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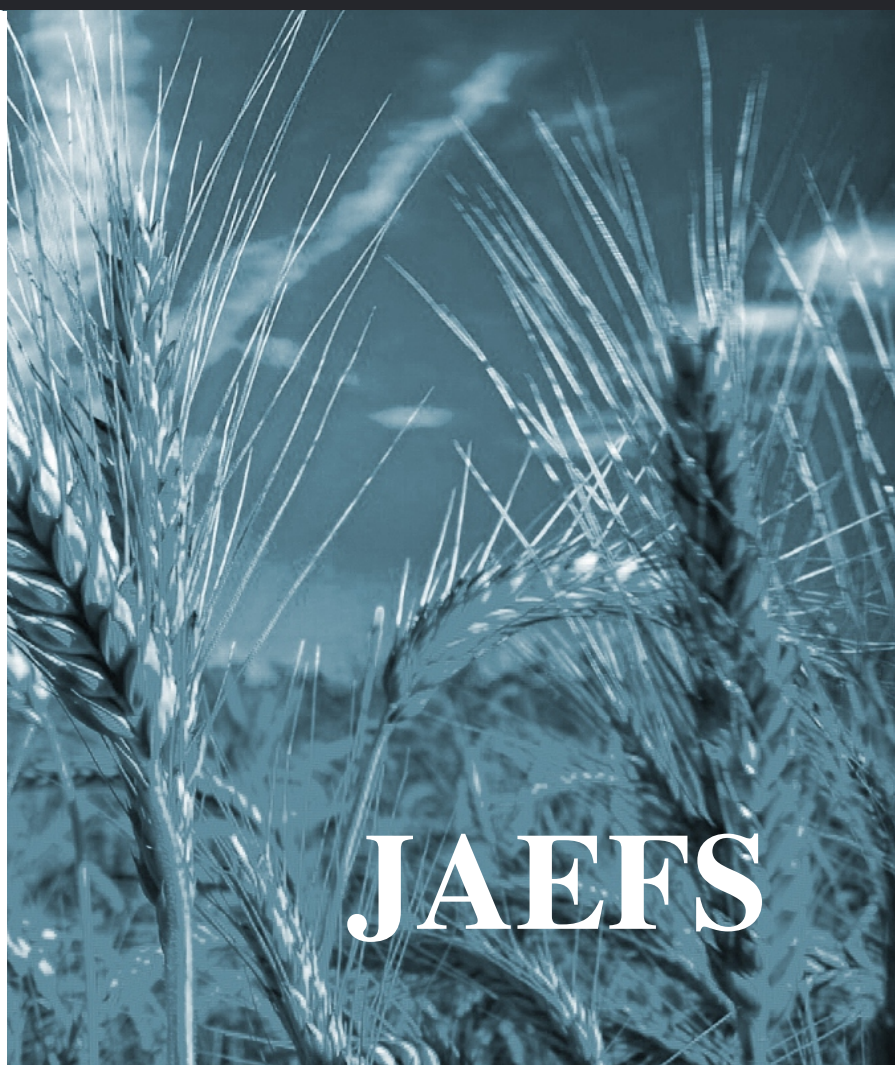

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Research Article Int J Agric Environ Food Sci 5 (4):433-442 (2021)

Effect of Different Organic Fertilizers on Bread Wheat (*Triticum aestivum L.*) Productivity

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Abstract

Organic fertilizers are the basis of sustainable agriculture and an important resource in plant nutrition as well. The research was conducted in the greenhouse conditions at the Dicle University Faculty of Agriculture, Diyarbakır, Turkey in 2020. The effects of 14 different organic fertilizers on grain yield, yield components, morphology and physiology properties of bread wheat (*Triticum aestivum* L) were examined. It was found that sheep manure, among other used fertilizers has a greater effect on both the growth and yield components of wheat in comparison with other organic fertilizers. The grain yield and biomass yield obtained from sheep manure have been determined to be competitive with commercial fertilizers. It was revealed that certain organic fertilizers had no effect on grain yield or biomass yield as compared to the control level of no fertilizers. According to the research results, it would be appropriate to use chicken and sheep manure in order to obtain an optimal wheat yield in the organic farming system.

Keywords: Organic fertilizers, Wheat, Yield, Morphology, Physiology

Introduction

Bread wheat (*Triticum aestivum* L.) is grown almost everywhere in the world due to its nutritional importance. Corn, rice, and wheat are the world's top three most-produced grains (Byerlee and Polanco, 1983). Wheat grain is consumed in various ways in many industries. It is also a cheaper feed source for livestock and poultry.

Overuse of chemical fertilizers has devastating effects on soil fertility. Moreover, even if chemical fertilizers are used in a balanced way, they negatively affect soil health in the long term. Nowadays scientists are trying to develop an agricultural system that not only reduces the cost of agricultural production but also protects natural resources (Abbas et al., 2012).

Organic farming is a production system that limits or largely eliminates the use of synthetic or inorganic fertilizers, pesticides, and hormones (Reddy, 2005). Using poultry and livestock wastes in organic agriculture makes this system a part of the integrated production system. The biggest obstacles to using these wastes are the insufficiency of waste-processing and the storage problem. However, due to the affordable cost of bio-resources, the demand for these resources is increasing day by day (Deksissa et al., 2008).

Turkish agricultural soils are deficient in organic matter and nitrogen, with only 6% having enough of these nutrients to support crop growth (Aygün and Acar, 2019). Using organic fertilizers in agriculture to eliminate plant nutrition and organic matter deficiency is of great importance.

Intensive farming applications cause a decrease in plant

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nutrients in the soil. If these decreasing plant nutrients are not recompensed by mineral and organic fertilizers, a serious decrease in agricultural production occurs. Each year about 6 million tons of chemical fertilizers and 38 thousand tons of plant protection products and hormones are used in agriculture in Turkey (Aygün and Acer, 2019). Unconscious use of these chemicals causes an increase in soil salinity and has a negative impact on soil pH and corruption of soil structure. Organic fertilizers should be utilized to maintain the soil's natural balance, improve its structure, increase yield and quality, and produce healthy products.

Using waste mushroom compost in bread wheat production has been found to be an alternative to conventional methods and if the compost is made from organic materials, it can be used as a fertilizer by organic farmers (Aydın et al., 2010). In a study using 3 different organic fertilizers and 8 bread wheat varieties, more yield was obtained in conventional cultivation and a 27% lower yield was observed in green manure application compared to conventional, but this difference could be covered by the price difference in organic (Kodaş et al. 2015).

It was the goal of this research to find out the effects of various organic fertilizers on wheat growth and potential yield.

Materials and Methods

The study was conducted in the greenhouse of Dicle University Faculty of Agriculture in 2020. In the study, 15 fertilizers (Table 1) and DZ7-59 bread wheat varieties were used as materials. The study was established on December 5, 2019, with 3 replications according to the randomized block experimental design. Bread wheat line was grown under greenhouse conditions in 8 liter pots with 4 plants per pot. The soil material used in the study was obtained from a field where no agricultural activity was carried out for a long time. Some physical and chemical contents of soil are given in Table 2. Seeds that were not exposed to any chemical treatment were used as plant material. All fertilizers were added to the pots at the time of sowing, solid fertilizers were mixed with the soil and liquid fertilizers were diluted with water and applied to the soil. Control pots with no fertilizer were created in order to compare the fertilizer applications. A drip irrigation system, controlled by a timer-based solenoid valve, had been set up for the precise application of irrigation. Each pot was irrigated to the field capacity at 2 days intervals. The name of organic fertilizer and their contents are given in Table 1.

Table 1. Fertilizer Sources and Contents

Fertilizer	Advised Dose	Applied Fertilizer	Total Nitrogen (%)	Organic Content (%)	Other Content
NPK	12 kg/da	3.6 g/pot	20	-	Phosphorus Pentaoxide 20%
COF-1	100-150 cc/100 lt su	1,5 ml/pot	2	30	Humic + Fulvic% 20; Potassium Oxide% 2; Phosphorus Pentaoxide% 0,4
COF-2	50-60 kg/da	1,2 g/pot	3	50	Humic + Fulvic% 12,5; Phosphorus Pentaoxide% 0,6; Potassium Oxide% 1,3; Kalsiyum Oxide% 0,3
COF-3	50-60 kg/da	1,2 g/pot	7	50	Humic + Fulvic Acid% 18; Phosphorus Pentaoxide% 1; Potasyum Oxide% 1
OC	120-150 kg/da	3 g/pot	2	65	Potassium Oxide% 2; Alginic Acid% 0,3
OSC	1000-2000 cc/da	0,004 g/pot	3	30	Humic + Fulvic% 24; Potassium Oxide% 5;
RL	50-75 kg/da	5 g/pot	1,35	40	Humic + Fulvic% 40
PL	40-60 kg/da	5 g/pot	1,3	40	Humic + Fulvic% 40
LV	1000-2000 cc/da	0,004 g/pot	0,8	10	Organic Carbon% 12; Potassium Oxide% 1
LSF	2-3 lt/da	0,06 g/pot	0,3	10	Humic + Fulvic% 6; Potassium Oxide% 1; Phosphorus Pentaoxide% 0,2
BG	50-100 kg/da	2 g/pot	5,65	26,4	Humic + Fulvic% 30; Potassium Oxide% 1; Phosphorus Pentaoxide% 1
SV	2000-3000 g/da	2 g/pot	1,5	40	Humic + Fulvic% 15; Phosphorus Pentaoksit% 3
FM	2 tons	40 g/pot	3,82	61,59	Organic Carbon% 25; Phosphorus Pentaoxide %4; Iron %0,3; Humic + Fulvic % 25
SM	2 tons	40 g/pot	4,98	68,3	Phosphorus Pentaoxide% 0,03
CM	500 kg/da	10 g/pot	4,09	57,89	Phosphorus Pentaoxide% 0,03

Table 2. Some physical and chemical properties of the soil used in the study

Analysis Results							
Results				Evaluation			
Analysis Name				Low	Middle	High	Very High
Saturation (%)	:	63,00	Clay loam				
Salinity (Saturation Sludge) (dS / m)	:	0,92	Without salt				
% Salt (by calculation) TS 8334	:	0,04	Without salt				
pH (Saturation Sludge)	:	8,11	Light Alkali				
Lime (Calcimetric) (%)	:	11,24	Middle				
Organic Matter (Walkley Black) (%)	:	0,71	Low				
Nitrogen (%)	:	0,04	Low				
Phosphorus (Olsen Spectrometer) (ppm)	:	4,00	Low				
Potassium (A. Acetate-ICP) (ppm)	:	314,45	Very High				
Calcium (A. Acetate-ICP) (ppm)	:	10717,8 9	Very High				
Magnesium (A. Acetate-ICP) (ppm)	:	471,78	Middle				
Sodium (A. Acetate-ICP) (ppm)	:	26,65	Low				
Iron (DTPA-ICP) (ppm)	:	9,29	Very High				
Copper (DTPA-ICP) (ppm)	:	1,61	Middle				
Manganese (DTPA-ICP) (ppm)	:	16,50	Middle				
Zinc (DTPA-ICP) (ppm)	:	0,08	Low				

Measurement and Statistical Analysis

Heading time (day): It represents the number of days between the date of the plant emergence and the date when the spike appeared on the flag leaf at the rate of 1/2, in 50% of the plants in the pot.

Plant height (cm): It was determined by measuring the length from above ground soil to the tip of the top spike.

Maturity time (day): It represent the number of days between the date of plant emergence and the date when the spike and upper stem of the plants in pots turn yellow by 90%.

SPAD value: It was measured by using the SPAD 502 Chlorophyll-Meter at the heading time of the plant, in the middle of the flag leaf.

Stem diameter (mm): The average value was calculated at the first node of the main stem with a digital caliper.

Spike length (cm): The length of the spikes from each pot was measured.

The number of spikelets per spike: The number of spikelets of the spikes from each pot was counted.

Number of Grain per Spike: The grains of spikes from each pot were counted.

Grain weight per spike: Spikes taken during the harvest period were threshed and weighed by precision scale.

Grain yield (g/plant): The grains acquired from the harvested plants were determined by weighing.

Biomass (g/plant): The weights of all plant parts taken from the pot were determined by weighing after drying.

The values obtained from the investigated parameters were analyzed by the JUMP Pro 13 statistical package program, and the statistical differences between the averages were determined by the LSD test.

Results and Discussion

The significant levels and the average values of the observations which show the effects of various organic fertilizers on the morphological, physiological, yield, and yield investigated parameters of bread wheat are given in Table 3.

The effects of fertilizers on all traits (except maturity time) of bread wheat were found to be statistically significant ($p < 0.01$).

While fertilizer applications significantly affected the heading time, they did not affect the maturity time. The average values ranged between 97 - 108 days for heading time, and 136.33 - 146 days for maturity time. Among the organic fertilizers, sheep manure encouraged earliness, whereas the control and OSC application extended the heading time and maturity time (Figure 1). In the study conducted by Subhan et al. (2017), the maximum heading time was determined as 115 days in the control treatment (no fertilizer). The minimum heading time was determined as 88.5 days in the NPK application.

Chicken manure application significantly increased plant height compared to the other treatment (Figure 1). Average values ranged between 62.83 - 80.67 cm for plant height. The maximum plant height was obtained in chicken manure application, while the minimum plant height was obtained in the control treatment. The previous studies stated that when

organic fertilizers are used regularly and at appropriate doses, there may be improvements in plant growth parameters (Dixit and Gupta, 2000, Selvakumari et al., 2000, Khoshgoftarmanesh and Kalbaşı, 2002). Delden (2001) reported that the plant height in wheat can be increased by the application of organic and inorganic fertilizers. Aksu (2017) found that farmyard manure application before wheat sowing in autumn had a positive effect on plant height.

Average values ranged between 27.70 - 47.83 for SPAD, 3.68 - 6.87 cm for spike length, 8.48 - 17.83 for the number of spikelets per spike, 8.05 - 22.22 for the number of grains per spike, 0.29 - 1.05 g for grain weight per spike. Maximum values for these parameters were obtained from conventional fertilizer (NPK) application, followed by sheep and chicken manure applications. The minimum values were obtained from the control treatment (no fertilizer). It was determined that sheep and chicken manure application increased plant height, spike length, chlorophyll content, number of spikelets, grains per spike, and grain weight per spike compared to the other treatment (Figure 1-2). It can be said that the main reason for this is the rich content of the sheep and chicken manure. Similar results were obtained in previous studies (Chattha et al. 2019; Joshi et al. 2013; Mazhar et al.2018). Hammad et al., 2011, obtained the highest spike length (9.28 cm) in traditional fertilizer application, while they reported the highest number of spikelets per spike (14.9) and the number of grain per spike (49.25) from the combination of different organic fertilizers. Kara and Gül (2013) reported that the highest results belonged to conventional fertilizer (NPK) practice and the lowest results was found liquid seaweed fertilizer in a two-year study. Aksu (2017) stated that the combination of nitrogen dose and farmyard manure can reach even higher levels of grain number per spike.

Grain yield, biomass yield, and stem diameter increased significantly with sheep manure and conventional fertilizer applications compared to the other treatment. Average values ranged between 1.09 - 3.56 g / plant for grain yield, 2.69 - 8.04 g / plant for biomass, 1.81 - 3.48 mm for stem diameter. The highest values of these parameters were obtained from the sheep manure and conventional fertilizer (NPK) application in the same statistical group, while the minimum values were obtained from the control treatment (no fertilizer) (Figure 3). The grain yield and biomass yield obtained from sheep manure among organic fertilizers have been determined to be competitive with commercial fertilizers. It was revealed that certain organic fertilizers had no effect on grain yield or biomass yield as compared to the control level of no fertilizers. According to these results, it can be said that nitrogen and phosphorus applied to the soil with conventional fertilization is taken by the plant more easily and accordingly it causes an increase in the yield value. The biggest disadvantage of organic fertilizers is the low nitrogen and other nutrients that the plant needs. Therefore, grain yield decreases as the plant cannot get enough nitrogen and other nutrients. Organic fertilizers can differ due to their slow decomposition (mineralization, transformation from organic form to inorganic form) in the soil and the variable distribution of nutrients they contain (Ameeta and Chetani, 2017). It has been reported that the application of nitrogen-rich organic fertilizers tends to high yield in wheat and increases biomass yield (Camara et al., 2003). Hole et al. (2005) reported that the available nutrients in organic fertilization are lower than in conventional fertilization. Hiltburunner et al. (2005), reported that farmyard manure increases the stem diameter during the grain filling period of wheat and higher protein is obtained due to the high nutrient accumulation in the stem. Öztürk et al., (2011), reported that farmyard manure increased the grain yield of wheat by improving the fertility of the soil. The results we obtained in the study have similarities with the results of the previous studies (Khanam et al., 2001; Rees and Castle, 2002; Ghosh et al., 2004; Sarwer et al., 2008; Reddy et al., 2005). Abbas et al. (2012) found that maximum grain yield was obtained when the appropriate dose of NPK was given with 6 t ha^{-1} chicken manure application. Jamal and Fawad (2018, 2019) stated that chicken manure significantly increased biomass yield. Phullan et al., (2017) concluded that farmyard manure at 6 tons ha^{-1} coupled with mineral fertilizer rate of 120-90 kg N-P_2O_5 ha^{-1} was the best source for sustainable soil health and wheat production. Aydın et al., (2010), reported that the highest grain yield was obtained as 3716 kg/ha with the application of 72.0 kg/ha spent mushroom compost and additionally grain yield obtained from conventional practice 27.69 kg/ha, 19.54 kg/ha of organic agriculture application respectively. Kodaş et al., (2015), found that the highest yield was obtained from conventional with 3290 kg/ha, the lowest yield was 1900 kg/ha obtained from farmyard manure.

Conclusion

According to the findings of this research, sheep manure treatment increased grain production and biomass capacity to levels comparable to commercial fertilizer. By overcoming the primary disadvantage of organic agriculture, low yield, the use of sheep manure may contribute to the expansion of organic farming areas. Encouragement of sheep manure production and application to agriculture will be critical in this regard. Given the scarcity of fertilizer in areas where sheep are primarily grazed, it is possible to recommend a mixture of chicken manure and sheep manure, which ranks second in terms of grain yield after sheep manure. From the organic fertilizers tested in this study, it was discovered that only sheep and chicken dung could be used successfully in organic agriculture. More testing will be essential to bring these findings into practical use by supporting them with further trials.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Remzi Özkan designed the study and collected the data with Levent Yorulmaz. Remzi Özkan, Merve Bayhan, Muhammet Öner and Mehmet Yıldırım made the statistical analysis and wrote the original draft of the article. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Table 3. Average values examined traits and theirs

Fertilizers	Heading time (day)	Maturity time (day)	SPAD value	Plant height (cm)	Spike Length (cm)
FM	98,00 d-f	138,67	31,00 e-g	78,50 a-c	5,90 bc
LSF	103,67 a-c	142,33	30,35 e-g	68,00 d-g	4,38 d-g
RL	104,33 a-c	141,33	32,40 d-f	65,17 e-g	3,77 g
PL	105,67 a-c	143,33	33,60 de	65,50 e-g	3,96 fg
SM	96,67 f	136,33	41,83 b	79,33 ab	6,87 ab
OC	101,33 c-f	137,33	29,10 fg	64,83 e-g	4,17 e-g
COF-1	102,33 b-e	144,00	32,75 d-f	73,33 a-d	5,40 cd
COF-2	102,33 b-e	139,67	32,20 d-f	73,67 a-d	5,00 c-f
COF-3	105,67 a-c	142,67	39,45 bc	74,50 a-d	4,67 d-g
LV	103,33 a-c	144,00	31,85 ef	71,00 c-f	4,50 d-g
SV	105,00 a-c	145,67	32,40 d-f	63,83 fg	4,17 e-g
CM	97,00 f	136,67	35,97 cd	80,67 a	6,13 bc
CF	97,67 ef	140,67	47,83 a	75,17 a-d	7,94 a
OSC	107,00 ab	146,67	40,90 b	72,00 b-e	5,25 c-e
BG	103,00 a-d	142,33	32,67 d-f	68,67 d-g	3,83 fg
Control	108,00 a	146,00	27,70 g	62,83 g	3,68 g
Average	102,56	141,73	34,5	71,06	4,97
%CV	2,94	2,88	6,93	6,37	10,77
LSD (P<0.05)	5,01	no	3,98	7,55	1,22

no: not important, **FM:** Farmyard manure, **LSF:** liquid Seaweed Fertilizer, **RL:** RawLeonardite, **PL:** Processed Leonardite, **SM:** Sheep Manure, **OC:** Organic Compost, **COF-1:** Commercial Organic Fertilizer-1, **COF-2:** Commercial Organic Fertilizer-2**, COF-3:** Commercial Organic Fertilizer-3, **LVF:** Liquid Vermicompost Fertilizer, **SVF:** Solid Vermicompost Fertilizer, **CM:** Chicken Manure, **CF:** Conventional Fertilizer (NPK). **OSC:** Organic Seed Coating, **BG:** Bat Guano.

Table 3. Average values examined traits and theirs (contunied)

Fertilizers	Number of spikelet per spike	Number of grains per spike	Grain weight per spike	Grain yield (g/ plant)	Biomass yield (g/ plant)	Stem diameter (mm)
FM	15,78 ab	13,75 b-d	0,40 cd	1,43 de	4,80 b-d	2,72 b-d
LSF	10e.g. cd	10,58 d	0,38 cd	1,51 b-e	3,52 e-g	2,13 d-f
RL	8,61 d	8,19 d	0,30 d	1,30 de	3,14 fg	2,08 d-f
PL	8,69 d	8,69 d	0,31 d	1,14 e	2,74 fg	1,95 ef
SM	16,10 ab	17,63 a-c	0,78 ab	3,56 a	8,04 a	3,48 a
OC	9,67 cd	8,42 d	0,30 d	1,19 e	2,94 fg	1,87 f
COF-1	13,03 bc	12,61 b-d	0,61 bc	2,19 bc	4,88 bc	2,63 c-e
COF-2	12,92 bc	10,67 d	0,38 cd	1,51 c-e	3,74 d-g	2,3 c-f
COF-3	11,72 cd	13,67 b-d	0,52 b-d	1,91 b-d	4,29 b-e	2,28 c-f
LV	10,92 cd	10,92 cd	0,39 cd	1,57 b-e	3,80 c-f	2,30 c-f
SV	9,67 cd	10,33 d	0,38 cd	1,53 b-e	3,40 e-g	1,96 ef
CM	15,58 ab	18,00 ab	0,71 b	2,20 b	5,21 b	2,90 a-c
CF	17,83 a	22,22 a	1,05 a	3,70 a	8,23 a	3,41 ab
OSC	11,17 cd	13,83 b-d	0,55 b-d	1,63 b-e	3,81 c-f	2,48 c-f
BG	8,70d	9,68 d	0,33 d	1,41 de	3,26 e-g	1,81 f
Control	8,48 d	8,05 d	0,29 d	1,09 e	2,69 g	1,81 f
Average	11,83	12,32	0,48	0,49	4,28	2,38
%CV	9,26	10,85	11,79	9,23	11,19	8,07
LSD (P<0.05)	3,79	6,75	0,28	0,68	1,09	0,71

no: not important,
FM: Farmyard manure, **LSF:** liquid Seaweed Fertilizer, **RL:** RawLeonardite, **PL:** Processed Leonardite, **SM:** Sheep Manure, **OC:** Organic Compost, **COF-1:** Commercial Organic Fertilizer-1, **COF-2:** Commercial Organic Fertilizer-2**, COF-3:** Commercial Organic Fertilizer-3, **LVF:** Liquid Vermicompost Fertilizer, **SVF:** Solid Vermicompost Fertilizer, **CM:** Chicken Manure, **CF:** Conventional Fertilizer (NPK). **OSC:** Organic Seed Coating, **BG:** Bat Guano.

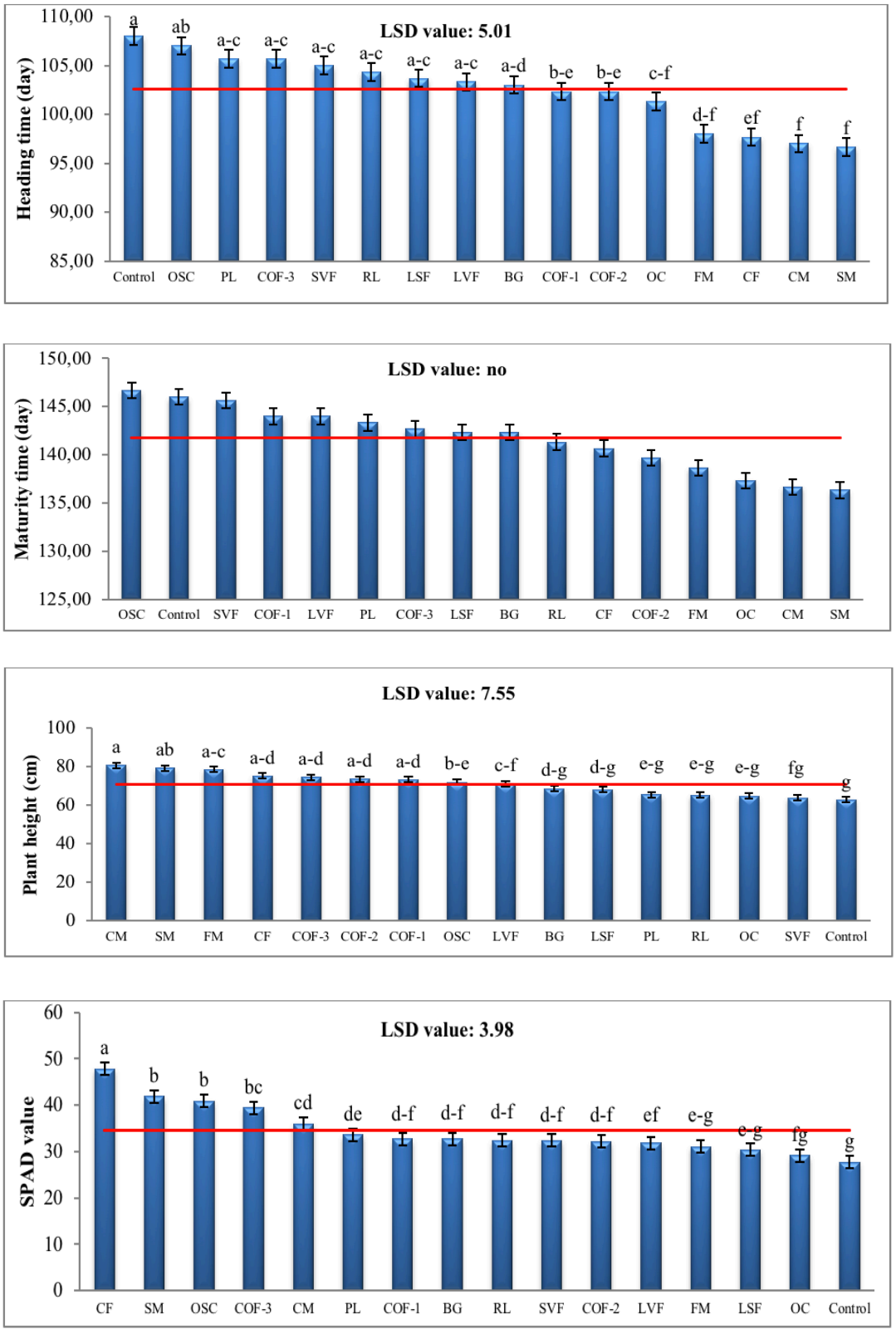

Figure 1. The effect of different organic fertilizers on the heading time (day), maturity time (day), plant height (cm), SPAD value in wheat. **Red line:** General Mean, **FM:** Farmyard manure, **LSF:** liquid Seaweed Fertilizer, **RL:** RawLeonardite, **PL:** Processed Leonardite, **SM:** Sheep Manure, **OC:** Organic Compost, **COF-1:** Commercial Organic Fertilizer-1, **COF-2:** Commercial Organic Fertilizer-2**, COF-3:** Commercial Organic Fertilizer-3, **LVF:** Liquid Vermicompost Fertilizer, **SVF:** Solid Vermicompost Fertilizer, **CM:** Chicken Manure, **CF:** Conventional Fertilizer (NPK), **OSC:** Organic Seed Coating, **BG:** Bat Guano

