developments in case of new pest organism. An effective and quick information system should be established among all stakeholders, and awareness of producers about new diseases and pests should be increased. By developing new early warning systems via artificial intelligence, it should be ensured that diseases and pests are controlled at the right time before they widespread. With a comprehensive and sustainable action plan, the negative effects of climate change and global warming can be minimized. Thus, by correctly planning the pest management in wide areas, both successful control could be made and resistance and residue problem could be managed with the rightest method.

The target is to take all stakeholders attention about the danger of coming global change not only will effect human being, it will also effect plant health with outbreak of pest organisms. In case of plant health badly affected, it means the scarcity of food and feed and maybe famine. The disruption of the natural balance. The use of more pesticide, consuming more effort, time and Money. The loss of yield and quality. Economic and social problems of producers. So many other problems both for producers and consumers and for other living organisms.

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GREEN MASS YIELD AND PLASTID PIGMENTS CONTENT IN WINTER FORAGE PEA LINES

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ABSTRACT

Forage peas hybrid lines №PL, №11, №12A, №13, Taskent, Tore and Mir variety (standard) were studied for green mass yield and plastid pigments content. 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. For the first experimental year green mass yield for location A ranged between 1270 and 2484 kg/da. Lines №13 and №11 showed higher green mass yield than the standard by 23.47% and 38.15%, respectively. Green mass yield for location B ranged between 2144 and 2873 kg/da, and Tashkent variety and №13 showed higher green mass yield than the standard by 6.12% and 7.87%, respectively. For the second year green mass yield for location A ranged between 2144 and 2873 kg/da. №PL and №13 lines showed higher green mass yield than the standard by 6.94% and 7.20%. Green mass yield for location B ranged between 1158 and 1420 kg/da and №13 only showed higher green mass yield than the standard by 10.94%. On average for the period green mass yield for location A ranged between 1960 and 2547 kg/da and №11 and №13 lines showed higher green mass yield than the standard by 13.51% and 13.73%, respectively. Green mass yield for location B ranged between 882 and 1049 kg/da and Tore variety and №13 showed higher green mass yield than the standard by 8.13% and 9.87%, respectively. As a whole green mass yield was found higher for location A as compared to location B. The data were in correspondence with plastid pigments content: for location A - 304.48 mg/100 g FW and for location B - 282.18 mg/100 g FW.

Key words: forage pea, green mass, plastid pigments

INTRODUCTION

The global challenge of agriculture in the 21st century is to meet the increasing food demand of the growing world population. The need for protein-rich crops for human food or farm animals has led to greater interest in these types of plants. Pea as a legume crop plays an important role as a source of vegetable protein in solving the protein problem of livestock production.

Peas are a crop that has long been grown and cultivated, and is certainly of growing importance for global agricultural production. The need for protein-rich products for food for both humans and farm animals has led to greater interest in this crop. It is resistant to adverse climatic conditions and is one of the main crops suitable for growing in different soil and climatic conditions. It uses abiotic factors very effectively, such as soil and atmospheric temperature, and soil and air humidity. Pea has a relatively short vegetation period and provides the possibilities for additional economic use of the agricultural land.

This crop has a high intensity of formation of green mass yield in April and May, and grain in May, as for the northern parts until early June. This part of the pea vegetation has a favorable

temperature and water regime, which determines the reliable productivity of the crop, even in adverse weather years. Peas leaves behind nitrogen in the soil, as well as accumulates root mass, the decomposition of which preserves and/or improves soil fertility. It has been found that winter forms accumulated more root biomass than spring ones (Vasileva and Kosev, 2015).
The biological characteristics of pea enable it to be successfully grown as a winter crop. Winter pea play an important role in the southern regions The intensive growth and development of winter pea varieties occur during the period May-June, when rainfall is sufficient to ensure an intensive growth without irrigation. Its advantage as a crop is not only to ensure high-protein value, but also as a stable source of green mass (Brezhneva and Brezhnev, 2014).
One of the most important factors determining productivity is the process of photosynthesis. Through it, plants accumulate organic matter and energy (Smirnova et al., 2013). Photosynthetic pigments - chlorophyll a and b, and carotenoids are responsible for the absorption and transformation of solar energy. Chlorophyll a is basic chlorophyll, which provides higher efficiency of the process of conversion of carbon dioxide and water into organic compounds. Carotenoids also perform a protective function against chlorophyll photooxidation and prevent destructive photooxidation of protoplasmic organic compounds in the presence of free oxygen (Gilmore and Govindjee, 1999).
The content of photosynthetic pigments is one of the indicators of the reaction of plants to changes in environmental factors and the degree of adaptation to new environment (Titova, 2010, Nurmakova, 2013). Thus, they are one of the indicators of the physiological status of plants. The work aimed at to study winter forage pea lines in reference to green mass yield and plastid pigments content.