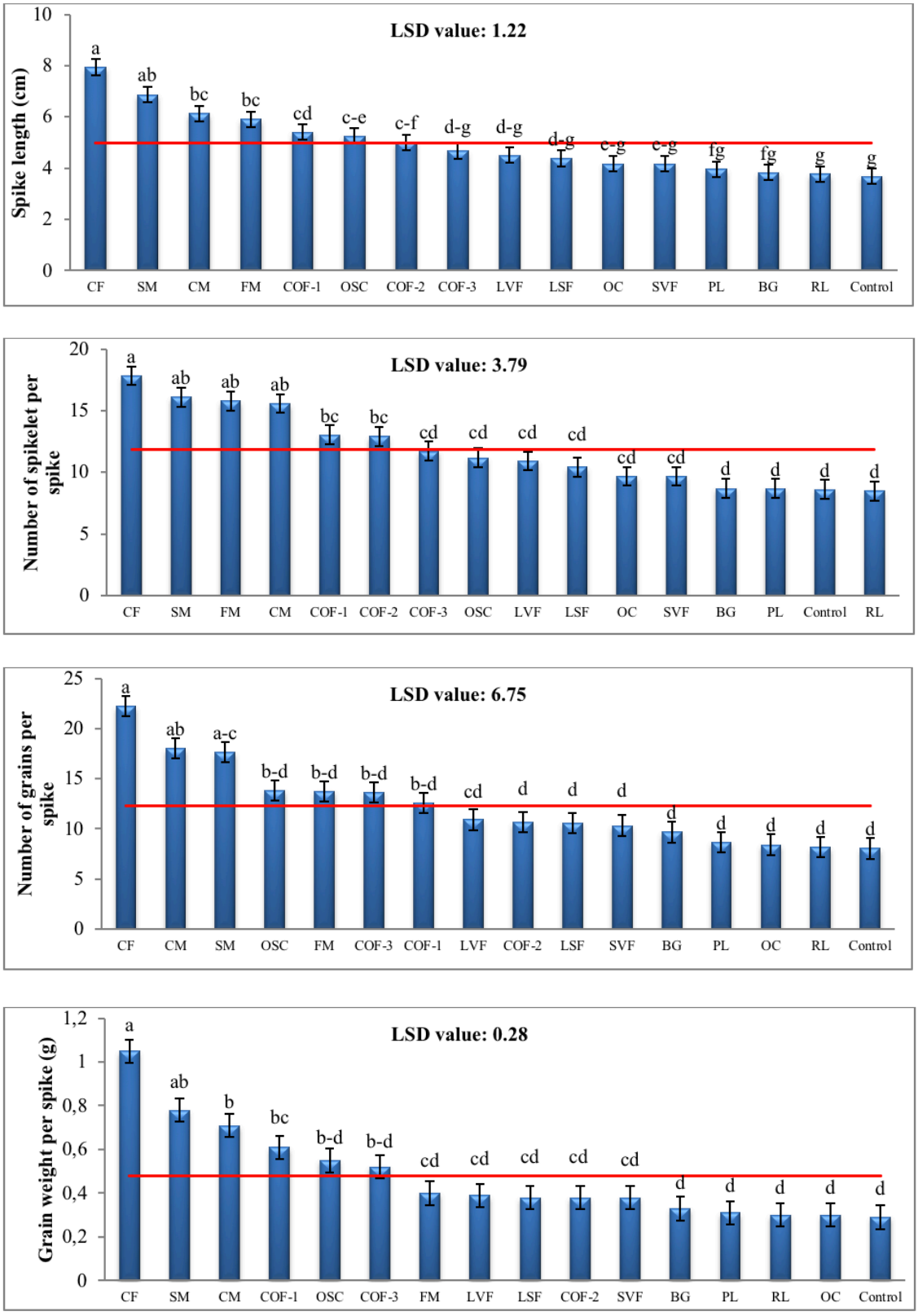

Figure 2. The effect of different organic fertilizers on the spike length (cm), and the number of spikelets per spike, number of grains per spike, grain weight per spike in wheat. **Red line:** General Mean, **FM:** Farmyard manure, **LSF:** liquid Seaweed Fertilizer, **RL:** RawLeonardite, **PL:** Processed Leonardite, **SM:** Sheep Manure, **OC:** Organic Compost, **COF-1:** Commercial Organic Fertilizer-1, **COF-2:** Commercial Organic Fertilizer-2**, COF-3:** Commercial Organic Fertilizer-3, **LVF:** Liquid Vermicompost Fertilizer, **SVF:** Solid Vermicompost Fertilizer, **CM:** Chicken Manure, **CF:** Conventional Fertilizer (NPK), **OSC:** Organic Seed Coating, **BG:** Bat Guano

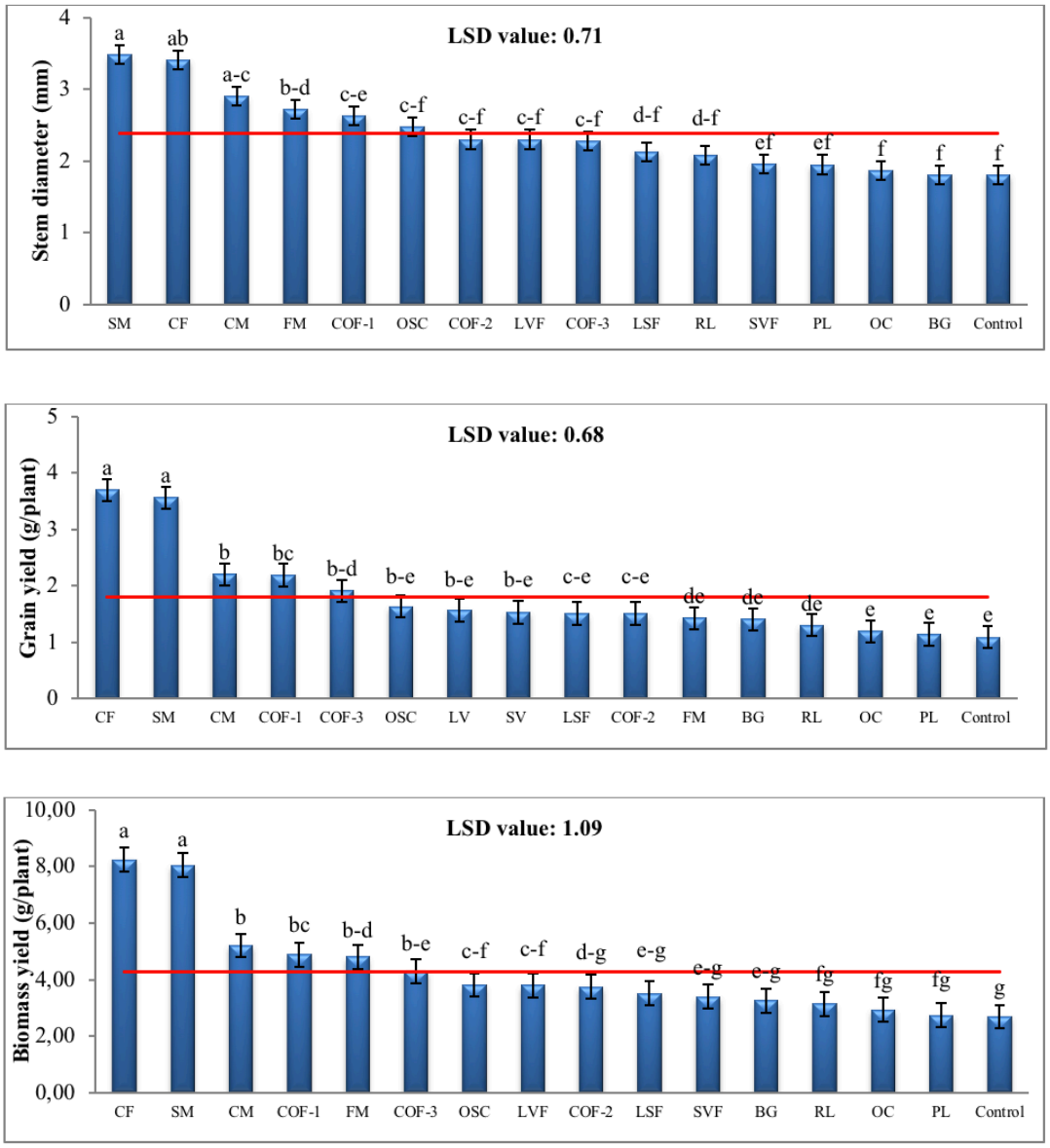

Figure 3. The effect of different organic fertilizers on the stem diameter (mm)and biomass yield (g/plant) in wheat. **Red line:** General Mean, **FM:** Farmyard manure, **LSF:** liquid Seaweed Fertilizer, **RL:** RawLeonardite, **PL:** Processed Leonardite, **SM:** Sheep Manure, **OC:** Organic Compost, **COF-1:** Commercial Organic Fertilizer-1, **COF-2:** Commercial Organic Fertilizer-2**, COF-3:** Commercial Organic Fertilizer-3, **LVF:** Liquid Vermicompost Fertilizer, **SVF:** Solid Vermicompost Fertilizer, **CM:** Chicken Manure, **CF:** Conventional Fertilizer (NPK), **OSC:** Organic Seed Coating, **BG:** Bat Guano

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Determination of Some Phytochemical Contents of Local and Standard Grape Varieties Grown in Diyarbakır Province

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Abstract

Grape (*Vitis vinifera* L.) has been the subject of many research studies because of its phenolic compounds and antioxidant properties which are known to have positive effects on health. In this research study, some phytochemical contents of local and standard grape varieties widely grown in Diyarbakır province were determined. Amount of total phenolic, total anthocyanin and total flavonoid were examined in the berry seed, berry pulp and berry skin of each variety. The statistical differences among Boğazkere, Öküzgözü and Kızıl Banki were obtained and with the addition of Şire variety all varieties were compared in terms of statistical differences.Content of total phenolic was between 389.15 mg GAE / kg and 4050.17 mg GAE / kg, while content of total anthocyanin was recorded between 25.61 mg / kg and 634.00 mg / kg.Total flavonoid content recorded from 2.34 mg CE /kg to 2402.00 mg CE/kg.With this study, it was determined that some phytochemical contents of different tissues of the grapes vary and this difference also occurs between the grape varieties.

Keywords: Grape, Phytochemical contents, Berry tissues

Introduction

In 2018 grapes are produced on 7.2 million hectares in the world and approximately with 417 thousand hectares area and 6 % ratio Turkey placed 5th row. In the same year, 79 million tons of grape were produced in the world, and with 3.9 million tons and a 5% ratio, Turkey placed 6th row. According to the data of the World Food Organization (FAO), the production amount increased by 8.4% and the area increased by 3.4% in 2018 compared to the previous year. (Tarım ve Orman Bakanlığı Bitkisel Üretim Genel Müdürlüğü, 2020).

The economy of Hittite;Phrygian,Urartian and Lydian which were important cultures of ancient Anatolia in the 2nd and 1st Millennium BC was based on agriculture and livestock which the main agricultural products of the Hittites were wheat and barley as well as peas,beans,onions, flax, figs, olives, grapes, apples and pomegranates(Bülbül, 2017) Viticulture is still one of the most important agricultural branches in Anatolia. (Söylemezoğlu et al., 2018). Viticulture was made in a total area of 175,387 da in Diyarbakır and 103,872 tons of production was realized in 2019. (TÜİK, 2019).

The number of standard and local grape varieties commonly used in grape production in Diyarbakır is 74 and Boğazkere, Kızıl Banki, Öküzgözü and Şire (sin. Mazrumi) take place among the varieties (Karataş et al.,2015). The synonym of the Kızıl Banki variety grown in Diyarbakır is Kızıl Vanki (Gürsöz,1993). Öküzgözü, Boğazkere and Şire grape varieties are widely grown in Diyarbakır, Elazığ and Mardin provinces (Özdemir and Sessiz,2018).

Grape varieties (*Vitis vinifera L.*) are considered to be

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beneficial for the health through their antioxidant effects,fatty acid contents and phenolic contents(Odabaşıoğlu and Gürsöz,2020). Researchers carry out studies in order to search grape positive effects on human health (Özdemir,et al.,2017). Scientists conduct studies to find grapes positive effects on lot of chronic diseases (Özden and Vardin,2009).Grape(*Vitis vinifera L.*)have positive effects on health thanks to their many various bioactive phenolic contents(Lutz,et al.,2011). Grapes are important in terms of sources of nutritional antioxidants and biologically active dietary components at the same time (Eshghi,et al.,2014). Grapes are rich in terms of resveratrol,phenolics and flavonoids that are claimed to be responsible on their benefits for the health(Yang,et al.,2009). This research study is aimed to examine the important phytochemical contents of grapes in terms of human health that are widely grown in Diyarbakır and to make a comparision between varieties and also grape berry organs.

Materials and Methods

In this study, 4 different grape varieties were examined. The varieties examined are Boğazkere, Öküzgözü, Şire and Kızıl Banki grape varieties grown within Diyarbakır province. Grapes were taken from different vineyards (modern and traditional vineyards). Samples of the varieties were taken when each variety reached harvest maturity. The samples for the study were kept at -20 ^{o}C and were taken off deep freezer before being used. The amount of total phenolic, total flavonoid and total anthocyanin were examined as three replications in berry skin, berry pulp and berry seed of each grape variety, and our study was realized in the Central Laboratory of Harran University.

Preparation of extract for total phenolic content

With modification, grape berries were selected as 5 g from grape sample and separated as pulp, skin and seed. It was treated with liquid nitrogen and was ground in the mortar. It was extracted by 50 mL of distilled water over 5 g. It was heated in the Soxhlet device until it reached the boiling temperature and 1 hour after the boiling started it was taken. When it came to room temperature they were placed in tubes. Analyzes for skin and seed were made after dilution of 1/10 from the extracted samples(Atak and Uslu,2018).

Preparation of extract for total flavonoid and total anthocyanin content

With modification, , grape berries were selected as 5 g from grape samples and separated as pulp, skin and seed. They were extracted with 50 mL of ethanol over 5 g, heated up to boiling temperature in the Soxhlet device and 1 hour after the boiling started it was taken and kept until it came to room temperature and placed in tubes. Analyzes were made after dilution of 1/10(Atak and Uslu,2018).

Total Phenolic Content Determination Method

Content of total phenolic was determined with some modifications and using gallic acid as standard by Folin-Ciocalteu (Singleton and Rossi, 1965). For the mixture 0.4 mL of diluted extract solution was used to mix with 2 mL of Folin-Ciocalteu reagent (the reagent was pre-diluted by distilled water 10 times) and 1.6 mL of sodium carbonate (7.5% w /v). At room temperature after 60 minutes of incubation, the absorbance was measured at 765 nm against the blank solution prepared using a UV-Visspectrophotometer (Akyurt et al., 2018).Our findings in our study evaluated as mg GAE/kg.

Total Anthocyanin Content Determination Method

Method of pH differential (Giusti and Wrolstad) was used. In the framework of this method,the spectrophotometric absorptions of extracts that incubated at room temperature for 15 minutes in the 0.025 M KCl buffer (pH 1.0) and 0.4 M CH3COONa buffer (pH 4.5) were measured at 520 and 700 nm and the absorbance values found by formula (Özden and Özden,2014).

Total Flavonoid Content Determination Method

Aluminum chloride colorimetric method (Zhishen et.al.,1999) was used. One portion (1 mL) of extract or standard catechin solution (20, 40, 60, 80 and 100 mg /L) was added to a 10 mL volumetric flask containing 4 mL dd H_2O. Also to the flask ,0.3 mL of 5% $NaNO_2$ was added . 5 minutes after, 0.3 mL of 10% $AlCl_3$ was dropped, and at the minute of 6th ,2 mL of 1 M NaOH was added to complete total volume to 10 mL .The solution was mixed good and then the absorbance was measured against the reagent blank which was prepared at 510 nm. The content of total flavonoid was expressed as mg catechin equivalent (CE) / 100 g fresh mass (Srivastava et al.,2013). In our study, the values were converted to mg CE / kg.

Statistical Analysis

Variance Analysis (ANOVA) of the data of the study was done by using the SPSS 20.0 package programme. Then Tukey test was used to determine the level of differences.

Results and Discussion

According to a research carried out with 22 grape varieties that grown in Marmara region of Turkey, total phenolic amount of pulp, seed and skin was respectively found between 9.26- 62.29; 162.29- 326.18 and 96.61- 167.42 mg GAE / 100 g fresh weight (Yılmaz et al., 2015). Furthermore ,some quality and the phytochemical properties of Bertiz Kabarcık grape which is important in Kahramanmaraş viticulture was examined and the total amount of phenolic substance was reported to vary between 44.3 mg GAE 100 g $^{-1}$ and 313.9 mg GAE 100 g $^{-1}$ (Balbaba and Bağcı, 2020). Also, total phenolic content of the seeds of two white (Emir and Gök grape) and one black (Kara Dimrit) grape variety were examined and the content of highest total phenolic was found with the seeds of the Gök grape variety with 87031.32 mg GAE / kg (Akın and Altındişli, 2010). When compared with this study the seed contents difference could be originated due to variety. In our study, the least total phenolic amounts were detected in berry pulp and the highest amounts were found in berry seed of varieties except Boğazkere variety. With this aspect, our findings shows similarity with previous studies .

In another study which anthocyanin, the tannin based phenolic compound profile and other phytochemical properties of Kalecik Karası grape variety which grown in Ankara and Nevşehir conditions were examined, amount of total anthocyanin varied between 323.08 mg kg^{-1}- 202.37 mg kg^{-1} (Toprak ,2011).In a study with sixteen red grape varieties total anthocyanin(TA)content ranged from 40.3 mg/L to 990.8

mg/L fresh weight (Orak,2007). In our study anthocyanin was determined between 25.60-634.00 mg / kg.Our study is in conformity with the studies.

A study carried out with 29 grape varieties in Şanlıurfa province, it was reported that when the tissues of grapes were compared, pulp contained very low flavonoid contents while the values of skin and seed were close to each other. It was stated that the highest amount of flavonoid was in the seed of Kızıl Banki among seed contents (0.371 g. kg^{-1}) (Polat, 2016) . In our study, the highest value was found in the seed of the Kızıl Banki variety and the lowest values were found in the berry pulp. Our study is similar to this study.

Where phenolic contents were examined as well as other parameters in the skin, pulp and seed parts of 15 grape varieties, it was reported that content of the total phenolic was the highest in the seed, then in skin and last in pulp (Harbi et al., 2013). In our study, the highest values were found in the berry seed except Boğazkere and the least values were found in the berry pulp. Our study is supported by the previous study findings.

Phenolic compounds of varieties of 3 table grape (Alphonse Lavallee, Red Globe and Hamburg Misketi) and varieties of 3 wine grape (Boğazkere, Cabernet Sauvignon and Kalecik Karası) were determined in a study, the highest total anthocyanin amount was found with the skin of Boğazkere variety and the total phenolic compound amount was found with Kalecik Karası seed (Tahmaz et.al.,2013). In our study, the highest values of anthocyanin were found in the berry skin of the varieties and are listed as Öküzgözü, Boğazkere and Kızıl Banki, Şire respectively.

A study which the contents of total phenolic and total flavonoid of Öküzgözü and Boğazkere varieties were examined in pulp, seed and skin for 2 years, the flavonoid content was found between 5.08 µg QUE / mg and 111.55 µg QUE / mg.And It has been reported that the contents of total phenolic (µg GAE / mg) and total flavonoid of Öküzgözü and Boğazkere cultivars differ significantly according to skin, seed ,pulp and years (Özdemir et al.,2017).In our study differences were seen as well in total flavonoid values in terms of berry tissues.

However, amount of total phenolic and total flavonoid substance and the antioxidant activities in the skin, seed, pulp and extracts obtained from whole berry were examined with 12 grape genotypes grown in Turkey. When compared the grape samples in terms of substance of total phenolic and total flavonoid, the highest amounts were obtained in grape seeds, then skin and then whole berry and pulp at the last (Bayır Yeğin and Uzun,2018). Our study showed that, the highest content of total phenolic was found with the seed of varieties except Boğazkere. Boğazkere variety has got thick skin ,and we think that made higher skin phenolic content And the lowest values were found with berry pulp. Our findings are generally supported by the findings of this study.

A study with 6 grape varieties obtained from Tunceli province, the highest content of anthocyanin was found with Koşkuran variety (1192.1 mg / kg) the lowest content of anthocyanin was found with Ulaş Siyahı variety (358.5 mg /kg) (Karaca Sanyürek et al.,2018). Also, a study which phytochemical properties and total antioxidant activities of some wine grape varieties that grown in Şanlıurfa conditions were determined it was reported that the total phenolic content, anthocyanin content, antioxidant activities and the phytochemical properties of grape varieties may vary based on the grape variety, growing climate and soil conditions, soil type, levels of ripening, cultural practices and amount of yield. Total anthocyanin contents of the varieties of Merlot, Chardonnay, Cabernet Sauvignon, and Şiraz were respectively 1144.9; 39.48; 723.3, and 1011.6 mg / kg (Özden and Vardin, 2009). Our study shows similarity with the previous findings. The highest anthocyanin value was found in the berry skin of Öküzgözü variety and values vary between 25.60-634.00 mg / kg.

In another study with Shiraz grape variety that grown in the district of Güney of Denizli which carried out in order to research the effects of four different cluster thinning (8- 16- 24 and 32 clusters / grapevine) being applied just after berry retention, on the yield, quality properties and on the biochemical properties of the berry ; highest total phenolic (285.20 mg GAE / 100 g) and total flavonoid (100.68 mg CTE / 100 g) substance amounts were found from 8 cluster / grapevine(Pehlivan and Uzun,2015). Total flavonoid content of Perricone grape variety in the higher peak concentration was 3233.29 ± 347.32 mg/kg in the dates of last harvest , and the total flavonoid content of Nero d'Avola grapes in maximum peak concentration was 2519.22 ± 66.91 mg/kg in the maturation of later stage reported in another study (Gervasi et.al., 2016). In the study carried out with 29 grape varieties in Şanlıurfa province ,total flavonoid contents of berry pulp of white varieties changed between 0.968 mg/kg-10.6 mg/kg and total flavonoid contents of berry pulp of colored varieties changed between 1.87 mg/kg and 20.0 mg/kg in terms of years of average(Polat,2016)In our study, total flavonoid values were between 2.34-2402 mg CE / kg and our findings are in conformity.

Table 1. Findings of Total Phenolic Content(mg GAE / kg)

Berry tissue	Boğazkere		Öküzgözü		Kızıl Banki		Şire	
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Berry skin	4004.32^{bc}	373.47	3437.51^{b}	26.89	2718.36^{b}	134.34	3228.35^{b}	108.57
Berry pulp	1200.88^{a}	9.32	457.56 ^{a}	20.93	435.89^{a}	9.07	389.15^{a}	20.13
Berry seed	3616.00^{b}	331.62	3907.86^{c}	243.17	3803.29^{c}	217.74	4050.17^{c}	215.77

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 2. Findings of Total Anthocyanin Content(mg/kg)

Berry tissue	Boğazkere		Öküzgözü		Kızıl Banki		Şire	
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Berry skin	457.55^{c}	11.69	634.00^{c}	22.24	227.10^{c}	43.80	94.63^{abc}	15.15
Berry pulp	115.78^{b}	2.55	134.15^{b}	25.12	123.57^{b}	24.71	85.16^{a}	7.28
Berry seed	80.15^{a}	6.02	25.60^{a}	8.40	47.87^{a}	5.86	87.95^{ab}	5.37

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 3. Findings of Total Flavonoid Content (mg CE/kg)

Berry tissue	Boğazkere		Öküzgözü		Kızıl Banki		Şire	
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Berry skin	173.94^{b}	4.87	144.30^{b}	3.57	70.98^{ab}	8.22	14.53^{ab}	3.70
Berry pulp	49.92^{a}	1.35	19.50^{a}	1.35	44.46^{a}	2.34	2.34^{a}	0.05
Berry seed	368.90^{c}	6.21	1457.03^{c}	61.42	2402.00^{c}	22.59	1436.00^{c}	118.05

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 4.Total Phenolic Content of Colored Varieties (mg GAE/kg)

Variety	Berry pulp		Berry seed		Berry skin	
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Boğazkere	1200.88 ^{c}	9.32	3616.00 ^{a}	331.62	4004.32 ^{bc}	373.47
Öküzgözü	457.56^{ab}	20.93	3907.86 ^{abc}	243.17	3437.51 ^{b}	26.89
Kızıl Banki	435.89^{a}	9.07	3803.29^{ab}	217.74	2718.36 ^{a}	134.34

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 5.Total Anthocyanin Content of Colored Varieties (mg/kg)

Variety	Berry pulp		Berry seed		Berry skin	
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Boğazkere	115.78^{a}	2.55	80.15^{c}	6.02	457.55^{b}	11.69
Öküzgözü	134.15^{abc}	25.12	25.60^{a}	8.40	634.00 ^{c}	22.24
Kızıl Banki	123.57^{ab}	24.71	47.87^{b}	5.86	227.10^{a}	43.80

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 6. Total Flavonoid Content of Colored Varieties (mg CE/kg)

Variety	Berry pulp		Berry seed		Berry skin	
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Boğazkere	49.92^{c}	1.35	368.90^{a}	6.21	173.94^{c}	4.87
Öküzgözü	19.50^{a}	1.35	1457.03^{b}	61.42	144.30^{b}	3.57
Kızıl Banki	44.46^{b}	2.34	2402.00^{c}	22.59	70.98^{a}	8.22

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 7. Total Phenolic Content of Varieties (mg GAE/kg)

Variety	Berry pulp		Berry seed		Berry skin	
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Boğazkere	1200.88^{d}	9.32	3616.00^{a}	331.62	4004.32^{d}	373.47
Öküzgözü	457.56 ^{bc}	20.93	3907.86 ^{abc}	243.17	3437.51^{bc}	26.89
Kızıl Banki	435.89 ^{b}	9.07	3803.29 ^{ab}	217.74	2718.36 ^{a}	134.34
Şire	389.15 ^{a}	20.13	4050.17^{abcd}	215.77	3228.35 ^{b}	108.57

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 8. Total Anthocyanin Content of Varieties (mg/kg)

Variety	Berry pulp		Berry seed		Berry skin	
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Boğazkere	115.78 ^{ab}	2.55	80.15 ^{c}	6.02	457.55^{c}	11.69
Öküzgözü	134.15^{bcd}	25.12	25.60 ^{a}	8.40	634.00^{d}	22.24
Kızıl Banki	123.57 ^{abc}	24.71	47.87 ^{b}	5.86	227.10^{b}	43.80
Şire	85.16 ^{a}	7.28	87.95 ^{d}	5.37	94.63^{a}	15.15

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 9. Total Flavonoid Content of Varieties (mg CE/kg)

Variety	Berry pulp		Berry seed		Berry skin	
	Mean	Std. deviation	Mean	Std. deviation	Mean	Std. deviation
Boğazkere	49.92^{d}	1.35	368.90 ^{a}	6.21	173.94^{d}	4.87
Öküzgözü	19.50^{b}	1.35	1457.03^{bc}	61.42	144.30^{c}	3.57
Kızıl Banki	44.46^{c}	2.34	2402.00^{d}	22.59	70.98^{b}	8.22
Şire	2.34^{a}	0.05	1436.00^{b}	118.05	14.53^{a}	3.70

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Conclusion

In our study contents of total phenolic, total anthocyanin and total flavonoid were detected in the berry pulp, berry skin and berry seed of Boğazkere, Öküzgözü, Kızıl Banki and Şire varieties which are commonly grown. The results showed significant differences both between the tissues of the variety and among the varieties. It is thought that the differences are caused by the factors such as cultivation, care conditions, irrigation, variety characteristics, climate, topography. Total phenolic content ranged from 389.15 mg GAE / kg to 4050.17 mg GAE / kg, while total anthocyanin content was recorded between 25.60 mg / kg and 634.00 mg / kg. Total flavonoid content ranged from 2.34 mg CE / kg to 2402.00 mg CE / kg,

Because of contents of these varieties they are considered to be an important resource for the research studies that will be carried out for human health. Phytochemical contents of Boğazkere,Öküzgözü,Kızıl Banki and Şire can be studied again,especially high phenolic content of Boğazkere skin and pulp is important for the upcoming studies. We think the grape varieties examined have an important potential in terms of phenolic compounds.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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The alkaloid content of poppy (*Papaver somniferum* L.) varieties in Turkey by their correlation and path coefficient relationships

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Abstract

The research was conducted with the aim of examining the alkaloids content of twelve poppies (*Papaver somniferum* L.) cultivars registered in Turkey by correlation and path analysis of relationships among alkaloids with capsule yield. The field experiments were set up according to a randomized complete block design with three replications in 2017-18 and 2018-19 under the semi-arid ecological conditions of Isparta, Turkey. The differences among the capsule yields and alkaloid contents of poppy varieties were statistically significant ($P \leq 0.01$) in both periods, and the capsule yields were determined to be between 253.4-556.2 kg ha^{-1} in the first year and 332.7-659.2 kg ha^{-1} in the second year. The morphine, codeine, oripavine, thebaine, papaverine, and noscapine contents of poppy cultivars varied between 0.46-1.84%, 0.08-0.30%, 0.009-0.057%, 0.031-0.093%, 0.002-0.047% and 0.017-0.094%, respectively. According to correlation analysis, the relationships between the morphine content with codeine (r=0.415*), oripavine (r=0.362*), and papaverine (r=0.624**) were significant and positive while noscapine (r=0.164^{ns}) and thebaine (r=0.043^{ns}) were insignificant and positive. In the path coefficient analysis, papaverine (p=0.631, 67.75%) had the highest direct effect on morphine content followed by thebaine, orpavine, and noscapine, respectively. Noscapine had the highest indirect positive effect on morphine content via papaverine while thebaine had negative and high indirect effects via papaverine. In the study, varieties that could be recommended for the semi-arid ecological conditions of Turkey were the TMO-1 and TMO-3 varieties due to higher capsule yields and the Ofis-1 variety due to the high alkaloid content.

Keywords: Poppy, Capsule yield, Morphine, Correlation, Path analysis

Introduction

The poppy (*Papaver somniferum* L.), belonging to the family *Papaveraceae*, is a valuable alkaloid plant. There are 36 species (58 taxa of which 15 are endemic) of poppies in Anatolia, and the *Papaver somniferum* L. species has traditionally been cultivated for thousands of years. Turkey is the world's important country in legal poppy cultivation and morphine supplier. Turkey has about 50% of the legal poppy planting area and supplies about 25% of legal morphine production. There are 15 poppy cultivars in the national varieties list of Turkey (SRCC, 2020). In Turkey, the poppy is cultivated over an area of 68 000 hectares with an annual production of 27 300 tons (TUIK, 2020), and the main cultivation regions are central Anatolia, the inner Aegean, and the western gateway zone. There are approximately 70000 registered poppy producers in 13 provinces of these regions.

Poppies contain approximately thirty different alkaloids with very high medicinal value (Prajapati et al., 2002); morphine, codeine, thebaine, noscapine, and papaverine are the main poppy alkaloids (Stranska et al., 2013). The most important of these is morphine ($C_{17}H_{19}O_3N$) due to its wide use as a powerful pain reliever and sedative, and its ratio in a dry capsule varies between 0.2-2.0%. The morphine, codeine, thebaine, noscapine, and papaverine content of Turkish poppy genotypes are 0.25-0.89%, 0.001-0.21%, 0.001-0.08%, 0.005-020%, and 0.004-021%, respectively (Arslan et al., 2009; Yazici and Yilmaz, 2017). Besides, poppy seeds contain approximately 40-55% oil and 20-30% protein, and seeds are used in Turkey in baked products such as donuts, bagels, and cakes (Kara, 2017).