MATERIAL AND METHODS

Forage peas hybrid lines №PL, №11, №12A, №13, Taskent, Tore and Mir variety (standard) were studied for green mass yield and plastid pigments content. 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 (43^{o} 23’N, 24^{o} 34’E, 230 m altitude), podzolized soil subtype; location B (43.41^{o} N, 24.61^{o} E), haplustoll soil subtype.
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. At the full bottom pods stage the green mass yield (kg/da) was recorded. In fresh plant samples plastid pigments content (chlorophyll a, chlorophyll b, and carotenoids) (mg/100 g FW), total pigments content [(chlorophyll a + chlorophyll b) + carotenoids) was determined according Zelenskii and Mogileva (1980). Chlorophyll a to chlorophyll b ratio and chlorophyll a + chlorophyll b to carotenoids ratio were calculated. Experimental data are presented by year and average for the study period. They were statistically processed using STATGRAPHICS Plus for Windows Version 2.1.

RESULTS AND DISCUSSION

It is common knowledge that the level of plant productivity depends on the interaction of the genotype with environmental factors. Essential for the formation of productivity in legumes, in particular in pea, is sufficient moisture during the growing season and the required amount of active temperature.
The sowing of the winter forage pea was carried out at the end of the third decade of the month of October. The whole month of October was characterized by precipitation (108.9 mm/m2), which exceeds by two times the mean value for the 20-year period, as well as normal daily

average temperatures (Figure 1). In February and March the amount of rainfall was above the norm for the month. The months of April and May were extremely dry. In May the temperatures were high, with a rainfall of economic significance. The stages of beginning of flowering to full bottom pods were running in these conditions. The many rainfall fallen in June have not influenced the green mass yield, as it has already been formed. In the first location the quantity of rainfall fell was greater, which determined the longer vegetation period, as well as the higher green mass yield of the late cultivars/lines.

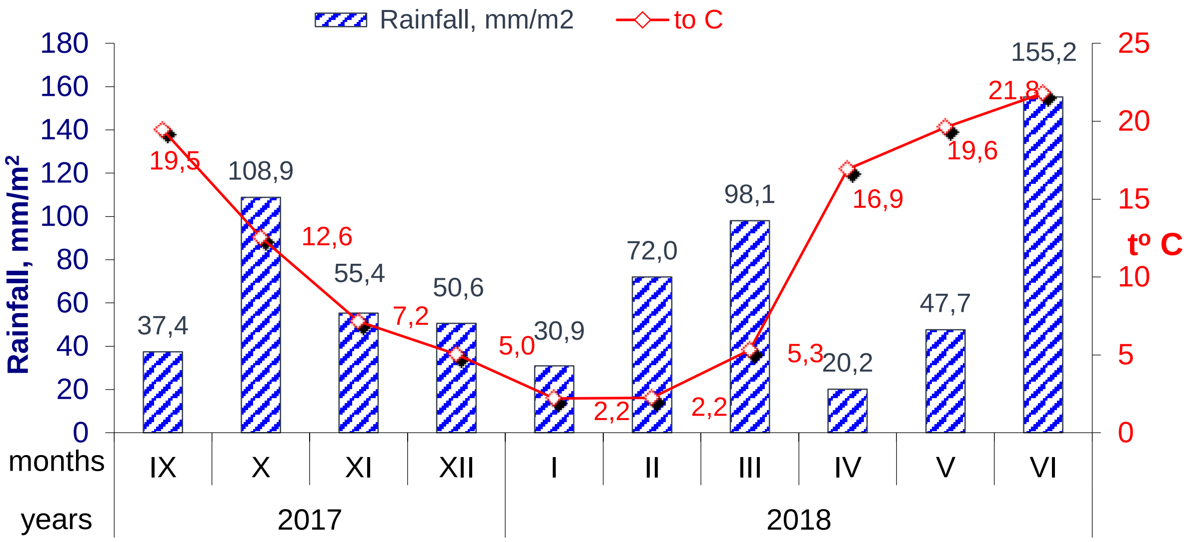

Figure 1. Agro meteorological conditions for the period of September 2017 to June 2018 (Pleven, Bulgaria)

The winter months of 2019 were characterized by below-normal rainfall compared to the 20-year period and higher average daily monthly temperatures (Figure 2). Small amounts of precipitation were reported in mid-March. This favored the initial growth of the crop. In April, a higher average temperature and precipitation was more than twice as high as for a twenty-year period.