The genetic, physiological, and morphological characteristics of poppy plants affect their production of capsule and seeds and their chemical content. In today's poppy breeding studies, the primary aim is to develop

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varieties with a high capsule yield and high morphine (or another opium alkaloid) content in the capsule. Capsule yield and alkaloid content of poppy are polygenic inheritances and are highly affected by the environment; therefore, it is crucial to know the direct or indirect relationships between traits to improve these characteristics. Generally, genetic and environmental factors that increased yield can have negative effects on quality (Baydar, 2020). Generally, capsule and seed yields of varieties with high morphine content are lower (Kara and Baydar, 2018) because poppies use a high level of photosynthetic assimilation and energy to synthesize more alkaloids. For this reason, breeders use correlation, regression, path, step-wise, and factor analysis to determine characteristics that showed a highly positive (direct and indirect) relationship with yield and quality for reliable selection criteria in searches (Baydar, 2020).

Plant breeding is used in correlation analysis to determine the relationship between yield and quality characteristics. However, correlation analysis only explains the relationships between independent characters, so it is necessary to use path analysis, which gives the relationship between the dependent variable and one or more independent variables. This research was conducted with aim of examining the relationships among alkaloids with the capsule yield and alkaloids contents of new poppy cultivars in Turkey by correlation and path analysis.

Materials and Methods

Experimental location

The field experiments were carried out during 2017-18 and 2018-19 under the semi-arid ecological conditions of Isparta, southwestern Anatolia, Turkey. The experiment conditions were typical of the continental climate with cold and snowy winters and dry and mild to temperate summers which are suitable for growing poppies with autumn production.

During the vegetative periods (from October to August) in 2017-18 and in 2018-19, there was an average temperature of 12.1 and 14.7°C, total precipitation of 431.0 and 574.3 mm, and average humidity of 63.4 and 63.1% (Table 1).

Table 1. Some climatic data of experimental area in growing seasons*

Climatic data	Years	Months											Mean total
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	
Mean temperature (°C)	2017-18	13.2	0.3	2.1	3.1	6.3	9.2	14.2	16.8	20.0	24.3	24.3	12.1
	2018-19	15.8	9.8	4.5	25	4.5	7.4	9.9	17.0	20.6	23.4	24.4	14.7
	Long term	12.9	7.4	3.5	1.9	2.9	6.2	10.7	15.6	20.2	23.6	23.2	11.6
Precipitation (mm)	2017-18	1.6	45.9	82.1	89.2	30.8	69.3	6.3	34.2	53.3	4.1	14.2	431.0
	2018-19	30.6	48.6	107.1	97.0	55.4	40.3	50.8	62.9	69.4	9.5	2.7	574.3
	Long term	38.0	46.3	84.9	72.2	64.7	54.2	56.0	51.4	29.8	14.6	10.5	522.6
Relative humidity (%)	2017-18	61.5	66.5	81.9	75.7	75.6	65.9	51.0	62.3	62.4	46.9	47.6	63.4
	2018-19	63.4	67.7	82.7	81.3	71.8	63.0	643	53.0	59.9	44.7	42.9	63.1
	Long term	62.0	68.5	74.7	73.2	70.2	65.4	61.3	57.4	51.1	45.4	46.3	61.4

*Meteorology Office Records

In the years 2017-18 and 2018-19, the soil at 60 cm was found to be sandy-loamy, low organic matter (1.92% and 1.70%, respectively), slightly alkaline (pH 7.3 and 7.5, respectively), and mid-limey (5.22% and 6.10% $CaCO_3$, respectively).

Seed material and experimental design

TMO-1, TMO-2, Ofis-95, and Ofis-96 with yellow seed; TMO-3, Ofis NM, Ofis-1, Ofis-2, and Ofis-3 with blue seed; Ofis-8 and Huseyinbey with white seed, and Ofis NP with seed pink were used for the genetic material in the research. The noscapine content of the Ofis NM and Ofis NP cultivars, the while morphine content of other cultivars are high. Generally, these cultivars are new varieties that have been registered in recent years in Turkey.

The field experiments were carried out in a randomized complete block design with three replicates in the period of the autumn sowing in 2017-18 and 2018-19. The seeds of each variety were sown at about a 0.5-1 cm depth, the spacing used was 0.40 x 0.15 m, and the plot length was 6 m with 6 rows on the 20th and 25th of October for the first and the second years, respectively. After emergence, one seedling was allowed to establish in each seedbed. The plants were non-irrigated at every growing stage. 100 kg nitrogen ha^{-1} (two equal doses at the sowing and at the 10-15 cm plant height stages) and 30 kg P_2O_5 ha^{-1} (all by sowing) fertilizers were applied to the form ammonium sulfate (21%) and triple super phosphate (43-46%), respectively (Aytekin and Onder, 2006). Plants were hoed at the time of the second fertilizing and were sprayed against aphids before flowering.

The capsules were manually harvested in the full ripeness period according to the maturity stage of the cultivars (moisture content of approximately 15-16%) from four rows in the center of each plot. After the harvest, capsules were dried at room temperature in the shade until reaching a moisture content of 10±0.10% (Kara, 2017). Then the seeds were separated from the capsules, and the capsule yield (kg ha^{-1}) was calculated by multiplying by 10000/plot sizes (m^2).

Alkaloids analysis

The alkaloid contents were analyzed only in the second year. Capsules of all varieties were ground after

drying at 70 ° C for 24 hours, and then the content (%) of morphine, codeine, oripavine, thebaine, papaverine, and noscapine was determined through HPLC-MS/MS analysis after the solvent extraction of poppy straw on dry matter at the laboratory of the Bolvadin Opium Alkaloids Factory (Karadavut and Arslan, 2006).

Statistical analysis

Capsule yield and alkaloid content data were analyzed according to the analysis of variance (ANOVA), and the significant differences between the group means were separated using the DUNCAN test. A matrix of simple correlation coefficients among the alkaloids was computed. The direct and indirect effects of traits on morphine content were made using path coefficient analysis. All analyzes were performed using the SPSS v.16.0 software (SPSS, Chicago, IL. USA).

Results and Discussion

Capsule yield

Capsule yield and the main alkaloid content of poppy cultivars are presented in Table 3. The mean capsule yield of poppy cultivars in the second year (543.8 kg ha^{-1}) was higher than the first year (392.3 kg ha^{-1}) (Table 2). These differences resulted from higher rainfall at the flowering and capsule growing periods (May and June) of plants in the second year compared with the first year (Table 1). In plants grown depending on natural precipitation (without extra irrigation), high rainfall significantly positively affects yield, because soluble nutrients elements are carried by water to growth points from the soil (Svobodova and Misa, 2004).

Differences between capsule yields of poppy cultivars were statistically significant ($P \leq 0.01$) in both years, varying between 253.4-556.2 kg ha^{-1} in the first year and 332.7-659.2 kg ha^{-1} in the second year. The highest capsule yields were obtained from the TMO-2, TMO-3, Ofis-3, Ofis-8, Ofis-95, Ofis-96, and Huseyinbey cultivars, while the lowest capsule yield was determined in the NP, Ofis NM, and Ofis-1 cultivars (Table 2). In a previous study, Kosar et al. (2014) reported that the capsule yield of poppies varied between 610-800 kg ha^{-1} in the Aksehir, 860-1170 kg ha^{-1} in the Bolvadin, 530-670 kg ha^{-1} in the Denizli, 610-740 kg ha^{-1} in the Eskisehir, and 760-1200 kg ha^{-1} in the Usak provinces of Turkey. It is true that our findings were lower than the results of Kosar et al. (2014), and these differences were probably due to climatic conditions, agronomic practices, genotype characteristics (root length, earliness or lateness, nutrient use efficiency) (Boydak and Kavurmaci, 2015; Kara, 2017).

Alkaloid content

The morphine, codeine, oripavine, thebaine, papaverine, and noscapine contents of poppies varied between 0.46-1.84%, 0.08-0.30%, 0.009-0.057%, 0.031-0.093%, 0.002-0.047%, and 0.017-0.094%, respectively. The morphine, codeine, oripavine, and papaverine content of Ofis-1, the thebaine content of Huseyinbey, and the noscapine content of the Ofis-2, Ofis-95, and Ofis-96 cultivars were higher. Generally, the lowest values for all alkaloids were determined to be in the TMO-3 cultivar (Table 2). Generally, varieties with high capsule yield (for example, TMO-2 and TMO-3) had low alkaloid content while varieties with low yield (for example, Ofis-1 and Ofis NM) had high alkaloid content. These varietal characteristics of varieties may vary depending on the spending on seeds or alkaloid synthesis of photosynthesis products during the capsule formation period. Dittbrenner et al. (2009) reported that the main alkaloid of the poppy is morphine, and other major alkaloids are noscapine, thebaine, oripavine, and papaverine. In the previous studies conducted in different regions of Turkey, the morphine, codeine, oripavine, thebaine, noscapine, and papaverine contents of the poppy varied between 0.593-1.453%, 0.000-0.237%, 0.000-0.104%, 0.000-0.523%, 0.000-1.793%, and 0.000-0.350%, respectively, in Ankara conditions (Ozgen et al., 2017); and 0.15-0.60%, 0.001-0.21%, 0-0.01%, 0.001-0.08%, 0.005-0.20%, and 0.004-0.21%, respectively, in Tokat conditions (Yazici and Yilmaz, 2017). The morphine, codeine, thebaine, papaverine and noscapine ranged from 0.110-1.140%, 0.005-0.27%, 0.005-0.134%, 0.001-0.440% and 0.006-0.418%, respectively in Afyonkarahisar conditions (Gumuscu et al., 2008). In our study, the morphine, codeine, oripavine, and papaverine content of the Ofis-1 variety and thebaine content of the Huseyinbey variety were significantly higher than in previous studies. The results showed that the alkaloid's content changed according to the varieties and climatic conditions. Similarly, Franz (1983) explained that the poppy alkaloid's contents were under the influence of genetic and environmental factors.

Table 2. Capsule yield and major alkaloid contents of poppy cultivars

Varieties	Capsule yield (kg ha^{-1})		Alkaloids (%)$^{+}$					
			Morphine	Codeine	Oripavine	Thebaine	Papaverine	Noscapine
	2017-18	2018-19						
TMO-1	367.3 d	564.2 b	0.52 de	0.16 bcd	0.014 fg	0.056 de	0.002 e	0.035 cd
TMO-2	556.2 a	589.3ab	0.46 e	0.17 bc	0.018 efg	0.053 e	0.003 de	0.053 bc
TMO-3	429.1cd	659.2 a	0.49 e	0.12 de	0.009 g	0.070 bc	0.004 de	0.057 bc
Ofis-1	272.4 e	338.1de	1.84 a	0.30 a	0.057 a	0.042 f	0.047 a	0.056 bc
Ofis-2	381.5d	435.7 c	1.19 b	0.15 bcd	0.013 g	0.080 b	0.035 b	0.086 a
Ofis-3	377.2 d	639.2ab	0.79 cde	0.14 cd	0.013 g	0.031 f	0.046 a	0.076 ab
Ofis-8	364.4 d	646.5ab	0.90 bcd	0.15 bcd	0.024 cde	0.071 bc	0.005 cd	0.017 d
Ofis-95	471.6bc	631.6ab	0.70 cde	0.13 de	0.035 b	0.032 f	0.003 de	0.087 a
Ofis-96	429.1cd	632.4ab	0.61 cde	0.08 e	0.033 bc	0.065 cd	0.005 de	0.094 a
Ofis NM	271.6 e	422.8cd	0.95 bc	0.28 a	0.029 bcd	0.048 e	0.005 cd	0.070 ab
Ofis NP	253.4 e	332.7 e	0.78 cde	0.13 cde	0.023 def	0.058 de	0.009 c	0.060 bc
Huseyinbey	534.7ab	618.1ab	0.54 de	0.19 b	0.010 g	0.093 a	0.003 de	0.038 cd
Year $_{Mean}$	392.3 B**	542.8 A	0.81	0.16	0.023	0.058	0.014	0.061
Mean square	288.8	461.9	0.296	0.123	0.057	0.012	0.093	0.162
F value $_{Cultivar}$	32.07**	31.48**	10.81**	27.16**	36.41**	54.73**	368.74**	12.92**
F value $_{Year\ x\ cult.}$	2.84^{ns}							
CV (%)	7.65	7.06	11.08	8.78	7.15	8.31	6.37	8.43

**: significant at P<0.01 probability levels, ns: non-significant

$^{+}$Alkaloids contents of poppy belongs to in 2018-19.

Means in the same columns followed by the same letters are not significantly different as statistically

Correlation and path coefficient analysis

The results of correlation analysis among the alkaloids of the poppy cultivars are shown in Table 3. According to the results of the correlation analysis, while there were significant and positive correlations between morphine with codeine (r=0.415*), oripavine (r=0.362*), and papaverine (r=0.624**), an insignificant and positive correlation was determined between noscapine (r=-0.164^{ns}) and thebaine (r=0.043^{ns}) in the poppies (Table 3). Significant and negative correlations were determined between oripavine and thebaine (r=-0.532**) while there were positive and insignificant correlations between papaverine (r=0.264^{ns}) and noscapine (r=0.207^{ns}). Significant and negative correlations were determined between thebaine with papaverine (r=-0.374*) while there was a non-significant correlation of thebaine with noscapine (r=0.228^{ns}). Insignificant correlations were determined between papaverine and noscapine (r=0.280^{ns}) in the poppy (Table 3).

Table 3. Correlation coefficient matrix of alkaloids

Alkaloids	Morphine	X_1	X_2	X_3	X_4	X_5
Codeine (X_1)	0.415*	1.000				
Oripavine (X_2)	0.362*	0.435**	1.000			
Thebaine (X_3)	0.043^{ns}	-0.204^{ns}	-0.532**	1.000		
Papaverine (X_4)	0.624**	0.317^{ns}	0.264^{ns}	-0.374*	1.000	
Noscapine (X_5)	0.164^{ns}	0.105^{ns}	0.207^{ns}	0.228^{ns}	0.280^{ns}	1.000

*, **: significant at P<0.05 and P<0.01 probability levels, respectively, ns: non-significant

In our research, morphine as a dependent variable and codeine, oripavine, thebaine, papaverine, and noscapine as determinative variables were used for the path coefficient analysis. The direct and indirect contributions to the morphine of the major alkaloids in the poppy cultivars are given in Table 4. The direct effects on morphine of the major alkaloids were positive. The highest positive direct effect on morphine content was papaverine (p=0.631, 67.75%) followed by thebaine (p=0.413, 47.54%), oripavine (p=0.343, 42.83%), codeine (p=0.152, 25.86%), and noscapine (p=0.027, 7.01%), respectively (Table 4). Papaverine had the highest indirect positive contribution to morphine content via noscapine (p=0.176, 45.82%) and codeine (p=0.200, 33.97%) (Table 4). Thebaine had an indirect negative effect on morphine content via all examined alkaloids (Table 4). These results indicated that the direct and indirect effect on morphine content of papaverine was higher than the other alkaloids. Bajpai et al (2001) stated that there was a significant and positive relationship between morphine and codeine. Shukla et al (2003) reported negative relationships between morphine and other poppy alkaloids. Yadav et al. (2006) reported that bilateral relationships between morphine, codeine, thebaine, and papaverine were positive while there was a negative relationship between morphine and papaverine; the same studies reported that alkaloids can transform into each other depending on the alkaloid synthesis pathway. Prajapati et al. (2002) reported that the morphine alkaloid was synthesized from codeine rather than oripavine, and there was a positive relationship between morphine and codeine. Psenak (1998) explained that oripavine alkaloid was the final product in the synthesis of morphine in some poppy species but that it is transformed into morphine via thebaine, codeinone, and codeine in *Papaver somniferum*. Besides, the same researcher reported that codeine was synthesized as a result of the reduction of

codeinone, and oripavine transformed into morphine by linking hydrogen to its chemical structure. Dittbrenner et al. (2009) stated that codeine was the pioneer of morphine synthesis and that sometimes the morphine pathway was blocked during enzymatic activity, and the conversion from codeine to morphine stops. We can report that the results of our study were similar to the findings of previous researchers.

Table 4. Path coefficient (direct and indirect effects) among alkaloids

Alkaloids	Direct effects	Indirect effects				
	Morphine	X_1	X_2	X_3	X_4	X_5
Codeine (X_1)	0.152	-	0.066	-0.031	0.048	-0.016
	25.86		8.27	3.57	5.17	4.27
Oripavine (X_2)	0.343	0.149	-	-0.182	0.090	0.071
	42.83	25.38		20.99	9.70	18.42
Thebaine (X_3)	0.413	-0.08	-0.219	-	-0.154	-0.094
	47.54	14.28	27.40		16.56	24.40
Papaverine (X_4)	0.631	0.200	0.166	-0.236	-	0.176
	67.75	33.97	20.77	27.17		45.82
Noscapine (X_5)	0.027	-0.002	0.005	-0.006	0.007	-
	7.01	0.49	0.69	0.70	0.81	

The first lines are path coefficient (pc) and the second lines are path percentage (%)

Conclusions

In this research, the highest capsule yield was obtained from the TMO-2 and TMO-3 varieties. Ofis-1 had the highest morphine, codeine, oripavine, and papaverine content while the thebaine content of Huseyinbey and the noscapine content of Ofis-2, Ofis-95, and Ofis-96 were higher.

There were significant and positive correlations between morphine content with codeine, oripavine, and papaverine while bilateral relations between alkaloids showed a difference. Papaverine had the highest direct and positive effect on morphine followed by thebaine, oripavine, and noscapine, respectively. Noscapine had the highest indirect positive effect on morphine via papaverine. Thus, relationships among alkaloids should be taken into consideration in selection breeding with high alkaloid content because the average morphine content in dry capsules of local varieties, which are widely cultivated in Turkey, is around 0.4%. However, the contents of morphine and its derivatives are around 1-2% in some countries; therefore, the breeding of varieties with both high capsule yield and high morphine content in Turkey should be studied.

Compliance with Ethical Standards

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Designed of the experiment and its conducted (Kara, N), prepared of plant materials and collected experiment data (Kara, N), performed of CS-MS analyses (Kara N), wrote the manuscript (Kara, N and Baydar, H). Both authors read and approved the final manuscript.

Ethical approval

Not applicable.

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Data availability

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Distribution of Water Footprint Components of University Students and Detecting the Factors that Affect Those Components

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Abstract

20% of world population face the risk of disease and death due to the lack of access to healthy drinking water. A certain portion of water resources can no longer be used because of being polluted while some other parts pose danger for public health because of substructure incapacity. Water footprint is a remarkably crucial concept in terms of sustainable water management. Within the context of this study, consumption habits of university students and related changes in water footprint values have been investigated. Water Footprint Survey has been administered to participant university students and water footprint profiles of the students have been designed based on water footprint values computed according to survey results. At the end of the conducted analyses in Istanbul University-Cerrahpasa Avcilar Campus, mean annual rate of water footprint per person has been computed as 1848.78 m^3 for students. Components of this water footprint has been designated as; green water footprint 1329 m^3/per person/year, blue water footprint 199 m^3/per person/year, grey water footprint 320.78 m^3/per person/year. In addition, by transferring the data attained from surveys to IBM SPSS environment, presence and/or absence of a significant relationship between variables has been analyzed. It was then observed that parallel to the rise in students' income level a corresponding climb emerged in general water footprint.

Keywords: Life habits, University campus, SPSS, Water consumption, Water footprint

Introduction

Water resources are being depleted by every new day and there has been a resultant increase in the number of societies facing water scarcity. Under these circumstances “Ecologic Footprint”, “Water Footprint” have gained wider acclaim as the concepts vital to prevent uncontrolled consumption of natural resources critical for living beings and essential to form a sustainable environment approach. In Turkey, available annual water quantity roughly equates to 112 billion m^3. Turkey is situated in a “semi-arid” location with high ratios of temperature. Falkenmark Water Scarcity Index reveals that to make a country water rich, annual volume of per person fresh water resource should exceed 1700 m^3. As of 2017-dated statistics issued by the State Hydraulic Works in Turkey, per person rate of fresh water resource is estimated around 1386 m^3. According to this Index,

Turkey is a country challenged with water stress. Besides, under the heading of “Water Stress”, it is projected that with a population expected to reach 100 million until 2023 there will be 1120 m^3/per year left per person; hence by the year 2050 Turkey will be a country facing “water scarcity”(TKSB, 2019).

Water footprint concept is an indicator of fresh water quantity consumed or polluted by unit of time and it is rooted back to ecological footprint. It was first discussed in the 2002-dated experts meeting named Potential Water Trade in the Netherlands. Next World Water Forums were held in 2003 in Japan and in 2006 in Mexico, the same topic has been discussed in various international conventions.

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Water footprint concept has surfaced because of higher pressures on the local or basin-based water resources and emergent problems in water management. In relation with higher pressures on water resources, water utilized in the production of goods and services attracted greater value. In addition to the kind of goods, that demand heavy consumption of water in production stages it was recognized that local fresh water resources employed in their production moved beyond geographical boundaries. Thus, it was mandated to perform water footprint computations. The said concept was, for the very first time, introduced in 2002 by Prof. Dr. Arjen Hoekstra and further developed by Water Footprint Network (WFN) and Twente University. Water footprint is defined as a measure of the cumulative virtual water content required for human consumption (Aldaya et al, 2012; Wang and Ge, 2020).

Water footprint is an indicator of direct and indirect water use in relation to consumer goods. Water footprint can provide links between use of water resources and consumption of goods (Hoekstra, 2003; Mirzaie-Nodoushan et al., 2020). It is possible to measure water footprint of an individual, a product, a business branch or a country. Water footprint of an individual refers to the total volume of water utilized for the consumed service and produced goods and products per person. It is computed by multiplying "virtual water contents" of generated service, goods and products by consumption volumes. Water footprint not only displays the volume of consumed water but also reveals when and where the said water was utilized and to which category it belongs; green, blue and grey water footprints (Mekonnen and Hoekstra, 2011).

The blue water footprint concept refers to the total amount of surface and underground freshwater resources required to produce any good or service. This concept refers to consumption volume that occurs when water extracted from groundwater resources or surface water resources evaporates and is utilized in production; hence, extracted water fails to return to its original water resource. Water utilized in agriculture, water used in production lines of plants and domestic use is categorized as blue water footprint (Hoekstra et al., 2011; Pellicer-Martínez and Martínez-Paz, 2016).

Green water footprint calls for the volume of total rainwater used in the production of any good or service. These resources are primarily used in gardening, agriculture and forestry operations. It is evident in cases when rainfall per unit area fails to penetrate into ground waters and remain on the surface or absorbed by plants use. This phenomenon is a measure of evaporated volume of water and amount of water used by plants. Green water footprint primarily comes to the scene in the stage of producing agricultural products (Hoekstra et al., 2011; Pellicer-Martínez and Martínez-Paz, 2016). Green and blue water footprint indicates fresh water consumption whilst grey water footprint is in indicator of pollution (Hoekstra and Chapagain, 2008; Hoekstra et al., 2009). Grey water footprint concept, on the other hand, refers to the sum of fresh water volume required to designate a specific criteria of water quality by lowering contaminant concentration directly discharged to water resources or indirectly released into wastewaters to threshold values through administering dilution method (Pellicer-Martínez and Martínez-Paz, 2016). In relevant literature, there is a scarcity of studies conducted to determine campus water footprint. One of the few studies was conducted by (Natyzak et al., 2017) and in this study water footprint of the University of Virginia (UVA) was computed as the sum of direct water consumption and virtual water consumption. By analyzing in tandem with Water Footprint Statistics (Water Stat) public services, food, transportation, paper, research animals and facility management water bills within the premises of university as well as purchase records that entailed purchases for the hospital were reported and water footprint could thus be estimated. 10.06% of total water footprint consisted of direct water consumption, 45.77% consisted of public service industry, 23.34% consisted of food production industry, 16.88% consisted of health sector and 3.95% consisted of paper, transportation and research animals' domains. Footprint due to direct water consumption was roughly computed as 1.7 million m^3 and virtual water footprint was computed as 15.2 million m^3.

In the study of Emory University (Allison et al., 2018), to achieve a campus-wide innovative water treatment, a re-use system also known as WaterHubt was operated. By this system, daily 151 liters of recovery was enabled thus two third of wastewater production of the university was recycled to its equivalent and campus water footprint could then be lowered as low as 40%.

In 2019 a research was conducted in Keele University to measure the total energy footprint, carbon footprint and water footprint values and in this particular study total water footprint of Keele University was computed as 532,415 m^3 (Gu et al., 2019).

Likewise, in another 2019-dated study, the water footprint of Valaya Alongkorn Rajabhat University due to electric-use was examined and it was concluded that vehicle fuel consumption was the reason for the highest level of water footprint (Kandananod, 2019).

In this particular study water footprint of university students was computed. In this case the aim was to determine water footprint components of same-age youngsters living in the same environment despite being raised in different cities and have different cultural formations. It was also aimed to unveil the factors affecting these components.

Materials and Methods

In this study, while water footprint values were designated (URL1), questions posted in a water

footprint calculation motor were printed in a document. This Survey document was shared with the participants and each of the responses was singly entered to this calculation motor in order to compute their footprints. Survey questions are as listed in Table 1. During 2018-2019 academic year this research was conducted among 559 students, studying in 3 faculties respectively listed as Faculty of Engineering, Veterinary School and Faculty of Sports Sciences located in Istanbul University-Cerrahpasa Avcilar Campus. In this study, the incomes of the participants are given in Turkish Lira (TL). When this study was done, 1 US Dollar was 6.96 TL. SPSS 15.0 software was used to explain statistical significance of digital data.

Results and Discussion

In this research, firstly, participants were categorized into groups based on their age. It was detected that age range of the participants was 18 – 24. Water footprints of the participants based on age are as shown in Figure 1 (Green, blue, grey water footprints and total water footprints). As displayed in Figure 1, an increase in age corresponded to higher ratio of total water footprint whereas green, blue and grey water footprints failed to perform a directly proportional rise. Maximum mean water footprint value (2572 m^3/year) and maximum green water footprint value (1878 m^3/year) were reported to belong to age 24. Maximum blue water footprint value (306 m^3/year) belonged to age – 21 group and maximum grey water footprint value (524 m^3/year) belonged to age-23 group (Figure 1).

Upon analyzing the connection between Age and Water footprint, water footprint components were explored by considering participants' income levels. Connection between income level-water footprint was examined with respect to each faculty and in a general context. As the connection between income level-water footprint components was examined with respect to each of the three faculties, obtained results are as exhibited in Figures 2, 3 and 4.

Water footprint results of the Faculty of Engineering students with respect to income level can be viewed in Figure 2. As it can be observed one unit rise in income level corresponded to a climb in water footprint components (green, blue and grey water footprint) and total water footprint values. It was realized that total water footprint value of the participants whose monthly income levels were above 10 thousand TL corresponded to the top rank in all income groups (3142.5 m^3/year) (Figure 2).

Water footprint results of Veterinary School students with respect to income level can be viewed in Figure 3. When compared to the increases in students' income levels it was seen that only green water footprint and total water footprint among all water footprint types elevated in direct proportion. It was realized that maximum green water footprint value (1808 m^3/year) and maximum total water footprint value (2306 m^3/year) belonged to the participants whose monthly income levels were above 10 thousand TL (Figure 3).

Water footprint results of the Faculty of Sports Sciences students with respect to income level are as shown in Figure 4. When compared with the increases in students' income levels it was detected that all of the water footprint components and total water footprints also rose.