Throughout May, precipitation was evenly distributed with amounts slightly above the average for the month and a favorable average daily temperature. This affected the growth and development of pea. The month of June was characterized by precipitation during all three days and the amount of precipitation for the month was slightly above the value for a twenty-year period.

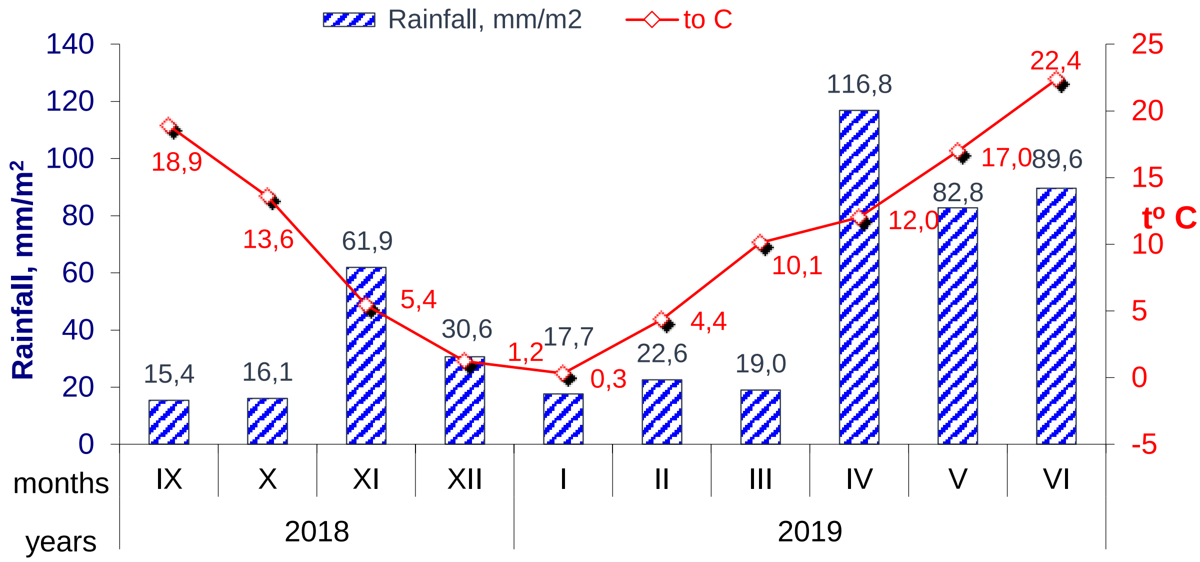

Figure 2. Agro meteorological conditions for the period of September 2018 to June 2019 (Pleven, Bulgaria)

As a complex quantitative trait, the formation of fresh biomass is directly dependent on changing climatic conditions. For the first experimental year green mass yield for location A ranged between 1270 and 2484 kg/da. Lines №13 and №11 showed higher green mass yield than the standard by 23.47% and 38.15%, respectively (Table 1). They managed in the unfavorable environment to fully exert their biological potential and to accumulate more green mass. They accumulate with 422.0 and 686.0 kg/da additional green mass yield as compared to the standard. In other study we found, the line No. 13 managed in the most unfavorable environment to fully manifest their biological potential and to accumulate more green mass (Vasileva et al., 2020). In addition, in previous study, the line No. 13 was identified as the latest flowering line.

Table 1. Green mass yield from winter forage pea cultivars/lines, 2018

Cultivar/Line	Green mass yield (kg/da)	
	Location A	Location B
Mir	1798	629
№PL	1620	605
№11	2484	617
№12A	1331	596
№13	2220	679
Taskent	1775	668
Tore	1270	729
Average	1785	646
STDEV	443	48
Max	2484	729
Min	1270	596
CV%	24.8	7.4

During the study period, favorable conditions for pea development were 2018, when the varieties and lines tested were able to reach their biological potential to a great extent. At the same time, it should be noted that different genotypes respond differently to stressful situations.

For the second year green mass yield for location A ranged between 2144 and 2873 kg/da (Table 2). №PL and №13 lines showed higher green mass yield than the standard by 6.94% and 7.20%. Green mass yield for location B ranged between 1158 and 1420 kg/da and №13 only showed higher green mass yield than the standard by 10.94%.

Table 2. Green mass yield from winter forage pea cultivars/lines, 2019

Cultivar/Line	Green mass yield (kg/da)	
	Location A	Location B
Mir	2680	1280
№PL	2866	1158
№11	2599	1233
№12A	2736	1219
№13	2873	1420
Taskent	2144	1302
Tore	2774	1336
Average	2667	1278
STDEV	251	86
Max	2873	1420
Min	2144	1158
CV%	9.4	6.7

According to Vozian et al. (2017) drought is one of the most dangerous natural phenomena in the climate due to the uneven distribution of atmospheric precipitation against the background of high air temperatures. The author finds that the likelihood of severe droughts during the growing season is 11-41%.