Table 1. Total iron concentrations in leaves of the grapevine genotypes grown in nutrient solution

1-Monthly water consumption	2-Monthly drinking water consumption	3-How often do you wash your car in a week?
4- Weekly meat consumption	5- Weekly poultry consumption	6- Weekly egg consumption
7- Weekly milk consumption	8- Weekly cheese consumption	9- Weekly yoghurt consumption
10- Weekly vegetables consumption	11- Weekly fruit consumption	12- Daily bread consumption
13- Weekly pasta consumption	14- Weekly rice consumption	15- Weekly potato consumption
16- Weekly legumes consumption	17- How many cups of tea do you drink in a day?	18-How much sugar do you add to one cup of tea?
19- How many cups of coffee do you drink in a day?	20- How much sugar do you add to one cup of coffee?	21- Monthly dessert spending
22- Monthly electricity bill	23- Monthly vehicle fuel expenditure	24- Monthly LPG expenditure
25- Monthly expenditure on attire	26-Monthly expenditure on electronic devices	

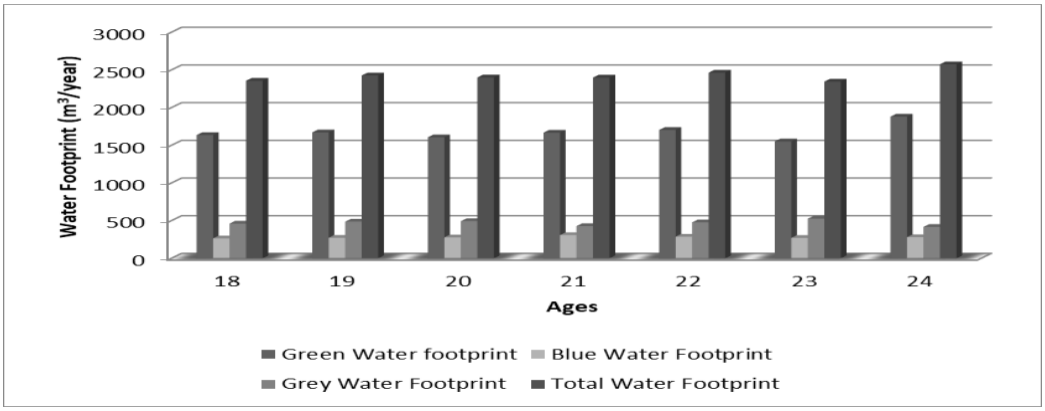

Figure 1. Mean annual water footprint results of the participants with respect to age

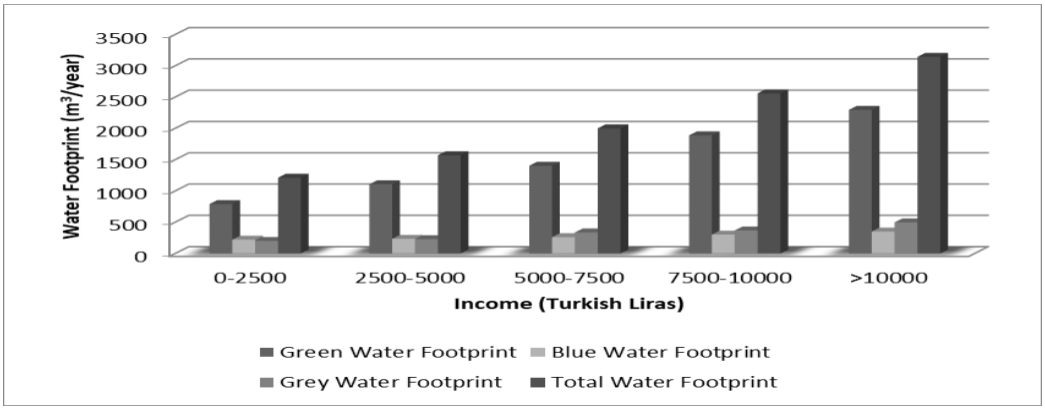

Figure 2. Water footprint results of the Faculty of Engineering students with respect to income level

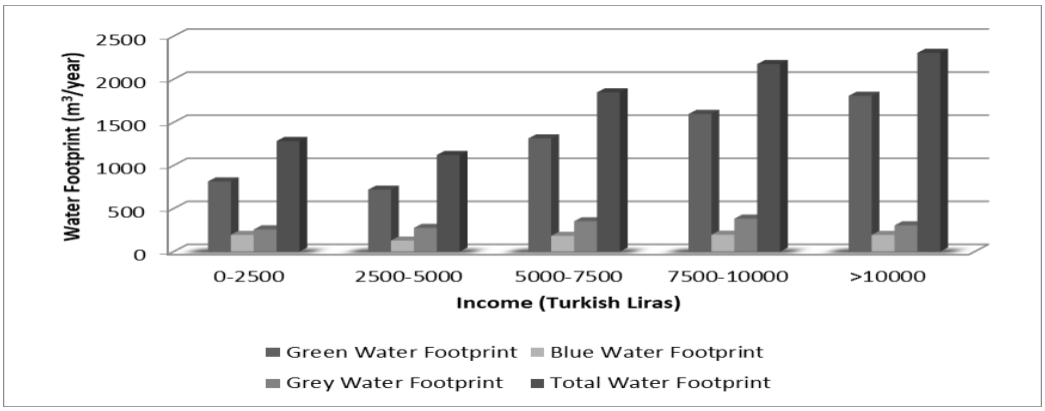

Figure 3. Water footprint results of Veterinary School students with respect to income level

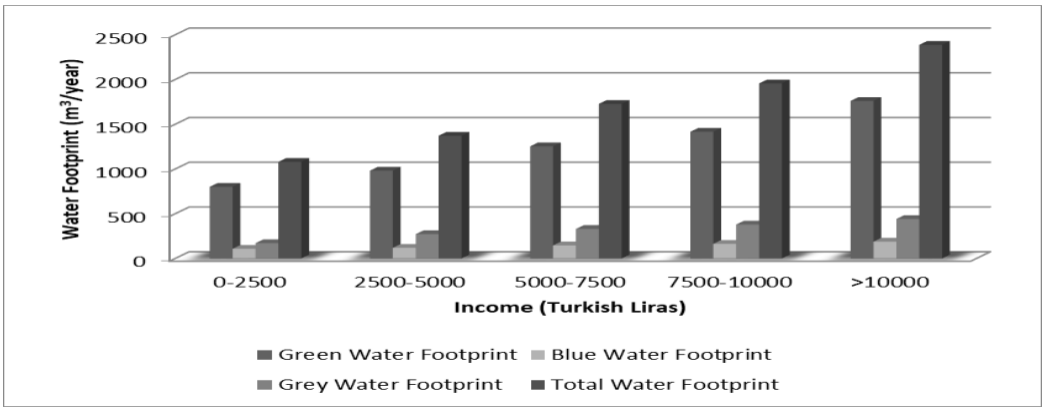

Figure 4. Water footprint results of the Faculty of Sports Sciences students with respect to income level

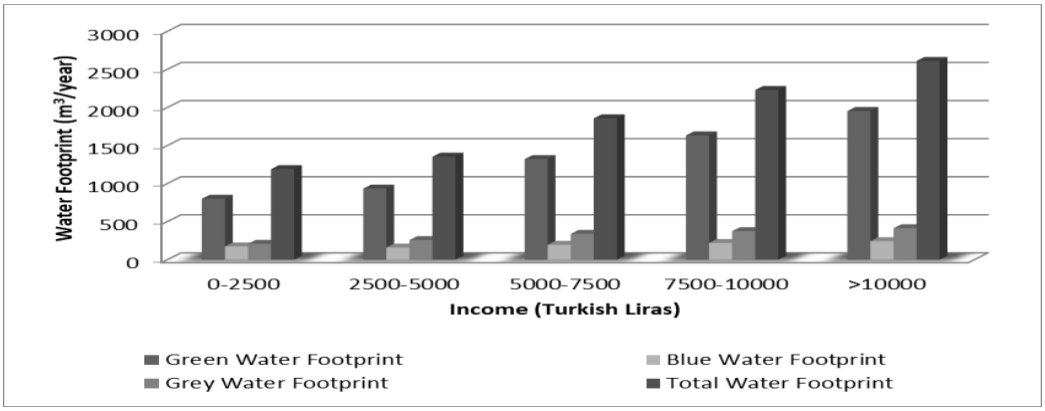

Figure 5. Mean annual water footprint and its components for Istanbul University – Cerrahpasa students with respect to income level

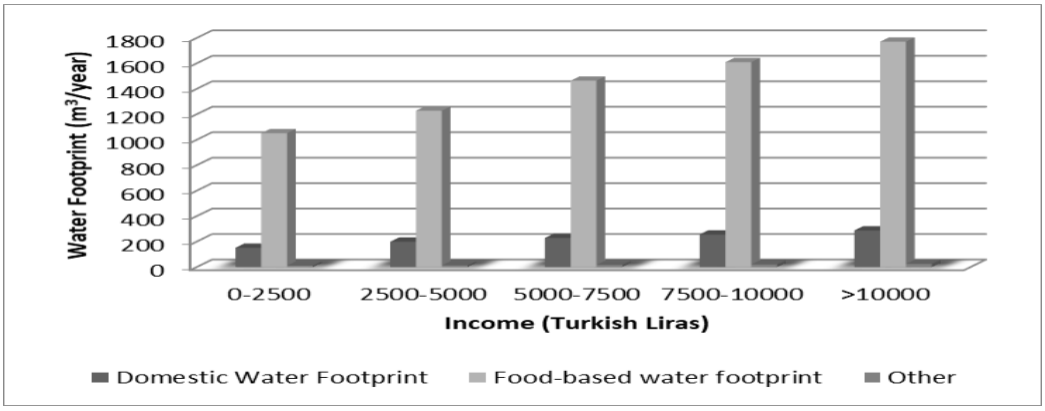

Figure 6. Water footprint distribution graphic with respect to income level

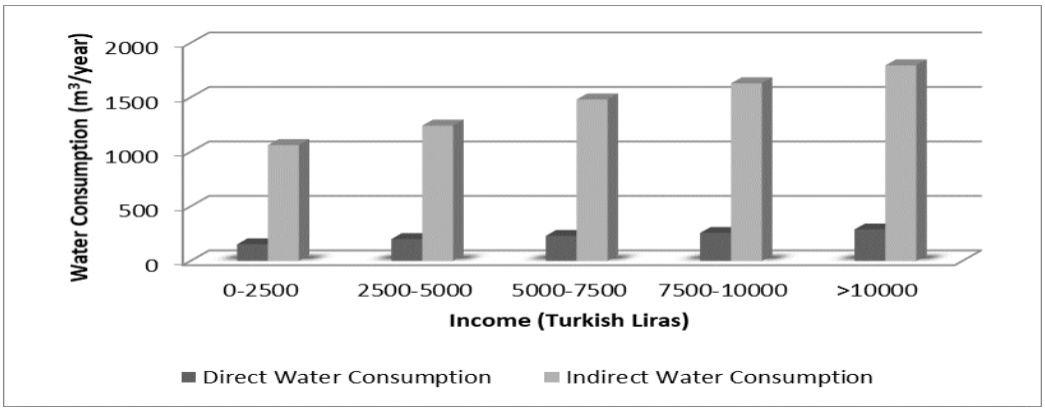

Figure 7. Distribution graphic of direct and indirect water consumption with respect to income level

Maximum green water footprint (1759 m^3/year), blue water footprint (187 m^3/year), grey water footprint (440 m^3/year) and total water footprint (2386 m^3/year) were computed (Figure 4). As water footprint values of the participants from three faculties were examined it was detected that in all faculties, maximum water footprint values belonged to Faculty of Engineering students partaking in the research (Figure 2, 3 and 4).

Figure 5 presents water footprint components with respect to income levels for all participants. As seen in Figure 5 a rise in income level heightened total water footprint. An analysis of water footprint components showed that maximum increase was evident in green water footprint while in blue water footprint the same increase was insignificant. Maximum total water footprint value (2611.63 m^3/year) belonged to the participants whose monthly income levels were above 10 thousand TL (Figure 5).

In Figure 6, income-level based distribution of water footprint inducing-factors that are related with domestic use, food and other consumptions are illustrated. Figure 6 evidenced that the largest components that constituted students' water footprint stemmed from foods. To explain this finding it was

suggested that water was most heavily used in the stage of producing food products.

Figure 7 manifested that as income level climbed, direct and indirect water consumption also increased. Relative highness of indirect water consumption compared to direct water consumption was explained with food consumption.

At the end of conducted analyses, mean annual water footprint per person in students of Istanbul University – Cerrahpasa Avcilar Campus was computed as 1848.78 m^3/per person/year. As similar researches in literature were analyzed it was revealed that in a different research published in 2019 and conducted among students from a different university in Turkey, mean water footprint was computed as 1490.1 m^3/year (Dursun, 2019). The said research was conducted in a sparsely populated Eastern city of Turkey and students in this city had lower income levels. Thus obtaining a lower value than the mean water footprint value computed in our study is in support of the suggestion of our study that income level is a major contributor for water footprint.

In Turkey, mean per person water footprint is 1977 m^3/year. Per person, consumption is 216 L/day. Yet based on virtual water, per person consumption equates to 5416 L/day (URL 2). As reported currently in Turkey per person mean water footprint is 4425 L/day (1422 m^3/per person/year) (URL 3). Computed average ratio in this analysis is 1849 m^3/per person/year value, whereas in Turkey water footprint value computed for present day is above 30%. Per person consumption computed as 1.422 cubic meter in 2015 was measured as 1.386 cubic meter in 2017(1) Mean indirect water consumption per person is computed as 1440 m^3 in this study and direct water consumption as 224 m^3. In that computation, total sum of per person water consumption was computed as 1664 m^3. Per person water (1386 m^3) value computed for Turkey evidences that water demand in Turkey exceeds 20%. In this study, it was detected that a rise in income level corresponded to higher spending for electronic devices (mobile phones etc.) and clothes. Besides, since people with high-income levels prefer to own cars they would also pay for additional costs like car washing and vehicle fuel expenditures. Meat consumption is also relatively higher than other income groups. Consequently, it is suggested that higher income level triggered greater total water footprint values.

Data obtained at the end of this survey conducted in Istanbul University – Cerrahpasa Avcilar Campus were transferred to SPSS program to conduct an analysis. Five different variables were selected respectively as age, income, green water footprint, blue water footprint, grey water footprint. Normalcy test was administered to the variables. Results indicated that none of the 5 variables could fit with normal distribution. Hence, Spearman Correlation Analysis was performed for further comparisons. Firstly, hypothesis on the direct relationship between increased age and water footprint was tested. Although a significant relationship between variables requires that significance level should be below 0.05 value, when the age and other variables were contrasted significance level was measured to be above 0.05 value; thereby indicating that no significant relationship existed between the variables. In the second analysis, income variable was included and partial correlation analysis was then conducted. Here, age was identified as the fixed variable. Results of the analysis evidenced that a significant and positive relationship existed between income and water footprints. As the correlation coefficients between income variable and water footprints were analyzed it was detected that a strong relationship existed between 0.7 correlation coefficient and green water footprint, an average relationship existed between 0.6 and 0.4 correlation coefficients respectively and between grey and blue water footprints respectively. It can thus be observed that as income level rose, water footprint types also increased in a significant way. In the third analysis, all of the variables were co-evaluated and a generic Spearman Analysis was applied. Obtained results remained the same and identical results were observed. It was revealed that as the income level increased, the water footprint values also increased.

Conclusion

Water indeed is the very soul of life and total amount of water on Earth is 1.4 billion 350 million km^3. Yet since 97.5% of water mass is salty water, it is unviable for human consumption. A large portion of remaining fresh water resources are underground waters or glaciers. Hence, available fresh waters that living beings can directly consume is at an alarmingly low level. On the other hand, due to gradual rise in world population and increased demand for water, fresh water resources are being depleted out of control despite being already scarce. To make water resources sustainable it is essential to conduct water footprint computations.

According to Falkenmark Index Turkey is not a water rich country but rather a country stricken with water stress. The size of domestic water consumption by the sum of used water is approximately 10% and this is the sector with the least amount of water loss. Yet taking precautions is quite important in the fight against water scarcity. Domestic water consumption can be lowered only after striving to accomplish our personal duties. Our conscious choices could be of use to prevent water scarcity and it can only be achieved through promoting awareness- raising initiatives.

Consequently, in this study university students were deliberately chosen since it is assumed that acquisition and awareness gained particularly during university education could be effective in initiating a life-long change. Therefore, by measuring water footprints of students in Istanbul University-Cerrahpasa Avcilar Campus it was aimed to use obtained findings to raise environmental awareness of university students.

At the end of the analyses, mean annual per person water footprint of 559 students in Istanbul University – Cerrahpasa Avcilar Campus was measured as 1848.78 m^3. As water footprints and income levels were compared, it was detected that a rise in income level heightened total water footprint. Maximum increase was measured in green and grey water footprint whilst in blue water footprint not any significant increase could be detected. This research evidenced that the biggest component responsible for students' water footprint belonged to food products. This finding suggested that green water footprint most dramatically emerged at the stage of producing food products. As income level rose, direct and indirect water consumption also increased. Compared to direct water consumption indirect water consumption was mostly associated with food consumption. As the age increased no proportional increase or decrease could be detected in water footprint. It is suggested that conducting the research among a narrow age band of 18 – 24 played quite an important role in this result.

In addition, by transferring the data attained from surveys to SPSS environment presence and/or absence of a significant relationship between variables was analyzed. Based on the results of the analysis it was seen that a significant and positive relationship existed between income level and water footprints. It can thus be observed that as income level rose, water footprint types increased in a significant way.

At the end of this study, it was identified that daily water need of university students moved further beyond available amount of water. Findings of this study evidenced that water footprint of university students should be diminished. Suggestions offered to reduce water footprint values can be listed as below:

- It is suggested to promote lower consumption of dietary meat products hence it can be feasible to create minimal footprint.
- Packaged foods always contain higher ratio of water footprint. Water footprint tends to increase in all stages from production to packaging until the period they reach to the market. Thus, it is suggested to promote consumption of natural goods.
- In our daily activities based on water consumption we should lower our water use to half to care more about the future.
- By avoiding renewing electronic devices unless absolutely necessary and rejecting consumption of luxurious goods it is also possible to decrease water footprint.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Acacia Karroo Pods and Leaves as Major Feed for Fattening of Goats

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Abstract

The objective of this study was to assess the effects of using *Acacia karroo* pods and leaves for fattening of goats. The experiment was carried out at a farm in Mashava, an area located in Masvingo Province within latitude 20° 2′ 43″ S and longitude 30° 40′ 29″ E in the south-eastern part of Zimbabwe. Mixed dried leaves and pods of *Acacia karroo* were ground using a 2 mm screen and then included at levels of 0, 20, 60 and 100%, replacing ground maize in the diets. Weaned goats (n=64) were allocated in weight order to groups of four animals and randomly assigned to the four treatments in a randomised block design. Growth rates of goats fed with diet containing 100% *A. karroo* had higher (15.48 ±0.069 kg) final weight compared to any other diets followed with goats fed diet containing 60% of *A. karroo* and results showed significant differences ($p<0.001$) between treatments. Feed intake over all treatments was comparable with around 500 g day^{-1}. Goats fed with control diet recorded highest voluntary feed intake of 504.5 g day^{-1} and lowest of 499 g/day was recorded from diet containing 60 % *A. karroo*. Goats which were fed with 60 % and 100% A. karroo had low feed conversion ratio (FCR) although the results show significant differences ($p<0.05$) among all treatments. Goats fed diets containing 0% *A. karroo* had the least average weekly weights as compared to all other diets. Average weekly weight gains for goats fed with 60 % *A. karroo* and 100% *A. karroo* diets rapidly increased in week 2 with those fed 100% *A. karroo* diets recorded a highest gain of 915.75 ±59.888 g and results were significantly different ($p<0.001$) between treatments. Farmers are recommended to use 60-100% A. karroo diets when pen fattening goats.

Keywords: Assessing, effects, Acacia karroo, pen fattening, goats

Introduction

Goat production is one of the major income generations for smallholder farmers in arid and semi-arid areas (Brown et al., 2018) due to low cost of production and short period of reaching maturity. Goats have a paramount role in human livelihoods in Zimbabwe and other southern African countries like South Africa (Ngambi et al., 2013; Brown et al., 2018). Goats provide milk, meat, manure, hide, skin and cash after selling them. Goat production is mainly limited by poor feeds during dry seasons in most dry regions. Feed availability has limited goat production in most communal areas (Alemu et al., 2014; Brown et al., 2016) in dry regions for example Southern Africa. Feed resources available during dry season are deficient in protein, minerals, vitamins and energy which negatively affect growth of goats (Brown et al., 2018). The vegetation in regions of low rainfall is associated with high densities of Acacia species such as Acacia karroo for which the browsing habit of goats is well adapted (Mapiye et al., 2011).

Acacia karroo is one of the most abundant indigenous legume trees in semi-arid and arid areas in Southern Africa (Halimani et al., 2005). Acacia karroo has been noted to supply high levels of crude protein (CP) to livestock production together with other legume trees such as A. nilotica and A. tortilis among other species. According to Mapiye et al. (2011) A. karroo leaves contain essential fatty acids such as linoleic and oleic acids which improve meat quality. The leaves also contain tannins which can improve the "bypass" characteristics of protein thus increasing its nutritive value (Brown et al., 2018).

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Condensed tannins contained in A. karroo (55-110 g/kg DM) have been reported (Mokoboki et al., 2005). Acacia karroo diets were mixed with fresh grass as means of diluting the effects of tannins. However, the presence of spines may reduce feed intake as these cause sores around the goat mouths. The pods and leaves can be harvested while still green, mixed together, dried and ground (to avoid problems of the spines).

Materials and Methods

The experiment was carried out at a farm in Mashava an area located in Masvingo Province located within latitude 20° 2′ 43″ S and longitude 30° 40′ 29″ E in the south-eastern part of Zimbabwe. The area receives on average 450 to 500 mm rainfall per annum. The area is dominated with Acacia species in the eastern part and *Colophospermum mopane* in the western part of the area. Acacia karroo leaves and pods were sun dried one month before used and ground using a hammer mill with a 2 mm screen, then mixed with ground maize grain at levels of 0, 20, 60 and 100% to form the 4 experimental diets. Weaned goats (n=64) were allocated in weight order to groups of 4 according to live weight and randomly assigned to the four treatments. The treatments were replicated four times to give a total of 64 goats. A complete randomised design was used. Goats were fed 5% feed of their body weights on daily basis. Goats were managed under intensive management system in pens for easy management and monitoring. All treatments were provided corresponding quantities of green grass to percentages of *Acacia karroo* as a source of energy. Goats were fed experimental diet together with green grass for one week (7 April -14 April 2019) before start of the experiment to allow them to adapt to the diet. The experiment was done for six weeks starting from 15 April 2019 to 27 May 2019. Goats were also vaccinated and dosed to prevent internal parasites. Weights of goats were measured on weekly basis and recorded to calculate average weekly weights and average weekly weight gain for each group. Weekly weights were measured on same day for all groups before feeding them to get accurate weights. Leaves and pods were analysed according to procedures of AOAC (2005).

Data collected was analysed using IBM SPSS version 25 and means which were different were identified using Duncan's multiple range of test at 5 %.

Results and Discussion

Proximate Analysis of *Acacia karroo* pods and leaves and growth performance of goats

The pods were richer than the leaves in crude protein (Table 1) but had higher proportions of NDF and ADF. Live weight gain increased with a curvilinear trend (Table 2) as the ground A. karroo pods was mixed leaves and green grass. In contrast, the feed conversion was declining with increasing percentage of A. karroo. The results showed that pods have high crude protein and a possibility of increasing growth rates of goats. Goats are ruminants and are also able to synthesise proteins hence this will combine with proteins from pods and leaves to increase growth and development. All experimental goats fed A. karroo pods and leaves showed higher average weight gains as compared to the control because the diets contained higher CP content which promotes microbial functioning (Masiku, 2013) and this increased feed intake. These results coincide with those of Halimani et al. (2005) and Mapiye et al. (2010) who reported that A. karroo leaves contain high levels of CP and essential amino acids. This result is similar to findings by Mapiye et al. (2009c) who reported that high CP in A. karroo increases growth rate of livestock animals especially ruminants. This also coincides with findings by Dube (2000) who reported that an increase of A. karroo leaf meal from 40 % to 60 % in goat diet significantly increased feed intake and body weight gain. This also coincides with results by Kahiya et al. (2003) and Halimani (2002) who reported an increased average weekly weight gain in goats and pigs respectively. Similar results were reported by Ngongoni et al. (2007) who indicated that A. karroo has high digestibility and this may increase voluntary feed intake, weight gain and growth rate of ruminants. The result also coincides with work by Marume et al. (2012a) who reported that high CP content in A. karroo leaves and pods increases digestibility and nutrient availability to goats leading to increased meat quality.

Table 1. Chemical composition of *A. karroo* pods and leaves (Dry matter basis, except for DM which is on air-dry samples)

A. karroo	DM	OM	Ash	CP	NDF	ADF
Pods	900	906	60.9	190.1	462.8	375.1
Leaves	919	897	67.8	127	296.5	123.5

Where: DM= Dry matter (g kg^{-1}); OM=Organic matter (g kg^{-1}); CP=Crude Protein (g kg^{-1}); Neutral Detergent Fibre (g kg^{-1}); Acid Detergent Fibre (g kg^{-1}).

Same superscripts in the same column denotes no significant different between treatments at $p \leq 0.05$.

Table 2. Growth performance of the goats during the 6 weeks experiment

Treatments	Initial Live Weight (LWT) (kg)	Final LWT (kg)	Average weekly Gain (g/goat)	Average Daily Gain (ADG) (g/goat/day)	Voluntary Feed Intake (VFI) (g/goat/day)	Feed Conversion Ratio (FCR) (kg feed/kg live weight gain)
AK0	10.09±0.114[a]	14.28 ± 0.18[c]	705.08±107.226[c]	100.72± 15.307[c]	504.5± 12.254	5.057± 0.032[a]
AK20	10.00± 0.136[a]	14.89± 0.116[b]	814.87±158.577[b]	116.43±22.641[b]	500.0± 12.032	4.294± 0.024[b]
AK60	9.98±0.142[a]	15.29± 0.103[a]	889.04±49.358[a]	126.97±7.065[a]	499.0± 10.98	3.947± 0.019[c]
AK100	9.995±0.092[a]	15.48 ± 0.069[a]	915.75±59.888[a]	130.87±8.57[a]	499.75 ± 11.15	3.827± 0.017[c]

Effects of A. karroo on weight gain and feed conversion ratio

The results showed that average daily gain was significantly different for all treatments with $p<0.001$. Feed intake was also not significantly different between all the treatments with $p>0.05$ feed conversion ratio was significantly different between treatments with $p<0.05$. Average daily gain (ADG) was high from goats fed with 100 % A. karroo diet but no significant differences with goats fed diet with 60 % A. karroo (Fig 1). Control treatment recorded the lowest ADG of 100.72 g and was 23.04% less than diet with 100% A. karroo content. Average daily weight gain was highly correlated ($R^2=0.99$) to increase in A. karroo level in the diet (Fig 1). Goats fed with control diet recorded highest voluntary feed intake of 504.5 g/day and lowest of 499 g/day was recorded from diet containing 60 % A. karroo. Goats which were fed with 60 % and 100% A. karroo had low FCR although there were significant differences ($p<0.05$) among all treatments. High tannin content in diets with high levels of A. karroo leaf and pod meal contributed to low FCR. The lowest FCR (3.827) was recorded from goats fed 100% A. karroo leaf and pod meal and highest FCR was recorded from goats fed control diet (0% A. karroo). Results on weight gain from this experiment were also similar to findings by Nyamukanza & Scogings (2008) who reported that feeding goats with diet containing A. karroo pods and leaves increased feed intake and weight gain. This was also similar to findings by Masiku (2013) who reported that supplementing Boar goats with A. karroo leaves increased body condition score, average daily gain and slaughter weight. Increase in inclusion of A. karroo pods and leaves in diet increased daily weight gain of goats. Results on average daily weight gain were in the same range with results by Masiku (2013) who reported higher average daily gain from goats fed with 25 % A. karroo. Higher average daily gain was noted in goats fed with a 100% A. karroo inclusion. This may have been caused by an increase in digestibility caused by increased protein content in the diet since body weight of goats is sensitive to protein and energy content in diet.

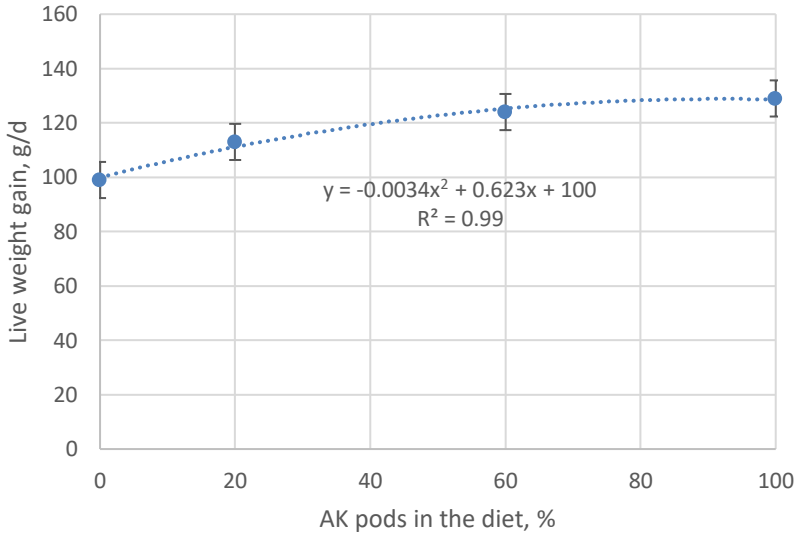

Figure 1. Effect of level of *A. karroo* pods-leaves replacing maize grain on live weight gain of the goats

Effects of *A. karroo* on weekly weight gain and average weekly weight gain of goats

Average weekly weights of goats increased with increase in inclusion of *A. karroo* in diet. Average final weight of goats fed with AK100 was 15.48±0.069 kg which was the highest with lowest from control being 14.28±0.18 kg (Table 3). Two weeks after feeding goats with different diets, their weights increased but not rapidly. After six weeks of feeding goats those in group AK100 fed with 100% *A. karroo* attained an average weight of 15.48±0.069 kg which was 1.2 % higher than those fed with 60%, 3.8% higher than those fed with 20% A. karroo and 7.8 % higher than those fed with 0% *A. karroo* (Table 3). Average weekly weight gain was higher in the first week for goats fed with AK100 diet which contained100% *A. karroo* but not different much to those fed AK60 diets which contained 60% *A. karroo*. The results also show that there was 100% weight gain for those fed AK100 diets (Table 4). There was no difference in average weekly weight gain for those fed with AK0 and AK20 diets in the second week of feeding. Goats fed with AK20 diets indicated an increase in average weekly weight gain in week 2 compared to all other groups which recorded a slight increase in weight gain. Goats fed AK100 diet showed a slight decline of weight gain in week 3 of feeding compared to all other treatments which had rapid declines in weight gain (Table 4). Goats fed with diet containing 100 % *A. karroo* recorded the highest average weekly weight gain (AWG) of 915.75 g which was 23% higher than AWG recorded from control diet (0% *A. karroo*). The results show significant different ($p<0.05$) between all treatments. Average weekly weight gain continue to decline rapidly for those fed with AK0-AK60 diets and only goats fed with AK100 diets showed a slight decline in average weekly weight gain from week 4 to week 6. Results on Fig 2 also indicate that average weekly weight gain for goats fed with AK0 and AK20 declined rapidly and were always below other treatments with AK0 being the lowest. Average weekly weight gain showed a decline for all goats fed diet containing *A. karroo* pods and leaves. This might have been caused by tannins which are contained in *A. karroo* leaves especially. This was also reported by Brown et al. (2016) as a way of diluting the effects of tannins. Tannins content was ranging from 3.8g/kg DM for diet with 20% A. karroo inclusion and 19.5g/kg DM for diet with100% A. karroo inclusion. Tannin levels less than 50g/kg DM can be easily tolerated by goats without any problem and values from 50g/kg DM have detrimental effects to ruminants (Brown et al., 2016). This also coincides with work by Dube et al. (2001) who reported that weight gain declined to goats fed with high levels of A. karroo due to presence of tannin which reduces protein digestibility. The same sentiment was rasied by (Ngongoni et al., 2007) who reported that tannin significantly reduces protein digestibility leading to reduced feed intake and weight gain. This coincides with report by Aganga et al. (2000) and Halimani et al. (2005) who indicated that A. karroo contains tannins which reduced digestibility and this may have effect on growth rate of animals. This also concurs with work by Dube et al. (2001) who reported that tannins have negative effect on growth and weight gain. These results were also in agreement with Kugedera and Chimbwanda (2018) who reported a decline in weight gain from broilers fed Red Swazi which contains condensed tannins.