On average for the period green mass yield for location A ranged between 1960 and 2547 kg/da and №11 and №13 lines showed higher green mass yield than the standard by 13.51% and 13.73%, respectively (Table 3).

Green mass yield for location B ranged between 882 and 1049 kg/da and Tore variety and №13 showed higher green mass yield than the standard by 8.13% and 9.87%, respectively. As a whole green mass yield was found higher for location A as compared to location B. The coefficient of variation was found higher for location A green mass yield.

Table 3. Green mass yield from winter forage pea cultivars/lines av. for 2018 and 2019

Cultivar/Line	Green mass yield (kg/da)	
	Location A	Location B
Mir	2239	955
№PL	2243	882
№11	2542	925
№12A	2034	908
№13	2547	1049
Taskent	1960	985
Tore	2022	1033
Average	2226	962
STDEV	242	63
Max	2547	1049
Min	1960	882
CV%	10.9	6.6

We also determined the content of plastid pigments in the green mass. The data were in correspondence with plastid pigments content: for location A - 304.48 mg/100 g FW and for location B - 282.18 mg/100 g FW (Table 4 and Table 5).

Table 4. Plastid pigments content in the winter forage pea cultivars/lines (Location A) (mg/100 g FW), average for 2018 and 2019

Cultivar/Line	Cl a	Cl b	Cl a+b	Carotin	(Cl a+b)+Car
Mir	133.14	108.57	241.71	31.29	273.00
№PL	155.10	134.37	289.47	39.90	329.37
№11	126.00	148.47	274.47	32.64	307.11
№12A	133.59	92.04	225.63	37.14	262.77
№13	133.35	147.78	281.13	37.35	318.48
Taskent	159.63	149.46	309.09	40.14	349.23
Tore	115.62	143.37	258.99	32.40	291.39
Average	136.63	132.01	268.64	35.84	304.48
STDEV	15.58	22.75	28.66	3.69	30.90
Max	159.63	149.46	309.09	40.14	349.23
Min	115.62	92.04	225.63	31.29	262.77
CV%	11.40	17.23	10.67	10.30	10.15

Table 5. Plastid pigments content in the winter forage pea cultivars/lines (Location B) (mg/100 g FW), average for 2018 and 2019

Cultivar/Line	Cl a	Cl b	Cl a+b	Carotin	(Cl a+b)+Car
Mir	121.31	100.95	222.26	29.61	251.87
№PL	121.59	131.27	252.86	36.09	288.95
№11	116.36	129.93	246.29	31.22	277.51
№12A	122.48	88.99	211.47	35.61	247.08
№13	144.32	125.66	269.98	30.99	300.97
Taskent	141.52	136.57	278.09	38.96	317.05
Tore	122.34	136.11	258.45	33.41	291.86
Average	127.13	121.35	248.49	33.70	282.18
STDEV	11.01	18.72	24.22	3.35	25.43
Max	144.32	136.57	278.09	38.96	317.05
Min	116.36	88.99	211.47	29.61	247.08
CV%	8.66	15.43	9.75	9.94	9.01

The degree of formation of the photosynthetic apparatus is judged by the ratio of chlorophyll a to chlorophyll b. As reported by Yokoya et al. (2007) and Zhao et al. (2016), these photosynthetic pigments are responsible for collecting and transmitting absorbed light to photosynthetic reaction centers, and their concentration is linked to the effectiveness of photosynthesis. In addition, according to Zhao et al. (2016), increased content of these pigments may be one of the factors increasing photosynthetic activity. This ratio is related to the activity of the basic chlorophyll a. It is a relatively constant quantity and is considered to be genetically determined (Petkova and Poryazov, 2007; Titova, 2010). The values obtained in our study for the ratio of chlorophyll a to chlorophyll b varied in the range 0.806 - 1.451 for location A and from 0.896 to 1.376 for location B (Figure 3 and Figure 4).

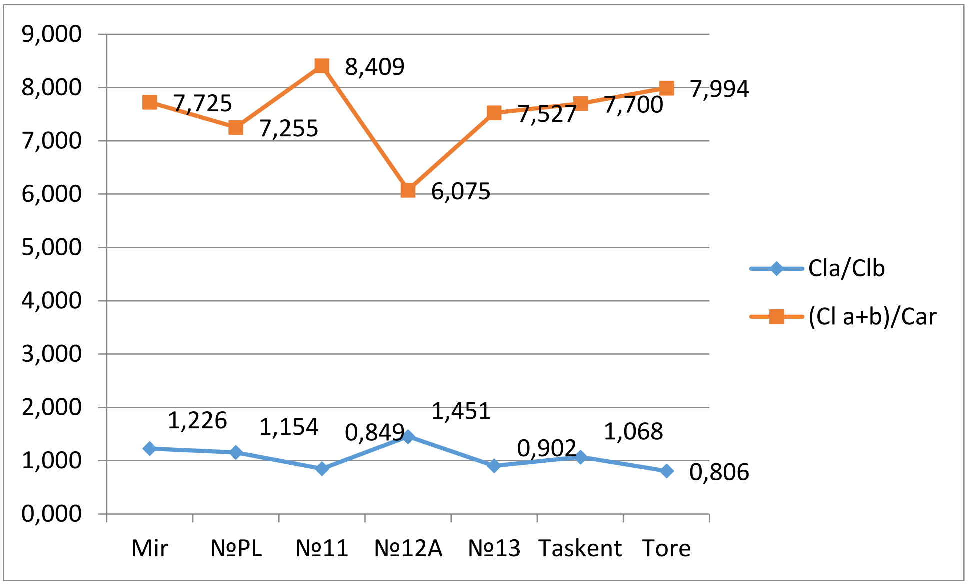

Figure 3. Chlorophyll a to chlorophyll b ratio and chlorophyll a + chlorophyll b to carotenoids ratio in the winter forage pea cultivars/lines (Location A)

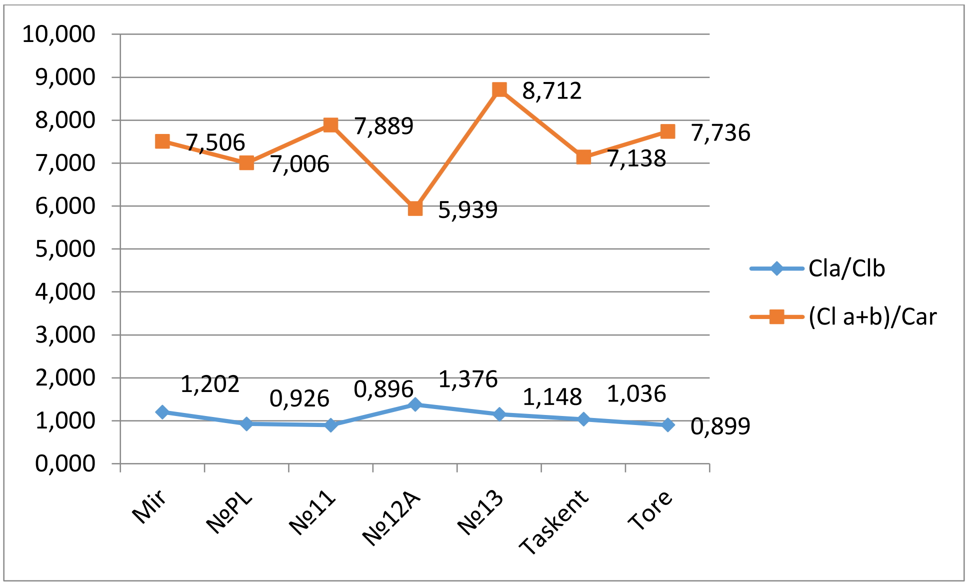

Figure 4. Chlorophyll a to chlorophyll b ratio and chlorophyll a + chlorophyll b to carotenoids ratio in the winter forage pea cultivars/lines (Location B)

Sherepitko et al. (1990) as a result of their research considers that correlation relationships allow to determine the criteria for selection of new idiotypes with greater adaptability to environmental conditions, including a modified leaf shape, reduction of vegetative branches on the main stem, changing the angle that the leaves make to the stem. We found significant correlation between green mass yield for location B and total plastid pigments content (r=0.510).

CONCLUSIONS

After the studying of forage peas hybrid lines №PL, №11, №12A, №13, Taskent, Tore and Mir variety (standard) for green mass yield and plastid pigments content in two locations we found for the first experimental year green mass yield for location A ranged between 1270 and 2484 kg/da. Lines №13 and №11 showed higher green mass yield than the standard by 23.47% and 38.15%, respectively. Green mass yield for location B ranged between 2144 and 2873 kg/da, and Tashkent variety and №13 showed higher green mass yield than the standard by 6.12% and 7.87%, respectively. For the second year green mass yield for location A ranged between 2144 and 2873 kg/da. №PL and №13 lines showed higher green mass yield than the standard by 6.94% and 7.20%. Green mass yield for location B ranged between 1158 and 1420 kg/da and №13 only showed higher green mass yield than the standard by 10.94%. On average for the period green mass yield for location A ranged between 1960 and 2547 kg/da and №11 and №13 lines showed higher green mass yield than the standard by 13.51% and 13.73%, respectively. Green mass yield for location B ranged between 882 and 1049 kg/da and Tore variety and №13 showed higher green mass yield than the standard by 8.13% and 9.87%, respectively. As a whole green mass yield was found higher for location A as compared to location B. The data were in correspondence with plastid pigments content: for location A - 304.48 mg/100 g FW and for location B - 282.18 mg/100 g FW.