Table 3. Average weekly weight (kg) for goats in different groups

Acacia level (B)	weekly weight (mean ± SD) (kg) (A)						Pooled mean ± SD
Treatments	**1**	**2**	**3**	**4**	**5**	**6**	**1-6**
AK0	10.85±0.21^{a}	11.67±0.24^{b}	12.4±0.22^{c}	13.09±0.18^{c}	13.72±0.17^{d}	14.28±0.18^{d}	12.67±1.21^{d}
AK20	10.82±0.14^{a}	11.92±0.12^{a}	12.71±0.1^{b}	13.49±0.12^{b}	14.23±0.13^{c}	14.89±0.12^{c}	13.01±1.41^{c}
AK60	10.87±0.15^{a}	11.82±0.15^{a}	12.74±0.15^{ab}	13.63±0.13^{a}	14.47±0.12^{b}	15.29±0.1^{b}	13.14±1.55^{b}
AK100	10.87±0.068^{a}	11.85±0.066^{a}	12.82±0.057^{a}	13.76±0.052^{a}	14.65±0.065^{a}	15.48±0.069^{a}	13.24±1.62^{a}
P-value: A							<0.001
B							<0.001
AB							<0.001

Same superscripts in the same column denotes no significant different between treatments at $p\leq0.05$.

Table 4. Average weekly weight gain (kg) for goats in different groups

Acacia level (B)	weekly weight gain (mean ± SD) g kg^{-1} (A)						Pooled mean (SD)
Treatments	**1**	**2**	**3**	**4**	**5**	**6**	**1-6**
AK0	805 ±80.22^{d}	820.75±75.039^{d}	729.75±26.42^{d}	692.5±53.15^{d}	630±34.64^{d}	552.5±26.3^{c}	705.08±107.27^{d}
AK20	824.25±11.79^{c}	1100±162.48^{a}	785±17.32^{c}	785±20.82^{c}	735±20.82^{c}	660±25.8^{b}	814.87±158.58^{c}
AK60	911.75±34.94^{a}	947.5±5^{c}	917.5±12.58^{b}	892.5± 17.08^{b}	845±12.91^{b}	820±39.16^{a}	889.04±49.36^{b}
AK100	897±36.092^{b}	975±10^{b}	975±10^{a}	935±12.91^{a}	895±12.91^{a}	817.5±43.49^{a}	915.75±59.89^{a}
P-value: A							<0.001
B							<0.001
AB							<0.001

Same superscripts in the same column denotes no significant different between treatments at p≤0.05.

Conclusion

Goats fed with AK100 diets recorded the highest average weight at week 6 compared to all other goats fed different diets. High CP content in *A. karroo* was noted to increase weight for goats fed with diets containing *A. karroo* pods and leaf meal. It was also noted that an increase in composition of *A. karroo* in diets increases weights of goats fed with that diet. Goats fed with AK0 diets had the lowest weights at week 6 and also lowest weekly gain compared to all other diets. Effects of anti-nutritional factors in *A. karroo* were also noted by causing a decline in weight gain to goats fed AK60 and AK100 diets. Effects of high CP content were also noted due to high weights for those goats fed with AK100 diets at week 6.

Recommendations

Farmers are recommended to fatten their goats with diets containing above 50% content of *A. karroo* due to high growth rate. Resource poor farmers in the smallholder farming areas are also recommended to use *A. karroo* to improve goat production because *A. karroo* is readily available in Zimbabwean smallholder farming areas.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Effects of different BA and IBA concentrations on proliferation and rooting of 'GARNEM' rootstock *in vitro* propagation

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Abstract

In this study, the regeneration shoot tip and nodal explants grown *in vitro* conditions of 'Garnem' hybrid rootstock were investigated, the effect of different phytohormone and concentrations on obtaining adventitious shoots from different explants was investigated, so an efficient protocol was developed for *in vitro* regeneration of 'Garnem' hybrid rootstock These outputs of the study can be a reference resource for future *in vitro* and biotechnological studies on the rootstock in question. The differences of PGR in MS medium culture containing node explants infinite (0.5-2.0 mg / l) BA (benzyladenine) were investigated. Upon the research, it was observed that the number of shoots andproliferationwere achieved in explants of nodal cuttings that were taken to culture in MS medium containing 2.0 mg / l BA. It has been determined that the most effective culture medium for the elongation of shoot length is MS medium containing 0.5 mg / l BA, 30 g / l sucrose and 5.5 g / l agar. Shoots growing in length were transferred to a new culture with ½ MS medium 0.5-2.0 mg / l IBA (indole-3-butyric acid) to be rooted. While rooting at a rate of 42.8% was achieved in ½ MS medium containing 2 mg / l IBA, 47.2% of rooted plantlets were acclimatized to ex- vitro conditions. Rooted plantlets obtained under *in vitro* conditions were transferred to plastic containers with 3 different environments in order to get accustomed to external conditions. At the end of the 8th week, the vitality rates of the plantlets were determined. While the viability rate of the plantlets transferred to the medium containing peat: perlite at the ratio of 1: 1 was found to be 47.2%, the viability rate of the plantlets in the environment containing only perlite was found to be 32.8%, and the viability rate of the plantlets in the environment containing only peat was found as 23.6%

Keywords: 'Garnem', Rootstock, Woody plants, Regeneration, *In vitro*, Micropropagation

Introduction

Almond cultivation has an important place in the World nut production. According to last decades data; world almond production, the United States of America (USA) ranks 1st by providing 58.8% of the total production. Spain, which provides 10.7% of the total World production, is in the second place, while Iran is in the third place with a production share of 4.4%. Turkey ranks 5th by providing 3.1% of the total World production (Eldogan, 2020). Almond, Turkey has adapted to the climate are among the important structures nuts (Akcay et al., 2005). Stone fruit species (*Prunus* sp.) are important and economically valuable fruit species in the *Prunoideae* subfamily of the *Rosaceae* family (Socias and Company, 1998; Arıcı, 2008). Although the number of almond trees grown is high, the low yield is due to non-compliance with standard production principles. The fact that old almond plantations consisted of more seeds causes variation between the types. Ensuring a standard production will only be possible with the determination of varieties suitable for ecological conditions and the use of standard rootstocks. If the new gardens to be established are established with standard varieties and these varieties are produced with grafting, a standard product and efficiency can be increased.

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When rootstocks are used in the reproduction of plants; growth of the variety grafted on rootstocks, resistance to diseases and pests, yield, fruit quality, earlyness, lateness, drought, frost, salinity, ground water resistance and plant nutrient uptake from the soil are affected. It is necessary to use a clonal rootstock for early fruiting and to obtain the maximum yield per unit area.

'Garnem' (*P. dulcis* x *P. persica*) is a hybrid rootstock obtained by crossing North American peach 'Nemared' and Spanish almond 'Garfi' in Spain. This rootstock was originally selected for almonds and showed excellent graft compatibility with peach and nectarine varieties. 'Garnem' (GN 15) rootstocks develop similar or stronger / better than GF677 rootstock. In the early stages of the growing season, they grow hairless or less hairy. The leaves are large and red, characteristically between almond and peach. It provides a high degree of shoot formation in all known varieties of almond, nectarine and peach. It is tolerant of drought and inefficient soil conditions. It has lower tolerance to iron chlorosis than GF677 rootstock. It has low tolerance to asphyxia in heavily irrigated soils. Plants inoculated with this rootstock show better graft compatibility than grafts made on GF677 rootstock. They need summer pruning to prevent excessive shoot growth and to reduce negative impact on fruit size and quality. It is resistant to root nematodes (*M. arenaria, M. hispanica, M. incognita* and *M. javanica*) which are effective in *Prunus* species.

In soils with nematodes, 'Garnem' rootstock can be used instead of GF677 rootstock. However, its resistance to overly irrigated and calcareous soils is lower than GF677 rootstock (Özden et al., 2011). The rapid production of plants under *in vitro* conditions using plant tissue culture techniques is routinely used in many plants nowadays. Since most of the fruit species have a heterozygous structure, it must be produced vegetatively. Although success in vegetative reproduction with classical methods varies according to each species and even variety, the reproduction coefficient is generally low. *İn vitro* reproduction with tissue culture techniques allows a faster production compared to conventional methods (Ak, 2018). A large number of disease-free plants can be produced in a short time, in a narrow area, out of the growing season by micropropagation.

Today, commercial production of some ornamental plants such as chrysanthemum, carnation, fuchsia, gladiol; some herbaceous (onion, peanut, asparagus, beet, brassica, vetch and chickpea species, soy, grass species, corn) and woody (such as *Malus, Prunus, Pyrus, Ribes, Atriplex, Betula)* and eucalyptus and poplar such forest trees can be produced by micropropagation. In woody plants used in fruit growing and ornamental plant cultivation, the aforementioned environments and sterilization stage may show some differences. At the same time, it is extremely important to use grafted saplings in both areas. In this study, the regeneration shoot tip and nodal explants grown *in vitro* conditions of 'Garnem' hybrid rootstock were investigated, the effect of different phytohormone and concentrations on obtaining adventitious shoots from different explants was investigated, so an efficient protocol was developed for *in vitro* regeneration of 'Garnem' hybrid rootstock These outputs of the study can be a reference resource for future *in vitro* and biotechnological studies on the rootstock in question.

Materials and Methods

Plant Material

'Garnem' (GN-15) (*P. dulcis* x *P. persica*) rootstock was used as plant material in experiment. Plant material had been given early spring time.

Explant sterilization and treatment

Plantlets brought from orchard and 20 mm green soft cuttings were obtained from the newly growing cuttings taken from 'Garnem' (*P. dulcis* x *P. persica*) rootstock, which were taken out springtime in optimum physiological cell division period. Then, after the leaves were cut and removed, with a bud on it. The cuttings kept under tap water for 30 minutes in the laboratory were kept in 70% ethyl alcohol for 1 more minute and then rinsed with sterile distilled water. After this stage, green cuttings are subjected to surface cleaning for 15 minutes in 8% commercial bleach (0.525% NaOCl) containing one or two drops of Tween 20 for surface sterilization of the plant material, and then 5 minutes each. Surface sterilization was completed by washing 3 times with sterile distilled water. After the sterilization was completed, explants were transferred to MS culture medium containing 1 mg / l BA, 30 g / l sucrose and 5.5 g / l agar and adjusted to pH 5.6 with 1 N NaOH or HCl. Then, 3 weeks after the beginning of the culture, the percentage of contaminated and healthy explants in the culture medium was determined as percent.

Culture conditions

The light intensity of the climate cabin used in this study was adjusted to 2500-3000 lux, it was programmed to apply a 16 hour (16 hours light / 8 hours dark) photoperiod, and the ambient temperature was kept at 24 ± 1 ° C.

Culturing explants in the initial environment

The buds on the green cuttings, whose superficial sterilization was completed, were separated from the wood tissue and planted in magenta plates containing 50 ml of initial culture medium. Each magenta containers were placed 5 nodes. Nodal explants were subcultured at 3-week intervals until the shape and number of leaves and shoots that can be used in regeneration experiments in the starting culture medium were obtained. The pH of the MS (Murashige and Skoog, 1962) culture medium containing 0.5, 1.0 or 2.0 mg / l benzyladenine (BA), 30 g / l sucrose, 5.5 g / l agar, minerals and vitamins used as the starting culture medium should be determined without autoclaving. just before it is set to 5.6. The prepared culture medium is then at 121°C for 20 min. sterilized. In order to maintain the shoot culture, newly obtained shoot clumps were separated as individual shoots and transferred onto fresh shoot culture medium.

In vitro rooting

Each shoot 2-3 cm long obtained from different explants was separated and transferred into media

containers. Shoot rooting medium consisted of ½ MS containing 0.5, 1.0 or 2.0 mg / l IBA (indole-3-butyric acid), 30 g / l sucrose, 5.5 g / l agar. The pH of the rooting medium was adjusted to 5.6 before sterilization.

Acclimatization to the external environment

Approximately 4-5 weeks later, the roots of the plantlets forming sufficient roots under *in vitro* conditions were washed and cleaned from agar and then they were transplanted into plastic containers containing sterile 1: 1 peat and perlite. Plantlets are covered with transparent containers in order to provide a humid outdoor environment. The pots were opened every day for increasing periods (5-10 minutes) 3 weeks after the beginning of the acclimatization process to the external conditions and the plantlets were accustomed to the external conditions. The leaves of the 'Garnem' rootstock can become green and red over time at the place where it was taken; this was seen in plants grown under *in vitro* conditions. Care has been taken to ensure that this is not particularly related to the effect of ambient pH.

Statistical evaluation

In the *in vitro* regeneration study, the statistical evaluation of the research results in terms of 'Garnem' hybrid rootstocks was made according to the randomized plot trial design, and the research was repeated 3 times independently and each independent trial was arranged to consist of 12 shoots. When the statistically significant processes were determined, the differences between the average data were subjected to LSD test at the P <0.05 level. Angle transformation was applied to the proportional (%) data obtained by counting.

Results and Discussion

One of the most important factors affecting the success in *in vitro* studies is the effective sterilization of plant materials. Therefore, the first and most important step to be done in the study is the superficial sterilization of the plant material to be used and the decision of the most appropriate sterilization method for this. The most commonly used disinfectants in the superficial sterilization of explants; ethanol, calcium, sodium hypochlorite, silver nitrate, hydrogen peroxide and mercury chloride (Babaoglu et al., 2002).

In general, surface sterilization of explants at the beginning of *in vitro* studies is done for 5 minutes after being shaken for 6- 20 minutes in 0.5-15% NaOCl, CaOCl or HgCl2 solution containing a couple drops of Tween-20 used to break the surface resistance of explants. It was completed by rinsing with sterile distilled water 3 times (Ainsley et al., 2001a; Channuntapipat et al., 2003). On the other hand, there are researchers who perform sterilization in several stages. Some researchers (Gürel and Gülsen, 1998b; Ainsley et al., 2000; Ainsley et al., 2001b; Pruski et al., 2005) used explant sources under tap water for different times (5 min-2 hours) before switching to explant superficial sterilization has done rough cleaning by keeping. Muna et al. (1999) did not consider keeping the explant sources under tap water sufficient for coarse cleaning, and after washing with a few drops of Tween-20 for 10-15 minutes, they switched to surface sterilization. Jain and Babbar (2003) wash the shoots that they will use in the same way with 10% (v / v) Teepoland 30 minutes. After keeping them under tap water, they started the actual superficial sterilization.

Espinosa et al. (2006); Jain and Babbar (2000) completed the superficial sterilization of the herbal material using commercial bleach after keeping the explants in ethanol (50-70%) for a short time (30 sec.- 5 min.). Matt and Jehle (2005), the buds they took from 1 old branches of 5 cherry varieties first in 1.5% (w / v) Benomyl for 10 minutes. After holding, they performed a more detailed sterilization by soaking in 10% (w / v) CaOCl for 20 minutes. In the study carried out by Jain and Babbar (2003), the shoots of that year taken from 30 old trees belonging to the Syzygiumcuminii species were brought to the laboratory in a container containing 50 mg / l ascorbic acid and 100 mg / l citric acid.) washed with Teepol, 30 minutes rinsed under tap water. 5 minutes in 50% (v / v) ethanol. after capture, 25 minutes with 0.2% (w / v) mercuric chloride, 20 minutes with 0.2% (w / v) bavistin (fungicide)has been treated. Finally, 10 minutes after being kept in 0.1% (w / v) NaOCl, the explant was superficially sterilized by rinsing with sterile distilled water several times. In this study, no contamination occurred in cultures in which the superficial sterilization was started with nodal explants in 8% NaOCl disinfectant solution, and they continued their development without any morphological change in *in vitro* shoots.

Therefore, it has been concluded that there is no need to apply the explant used in the superficial sterilization of this study, in which different sterilization methods used in the above studies were carried out. For the superficial sterilization of the explants used in this study, 8% NaOCl disinfectant solution was found to be the most appropriate concentration and this concentration was used in the superficial sterilization of the explants during the research process.

The medium prepared for shoot development of shoot tip explants taken from 'Garnem' rootstock contains MS medium supplemented 30 g / l sucrose and 5.5 g / l agar. The effects of node explants taken from 'Garnem' hybrid rootstock on shoot development by adding 0.5, 1.0 or 2.0 mg / l BA to this combination were investigated. 3 weeks after the initiation of culture, the explant and undeveloped explant rates that can be subcultured were determined (Table 1).

According to the findings obtained, it was determined that BA administration doses of 2.0 mg / l were more appropriate than the others. The rate of explants that can be subcultured was obtained from the best 2.0 mg BA application with 47.2%, while the lowest rate (20.2%) was found to be at 0.5 mg BA application. As with shoot tip explants, explants taken from the lateral buds of the plants were also grown in the same environment. Again, the effects of node explants taken from 'Garnem' hybrid rootstock on shoot development by adding 0.5, 1.0 or 2.0 mg / l BA in the same combination were investigated. 3 weeks after the beginning of the

culture, explants that can be subcultured, the rate of undeveloped explants (%) was determined (Table 2).Both shoot tip and nodal explants taken into culture started to persist from the 4th day in MS medium containing 2.0 mg / l BA, at the end of the 3rd week, 52.3% of the explants reached the size that can be subcultured. The nodal explants cultured in a MS medium containing 0.5 and 1.0 mg / l BA are only 7-10 and at the end of the 3rd week 41.8% of the explants taken into culture in MS medium containing 1.0 mg / l BA and 18.3% of the explants taken in culture in MS medium containing 0.5 mg / l BA reached a size that can be subcultured. The data obtained from the results of 3 separate trials conducted independently from each other were evaluated and the shoots obtained in 0.5, 1.0 and 2.0 mg / l BA culture medium were subcultured in MS medium containing the same PGR (Plant Growth Regulators) concentration and the experiment was continued (Figure 1).

At the end of the three-week development period, proliferation numbers were determined in explants taken into subculture. The effect of 0.5, 1.0 and 2.0 mg / l BA on proliferation was demonstrated by finding different proliferationnumbers of *in vitro* shoots cultured in MS medium containing 0.5, 1.0 and 2.0 mg / l BA. According to the findings obtained, it is seen that the number of proliferationis 6.2 in the 3rd subculture, 4.8 in the 2nd subculture and 2.9 in the 1st subculture in MS medium containing 2.0 mg / l BA (Table 3).

According to various sources, the rate of proliferations in subculture is low at the beginning, but it increases especially in the later periods. Studies show that when done up to the 8th subculture, the rate of proliferationincreases rapidly. However, under the current conditions, 3 subculture applications could be made. At this stage of the research, the effect of different levels of IBA in rooting *in vitro* shoots was investigated, and *in vitro* shoots were cultured in ½ MS medium containing 0.5-2.0 mg / l IBA. In the study, the effects of different levels of IBA on the rooting rates of *in vitro* shoots were found to be statistically significant (Table 4).

As seen in Table 4, the rooting rate in micro shoots cultured in ½ MS medium containing 2.0 mg / l IBA was 42.8%, and the rooting rate in micro shoots cultured in MS medium containing 1.0 mg / l IBA was determined as 31.6%. The rooting rate of the shoots rooting in the MS medium containing the lowest concentration of IBA decreased up to 23.5% (Figure 2). In this study, it was determined that the effect of 1.00 mg / l IBA added to the rooting medium on the root length of micro shoots was better and statistically different from the others.

Antonopoulou et al. (2005) stated that MS MS medium and different concentrations of auxin are generally used in rooting *in vitro* shoots of varieties belonging to the genus Prunus. In this study conducted on GF-677 almond rootstock, *in vitro* shoots obtained 100% rooting when cultured in a MS media containing 1 mg / l IBA. Fotopoulos and Sotiropoulos (2005) determined the effects of ½ MS or MS media containing different IBA concentrations on the rooting rate in *in vitro* shoots in their study on PR 204/84 peach-almond hybrid. 100% rooting was obtained in micro shoots that were cultured in MS medium containing 1.0 mg / l IBA or ½ MS media containing 0.5 mg / l IBA. Durkovic (2006), in his study, obtained 73.3% rooting by culturing *in vitro* shoots from the nodal segment explants of cherry in MS medium containing 0.3 mg / dm3 IBA. Osterc et al. (2004) in the study of cherry, 75-100% rooting rate was obtained when *in vitro* shoots were cultivated in ½ MS medium containing 1 mg / l IBA. Rooted plantlets obtained under *in vitro* conditions were transferred to plastic containers with 3 different environments in order to get accustomed to external conditions. At the end of the 8th week, the vitality rates of the plantlets were determined. While the viability rate of the plantlets transferred to the medium containing peat: perlite at the ratio of 1: 1 was found to be 47.2%, the viability rate of the plantlets in the environment containing only perlite was found to be 32.8%, and the viability rate of the plantlets in the environment containing only peat was found as 23.6% (Table 6).

When transferred to larger pots in the 8th week, the adaptation of the plantlets grown in an environment containing peat: perlitewas faster and the plantlets continued to grow and develop in a short time. Leaf colors of the plantlets transferred only to perlite medium were observed to lighten. One of the reasons for the low viability of the plantlets transferred to the peat environment is that the roots may have been damaged during planting. Therefore, in order to prevent damage to the roots during planting, the roots of the plantlets should be transferred to the lapping environment without exceeding 12-15 mm on average. Again, in parallel with this research output, Muna et al. (1999) in the study conducted on Maxma-14 cherry variety, it was emphasized that plantlets get used to external conditions more easily when they are transferred to pots shortly after rooting. The most important point in the acclimation of rooted plantlets obtained in *in vitro* studies to external conditions is to prevent water loss. It is assumed that water and nutrient insufficiency is the main reason for the low viability of the plantlets transferred to the perlite environment. While *in vitro* plantlets are accustomed to external conditions, it is also necessary to keep them in climatic rooms with high humidity and gradually reduce the humidity.

Table 1. Shoot development from shoot tip explants

BA (mg/L)	Explant that can be subcultured (%)	Undeveloped explant (%)
0.5	20.20 c	16.50 b
1.0	37.30 b	8.40 c
2.0	47.20 a	21.30 a

*The difference between the means with different letters on the same column is statistically significant(P<0.05)

Table 2. Rooting percentage of PMPC hybrid rootstock candidate (%)

BA (mg/L)	Explant that can be subcultured (%)	Undeveloped explant (%)
0.5	18.30 c	28.30 b
1.0	41.80 b	12.10 c
2.0	52.30 a	21.80 a

*The difference between the means with different letters on the same column is statistically significant(P<0.05)

Table 3. The effect of BA applied in different concentrations on the number of proliferationobtained from nodal segment explants

CYTOKINE	PROLIFERATION NUMBERS			
BA (mg/L)	1st Subculture	2nd Subculture	3rd Subculture	Avarege
0.5	1.30	1.90	3.30	2.17 b
1.0	2.10	4.30	5.30	3.90 a
2.0	2.90	4.80	6.20	4.63 a

*The difference between the means with different letters on the same column is statistically significant(P<0.05)

Table 5. *İn vitro* rooting

IBA (mg/L)	Rooting rate (%)	Number of Roots (pieces)
0.5	23.50 c	2.80 c
1.0	31.60 b	3.10 a
2.0	42.80 a	2.20 b

*The difference between the means with different letters on the same column is statistically significant(P<0.05)

Table 6. Acclimatization of rooted plantlets ex vitro conditions

MEDIUMS		
Perlite	Peat	Peat:Perlite (1:1)
32.80 b	23.60 c	47.20 a

*The difference between the means with different letters on the same column is statistically significant(P<0.05)

Figure 1. Shoot development from explants

Figure 2. Views from proliferated and rooted explants

Conclusion

'Garnem' clone rootstock was chosen as rootstock because it shows good graft compatibility with almond, peach and nectarine. In the rootstock nursery, it shows a very good development at half the strength of GF 677 rootstock. The success of the budding is high with all known almond, peach and nectarine varieties. It is tolerant of poor soil conditions and arid conditions. In this study, the effects of different PGR and their concentrations on obtaining adventitious shoot regeneration of 'Garnem' rootstock, which is a hybrid of almond and peach rootstock, which has adapted to the general high lime ratio where almond growing areas in the World, were investigated. This study can be grouped under 4 main headings: 1. Surface sterilization of plant material, 2. Culturing of the node explants in the starting environment and shoot propagation, 3. Rooting of the shoots, 4. The acclimatization of rooted plantlets to external conditions. Very high values could not be obtained in terms of rooting and acclimatization. Because it can be said that especially the plant growth medium is very effective in this part of research. In the light of previous sources and information, it is thought that better results can be obtained by adding GA3 to the rooting medium and making applications in the dark in the light of some information. It may be suggested to apply them in future studies. In this study, it was determined that the ratio of explants to be subcultured was better in lateral explants when plants obtained from shoot tip and lateral (node) explants were compared. However, in general, the plants obtained from the shoot tip give better results in the reproduction of many fruit species or rootstocks. In order to be successful in plant tissue culture studies, it is essential that the laboratory and climate room conditions are complete. On the other hand, care should be taken to determine the choice of explants, PGR concentrations and combinations. The choice of media is very important in tissue culture studies. As a result of the studies, it has been determined that the reactions of each species and variety to tissue culture techniques are different. Factors such as the age of the explant, the physiological condition of the donor plant, the source of the explant affect the regeneration rate and may differ significantly. The type of culture media, its components, and the most suitable culture conditions differ for each genotype.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the aurhors to the present study is equal. All the authors verify that the text, figures, and tables are original. The authors read and approved the final manuscript.

Ethical approval

Not applicable

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Data availability

Not applicable

Consent for publication

Not applicable

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Fertilizers, Mycorrhizal Inoculation and Atrazine Interactions on Weed Biomass and Yield of Maize

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Abstract

The decline in soil fertility and poor weed management are the dominant limitation on the production of maize in Nigeria. Improving the efficiency of fertilizers through AMF inoculation and atrazine application may be a sustainable way to increase land productivity. Using completely randomised design with three replications, combinations of Organomineral Fertilizer (OF) at 0, 50, 100, 150 kg N/ha and NPK 15-15-15 (400 kg/ha); Arbuscular Mycorrhizal Fungi (AMF, with and without); and atrazine (0, 1.5 kg a.i/ha) were evaluated in 30 kg pot experiment. Maize grain yield ranged from 0.07-101.20 g/plant. Sole applications of OF, AMF and atrazine increased maize growth and yield. Combining atrazine with AMF inoculation improved maize growth but significantly reduced grain yield (37.13 g/plant) compared to sole applications of AMF (75.13 g/plant) or atrazine (62.97 g/plant). The application of OF did not alter the AMF-atrazine interaction, except under NPK fertilizer application, where the interaction enhanced grain yield. All treatments involving atrazine produced lower total dry weed biomass. However, the total dry weed biomass produced across fertilizer applications increased non-significantly with AMF inoculation, while AMF colonization reduced with atrazine application. Therefore, combining 100 kg N/ha OF with AMF inoculation or atrazine was suggested under organomineral fertilizer application.

Keywords: Organomineral fertilizer, Arbuscular mycorrhizal fungi, Atrazine, grain yield, Dry weed biomass

Introduction

Nigeria has 20.6 million people in 2020 with an annual growth rate of 2.58% (Worldometers, 2020), produced 11.55 million tons of maize (FAOSTAT, 2018). The average estimated yield in 2014 was 2 tons per hectare which is just between 30 – 50% of expected yield. This is majorly attributable to decline in soil fertility (Sileshi et al., 2010). Despite smallholder's adoption of the high-yielding maize genotypes, national per-hectare yield of 1593.3 kg/ha for 2017 in maize productivity is not encouraging. Presently, the challenge of sustaining and improving maize productivity have increased the need to combine improved genotypes with management practices (Ibitola et al., 2019).

Application of inorganic fertilizers are too expensive and not profitable for smallholders (Sileshi et al., 2010). Blanket fertilizer recommendations which ignored the soil and climatic variations have made it unattractive to smallholder farmers (Ibitola et al., 2019). In view of the challenges with fertilizers, arbuscular mycorrhizas (AM) are attracting interest to promote more efficient use of soil mineral resources (Perez-Montano et al., 2014). The contributions of AMF to plant growth with species variation through enhanced nutrients absorption have made it essential components of sustainable crop production (Wenke, 2008; Fitter et al., 2011). Factors such as mycorrhizal diversity/density, soil type, nutrient status, crop and management including herbicide (atrazine) application affect mycorrhizal dependency (Karagiannidis and Hadjisavva-Zinoviadi, 1998; Vatovec et al., 2005; Swanton et al., 2007). For instance, application of atrazine (a selective pre- or post- emergence herbicide applied for the control of weeds in maize) is known to

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influence the populations of certain microbes and crop performance (Ros et al., 2006; Williams et al., 2010). However, the application of organic matter to the soil may modify the effect of atrazine as they adsorb and alter the efficacy of the herbicide (Takeshita et al., 2019). Lack of proper understanding of the interactions among these inputs for enhanced However, the application of organic matter to the soil may modify the effect of atrazine as they adsorb and alter the efficacy of the herbicide (Takeshita et al., 2019). Lack of proper understanding of the interactions among these inputs for enhanced plant benefit require attention. Hence, these studies evaluate the influence of organomineral fertilizer, AM inoculation and atrazine on leaf nutrients concentrations and performance of maize. Also, the effect of these treatments on weed biomass were assessed.

Material and methods

The study site was at Ayepe On-Farm Research (7°17'29.83''N and 4°16'31.88''E) for the Department of Agronomy, University of Ibadan, which was located at Ayepe, Apomu (Isokan LGA), Osun State. Nigeria.