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EVALUATION OF UNIVERSITY FEMALE STUDENTS IN BREAST CANCER SCREENING WITH CHAMPION'S HEALTH BELIEF MODEL SCALES

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ABSTRACT

Among all cancers, breast cancer has very high cancer-related morbidity and mortality rates worldwide. Our study is an example of Trakya University Health School of the latest version of Champion's Health Belief Model Scales (CHBMS) adapted to Turkish for breast cancer screening. Young women's beliefs about "breast self-examination" and their behavior was measured in our study. For this reason, three dimensions of the scale related to BSE and common dimensions were used. When the breast cancer screening behaviors and beliefs of women are measured; all dimensions of the scale were used. 233 female university student volunteers were included in the study. The study was conducted with online interviews with the participants. The results obtained from the scale; It was evaluated at the level of 3 basic sub-dimensions. The first sub-dimension; It was a breast self-exam. 2nd sub-dimension; It was based on sensitivity, caring and health motivation. The third sub-dimension was the level of knowledge about breast pathology imaging techniques. It was observed that they did not have sufficient knowledge about mammography and breast ultrasonography. Those who did breast self-examination had higher scores from the 3 sub-dimensions of the scale. Those living in an extended family compared to those living in a nuclear family; their scores on the scale were lower. The scores of those with low socioeconomic status from the scale were lower than those with high socioeconomic status. In our study, with the CHBMS-Turkish scale; The breast cancer awareness levels of young University Turkish women and the factors affecting their breast cancer screening behaviors were evaluated. It has also been shown that CHBMS-Turkish forms can be used in experimental research designs to raise awareness and raise the quality of young women's beliefs and behaviors about breast cancer and screening behaviors. Thus, it can be used in screening studies for early diagnosis of breast cancer in the field of women's health. The data of our study; It will also be useful for healthcare professionals as a pre-test-post-test data collection tool to evaluate the effectiveness of breast cancer screening projects.

Keywords: Breast cancer, University Students, Health Beliefs, Women's Health, Champion's Health Belief Model Scale

INTRODUCTION

Of all cancers, female breast cancer has very high cancer-related morbidity and mortality rates worldwide. Breast cancer is the most common malignancy in women worldwide (Cancer, 2019). Women with breast cancer are usually diagnosed in advanced stages. Many women miss the opportunity for early diagnosis and treatment due to the lack of knowledge, experience and awareness about breast cancer, as well as the lack of care in cancer screening practices (Cancer, 2019; Gözüm et al., 2004). Even in our country, which is a country with free health services, very low breast cancer screening rates are reported compared to the population (Özdemir et al., 2020).

To improve breast cancer screening and address the barriers to breast cancer screening, young college-educated and continuing health education women in the community should be evaluated. Breast self-exam (BSE), mammography and clinical breast examination (CBE) are accepted as screening methods for early detection of breast cancer. Although we are one of the countries where mammography and clinical breast examinations can be done easily, breast cancer screenings cannot reach the desired levels.

Aim

Our study was designed to examine the perception of breast cancer and BSE among young women in undergraduate health education through the following methods:

1) Evaluation of young women's beliefs and attitudes about breast cancer and breast self-examination using the health belief model,

2) Determining the level of knowledge about breast cancer and practices related to breast cancer,

3) Identifying behaviors related to breast cancer and

4) Determining the factors affecting the BSE practice.

MATERIAL AND METHOD

Our study was carried out in Trakya University Health Sciences University volunteer students between the ages of 18-25. Age, gender, sociodemographic characteristics were recorded in order to evaluate the participants. Trakya University School of Health is an example of the latest version of Champion's Health Belief Model Scale (CHBMS) adapted to Turkish.

The validity and reliability of the Turkish version of the CHBMS were also found sufficient in the studies. Turkish versions of the scale are used safely to identify and develop beliefs and behaviors related to breast cancer.

Turkish versions of the scale are used safely to identify and develop beliefs and behaviors related to breast cancer. The Champion's Health Belief Model Scale on breast cancer screening has been adapted into Turkish by different studies almost in recent times, unaware of each other (GÖZÜM et al., 2004; Gözüm, Karayurt, Kav, & Platin, 2010). Although it is difficult to carry out a methodological study several times for a country with limited economic opportunities, the extensive database obtained on the subject has also been an advantage as it provides a lot of evidence for the validity and reliability of this adapted measurement tool. Evidence for the validity and reliability of the Turkish version of the scale has been provided by these adaptation studies conducted in different regions and different samples in Turkey.

General features of Champion's Health Belief Model Scale:

In CHBMS (health belief model); It is a scale consisting of dimensions for the concepts discussed. The scale, developed by Victoria Champion in 1984 to measure beliefs about breast cancer and BSE, includes five sub-dimensions and 39 items (Champion & Scott, 1997; Champion et al., 2004). In his study of 581 women selected randomly in 1993, Champion revised the five sub-dimensions of the scale related to breast cancer and BSE and added the sub-dimension of trust/self-efficacy. In 1997, Champion revised the BSE-related dimensions of the scale in a sample group of 329 African-American women and added two new mammography-related sub-dimensions (mammography barriers and mammography benefits). (Champion & Scott, 1997). The revision of the scale that we could reach; It was done in 1999 in mammography and sensitivity dimensions (Champion, 1999).

Ethical approval was obtained from the scientific research ethics committee of Trakya University Faculty of Medicine for the study. Informed consent was obtained from the students participating in the study.

RESULTS

The study was carried out between February 2021 and June 2021 with a volunteer student studying health sciences at Trakya University in Turkey. 233 female university students were included in the study. The age range of the participants was 19-25. After informing the participants about the study, informed consent was obtained from each of them. Volunteer participants; An interview form was filled during the interview. An online interview was held with the participants. Then, they were asked to fill out the prepared questionnaires via Microsoft Forms. In this form, sociodemographic characteristics (age, height, body weight, education, family income level) were asked and scale questions were asked. For the participants; Those with known internal chronic diseases, psychiatric diseases, cigarette and alcohol users were not included in the study.

Our study is an example of Trakya University Health School of the latest version of the Champion's Health Belief Model Scale (CHBMS) for breast cancer screening, adapted into Turkish. For the girls in our study, p values were recorded for each sub-dimension. Among the subtests, only first-degree relative was statistically different (p=0.004).

“Sensitivity”, “caring/seriousness” and “health motivation”, “barriers” related to BSE, “benefits” “self-efficacy/confidence”, “benefits” related to mammography, evaluating the judgment of the individual about breast cancer and general health of the scale. and “barriers” dimensions. The results were evaluated at the level of 3 main sub-dimensions. The first sub-dimension; It was a breast self-exam. The "barriers", "benefits" and "self-efficacy/confidence" of BSE were evaluated. The second sub-dimension; It was based on sensitivity, caring and health motivation. The third sub-dimension was the level of knowledge about breast pathology imaging techniques. It was observed that they did not have sufficient knowledge about mammography and breast ultrasonography. In our study, the beliefs and behaviors of young women about "breast self-examination" were measured. Three dimensions of the scale related to BSE and common dimensions were used.

When the breast cancer screening behaviors and beliefs of the participants were measured; All dimensions of the scale were used. In the evaluation of the scale, 5-point Likert-type scaling ranging from 1 to 5- “strongly disagree” (1), “disagree” (2), “undecided” (3), “agree” (4), “strongly agree” (5)- method was used. Each dimension of the scale was evaluated separately, not combined into a single total score. Accordingly, scores were obtained as the number of dimensions used for each individual. The scale took approximately 12–15 minutes to complete.

The participants in our study; The distribution of breast cancer (BC) and BSE according to their knowledge and practices was examined. Most of the women in the study (73.4%) were from public campaigns, mostly for educational purposes; (24.6%), media (20.7%) and social media (28.1%) had heard of BSE. Regarding the participants' BSE information, about half of all women (44.7%); BSE reported onset age as ≥20. Only 33.6% of the participants stated that monthly BSE should be done. The reasons for performing BSE, as reported by 50 women, were: regularly examining their breasts (52.3%) and checking the progression of some abnormal changes (37.6%) or doctor's recommendation (10.1%).

As reported by 183 women, the reasons for not performing BSE were: not knowing how to examine their breasts (52.8%) or not trusting that they could do it (47.2%). Those who did breast self-examination had higher scores from the 3 sub-dimensions of the scale. Those living in an extended family compared to those living in a nuclear family; their scores on the scale were lower. Participants with low socioeconomic level; their scores on this scale were lower than those of the higher ones.