Soil Sampling and Analysis

Composite samples of the topsoil (0 – 15 cm) were collected from the soil gathered from experimental sites before the start of the experiment. The soil samples were bulked and air-dried. The soil samples were passed through a 2 mm mesh sieve for physical and chemical analysis. The soil samples were analysed in the Department of Agronomy service laboratory as described in the IITA laboratory manual (IITA, 1982). Particle size distribution was determined by the hydrometer method as described by Bouyoucos (1951). Soil pH was determined with a glass electrode pH meter (Coleman pH meter) on a 1:1 soil-water ratio. Organic carbon and was determined following the wet oxidation method by Walkey and Black (1934).

Determination of Nutrient Elements in Pacesetter Manure

The Pacesetter Organomineral material was dried at 70 ˚C for 48 hours and passed through 1 mm sieve in a Willey mill. Total nitrogen was analyzed by wet digestion (micro Kjeldahl method) using a mixture of H_2SO_4 and H_2O_2 (30%) as described by Jackson (1958) and Black (1965). Phosphorus extraction was by Blancher et al. (1965) procedure and the concentration determined in the Technicon Autoanalyzer. Other nutrients determination (Ca, Mg, K) were by using a mixture of HNO_2, HCO_4 and HCl. Potassium concentration was read with a flame photometer while the others were with Atomic Absorption Spectrophotometer.

The values of the chemical properties of the organomineral fertilizer used for this study are presented in Table 2. The N, P and K contents in the organomineral fertilizer were 1.88, 0.23 and 1.01, respectively. The values of Ca and Mg were 0.64 and 0.23, respectively while the organic carbon content was 39.3.

Experimental design

It was a factorial combination experiment in completely randomized design replicated three times. The factors were fertilizers (control, organomineral fertilizer at 50, 100 and 150 kg N ha^{-1} and NPK 15-15-15 at 400 kg/ha as recommended by IITA, 2007); Arbuscular Mycorrhizal Fungi (AMF) inoculation (no AMF inoculation and with AMF inoculation); and pre-emergence herbicide (no atrazine and with atrazine).

Experimental materials

The mycorrhizal fungus used in this study was *Glomus clarum*. It was obtained from the stock kept and maintained in the Soil Microbiology Laboratory

The nitrogen content of the soil was determined by the Kjeldahl method as adapted by Jackson (1958). The determination of available P in the soil samples was by Bray P-1 method (Bray and Kurtz, 1945). The Ca, Mg, K and Na) were extracted with ammonium acetate ($NH_4C_2H_3O_2$) as described by IITA (1982) and the concentrations determined using flame photometric method for K and Na, while Ca and Mg were determined using Atomic Absorption Spectrophotometer.

The physical and chemical properties of the soil used in the study are shown in Table 1. The soil physical fractions indicated that the soil was loamy Sand in texture. The soil pH was 6.3, while the N, P and K values were 0.78, 5.83 and 0.5 g/kg, respectively. The soil Ca and Mg concentrations were respectively 5.2 and 2.4 cmol/kg.

Table 1. Physical and chemical properties of soils in the experimental location

Parameters	Values
Soil physical fractions (g/kg)	
Sand	844.0
Silt	80.0
Clay	76.0
Soil texture	loamy Sand
Soil chemical properties	
pH (H_2O)	6.3
Exchangeable acidity	0.50
Organic carbon (g/kg)	11.5
N (g/kg)	0.78
P (mg/kg)	5.83
Exchangeable Bases (cmol/kg)	
Ca	5.2
K	0.5
Mg	2.4
Na	0.6
% Base Saturation	56.1
ECEC (cmol/kg)	9.1

Table 2. Chemical properties of the organomineral fertilizer used for the study

Properties	Organomineral fertilizer
Nitrogen (%)	1.88
Organic carbon (%)	39.3
C: N	20.9
Phosphorus (%)	0.23
Potassium (%)	1.01
Calcium (%)	0.64
Magnesium (%)	0.23

of the Department of Agronomy University of Ibadan, Ibadan. The inoculums used consisted of soil containing spores, hyphal fragments, and fine roots of maize infected with G. clarum. Maize (*Zea mays* L.) variety used was open-pollinated yellow Suwan-1, grown by farmers in the locality.

The preemergence herbicide was atrazine applied at 1.5 kg a.i.\ha (ICS-Nigeria, 2011). The organomineral fertilizer was commercially produced (Pacesetter organomineral fertilizer). The NPK 15-15-15 was obtained from the Department of Soil Resources Management, University of Ibadan.

Management

Topsoil (low in phosphorus and nitrogen) was collected from the experimental area at Ayepe. Thirty kilograms of the soil were filled into each pot after sieving with a 2 mm mesh sieve. Each pot was 50 cm in height and 45 cm in diameter.

Organomineral fertilizer was applied at planting, while the inorganic fertilizer was applied at 2 Weeks After Planting (WAP). The fertilizer was applied to maize by ringing around the maize plant. The inoculations in treatments containing AMF consisted of 20 g root placed 1/3 depth of the pot before maize planting (Fagbola et al., 1998). The treatments were arranged randomly in the open field at Ayepe.

Data Collection: Maize: Maize plant height and leaf area were measured fortnightly, from 2-8 WAP. At harvest, shoots dry weight were determined after oven drying the sample at 70 °C to a constant weight. Yield component such as number of kernels/ear, shelling percentage, cob weight/pot, and grain yield/pot (at 14% moisture content) were determined.

Arbuscular mycorrhizal fungal colonization: The colonization of AM fungi was determined using the Giovannetti and Mosse (1980) process. The root samples were cleared for 15 min in 10% KOH at 121 °C and then stained in trypan blue solution. Using a grid-line intersect method, 0.2 g fresh root samples were used in assessing AMF colonization and estimation (Giovannetti and Mosse, 1980).

Weed: Weed biomass (using quadrant) was taken during each weeding operation at 4, 8, and 12 WAP. Afterwards, the frequencies of weeding depended on visual observation. Weeds obtained from the pots were harvested, oven-dried to constant weight for dry weight determination using Binatone weighing balance EK 5055.

Statistical analysis: The collected data were subjected to analysis of variance (ANOVA)and the means compared using Duncan's Multiple Range Test (DMRT) at $P < 0.05$ level of significance, where F-ratio was significant. Pearson correlation analysis was conducted to determine the relationship between the monitored parameters.

Results

The plant height increased consistently with the plant height increased consistently with increase in plant age in all the treatments (Table 3). The highest plant height at 2 WAP was observed in the treatment involving 100 kg N ha^{-1} OF with AM inoculation and no atrazine application. The lowest plant height was observed in the treatments involving 0 kg N without AM inoculation and no atrazine or with atrazine. The values differed significantly from each other. The treatment involving 100 kg N ha^{-1} with AM and atrazine application gave the highest plant height at 4 WAP, while the treatment with NPK 15-15-15 gave the lowest plant height. However, at 0 kg N, 50 kg N ha^{-1} OF and NPK 15-15- 15 fertilizer treatments, combination involving atrazine excluding AM inoculation gave higher plant height than the other treatment combinations. At 100 and 150 kg N ha^{-1} OF, on the other hand, treatment combination involving AM inoculation with atrazine application gave the highest plant heights. Applying 150 kg N ha^{-1} OF with no AM inoculation or atrazine and NPK 15-15-15 with AM inoculation and atrazine gave significantly higher plant height compared to the control at 6 WAP. Similar trend was observed at 8 WAP, with NPK 15-15-15 combined with AM and atrazine and 150 kg N ha^{-1} OF with no AM or atrazine treatments having significantly higher plant height compared to the control. Within 0 kg N, 50 and 150 kg N ha^{-1} fertilizer treatments at 6 and 8 WAP, treatment with AM inoculation alone gave the highest plant height, while 100 kg N ha^{-1} with atrazine and no AM inoculation gave the highest plant height.

Maize leaf area measured increased with the increase in OF application at 2 WAP with significantly higher leaf area observed in plants treated 100 kg N ha^{-1} combined with atrazine alone compared to many other treatments (Table 4). The highest and significant leaf area at 4 WAP was observed in the maize plants treated with atrazine alone at 0 kg N, while the lowest leaf area was observed in the treatment that had NPK 15-15-15 fertilizer alone. Applying 100 kg N ha^{-1} OF alone significantly increased maize leaf area at 6 WAP compared to plants treated with NPK 15-15-15 fertilizer combined with AM inoculation. At 8 WAP, varying degrees of significance in leaf area measured were observed among the treatments. However, combining AM inoculation with atrazine application at 0 kg N gave the plants with the highest leaf area while the lowest was observed in treatment with 50 kg N ha^{-1} OF combined with AM alone. Across the fertilizer levels including NPK 15-15-15, treatments combining AM inoculation with atrazine application gave the highest leaf area. These treatments were followed by treatments combining atrazine alone across all the fertilizer treatments except at 150 kg N ha^{-1} OF.

Table 3. Response of maize plant height (cm) to the interactions of fertilizers, AM inoculation and atrazine application

Treatments			2 WAP	4 WAP	6 WAP	8 WAP
0 kg N	x No AM	x No atrazine	4.33c	21.83a-c	44.33c	81.00c
		With atrazine	4.33c	22.50a-c	57.00a-c	100.00bc
	With AM	x No atrazine	6.33ab	22.43a-c	64.00ab	133.67a-c
		With atrazine	5.33a-c	22.73a-c	56.33a-c	132.67a-c
50 kg N ha^{-1} OF	x No AM	x No atrazine	6.33ab	20.60bc	55.00a-c	74.33c
		With atrazine	5.33a-c	24.33a-c	63.00a-c	123.00bc
	With AM	x No atrazine	5.33a-c	21.90a-c	59.00a-c	131.33a-c
		With atrazine	5.00a-c	20.00c	55.00a-c	127.67bc
100 kg N ha^{-1} OF	x No AM	x No atrazine	5.67a-c	25.57ab	62.00a-c	143.00a-c
		With atrazine	5.33a-c	25.23ab	68.00a	150.33a-c
	With AM	x No atrazine	6.67a	23.13a-c	60.33a-c	127.33bc
		With atrazine	6.33ab	26.57a	62.00a-c	145.00a-c
150 kg N ha^{-1} OF	x No AM	x No atrazine	5.33a-c	25.43ab	71.00a	181.33ab
		With atrazine	6.00a-c	22.17a-c	60.00a-c	126.67bc
	With AM	x No atrazine	6.33ab	22.07a-c	56.00a-c	138.00a-c
		With atrazine	4.67bc	25.60ab	63.67a-c	132.00a-c
NPK 15-15-15	x No AM	x No atrazine	4.67bc	21.00bc	48.00bc	104.00bc
		With atrazine	5.33a-c	24.13a-c	65.67ab	155.33a-c
	With AM	x No atrazine	5.67a-c	23.73a-c	56.33a-c	123.00bc
		With atrazine	6.33ab	22.60a-c	72.67a	220.00a
SE			0.66	1.77	6.81	31.61

AM = arbuscular mycorrhizal inoculation; Values within the same column, followed by similar letter(s) are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

Table 4. The interactions of fertilizers, AM inoculation and atrazine application on the leaf area (cm^2) of maize

Treatments			2 WAP	4 WAP	6 WAP	8 WAP
0 kg N	x No AM	x No atrazine	16.66bc	177.82b-d	289.90 a-c	312.21fg
		With atrazine	20.55a-c	251.07a	332.18 a-c	456.82 b-e
	With AM	x No atrazine	17.95bc	189.83a-d	284.19bc	374.14d-g
		With atrazine	25.50ab	187.87a-d	288.16 a-c	631.86a
50 kg N ha^{-1} OF	x No AM	x No atrazine	18.77bc	154.92b-d	262.29bc	289.79g
		With atrazine	14.81c	166.81b-d	245.99bc	407.47c-f
	With AM	x No atrazine	15.78c	141.22cd	361.73a-c	284.54g
		With atrazine	25.65ab	204.05a-c	280.16bc	410.67c-f
100 kg N ha^{-1} OF	x No AM	x No atrazine	19.25bc	171.64b-d	450.99a	429.81 b-e
		With atrazine	28.59a	154.91b-d	264.34bc	459.14 b-e
	With AM	x No atrazine	20.40a-c	181.16a-d	264.42bc	411.75c-f
		With atrazine	17.46bc	189.30a-d	284.27bc	466.39b-e
150 kg N ha^{-1} OF	x No AM	x No atrazine	19.50a-c	174.27b-d	292.95 a-c	497.36bc
		With atrazine	15.66c	181.54a-d	293.60 a-c	448.16 b-e
	With AM	x No atrazine	17.58bc	154.85b-d	283.54bc	397.00c-g
		With atrazine	15.08c	210.62a-c	409.29ab	482.63b-d
NPK 15-15-15	x No AM	x No atrazine	15.43c	125.35d	250.64bc	354.43e-g
		With atrazine	15.61c	174.06b-d	308.31 a-c	497.69bc
	With AM	x No atrazine	21.31a-c	204.43a-c	240.41c	425.54b-f
		With atrazine	18.72bc	218.15ab	298.76 a-c	541.66ab
SE			3.21	25.21	57.18	40.63

AM = arbuscular mycorrhizal inoculation; Values within the same parameter grouping and column, followed by similar letter(s) are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

The highest and significant dry shoot weight was observed in plants treated with NPK 15-15-15 combined with AM inoculation and atrazine application compared to the lowest observed at 50 kg N ha^{-1} OF without AM inoculation or atrazine (Table 5). At 50 and 150 kg N ha^{-1} OF, combination involving AM inoculation alone gave higher maize dry shoot weight compared to the other treatments

within each level. There were varying levels of significance with respect to cob yield differences observed. The maize cob yield varied from 1.60 – 116.27 g plant^{-1} with significantly higher cob yield observed at 100 kg N with AM inoculation alone compared to the control. The 100 kg N ha^{-1} OF combined with AM inoculation alone significantly increased number of kernels per cob observed compared to the control, 50 kg N ha^{-1} OF alone or 50 kg N ha^{-1} OF combined with AM and with or without. All other treatments were not significantly different from the highest value observed. The observed shelling percentages varied in their degree of significance with highest and lowest shelling % observed at 100 kg N combined with AM inoculation alone and the control, respectively. There are varying degrees of significance with respect to grain yield observed. However, the treatment with AM alone at 100 kg N gave the highest grain yield observed, while the control gave the lowest grain yield. Similarly, plants treated with AM inoculation or atrazine at 0 kg N gave significantly higher maize grain yield compared to the control. Furthermore, the application of OF alone above 50 kg N ha^{-1} significantly improved maize grain yield compared to the control. Among the OF levels, combining 100 kg N ha^{-1} OF with AM inoculation alone gave the highest grain yield. The combination involving AM and atrazine lowered maize grain yields across the OF levels. However, NPK 15-15-15 treatment combined with AM and atrazine significantly improved maize grain yield compared to the control.

All the fertilizer treatments combined with atrazine and with/without AM inoculation had significantly lower dry weed biomass compared with other treatment combinations at 4 WAP (Table 6). At 8 WAP, significantly higher dry weed biomass was observed in the treatment combining AM inoculation with atrazine at 0 kg N compared to all sole fertilizer treatments and treatments with AM inoculation alone. The application of 50 kg N ha^{-1} OF combined with AM inoculation alone significantly increased dry weed biomass compared to the control and all treatments without dry weed biomass value at 12 WAP. The total dry weed biomass indicated that there were varying degrees of significance observed among treatments. The total dry weed biomass ranged from 51.06-9.77 g plant^{-1}. The NPK 15-15-15 fertilizer treatment without AM or atrazine gave the highest total dry weed biomass observed, while combining AM inoculation with atrazine at 100 kg N ha^{-1} OF and 150 kg N ha^{-1} OF combined with atrazine alone gave the lowest total dry weed biomass. Across all the fertilizer treatments, combinations involving atrazine significantly reduced total dry weed biomass observed compared to other treatment combinations. However, the combinations of AM inoculation with atrazine further increased total dry weed biomass, except at 100 kg N ha^{-1} OF.

Table 5. Influence of the interactions of fertilizers, AM inoculation and atrazine application yield components and yield of maize

Treatments	Dry shoot (kg plant^{-1})	Cob yield (g plant^{-1})	Shelling %	Grain yield (g plant^{-1})
0 kg N x No AM x No atrazine	0.15cd	1.60f	3.13e	0.07e
With atrazine	0.24a-d	81.90a-e	53.76a-d	62.97a-d
With AM x No atrazine	0.24a-d	104.63a-c	65.33ab	75.13ab
With atrazine	0.23a-d	48.70a-f	69.28ab	37.13b-e
50 kg N ha^{-1} OF x No AM x No atrazine	0.13d	26.90d-f	47.51b-d	22.73b-e
With atrazine	0.22a-d	45.90a-f	65.94ab	36.97b-e
With AM x No atrazine	0.26a-d	10.43ef	28.19c-e	8.97de
With atrazine	0.23a-d	19.93d-f	26.10de	15.60c-e
100 kg N ha^{-1} OF x No AM x No atrazine	0.25a-d	76.80a-e	72.06ab	63.40a-d
With atrazine	0.27a-d	34.83c-f	63.62ab	28.50b-e
With AM x No atrazine	0.35a-c	116.27a	81.09a	101.20a
With atrazine	0.26a-d	45.73a-f	71.10ab	34.50b-e
150 kg N ha^{-1} OF x No AM x No atrazine	0.22a-d	84.87a-d	70.76ab	68.77a-c
With atrazine	0.22a-d	48.50a-f	69.40ab	38.77b-e
With AM x No atrazine	0.38ab	36.17b-f	67.09ab	26.37b-e
With atrazine	0.23a-d	43.20a-f	76.09ab	34.67b-e
NPK 15-15-15 x No AM x No atrazine	0.18b-d	45.73a-f	72.74ab	35.83b-e
With atrazine	0.27a-d	84.53a-d	73.60ab	61.80a-d
With AM x No atrazine	0.21a-d	43.20a-f	59.56a-c	32.23b-e
With atrazine	0.41a	109.03ab	71.22ab	80.23ab
SE	0.08	25.81	11.44	20.60

AM = arbuscular mycorrhizal inoculation; Values within the same column, followed by similar letter(s) are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

Table 6. Dry weed biomass (g plant^{-1}) as influenced by the interactions of fertilizer, AM inoculation and atrazine application

Treatments			4 WAP	8 WAP	12 WAP	Total weed biomass
0 kg N	x No AM	x No atrazine	31.30ab	0.00c	1.33bc	41.66ab
		With atrazine	0.00c	2.10bc	0.00c	13.45d
	With AM	x No atrazine	28.07ab	0.00c	0.90bc	37.85ab
		With atrazine	0.00c	6.93a	0.00c	18.01cd
50 kg N ha^{-1} OF	x No AM	x No atrazine	20.07b	0.00c	0.63bc	28.07b-d
		With atrazine	0.00c	0.00c	1.50bc	10.32d
	With AM	x No atrazine	23.27b	0.00c	6.47a	40.82ab
		With atrazine	0.00c	0.00c	4.70ab	13.64d
100 kg N ha^{-1} OF	x No AM	x No atrazine	33.83ab	0.00c	0.83bc	42.52ab
		With atrazine	0.00c	1.47c	0.00c	10.44d
	With AM	x No atrazine	25.10ab	0.00c	1.07bc	35.06a-c
		With atrazine	0.00c	1.63c	0.00c	9.80d
150 kg N ha^{-1} OF	x No AM	x No atrazine	24.40ab	0.00c	0.83bc	34.94a-c
		With atrazine	0.00c	1.37c	0.00c	9.77d
	With AM	x No atrazine	34.70ab	0.00c	0.80bc	44.02ab
		With atrazine	0.00c	5.50ab	0.00c	12.93d
NPK 15-15-15	x No AM	x No atrazine	39.73a	0.00c	1.90bc	51.06a
		With atrazine	0.00c	2.03bc	0.00c	10.36d
	With AM	x No atrazine	39.40a	0.00c	0.93bc	48.37a
		With atrazine	0.00c	5.57ab	0.00c	12.52d
SE			5.53	1.31	1.46	6.59

AM = arbuscular mycorrhizal inoculation; Values within the same column, followed by similar letter(s) are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

Figure 1 showed maize roots AM colonization after the completion of the study. Arbuscular mycorrhizal colonization was observed in all the treatments. The AM colonization of the maize roots inoculated with *G. Clarum* (62.63-86.63%) was significantly higher than non-inoculated (22.00-41.93%). The application of organomineral and NPK fertilizers improved AM colonization in maize than the control. Also, across the fertilizer levels, AM inoculation alone increased AM colonization of maize roots than when AM inoculation was combined with atrazine application. Atrazine application to soil had little suppressive effects on AM colonization in the maize roots, regardless of the type of fertilizer or level of OF applied, except at 150 kg N ha^{-1} OF.

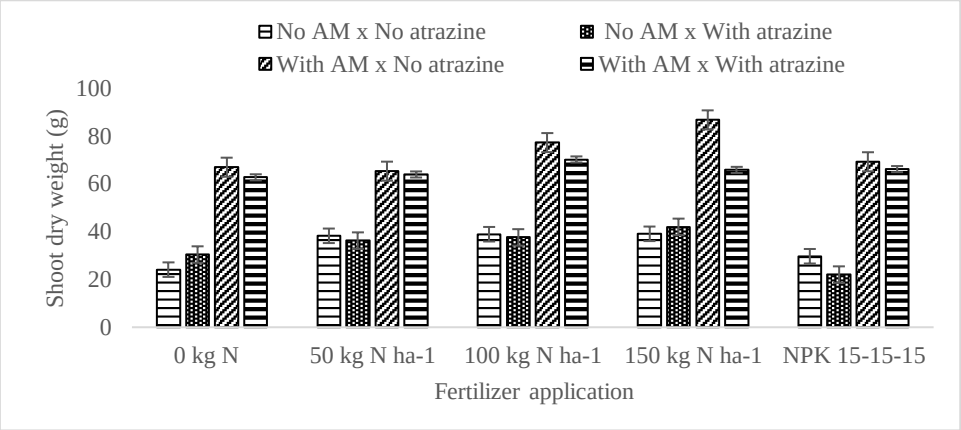

Figure 1. Arbuscular mycorrhizal colonization of maize root as influenced by the interactions of fertilizers, AM inoculation and atrazine application

There was varying degrees of significance in the concentration of N in maize ear leaf (Table 7). The highest N nutrient concentration in maize ear leaf was observed in the treatment involving 150 kg N/ha combined with AM inoculation alone, while the lowest was observed at 0 kg N combined with AM inoculation and atrazine. The concentration of N in maize ear leaf at 0 kg N showed that AM inoculation alone significantly increased N concentration compared to when AM inoculation was combined with atrazine. Similar observations were made at 50 and 150 kg N/ha OF, but without significance. The reverse was however observed at 100 kg N/ha OF and the application of NPK 15-15-15. Significantly higher P nutrient concentration in maize ear leaf was observed at 0 kg N combined with AM inoculation and atrazine compared to the other treatments. Across the fertilizer levels including NPK, the concentration of P in maize ear leaf were highest in the interactions involving atrazine alone than the other treatments. The concentration of K in maize ear leaf were significantly higher in the treatments involving 150 kg N/ha OF or NPK 15-15-15, each combined with AM inoculation and atrazine. The lowest K nutrient concentration in maize ear leaf was observed in the control. Across the OF levels,

including fertilizer, combining AM inoculation with atrazine application improved K nutrient concentration in maize ear leaf than the contemporary treatments. There were significant variations among treatments with respect to Ca concentration in maize ear leaf. The highest concentration of Ca in maize ear leaf was observed at 50 kg N/ha OF combined with atrazine alone, while the lowest was observed in the control. The highest concentration of Ca at 0 kg N was observed in the treatment with AM inoculation alone. However, across the OF levels, combinations with atrazine alone gave higher Ca concentration in maize ear leaf than the other treatment combinations. The highest Ca nutrient concentration in maize ear leaf was observed in the treatment combining AM inoculation with atrazine. Magnesium nutrient concentration in maize ear leaf varied in their level of significance with respect to treatments applied. Applying 100 kg N/ha with AM inoculation and atrazine gave the highest Mg nutrient concentration in maize ear leaf, while the lowest was observed in the control. At 0 kg N and NPK fertilizer, combination with AM inoculation alone gave plant with higher Mg nutrient concentrations in maize ear leaf than the other treatments. However, at the other OF levels, combining AM inoculation with atrazine improved Mg nutrient concentration than the other treatments.

Table 7. Influence of fertilizers, AM inoculation and atrazine applications interactions on maize ear leaf nutrients concentration (%)

Treatments	N	P	K	Ca	Mg
0 kg N x No AM x No atrazine	7.33a-c	0.80c	1.37f	0.93h	1.03i
With atrazine	8.00a-c	1.54b	8.93de	2.40bc	1.80d-f
With AM x No atrazine	8.67a	1.19bc	10.67a-e	2.67b	2.30bc
With atrazine	6.00c	2.47a	10.87a-e	1.57fg	1.57f-h
50 kg N/ha OF x No AM x No atrazine	8.00aa-c	1.18bc	9.47c-e	1.60fg	1.40h
With atrazine	8.33ab	1.62b	12.43a-c	3.97a	1.80d-f
With AM x No atrazine	8.67a	1.41bc	11.50a-d	2.00c-f	1.67e-h
With atrazine	8.00a-c	1.52b	9.10de	1.80d-f	2.23bc
100 kg N/ha OF x No AM x No atrazine	6.33bc	1.01bc	10.00b-e	1.53fg	1.60e-h
With atrazine	8.33ab	1.45bc	12.97ab	1.93c-f	1.73d-g
With AM x No atrazine	8.00a-c	1.30bc	10.77a-e	1.80d-f	2.53ab
With atrazine	9.00a	1.25bc	12.67ab	1.97c-f	2.70a
150 kg N/ha OF x No AM x No atrazine	7.33a-c	1.34bc	11.83a-d	1.70ef	1.90de
With atrazine	8.67a	1.44bc	11.57a-d	2.20b-d	1.60e-h
With AM x No atrazine	9.33a	1.34bc	11.07a-e	1.80d-f	1.80d-f
With atrazine	8.00a-c	1.38bc	13.27a	2.10c-e	2.00cd
NPK 15-15-15 x No AM x No atrazine	8.00a-c	1.23bc	8.33e	1.13gh	1.63e-h
With atrazine	8.33ab	1.31bc	12.40a-c	1.83d-f	1.67e-h
With AM x No atrazine	8.00a-c	1.30bc	11.77a-d	1.20gh	1.90de
With atrazine	9.00a	1.19bc	13.13a	2.13c-e	1.47gh
SE	0.71	0.24	1.09	0.17	0.11

AM = arbuscular mycorrhizal inoculation; Values within the same column, followed by similar letter(s) are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

There was no significant difference in the response of maize to the residual effects of the interactions of the application of fertilizers, AM inoculation and atrazine (Figure 2). However, the residual effect of 100 kg N ha^{-1} combined with AM inoculation and atrazine gave the highest maize shoot dry weight. The residual effect of the control treatment gave the lowest maize shoot dry weight. Across the fertilizer levels, the residual effect of combining AM inoculation and atrazine gave higher maize shoot dry weight than the use of atrazine alone.

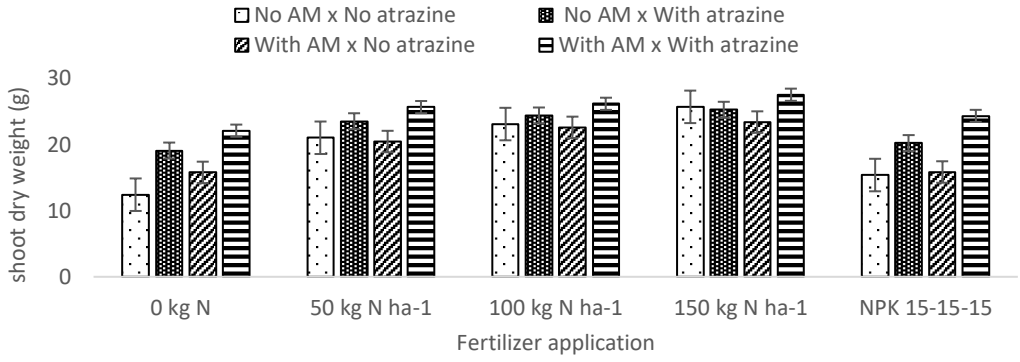

Figure 2. Residual maize shoot dry weight as influenced by the interactions of fertilizers, AM inoculation and atrazine application

Discussion

The textural classification of the soil physical fraction showed that the soil is loamy sand, indicating that it has a high amount of sand. According to Warncke et al. (2004), applying N fertilizer on such fine-textured soils have some economic advantages, but the environmental risks (leaching) generally out-weighed the economic benefits. The soil pH is high implying that it is alkaline in nature. Soil pH is critical to the efficacy of some herbicides (Warncke et al., 2004). Herbicide persistence in the soil is linked to the relationship between edaphic, climatic, and nature of herbicide (Fuscaldo et al. 1999). Findings suggest that persistence of atrazine, simazine and metribuzin in soil increased with high rates, low OM content and high pH (Fuscaldo et al., 1999; Warncke et al., 2004). Similarly, early reports have indicated that the pH of the soil has an impact on AM fungal density and diversity (Ouzounidou et al., 2015). Root colonization by inoculated fungi was stimulated at higher pH levels and inhibited at lower pH levels (Ouzounidou *et al*., 2015). It is therefore expected that the soil pH should favour root colonization by inoculated fungi. The low organic carbon observed in the soil is a characteristic of tropical soils (Kotschi, 2015). The pre-cropping soil organic carbon, nitrogen, available and exchangeable cation exchange capacity for the maize cultivation in Nigeria was considered low (Adeoye and Agboola, 1985). Similarly, Silva et al. (2011) demonstrated a direct relationship between reduced maize yield and soil nitrogen deficiency. For optimum maize production, N must be adequate during the growing season (Sileshi et al., 2010). As a consequence of these differences, it is logical to assume that there will be positive response of maize plants to the applied treatments.

The organomineral fertilizer used has N, K, Ca and Mg but low in P which on mineralization would be released for crop uptake. The values of organic carbon and C:N ratio of the material imply it is a good source of organomineral fertilizer to enhance crop performance through its effects on the soil's physical properties (Celik et al., 2004). According to Mooshammer et al. (2014), C:N ratios as observed in the OF may affect the dynamics of N, thereby, facilitating the rapid decomposition of organic matter and mineralization of N in the soils by microorganisms.

The reduction in the plant height at early growth stage (2 WAP) in all the treatments combined with atrazine but later increased at maturity was in support of Adigun and Lagoke (2003). They demonstrated atrazine depressive impact on the initial growth phases of maize plants, which showed a depressing impact of atrazine herbicides on maize plant height at the initial growth stages. They further supported the reports that at maturity, the depression disappeared and there was complete recovery in plant height at maturity. The results at maturity are also supported by the findings of Stefanovic et al. (2004), who reported that the use of herbicides not only suppresses weeds but also increased the height of maize plants. Across the OF levels including NPK 15-15-15 fertilizer at the early stage of growth, applied fertilizer did not suppress the influence of atrazine on maize plant height.

Unlike the responses observed on the other growth parameters, atrazine or its interactions with AM inoculation or fertilizers had no depressive effect at the early or maturity stages of the maize leaf area. This result was in support of findings made by Evans et al. (2003) that weed control increased leaf area. They reported that lower leaves of maize are suppressed by inappropriate weed control, which contributes to their early senescence, thereby leading to fewer numbers of leaves and consequently lower leaf area. Larbi et al. (2013) also reported similar finding on the weed competition for maize crops.

The highest dry shoot biomass observed in the interactions involving inorganic fertilizer suggests improved cell activity, enhanced cell multiplication and development of luxuriant vegetative plant compared to the organomineral fertilizer. However, the observed value was not significantly different from the values observed at 100 or 150 kg N/ha OF combined with AMF inoculation alone. This implies that the combinations were at par with inorganic fertilizer effect on maize dry shoot weight.

Higher grain yield observed in maize plants inoculated with AM fungi alone or atrazine alone compared to their interactions suggested their better influence when singly used to improve maize performance. The finding was in support of earlier reports by Celik et al. (2004) and Salami et al. (2005). They reported that the influence of AMF in improving plant growth diminishes with improvement in soil fertility status. Under Low P availability there is increases in plant demand for P and consequently increases in carbon allocation to AMF leading to improved AMF colonization and extra-radical hyphal development (Bending et al., 2004)., However, high P supply with high availability of other mineral nutrients, plants allocate relatively more photosynthate to shoots and leaves and less to roots and AMF, subsequently depressing AMF development (Liu et al., 2000; Treseder and Allen, 2002)

The improvement in N, Ca and Mg nutrition in maize by the application of AMF inoculation under the 0 kg N is in support of earlier report that inoculation with AMF increases these nutrients in crops (Liu et al., 2002; Fitter et al., 2011). Furthermore, the increase in N and K nutrient concentration observed in the treatment involving 150 kg N ha^{-1} with AMF inoculation implied a luxuriant consumption of N which resulted in vegetative growth at the expense of grain yield increment. The improved ear leaf nutrient concentration by atrazine application confirmed the report that maize has the ability to metabolize atrazine thereby improving its nutrition compared to where atrazine was not applied. Although combined effect of AMF and atrazine application improved K concentration, the lowest N nutrient concentration observed at 0 kg N combined with AMF inoculation

and atrazine treatment must be due to the antagonistic effect of the combining AMF inoculation with atrazine. This finding is in support of Huang et al., 2007) that AMF hyphae trap atrazine in the root system of maize (Nedumpara et al., 1999), thereby not mobilized into the site where it can be metabolized to improve the plant growth and development. This trend of result continued across the different levels of OF application. This implied that the application of organomineral fertilizer had no substantial effect in altering the observed occurrence in maize. This probably explained the higher performances observed in these treatments during the residual planting, whereby the residual effects from combined application of AMF inoculation performing relatively better than the other treatment combinations across all fertilizer levels. The N, P, K, Ca and Mg nutrient concentrations observed at 100 kg N ha^{-1} OF with atrazine likely suggest a balanced nutrition for improved maize performance, as this was the treatment that optimizes maize yield.

The ability to check weeds at the early stage of growth has been reported to favour maize growth and development. The control of weeds through the application of atrazine or in combination with AMF inoculation supported this principle. Hence, the treatments favoured early weed suppression across all fertilizer treatments, thereby encouraging maize growth and yields. The relative increment in the total weed biomass resulting from sole application of mycorrhizal inoculation or combined with atrazine across the fertilizer levels suggest that AMF enhanced weed infestation despite the influence of atrazine. This may imply that the weeds species in the soil were sensitive to AMF inoculation, thereby promoting their growth and development. This finding was supported by the report published by Vatovec *et al*. (2005), that AMF inoculation increase some weed species performance. However, this growth improvement did not reduce maize performance, thereby diminishing yield as evident in the study.

Conclusions

The interactions of various agronomic practices with respect to crop performance is affected by soil amendments and the need for weed management in order to increase crop production require understanding. The application of organomineral fertilizer, NPK 15-15-15, AMF inoculation and atrazine enhanced ear leaf concentration, growth and yield in maize. Combining atrazine with AMF inoculation improved maize growth, reduced grain yield under organomineral fertilizer but the interaction differed under NPK fertilizer application. Atrazine application reduced AMF colonization in maize root. Dry weed biomasses at 4, 12 WAP and total dry weed biomass produced reduced in all treatments involving atrazine. The total dry weed biomass produced increased with AMF inoculation across fertilizer applications. Consequently, combining 100 kg N/ha OF with AMF inoculation or atrazine was recommended.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the aurhors to the present study is equal. All the authors verify that the text, figures, and tables are original. The authors read and approved the final manuscript.

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Evaluation of Yield and Quality Performance of Groundnut Varieties under the Eastern Mediterranean Condition

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Abstract

High yielding superior groundnut cultivar development is the main objective of groundnut breeding programs. A four-year study was conducted in the Eastern Mediterranean Agricultural Research Institute in 2001, 2002, 2003 and 2004 growing seasons to evaluate for yield and quality characteristics of 80 groundnut varieties. The field experiments were laid out in a 3 replicated randomized complete block design. In the research, row spacing was 70 x 25 cm and 80 kg/ha nitrogen and phosphorus fertilizers were applied. The investigated plant parameters were plant growth form, seed husk color, flower color, number of seeds in capsule, shelling percentage, 100 seed weight, number of pod per plant, pod weight per plant, pod yield and seed oil content. The groundnut varieties GK-3 and VAC-92R could be cultivated as main crop in the Eastern Mediterranean Region due to their higher yield performance then the standard varieties Com and NC-7. The groundnut genotypes PI 361753, PI 288153, AT-108, AT120 and March 1 could be used to develop large seeded confectionery type groundnut varieties in the breeding programs.

Keywords: Groundnut, adaptation, cultivar, line, pod yield.

Introduction

Groundnut is an important nutritional source for human being and animals due to its high oil, protein, carbohydrate, vitamins and mineral contents (Arioglu, 2018). Most of the produced groundnuts are domestically consumed as roasted-in-shell nuts, raw nuts, salted nuts, and confectionaries. Peanut seeds contain up to 56% oil, 30% protein, 19.0% carbohydrates. Also, it has a good source of minerals, antioxidants, essential fatty acids (linoleic) and vitamin E, K, and B (Andrea and Palafoxdela, 1986; Eskalen and Yılmaz, 1993; Jagannathan et al., 1974; Sebei et al., 2013). Groundnut oil is much more superior then many other vegetable oils in terms of taste and shelf life (Arioglu, 2014). After extraction of the oil, the remaining pulp has approximately 45% crude protein, 24% nitrogen-free essence substances and 5.5% minerals. Therefore, groundnut pulp is added into animal feed in most of the developed countries.

The world shelled groundnut production was around 47 million tons in 27.8 million ha with an average yield of 1.78 t/ha (Anonymous, 2019a). The groundnut cultivation area in Turkey was 41.950 ha and the production was 165.330 tones (Anonymous, 2019b). The production of oilseed crops is not enough in Turkey, therefore, a certain amounts of oil seeds are imported. Every year, Turkey imports vast amount of oilseeds and raw oils and pays millions of foreign currency. According to data in 2016, five million tons of oilseeds were produced in the world and 43.9 million tons of them were obtained from groundnuts. On the other hand, 2.6 million tons of oilseeds and 780 thousand tons of vegetable raw oil were produced in Turkey. The production of vegetable raw oil in the world was 187 million tones (Anonymous, 2016). Chine, USA, Nigeria and Indonesia are the leading countries in world groundnut production.

The groundnut variety performance results in the Mediterranean Region showed that groundnut yield varied between 2340 and 8796 kg/ha (Gulluoglu et al., 2017a; Asik et al., 2018; Arioglu et al., 2016; Kurt et al., 2009; Onat et al., 2017; Arioglu et al., 2018; Gulluoglu et al., 2018; Onceler, 2005; Kadiroglu, 2012; Yılmaz, 1999). Variety NC-7 is widely cultivated in the groundnut cultivated areas of Turkey due to its high yield. Canavar, (2011) obtained up to 5210 kg/ha yield from NC-7 type groundnut varieties in Aydın province. Similarly Aytekin and Calıskan,

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(2016) obtained up to 5022 kg/ha seed yield in Nigde province. Shelling rates of groundnuts were reported as 62-76% in Turkey (Asik et al.,, 2018; Onceler, 2005). 100 seeds weight values for different varieties were reported between 53 and 137 grams (Asik et al., 2018; Arioglu et al., 2016; Gulluoglu et al., 2018; Canavar, 2011). Pod number per plant varied between 10 and 96 (Kurt et al., 2008; Kurt et al., 2009; Asik et al., 2018; Onat et al., 2017; Gulluoglu et al., 2017b; Onceler, 2005; Canavar, 2011) and the pod weight per plant varied between 35-120 g (Kurt et al., 2008; Onat et al., 2017; Gulluoglu et al., 2017b; Gulluoglu et al., 2018; Canavar, 2011). Oil content of groundnut varieties grown in Turkey varied between 29.43 and 55.60% Kurt et al.,, 2008; Kurt et al.,, 2009; Arioglu et al.,, 2016; Campos-Mondragon et al., 2009; Onceler, 2005; Aytekin and Calıskan, 2016; Gulluoglu et al., 2017b; Canavar, 2011; Yol et al., 2017).

About 95% of the groundnut is produced in the Mediterranean region of Turkey. Therefore, it is quite important to determine groundnut varieties with superior yield and quality features for the Mediterranean region.

This study was carried out to determine the performance of some selected groundnut cultivars and lines and determine their potential as breeding materials in the breeding programs.

Materials and Methods

In this study, 80 large seeded Virginia type groundnut varieties and lines were used as plant materials (Table 1). Two experimental plots in the Mediterranean Agricultural Research Institute and one in Osmaniye province, were selected for test locations. The field trials were conducted in two locations (Tasci in 2001 and 2002 and Dogankent 2001, 2002, 2003 and 2004) in the Mediterranean Agricultural Research Institute research fields. The groundnut varieties and lines were also tested in a farmer field in Osmaniye province in 2004. The selected varieties and genotypes (superior than the standard varieties in terms of yield or shelling percentage) of 2001 growing season were planted in the yield trials in 2002, 2003 and 2004.

In all locations, the seeds were sown by hand in the first half of April. The seeds were sown by hand in 4-row plots, 5 m long with the spacing of 70 cm between rows, and at a rate of 4 seeds per meter of row. Before sowing, 45 kg/ha N and 35 kg/ha P_2O_5 was applied. At flowering and pod formation (before first and third irrigation) 400 kg/ha Ammonium nitrate was applied. Standard cultural practices for groundnut (hoeing, irrigation, pest and disease control) were applied for all locations. Pod yields were determined by harvesting the middle two rows of each plot at all locations. The measured plant parameters were plant growth form, seed husk color, flower color, seed number per pod, shelling percentage (%), 100 seed weight (g), pod number per plant, pod weight per plant (g), pod yield (kg/ha) and oil content (%).

Data for investigated plant parameters were statistically analyzed using a standard analysis of variance in randomized blocks experimental design using the general linear model (SAS Institute, 1996). Means were separated using by Duncan.

Results and Discussion

In 2001, field variety performance trials were conducted in Dogankent and Tasci locations in the Eastern Mediterranean Agricultural Research Station. Compared to Dogankent, Tasci location, had fine textured soil such as clay, clay loam and silt loam.

During the growing seasons, some phenological observations like plant growth habit and flower color were recorded. The phenological observations showed that, 11 genotypes had prostrate (flat) growth habit, 43 genotypes had semi prostrate growth habit and 26 had runner growth habit. The seed husk color varied greatly among groundnut genotypes, particularly in red, shaded-red, dark red, bright dark red, pink, shaded pink, claret red, dark claret red, shaded claret red, tawny-brown, light red, bright orange. The flower colors varied from yellow, light yellow, dark yellow, bright dark yellow, dark matt yellow, orange, the mix of orange and yellow, the mix of yellow and bright orange, to matt orange yellow. The number of seed in the pod was two in general for all tested groundnut genotypes.

Shelling percentage, 100 seeds weight, the number of pods per plant, the pod weight per plant and pod yield per hectare in Dogankent and Tasci locations in 2001 growing season were given in Table1.

Table 1. Shelling percentage, 100 Seed weight, Pod number/plant, Pod weight/plant and pod yield of tested groundnut genotypes in Dogankent and Tasci in 2001.

Genotypes	Pod Number /Plant)		Pod Weight (g/plant)		100 Seed Weight (g)		Pod Yield (kg/ha)		Shelling Percentage (%)	
	DK	TS	DK	TS	DK	TS	DK	TS	DK	TS
1- PI 121071	125	44	115	45	85	81	5730	4090	57	64
2- PI 313361	78	71	88	64	83	76	4990	4340	58	66
3- PI 378013	146	50	124	30	92	93	4870	1690	54	64
4- PI 269704	112	70	107	60	83	73	5470	4300	57	65
5- PI 393525	125	33	139	39	68	63	1990	2200	54	56
6- PI 343361	98	55	83	63	78	89	4550	4160	57	66
7- PI 370326	61	58	61	57	78	73	4870	3790	53	63

8- PI 315624	98	59	97	59	85	79	5070	3400	56	63
9- PI 269699	72	36	71	44	85	83	2520	2370	60	67
10-PI 196732	46	39	46	41	71	85	4620	3250	55	67
11-NC-lOC	285	45	54	50	74	85	4280	2730	59	67
12-PI 221067	76	30	72	33	92	85	4250	3500	55	68
13-PI 361753	95	27	90	66	82	74	5760	5440	53	64
14-PI 295208	84	36	87	33	79	73	5120	2380	55	64
15-PI 259802	98	61	93	61	109	78	5770	3580	69	64
16-PI 319177	81	58	55	67	94	82	5800	4070	63	69
17-PI 269068	57	29	55	32	77	81	4140	2260	61	68
18-GK-3	130	113	112	86	76	77	5890	4350	56	64
19-PI 259861	94	63	74	58	70	67	4850	3690	63	70
20-PI 337455	58	55	58	50	84	77	5890	2340	65	66
21-PI 268882	97	51	89	42	98	70	6330	2750	67	63
22-H-3	96	38	94	47	94	84	5980	3510	55	60
23-PI 313197	57	42	55	31	112	72	4610	2860	74	62
24-PI 269082	65	65	53	72	98	78	4620	2180	63	64
25-PI 268885	48	22	45	20	92	68	2570	2480	70	61
26-PI 315609	82	45	88	55	85	88	4930	4700	60	70
27-PI 269722	48	47	47	70	80	88	4260	3590	63	65
28-PI 288153	119	54	116	59	89	79	6120	4410	60	67
29-PI 259649	140	72	142	71	89	71	5430	3180	58	64
30-PI 269723	30	37	29	39	111	91	3310	2510	65	65
31-PI 259815	156	88	141	79	75	78	5190	3500	63	67
32-PI 315616	103	51	109	44	93	72	5940	3520	57	62
33-PI 268883	160	68	159	56	76	61	5180	3620	59	58
34-PI 215628	212	71	119	49	61	60	3730	3840	62	74
35-NY-7	58	89	51	59	65	62	2860	3350	60	68
36-PI 124681	59	53	52	42	69	74	5040	3290	55	71
37-Shulamit	86	56	74	67	83	89	6140	4090	61	69
38-PI 291985	85	58	74	56	86	84	3950	3410	60	68
39-H-5	93	75	90	79	77	86	6090	4490	59	65
40-PI 378012	74	67	69	66	88	88	6080	3520	58	63
41-PI 343400	69	36	85	39	91	95	4630	3570	57	63
42-7 Selection H-1	106	47	88	48	71	82	5550	3530	60	70
43-Homobay	52	48	44	45	74	83	5490	3130	61	71
44-PI 378017	88	71	65	58	79	81	4950	3920	59	64
45-PI 259510	87	60	85	54	85	76	5680	3450	59	65
46-PI 315621	96	38	90	44	85	93	5540	3490	58	65
47-PI 378015	48	36	44	38	92	85	5110	3760	61	62
48-Turkmenistan	84	28	54	17	42	44	3280	1880	63	65
49- Edirne Tag 24	136	73	78	47	52	50	4310	4390	70	64
50- E VA 910212	63	89	58	87	84	82	4770	4160	65	67
51- Edirne TG-17	127	98	67	63	63	76	4120	5410	60	68
52-PI 346385	77	93	61	90	83	81	3670	3890	60	66
53-PI 315633	58	74	50	77	88	78	3520	4200	56	67
54-Edirne 138	60	67	68	45	49	51	5180	4750	63	70
55-Edirne 80	47	64	20	39	40	41	2150	2780	60	62
56- Edirne (CTGS)	55	56	37	34	46	46	3560	2590	63	62
57-Edirne 53	135	129	60	77	42	62	4150	5040	65	71
58-GA Runner	220	36	71	25	44	71	4750	3990	66	69

59-Florunner	83	16	91	17	83	92	5240	3680	64	71
60-GA Browne	72	78	34	27	65	41	3940	3250	72	70
61- 108ADV7	68	51	45	49	86	60	6210	4990	71	73
62-GA Green	55	88	28	54	54	60	5540	4780	68	68
63- AT120	87	29	73	23	97	69	5640	4450	75	75
64-GA Brown	95	60	69	46	61	73	4890	3900	71	71
65-Florunner	128	120	59	66	57	53	5940	3550	65	75
66- Sunoleic 95R	39	51	18	30	44	49	4140	2830	64	70
67-Andru 93	55	12	40	10	66	75	2950	1490	58	60
68-GA Runner	53	26	30	14	53	51	4140	2410	63	65
69-Southern Runner	93	66	45	33	44	48	4610	2460	68	69
70-March 1	82	131	54	86	59	63	7014	5080	66	75
71- VAC 92 R	47	58	44	57	93	89	3440	3400	63	68
72-269084	105	59	88	70	87	89	6000	3580	62	63
73-Adana	58	86	53	82	89	77	3600	3110	60	65
74-Çom	53	75	52	69	77	79	3840	4020	60	65
75-7511073	144	49	135	52	81	77	5740	3340	58	63
76-NC-7	52	78	63	81	94	91	5520	4320	68	70
77-7X	104	48	85	55	62	96	5230	3770	62	69
78-ATVCI	49	73	46	63	78	77	3190	3380	63	68
79-PI 346385	35	48	34	45	74	80	5440	3450	56	65
80-PI 372317	34	64	38	68	81	80	3100	2630	48	59

DK: Dogankent, TS: Tasci

As can be seen in Table 1, the highest pod number per plant was obtained from NC-lOC with 285 number/plant, and the lowest pod number per plant was obtained from Andru 93 with 12 number/plant in Dogankent and Tasci, respectively. The pod weight values of the tested groundnut genotypes were between 10 and 159 g and the highest and lowest pod weights were obtained from PI 268883 and Andru 93, respectively. The highest 100 seed weight was observed from PI 313197 with 112 g, and the lowest was obtained from Edirne 80 with 40 g. The highest pod yield was obtained from March 1 with 7014 kg ha^{-1}, and the lowest was obtained from Andru 93 wşth 1490 kg ha^{-1} in Dogankent and Tasci, respectively. Shelling percentage values in Dogankent and Tasci locations varied between 48% and 75%, the highest values were obtained from AT120, Florunner and 7511073 and the lowest value was obtained from PI 372317.

Groundnut variety Çom which is one of the standard varieties had pod yields with 3840 and 4020 kg/ha in Dogankent and Tasci locations, respectively. Additionally, 5520 and 4320 kg/ha pod yields were obtained from NC-7 v in Dogankent and Tasci locations, respectively. In Dogankent and Tasci, PI 361753, GK-3, PI 288153, 108ADV7, GA Brown, AT120, and March 1 had higher yielding groundnut genotypes than the standard varieties.

In 2002, 8 groundnut genotypes were chosen for further yield evaluations in two locations, since they were superior to the control genotypes. When Dogankent and Tasci locations was compared, pod yield of groundnut genotypes were higher than Tasci. The yield differences between two locations could be attributed the soil structure. Since Dogankent has heavier textured soil structure than Tasci location. Yield is higher in heavy textured soils; however, harvest is more difficult and harvest losses are high in heavy textured soils. Out of 8 genotypes, 4 genotypes were in the semi prostrate, 3 in runner and 1 in prostrate growth habit. The seed husk color of those genotypes PI 361753, GK-3, PI 288153 and GA Green were pink; genotypes in H-5 and 108 ADV7 were shaded pink, genotype AT120 was bright orange, genotype March 1 was shaded brown. The shaded seed husk color is not preferred by Turkish groundnut consumers. Based on evaluated yield and quality parameters, the groundnut variety March 1 had great performance in both locations.

Pod number per plant, pod weight per plant,100 seeds weight, pod yield and shelling percentages of genotypes in Dogankent and Tasci in 2002 were given in Table 2. The yield trials were conducted with 10 genotypes including standard ones in 2002. Groundnut genotypes PI 361753, GK-3, PI 288153, H-5, At-108ADV7, GA Green, AT120 and March 1ADV had higher pod yields than the standard varieties (Çom and NC-7) in Dogankent and Tasci locations in 2002.

Table 2. Shelling Percentage, 100 Seed weight, Pods Number, Pod Weight and Pod Yield of Groundnut Genotypes in Dogankent and Tasci in 2002

Genotypes	Pods Number /Plant		Pod Weight (g/plant)		100 Seed weight (g)		Shelling Percentage (%)		Pod Yield (kg ha^{-1})	
	DK	TS	DK	TS	DK	TS	DK	TS	DK	TS
1- PI 361753	54.9 c	87.3 a	55.3 cde	60.1 bc	74 c	73.3 cd	61.5 e	61.3 c	3230 bc	3650 bcd
2- GK-3	80.4 bc	54.3 b	66.3 abc	78.3 b	82.0 b	88.0 b	64.9 cde	66.0 b	4510 a	4600 a
3- PI 288153	50.1 c	85.4 a	74.8 ab	64.1 bc	74.7 c	68.7 d	62.9 de	60.9 c	3940 abc	3910 b
4- H-5	70.3 bc	88.9 a	78.4 a	99.9 a	86.0 ab	74.0 cd	66.2 bcd	62.0 c	4240 ab	4160 ab
5- AT-108	100.1 ab	85.7 a	44.9 de	61.0 bc	53.3 e	48.7 f	70.4 a	73.5 a	3140 bc	3260 cd
6- GA Green	119.3 a	106.4 a	54.7 cde	58.3 bc	53.3 e	50.0 f	69.5 ab	69.3 b	3010 c	3520 bcd
7- AT 120	76.8 bc	94.0 a	63.3 abc	57.2 bc	64.0 d	62.0 e	69.1 ab	69.1 b	3200 bc	3050 d
8- March1	51.3 c	57.3 b	40.1 e	45.7c	58.0 e	60.0 e	70.0 a	73.7 a	2940 c	3230 cd
9- ÇOM	84.1 bc	85.5 a	68.0 abc	73.7 b	75.3 c	75.3 c	62.9 de	66.0 b	3590 abc	3800 bc
10- NC-7	55.9 c	51.9 b	59.3 bcd	60.7 bc	89.3 a	98.0 a	67.6 abc	68.4 b	3790 abc	3910 b
CV (*:%5, **:%1)	14.25**	16.81**	25.31**	18.35**	4.60**	5.05**	2.84**	2.78**	17.0*	9.09**

DK: Dogankent, TS: Tasci

As can be seen in the Table 2, according to analyzed characters in 2002, in both locations the highest shelling percentage was obtained in AT-108ADV-7with the rate of 70.4% and then in March 1 ADV-6 with the rate of 73.7%; additionally, the lowest shelling percentages were observed from PI 288153 (% 60.9) and PI 361753 (% 61.5), respectively. Furthermore, the highest 100 seed weight was found in standard variety NC-7 and then GK-3 with 82-88 g. The lowest 100 seed weights were obtained from AT-108ADV-7 and GA Green with 48-53 g. The shelling percentages are similar with the other studies (Asik et al., 2018; Onceler, (2005). Whereas 100 seed weight was between 53.3-89.3 g in Dogankent and it was between 48.7 and 98 g in Tasci location. In both locations, the lowest was obtained from AT-108ADV-7 and GA Green. The highest value was obtained from standard variety NC-7. When 100 seed weight was in consideration, results were consistent with the findings of other studies (Asik et al., 2018; Arioglu et al., 2016; Gulluoglu et al., 2018; Canavar, 2011). The number of pod results was close to the finding of other researches (Kurt et al., 2008; Kurt et al., 2009; Asik et al., 2018; Onat et al., 2017; Gulluoglu et al., 2017b; Onceler, 2005; Canavar, 2011). Groundnut genotype H-5 had highest pod weight per plant with 78.4-99.9 g in both locations March 1 had the lowest value with 40.1-45.7 g. The pod weight per plant was compatible with other researchers' findings (Kurt et al., 2008; Onat et al., 2017; Gulluoglu et al., 2017b; Gulluoglu et al., 2018).

Whereas GK-3 had the highest pod yield in both locations (4510 and 4600 kg/ha), March 1 (2940 kg/ha) had the lowest in Dogankent and AT120 (3050 kg /ha) in Tasci. Pod yield obtained from the yield test conducted in Turkey had similar results (Gulluoglu et al., 2017a; Asik et al., 2018; Arioglu et al., 2016; Kurt et al., 2009; Onat et al., 2017; Arioglu et al., 2018; Gulluoglu et al., 2018; Onceler, 2005; Kadiroglu, 2012; Yılmaz 1999).

Variety yield test in 2003 were conducted with 13 genotypes including standard varieties in Dogankent location. PI 378017, VAC-92R and 7X had lower pod yield values lower than the standard varieties, but which have promising results in the yield experiments conducted by Çukurova University and which are also proper for appetizer consumption, were included in our studies on the recommendation of advisor.

Pod number per plant, pod weight per plant, 100 seed weight, pod yield, the shelling percentage and oil content of genotypes sowing in Dogankent were given in Table 3.

Table 3. Pods Number, Pod Weight, Pod Yield, 100 Seed weight, Shelling Percentage, and Oil Content of Groundnut Genotypes in Dogankent in 2003

Genotypes	Pods Number /Plant	Pod Weight (g/plant)	Pod Yield (kg/ha)	100 Seed weight (g)	Shelling Percentage (%)	Oil Content (%)
1- PI 361753	30.6 d	41.3 c	4100 d	90.0 b	61.0 c	50.17
2- GK-3	54.0 abc	69.3 a	5110 ab	100. ab	62.2 c	-
3- PI 288153	42.6 abcd	46.6 c	4970 abc	96.6 ab	61.0 c	50.68
4- H-5	38.0 cd	54.0 bc	4550 bcd	96.6 ab	63.3 bc	-
5- AT-108	66.6 a	52.0 bc	4260 cd	60.0 c	72.2 a	51.58
6- GA Green	62.3 ab	49.3 bc	4650 bcd	63.3 c	73.3 a	-
7- AT 120	57.3 abc	63.3 ab	4150 d	70.0 c	68.8 ab	50.65
8- March 1	45.6 abcd	50.6 bc	4550 bcd	70.0 c	68.8 ab	51.53
9- PI 378017	50.6 abcd	52.0 bc	4690 abcd	100.0 ab	65.50 bc	49.14
10- VAC-92R	62.0 ab	63.3 ab	5460 a	100.0 ab	63.30 bc	
11- 7X	51.6 abcd	63.3 ab	4640 bcd	100.0 ab	63.30 bc	49.27
12- ÇOM	41.0 bcd	53.3 bc	4790 abcd	90.0 b	61.07 c	-
13- NC-7	45.3 abcd	46.4 c	5150 ab	110.0 a	65.50 bc	-
CV (*:%5, **:%1)	19.31**	16.3*	9.99*	8.6**	4.00**	-

Pod number per plant varied between 30 and 66 number/plant. The lowest pod number was obtained from PI 361753, and the highest was obtained from AT-108ADV-7. Our findings for pod number was similar to the findings of the others (Kurt et al., 2008; Kurt et al., 2009; Asik et al, 2018; Onat et al., 2017; Gulluoglu et al., 2017b; Onceler, 2005; Canavar, 2011). The values of pod weight varied between 41.3 and 69.3 g, and also the lowest value was obtained from PI 361753 while the highest one was obtained from GK-3. Those values were compatible with the values reported by Kurt et al. (2008), Onat et al. (2017), Gulluoglu et al. (2017b) Gulluoglu et al. (2018).

Shelling percentage varied between 61 and 73.3% in the experiment carried out in Dogankent in 2003 and PI 361753 had the lowest and GA Green had the highest shelling percentage (Table 3). Our findings were compatible with the findings of Asik et al. (2018), Onceler (2005). While 100 seed weight was varied between 60 and 110 g, the lowest was obtained from AT-108 and the highest was obtained from NC-7. Seed weight values correspond to the values reported by Asik et al. (2018), Arioglu et al. (2016), Gulluoglu et al. (2018), Canavar (2011).