Table 1. Variation of the scores obtained from the Champion's Health Belief Model Scale according to the geographical regions of the participants

Subscales	Those coming from the Marmara, Aegean and Mediterranean Regions Mean (SD)		Those coming from Eastern Anatolia, Southeastern Anatolia, Black Sea Regions Mean (SD)		*p*
	Marmara, Aegean (n=58)	Mediterranean, Central Anatolia (n=70)	Eastern & Southeastern Anatolia (n=40)	Black Sea region (n=65)	
1.1	9.50 (2.06)	11.7 (2.1)	10.22 (2.50)	12.3 (3.5)	0.053
1.2	4.64 (1.14)	4.3 (1.4)	4.51 (0.84)	4.0 (0.9)	0.453
1.3	5.84 (0.99)	5.5 (0.0)	5.93 (1.08)	5.9 (1.0)	0.582
2.1	6.99 (1.36)	6.7 (1.1)	2.42 (1.24)	6.4 (0.9)	0.856
2.2	2.33 (0.51)	…	7.03 (1.45)	…	0.476
3.1	3.80 (0.62)	3.1 (0.5)	3.50 (0.53)	3.0 (0.4)	0.004*
3.2	4.60 (0.80)	3.2 (0.5)	4.45 (0.63)	3.0 (0.5)	0.240

Information about breast cancer (BC); The mean score of the percentage of general knowledge was (44.2%), indicating a low level of knowledge. BC was highest (54.6%) for risk factors information and lowest (31.6%) for warning signs information. Subtests 2.1 and 1.3 in our study; the results obtained from the third year students; It was seen that they scored better than the first and second graders. Regarding knowledge of screening measures, nearly half of the participants reported that they knew how to do BSE, and less than half (31.1%) of mammography (31.1%) and clinical breast examination (24.2%).

Regarding breast cancer (BC) information, most of the participants (68.2%) reported that they thought it was treatable in its early stages. 40.4% of the participants considered it fatal if left untreated. Only 17.2% of the participants stated that they knew it was common in obese people. 16.1% reported that it can occur in both breasts and should be evaluated.

Table 2. For some subtests of the CHBMS, the scores of the participants were evaluated according to their grade levels.

SUBTESTS		First Year students	Second Year students	Third Year students
		Mean (SD)	Mean (SD)	Mean (SD)
Subtest 1.1.				
		4.88 (1.46)	4.80 (1.1)	4.34 (1.05)
Subtest 1.3				
		6.27 (1.11)*	6.00 (1)	6.61 (1.02)
Subtest 2.1				
		2.70 (2.38)*	3.80 (0.7)	3.95 (0.84)
Subtest 2.2				
		3.70 (0.60)	3.30 (0.6)	3.12 (0.59)
Subtest 2.3				
		4.89 (1.34)	3.3 (0.5)	3.25 (0.53)

Some areas of the participants' sociodemographic characteristics were examined. The difference between the health vocational high school graduates and the non-vocational high school graduates was not statistically significant. However, in the results obtained from the health vocational high school graduates, it was seen that they completed the test in a shorter time. The first two sub-dimension scores of those who used the internet more than 3 hours in a day were better than those who used the computer less (p=0.018 and p=0.011). İ

No significant difference was found in other sociodemographic characteristics examined. There was no difference in terms of family income level. There was no difference in scale results between the part-time and non-working participants.

DISCUSSION

In our study, with the CHBMS-Turkish scale; The factors affecting breast cancer and breast cancer screening behaviors of young university Turkish women were evaluated. The main reasons for not doing BSE, not knowing how to examine their breasts, were the main reasons for poor practice. Women with a high perception of BSE confidence have a lower risk of not performing BSE, and women with low BSE barriers have a higher potential to perform BSE. Education is an important supporter of BSE practice.

It has also been shown that the CHBMS-Turkish forms can be used in experimental research designs to increase the quality of young women's beliefs and behaviors about breast cancer and screening behaviors and to raise awareness. Thus, it can be useful as a pre-test-post-test data collection tool to evaluate the effectiveness of the projects for health professionals who will be involved in screening studies for the early diagnosis of breast cancer in the field of women's health.

CONCLUSIONS

The results of the study may provide a baseline for future intervention programs to promote early detection and early management of breast cancer. In this study, it was observed that women had insufficient knowledge about breast cancer, they reported negative attitudes towards BSE, and their practices were weak.

The role of health providers was limited, reflecting the need for health professionals to develop awareness programs. Our findings show that continuing education programs are needed to increase knowledge and change attitudes, trust and behavior towards BSE. BSE should be emphasized in undergraduate and graduate courses.

Limitations

Our study was conducted on a limited number of university students. We recommend that future studies be conducted in more universities.

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Scientific confirmation

Approval for the study was obtained from the Scientific Research Board of the Ministry of Health of the Republic of Turkey before the study.

Conflict of interest

There is no conflict of interest.

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