Pod yield of groundnut genotypes varied between 4100 and 5460 kg/ha, the lowest pod yield was obtained from PI 361753 and the highest was obtained from VAC-92R. Pod yields were similar with the findings of Gulluoglu et al. (2017b), Asik et al. (2018), Arioglu et al. (2016), Kurt et al. (2009), Onat et al. (2017), Arioglu et al. (2018), Gulluoglu et al. (2018), Onceler (2005), Kadiroglu (2012) and Yılmaz (1999). The seed oil contents were not analyzed for all genotypes. The highest oil ratio was obtained from AT-108ADV with 51.58% and the lowest was obtained from PI 378017 with 49.14%. The seed oil content results were close to finding of other researchers (Kurt et al., 2008; Kurt et al., 2009; Arioglu et al., 2016; Campos-Mondragon et al., 2009; Onceler, 2005).

In the 2003 yield test, some of the high yielding genotypes were eliminated due to their lower quality characteristics. Since they could not be preferred by groundnut farmers. GK-3, H-5 and VAC-92R were further tested one more year in Osmaniye and the the Eastern Mediterranean Agricultural Research Institute with standard varieties by paying attention to seed rate and 100 seed weight and yield in 2004.

In the yield tests carried out in 2004, variety yield experiments was established in Dogankent field of institute and the village of Çona in Osmaniye by using 5 varieties and lines together with standard varieties in field of farmers. The yield experiments were conducted in Osmaniye for a year in order to see the performance of lines in the province of Osmaniye wich has the largest groundnut cultivation areas.

Pod number per plant, 100 seed weight, shelling percentage of genotypes tested in Dogankent and in farmer's field in Osmaniye were given Table 4.

Table 4. Shelling Percentage, 100 Seed Weight and Pod Number in Groundnut Genotypes in Dogankent and Osmaniye in 2004

Genotypes	Pods Number/Plant		100 Seed Weight (g)		Shelling Percentage (%)	
	DK	OMY	DK	OMY	DK	OMY
1- GK-3	190.7 a	217.3 a	78.6 c	74.6 c	66.0 bc	54.6a
2- H-5	147.7 b	145.3 b	74.6 c	80.0 bc	58.0 d	52.6 a
3- VAC-92R	125.7 b	144.3 b	104.0 a	88.0 ab	68.67 a	55.3 a
4- ÇOM	213.3 a	189.7 ab	77.3 c	73.3 c	64.0 c	48.6 b
5- NC-7	135.0 b	160.7 b	85.3 b	93.3 a	68.0 ab	54.6 a
CV (*:%5, **:%1)	12.56**	14.26*	3.40**	5.43**	1.87*	3.70*

DK: Dogankent, OMY: Osmaniye

The shelling percentage values significantly varied in both Dogankent and Osmaniye locations (Table 4). In Dogankent, the highest shelling percentage was obtained from VAC-92R with 68.67% , and the lowest was obtained from H-5 with 58.0%. In osmaniye location, shelling percentage varied between 48.6 and 55.3%, and the lowest and the highest values were obtained from Çom and VAC-92R, respectively. The shelling percentages in Dogankent location are close to findings obtained by Aşık et al., (2018) and Onceler, (2005); however, lower shelling percentage was obtained in Osmaniye location. When 100 seed weight was in consideration, there were significant 100 seed weight differences among groundnut genotypes in both locations. In Dogankent location the highest 100 seed weight was obtained from VAC_92R with 104.0 g and the lowest was obtained from H-5 with 74.6 g. Similarly, 100 seed weight significantly varied among groundnut genotypes. In Osmaniye location the highest 100 seed weight was obtained from NC-7 with 93.3 g and the lowest was obtained from Com with 73.3 g. The 100 seed weight values are similar to the finding of Asik et al.,(2018); Arioglu et al., (2016); Gulluoglu et al., (2018); Canavar, (2011). Pod number per plant significantly varied among groundnut genotypes in both locations. In Dogankent, Çom had the highest pod number with 213.3 and VAC-92R had the lowest pod number per plant with 125.7. In Osmaniye location pod number per plant varied between 217.3 and 144.3 among groundnut genotypes. The highest and the lowest pod number plant was obtained from GK-3 and VAC-92R, respectively. Our finding for pod number per plant were higher than the finding of Kurt et al., (2008); Kurt et al., (2009); Asik et al.,

(2018); Onat et al., (2017); Gulluoglu et al., (2017b); Onceler, (2005); Canavar, (2011).

Pod weight per plant, pod yield and seed oil content of the selected groundnut genotypes were given n Table 5.

Table 5. Pod Weight, Pod Yield and Oil Content of Selected Groundnut Genotypes in Dogankent and Osmaniye in 2004.

Genotypes	Pod Weight Per Plant (g/plant)		Pod Yield (kg ha^{-1})		Oil Content (%) (Dogankent)
	DK	OMY	DK	OMY	DK
1- GK-3	152.3 ab	160.3	5815 ab	3435 ab	48.4
2- H-5	124.7 bc	123.3	4826 c	3175 b	52.0
3- VAC-92R	130.3 bc	126.7	6343 a	3332 ab	48.2
4- ÇOM	180.7 a	140.0	5252 bc	3530 a	48.8
5- NC-7	114.0 c	144.0	5085 bc	3760 a	49.0
CV (*:%5, **:%1)	12.55**	11.68	9.49**	8.50**	

DK: Dogankent, OMY: Osmaniye

In Dogankent locations, pod weight per plant varied significantly among the tested groundnut genotypes. The highest and the lowest pod weight obtained from Çom and NC-7 with 114.0 and 180.7 g, respectively (Table 5). Osmaniye location, pod weight per plant varied between 123.3 and 160.3 g, however, pod weight per plant did not significantly vary among the groundnut genotypes. The reason for the high pod weight per plant is attributed to pod size in each genotype. Our results for pod weight were higher than the values reported by Kurt et al. (2008); Onat et al. (2017), Gulluoglu et al. (2017b), Gulluoglu et al. (2018). When pod yield was in consideration, the lowest was obtained from H-5 in both Dogankent and Osmaniye locations. The pod content was obtained from H-5 and the lowest was obtained from VAC-92R. Our results for oil content are similar to the findings obtained by (Kurt et al., 2008; Kurt et al., 2009; Arioglu et al., 2016; Campos-Mondragon et al., 2009; Onceler, 2005).

Conclusion

In this experiment, 80 groundnut varieties and lines were tested for yield and yield characteristics at three locations (Dogankent, Tasci and Osmaniye) under the Eastern Mediterranean condition. In the first year, high yielding groundnut genotypes were chosen to further evaluation for their yield and quality performance. The result showed that pod yield varied between 2940 and 6340 kg ha^{-1}, shelling percentage varied between 48% and 73.7%; 100 seed weight varied between 48 and 110 g, pod number varied between 30 and 217 number/plant, pod weight varied between 40 and 180 g/plant and finally the seed oil content varied 48.2% and 52.0%. (Table 2. 3, 4, 5). The groundnut varieties GK-3 and VAC-92R had higher pod yield and larger seed size, therefore, these two varieties cold be cultivated in the Mediterranean region for confectionery purposes. The groundnut genotypes PI 361753, PI 288153, AT-108, AT 120 and March could be used in the groundnut breeding programs to develop new superior varieties for confectionery purposes. Groundnut genotypes over 4000 kg ha^{-1} pod yield and over 50% oil content could be used to develop new groundnut varieties for oil industry.

yields varied between 6343 and 4826 kg/ha, and the lowest and the highest pod yields were obtained from VAC-92R and H-5, respectively in Dogankent. Compared with Dogankent, pod yields were lover in Osmaniye. The highest pod yield was obtained from NC-7 with 3760 kg ha^{-1}, and the lowest was ontained from H-5 with 3175 kg ha^{-1}. The pod yield results of the current study are similar to the results obtained by different researchers in the same region (Gulluoglu et al., 2017; Asik et al., 2018; Arioglu et al., 2016; Kurt et al., 2009; Onat et al., 2017; Arioglu et al., 2018; Gulluoglu et al., 2018; Onceler, 2005; Kadiroglu, 2012; Yılmaz, 1999). Seed oil content of groundnut genotypes varied between 48.2 and 52.0%, and the highest oil

Compliance with Ethical Standards

Author contribution

Author read and approved the final manuscript. Author verify that the Text, Figures, and Tables are original and that they have not been published before.

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Not applicable.

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Evaluation of Eggplant Cultivars for Tomato Spotted Wilt Orthotospovirus (TSWV) Disease Tolerance in Greenhouse Conditions

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Abstract

Eggplant (*Solanum melongena* L.) is widely consumed vegetables like potato and tomato. Worldwide, the eggplant is cultivated in all regions and Turkey is the fourth largest eggplant-producer. It is a rich source of minerals and as a low-calorie fruit. Eggplant plays a vital role having high phenolic content which enhance the radical absorbing capacity. Objective of this study was to evaluate the effect of tomato spotted wilt orthotospovirus (TSWV) on five different eggplant cultivars (Kemer, Aydın Siyahı, Halep Karası, Topan and Silindirik) under greenhouse conditions. Eggplant cultivars were mechanically inoculated with TSWV isolates and tested by DAS-ELISA method. According to DAS-ELISA and visible virus-like symptoms, all tested cultivars were susceptible to TSWV infection and showed typical tospo-like symptoms including concentric ringspot, necrosis, chlorotic ringspot, and necrotic ringspot. The highest infection rate was observed in Kemer (58%) followed by Topan (52%) whereas, the lowest infection rate was noticed in Silindirik (38%). Infection of TSWV caused significant ($p \leq 0.05$) reduction in fruit number (32.99-59.34%), fruit length (17.12-49.76%), fruit diameter (12.44-38.30%), fruit weight (31.31-67.70%), flesh thickness (18.11-46.05%), total soluble solid (16.83-40.69%), fruit color, fruit firmness (4.88-29.25%) and yield (50.22-84.22%) in infected plants. According to the results obtained, the cultivar Silindirik performed better performance against TSWV among all the tested cultivars. Whereas the performance of the Kemer and Topan was poor making them more sensitive to TSWV. These results will help breeders for the development of TSWV resistant varieties by using these tolerant cultivars.

Keywords: Eggplant, cultivar, ELISA, *Tomato spotted wilt orthotospovirus*, Yield, Quality

Introduction

Eggplant (*Solanum melongena* L.) belongs to the family *Solanaceae*, which is widely consumed vegetables like potato, tomato, and pepper. Globally, the eggplant is cultivated in all regions including the subtropical, tropical, and temperate regions (Sihachakr et al., 1994). It is considered as a rich source of minerals and as a low-calorie fruit. Among the top ten vegetables, the eggplant plays a vital role having high phenolic content which enhance the radical absorbing capacity (Cao et al., 1996; Caguiat and Hautea, 2014). Turkey is well-known for its widely cultivated vegetables; eggplant is one of them, which was introduced from Europe by traders during 16th century. Turkey is the fourth largest eggplant-producing with the annual production of 822.659 tons (FAOSTAT, 2019).

Several biotic and abiotic stress factors affect the yield of eggplant. The biotic factors include insect pests and pathogens. Different diseases are developed by bacteria, phytoplasmas, fungi and viruses either in roots or shoots of eggplant (Tsitsigiannis et al., 2008). Eggplant is prone to different kind of diseases (bacterial, fungal, and viral) that affect its productivity and yield. Among the viral diseases which affect eggplant are *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Cucumber mosaic virus* (CMV) and *Tomato spotted wilt orthotospovirus* (TSWV) whereas, *Tomato yellow leaf curl virus* (TYLCV) affects tomato and pepper, but eggplant is resistant

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to this virus (Czosnek et al., 1993). TSWV belongs to the genus *Orthotospovirus* of the family *Bunyaviridae* (Salamon et al., 2016). TSWV disease was reported in 1915 for the first time (Best, 1968). There are a lot of plant families including *Solanaceae*, *Asteraceae*, *Brassicaceae*, *Bromiliaceae* and *Leguminaceae* that are prone to TSWV infections (Momol and Pernezny, 2006). TSWV can be transmitted with the help of different thrips species including *Frankliniella occidentalis*, *F. schultzei*, *F. intonosa*, *F. bispiosa*, *F. fusca*, *Thrips setosus*, and *T. tabaci* and there is no report of it to be seed transmitted (Tsuda, 1999; Mound, 1996). The effect of TSWV on plants varies with type of plant, time, and duration of infection. General symptoms of its infection on plants are wilting, ringspots, stunting of leaves, necrosis, and chlorosis (Holguin-Pena and Rueda Puente, 2007). In eggplant, symptoms such as deformed leaves, necrosis of leaves, molted and stunted plants have been observed (Kamberoglu et al., 2009).

Among the viruses, TSWV can cause systemic infection which lead to yield loss resulting in producing un-marketable plants, flower, and fruit (Ramkat et al., 2006). This virus destroys all flowering crops, and it is currently causing the most severe effects on its host plants around the globe (Pfleger et al., 1989). TSWV was reported to cause 100% yield losses in tomato (Rosello et al., 1996). Currently, the occurrence of TSWV is causing severe problems in vegetable growing areas of Turkey, also in other parts of the world (Kilic et al., 2017). Due to these losses; the current study was conducted to evaluate the performance of selected Turkish eggplant cultivars in response to TSWV effects on yield and quality traits.

Material and Methods

Experimental Site Description

This research work was carried out in the greenhouse of the Faculty of Agricultural Science and Technologies at Niğde Ömer Halisdemir University, Niğde, Turkey during 2019-2020. It is located at 37.97 latitude and 34.68 longitude and 1243 m above the sea level in the Central Anatolia region of Turkey.

Plant Materials and Growth

The most commonly grown five cultivars of eggplant including Topan, Kemer, Halep Karası, Aydın Siyahı and Silindirik were used to evaluate the effects of *Tomato spotted wilt orthotospovirus* infection on eggplants. First, the seeds were sown in trays for germination (n=2). Later seedlings were transplanted to the 10L pot one plant per pot filled with turf and perlite (3:1) and were maintained in greenhouse with daytime and nighttime temperature of 24°C and humidity 60-70%.

Isolation of TSWV and Experimental Design

For TSWV isolate of tomato plant was obtained from the Turkish Ministry of Agriculture and Forestry, Ankara Directorate of Agricultural Quarantine. After transplanting the plants were rub-inoculated with TSWV two times: first at 7th days at three leaf stage and second at 14th day after transplanting the eggplants. Twenty-five plants were used for inoculation and 25 plants were used as a control for each cultivar had total 50 plants; 25 inoculated with TSWV and 25 buffer inoculated (i.e., mock-inoculated control).

Mechanical Virus Inoculation

Mechanical virus (rub)-inoculation method was used for inoculating eggplants. Inoculation buffer having pH: 7.4, containing 0.199 g/l KH_2PO_4, 1.14 g/l Na_2HPO_4 and 0.1% Na_2SO_3 and 1% PVP-40 were prepared. TSWV isolate of tomato plant fresh leaf samples were grinded by mortar and pestle to get leaf extract for inoculum sources and mix these extracts with inoculation buffer in 1/10 ratio. Seedlings were irrigated well and kept under dark conditions one day before inoculation. Plants were inoculated in the morning because the stomatal opening and absorption rate is generally higher as compared to evening. Before starting the inoculation, carborundum was sprinkled on the surface of leaves to cause abrasion of the cells and the virus inoculum source was rubbed over the surface of eggplants leaf by using cotton-swab. Tap water was sprayed on the surface of inoculated leaves after 5 mins of inoculation. For mock inoculations, only the buffer was rubbed on the surface of leaves and these plants were used as mock-control. After 10-15 days of virus inoculation, plants show symptoms of TSWV and for confirmation of the virus; the leaves were collected randomly and tested by ELISA method.

Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA)

DAS–ELISA method with the monoclonal antisera of TSWV was performed for detection of TSWV in eggplant according to Clark and Adams (1977). Specific antibodies for applied according to manufacturer instructions (Bioreba AG, Switzerland).

Calculation of Infection Rate of TSWV (%)

Leaf samples were collected from all cultivars as shown in Table 1. Following formula was used for calculation of TSWV infection rate in eggplants to find the infection ratio.

$$TSWV\ (\%) = \frac{Z1}{Z2} \times 100$$

Z1= Positive samples of eggplant tested by ELISA,
Z2= Total samples of eggplants tested by ELISA

Yield and Quality Parameters of Eggplant

Fruits of inoculated and mock-control plants were harvested at the full maturity. Fruit morphological traits related to quality were evaluated for 7-10 ripened fruits per cultivar. The ripened fruits from infected and uninfected plants were harvested by hand and put in plastic bags and the following parameters were analyzed. Fruit numbers (FN) were counted manually. The fruit length (FL) was measured by using measuring tape and data was recorded in centimeter (cm), whereas the fruit diameter (width) was measured by using vernier caliper and data was recorded in millimeter (mm). Fruit fresh weight (FW) and dry matter (DM), the digital scale was used for fresh weight fruits were weighed immediately after harvesting

and for dry matter the samples were put in oven for drying at 70ºC for 48 hours. Fruit color (FC) was determined by Chroma meter describes the color distribution in eggplant cultivars in three dimensions "L", "a" and "b". The dimension "L" was considered since it explains the alteration of color from dark/black to white (light), commonly observed in fruit skin of eggplant Fruit firmness (FF) was measured by using penetrometer (LANATECH-GY-3). Total soluble solid (TSS) (brix) was measured by refractometer. Thickness of fruit flesh was calculated by vernier caliper. Yield (g) per plant was measured by using digital scale.

Statistical Analysis

Analysis of Variance (ANOVA) statistical test was performed using the Statistix 8.1. Duncan multiple comparison test was used to compare the differences between the averages which were statistically significant according to the variance analysis results. Principal component analysis (PCA) and correlation analysis were done by XLSTAT-2014.

Results

Disease Symptoms

The observed symptoms were deformation of the leaves, ringspot, necrosis, necrotic ringspot, and concentric ringspot on inoculated plant leaves as shown in Figure 1. The symptoms of TSWV started to appear on fresh young leaves after 10 to 14 days of first inoculation. Samples were collected from the inoculated plants randomly and tested by DAS-ELISA for the confirmation of TSWV infection. No symptom was developed on infected plants' fruits and mock-control plants.

DAS-ELISA Results

Randomly collected leaf samples from inoculated eggplant cultivars were tested by DAS-ELISA method. According to the ELISA test, the highest infection result was detected from Kemer cultivar 58% followed by Topan 52%, whereas the lowest positive result was observed from Silindirik cultivar 38%. The results of ELISA tests are summarized in the Table 1.

Quantity Traits

Number of Fruits per Plant

It was observed that among the infected plants, cultivar Silindirik resulted in highest number of fruits (5,26), whereas the lowest numbers of fruits were recorded for infected plants of cultivars Aydın Siyahı (1.79), Topan (1.87) and Halep Karası (1.90) which were statistically non-significant from each other. Whereas, for mock-control plants the highest number of fruits was observed for Silindirik (7.85) followed by Kemer (6.47). It was observed that infection of virus significantly reduced the fruit number. Highest percent of reduction in fruit number over control in infected plants was recorded for Topan 59.34%, followed by Kemer 55.33% whereas the lowest percentage of reduction 32.99% was recorded for Silindirik cultivar (Figure 2-A).

Fruit Length

Fruit length of the eggplant cultivars was significantly ($p \leq 0.05$) affected by TSWV. The infected plants comparatively showed lower fruit length while the control showed higher fruit length. Among the infected plants the highest fruit length was recorded for Silindirik (12.59 cm) followed by Aydın Siyahı (8.7 cm). Whereas, among the mock-control plants the highest fruit length was recorded for Silindirik (15.19 cm) and lowest was recorded for Topan (10.36 cm). Tested eggplant cultivar Kemer resulted in highest percent of reduction in fruit length (49.76%), followed by Topan (45.95%) and Aydın Siyahı (41.53%) in infected with respect to their mock-control. Lowest percent decrease in fruit length (17.12%) was recorded for Silindirik which was least effected by TSWV (Figure 2-B).

Fruit Diameter

Significant ($p \leq 0.05$) reduction in fruit diameter was also observed in infected plants as compared to their respective mock-control. Among the infected plants highest fruit diameter (40.96 mm) was recorded from Silindirik followed by Topan (38.93 mm) and lowest was recorded for Kemer (21.31 mm).Whereas, among the mock-control plants the highest fruit diameter was recorded for Topan (52.76 mm) followed by Silindirik (46.97 mm) and lowest was recorded for Halep Karasi (32.72 mm). The highest percent reduction of fruit diameter was calculated from Kemer cultivar (38.30%) followed by Topan (26.21%), whereas Silindirik was least affected and resulted in lowest percent decrease in fruit diameter (12.80%) (Figure 2-C).

Fruit Weight

Fruit weight was significantly ($p \leq 0.05$) affected by TSWV. Among the infected plants the highest fruit weight was recorded for Kemer cultivar exhibited lowest fruit weight (39.45 g) followed by Topan (51.78 g) and Halep Karası (53.65 g) while among the mock-control plants the cultivar Silindirik conferred highest fruit weight (169.49 g) followed by Topan (145.53 g), Aydın Siyahı (127.74 g) and Kemer (122.12 g). The highest percent reduction of fruit weight was calculated from Kemer 67.70% followed by Topan 64.42%, Aydın Siyahı 55.74% and Halep Karası 45.70%, whereas lowest percent reduction was recorded for Silindirik cultivar 31.31% which was less affected by TSWV (Figure 2-D).

Flesh Thickness of Fruit

Flesh thickness was significantly ($p \leq 0.05$) affected by TSWV. Among the infected plants the highest flesh thickness was recorded for Silindirik (8.00 mm) followed by Aydın Siyahı (4.65 mm) and lowest was recorded for Topan (3.80 mm). Whereas, among the mock-control plants the highest flesh thickness was recorded from cultivar Silindirik (9.77 mm) followed by Kemer (7.60 mm) and lowest flesh thickness was observed from Halep Karası (6.29 mm). The highest percent reduction over control was recorded for Kemer 46.06% followed by Topan 40.90% and lowest reduction was recorded for Silindirik 18.11% (Figure 2E).

Quality Traits

Total Soluble Solid (Brix value)

TSWV significantly ($p \leq 0.05$) reduced the TSS content in infected eggplant cultivars compared to mock-controls. Among the infected plants highest mean value of brix was recorded for Silindirik (5.09%) followed by Halep Karasi (4.24%) and lowest was recorded for Kemer (3.06%) and Topan (3.10%) infected cultivars. Whereas among the mock-control plants highest mean value for brix was recorded for Silindirik (6.12%) followed by Aydın Siyahı (5.51%), Halep Karası (5.45%), Kemer (5.16%) and Topan (4.91%), which is statistically similar (Figure 2-F).

Fruit Color

Fruit color of eggplant was examined for five different cultivars on infected and mock-control (non-infected) plants. The infected cultivar Topan showed light fruit skin color (51.23) in comparison to dark fruit skin color (38.91) on mock-control plants. Same results were observed for Aydın Siyahı, which showed a significantly different ($p \leq 0.05$) light color (38.29) under infected condition as compared to its mock-control (36.00). Contrarily, infected Halep Karası exhibited dark fruit color (35.11) as compared to the light color (38.95) displayed under mock-control conditions. The eggplant cultivars Kemer and Silindirik did not show significant difference ($p > 0.05$) in fruit skin color under infected and mock-control conditions (Figure 2-G).

Fruit Firmness

Fruit firmness was significantly ($p \leq 0.05$) affected by TSWV. Among the infected cultivars the lowest fruit firmness mean value was recorded from infected plants Kemer (2.77) and Topan (3.24) followed by Aydın Siyahı and Halep Karası (3.85), (4.25). Whereas for the mock-control cultivars highest mean value for fruit firmness was recorded for Silindirik (5.32) followed by Halep Karası (4.60), Topan (4.58), Aydın Siyahı (4.56), which was statistically non-significant from each other. The highest percent reduction over control was recorded for Topan 29.25% followed by Kemer 28.97% and lowest was recorded for Silindirik 4.88% (Figure 2-H).

Dry Matter Contents

Results regarding dry matter content, showed non-significant ($p > 0.05$) difference among the cultivars with the infected and uninfected mock-control plants. The cultivar Kemer showed dry matter content of 10.05% in infected one which was also statistically similar to its control (10.24%). Similarly, for the cultivar Aydın Siyahı (10.52%), Silindirik (10.27%), Halep Karası (10.36%) and Topan (10.57%) dry matter content was statistically similar to their mock-control plants (Figure 3-I).

Yield (g plant^{-1})

Yield (g plant^{-1}) was significantly ($p \leq 0.05$) affected by TSWV. Among the infected cultivars the highest yield was recorded for Silindirik (655.4 g) followed by Kemer (124.2 g) and lowest was recorded for Topan (101.7 g). Whereas from mock-control plants the lowest yield (g plant-1) was recorded from Halep Karası (364.0 g) followed by Aydın Siyahı (541.4 g) and highest was recorded from Silindirik (1316.7 g). The highest percent reduction over control was recorded for Topan and Kemer 84.22%, 83.88 followed by Aydın Siyahı 80.32% and lowest percent reduction was recorded for Silindirik 50.22% (Figure 3-J).

Correlation

The correlation study among the yield and quality variables of eggplants (Table 2) showed positive correlations between fruit length and fruit number ($r=0.84^{*}$), fruit weight and fruit length ($r=0.90^{*}$), flesh thickness with fruit weight ($r=0.97^{**}$), whereas strongly positive correlation of fruit firmness to total soluble solids ($r=-0.98^{**}$). The fruit yield was strongly correlated with fruit number ($r=-0.96^{**}$), fruit length ($r=-0.90^{**}$), fruit weight ($r=-0.97^{**}$), and flesh thickness ($r=-0.98^{**}$) of eggplant tested in this study.

Principal Component Analysis

The interrelationship among selected eggplant cultivars along with the tested variables were analysed by biplot principal component analysis (PCA) as shown in Figure 3. It revealed that the biplot for yield and quality variables PC1 and PC2 explained 91.30% variance (contributed by PC1 68.89%, and PC2 22.41%) among the eggplant cultivars for the measured traits. PCA biplot grouped the eggplant cultivars based on their response to the observed yield and quality variables. The cultivar Silindirik showed positive PC1 values. The cultivar Silindirik showed better performance for fruit firmness, fruit weight, total soluble solids, fruit yield, fruit thickness, fruit length, and fruit numbers, whereas the cultivars Kemer and Topan were sensitive. The cultivar Halep Karası and Aydın Siyahı showed average response (Figure 4).

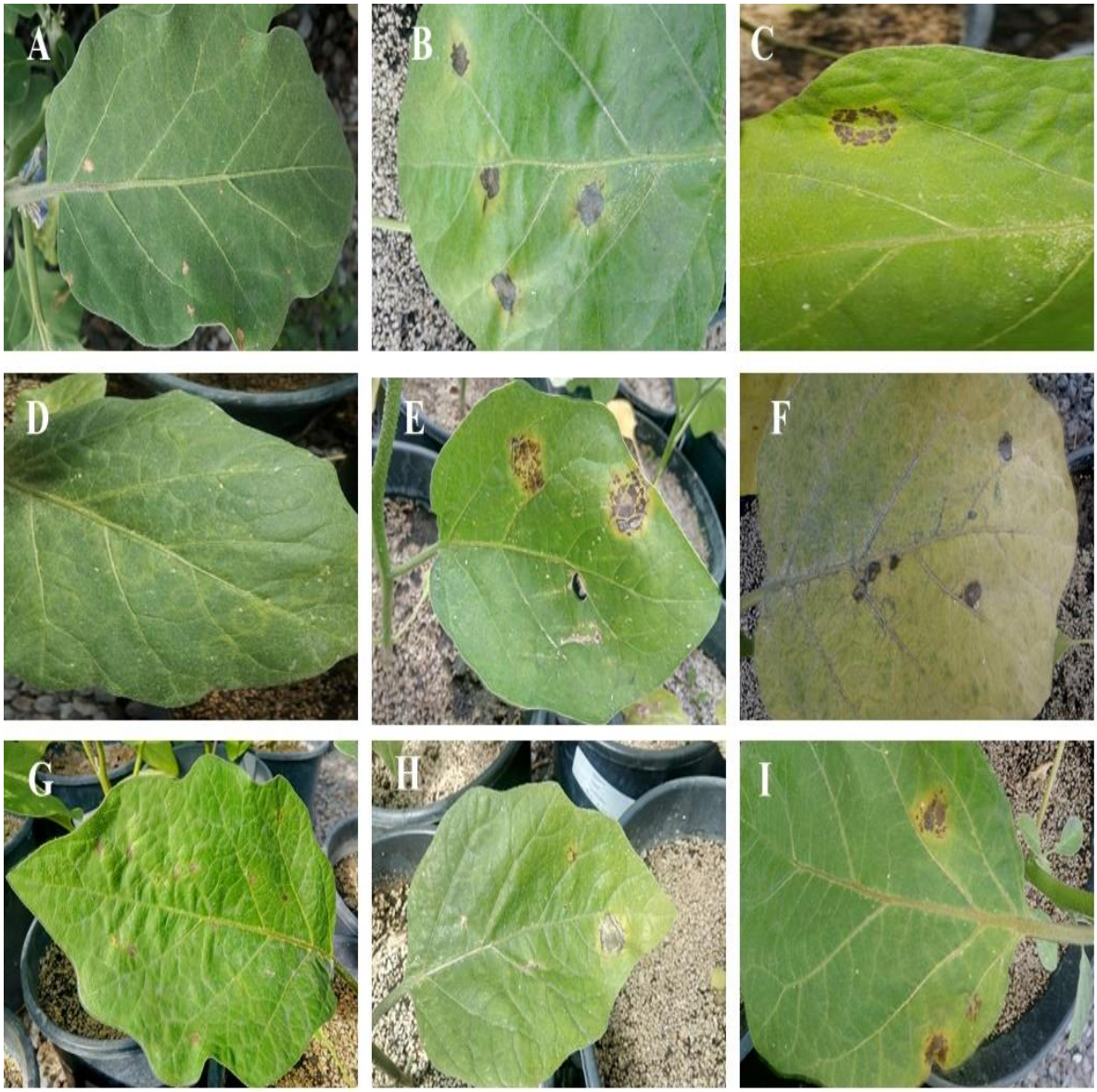
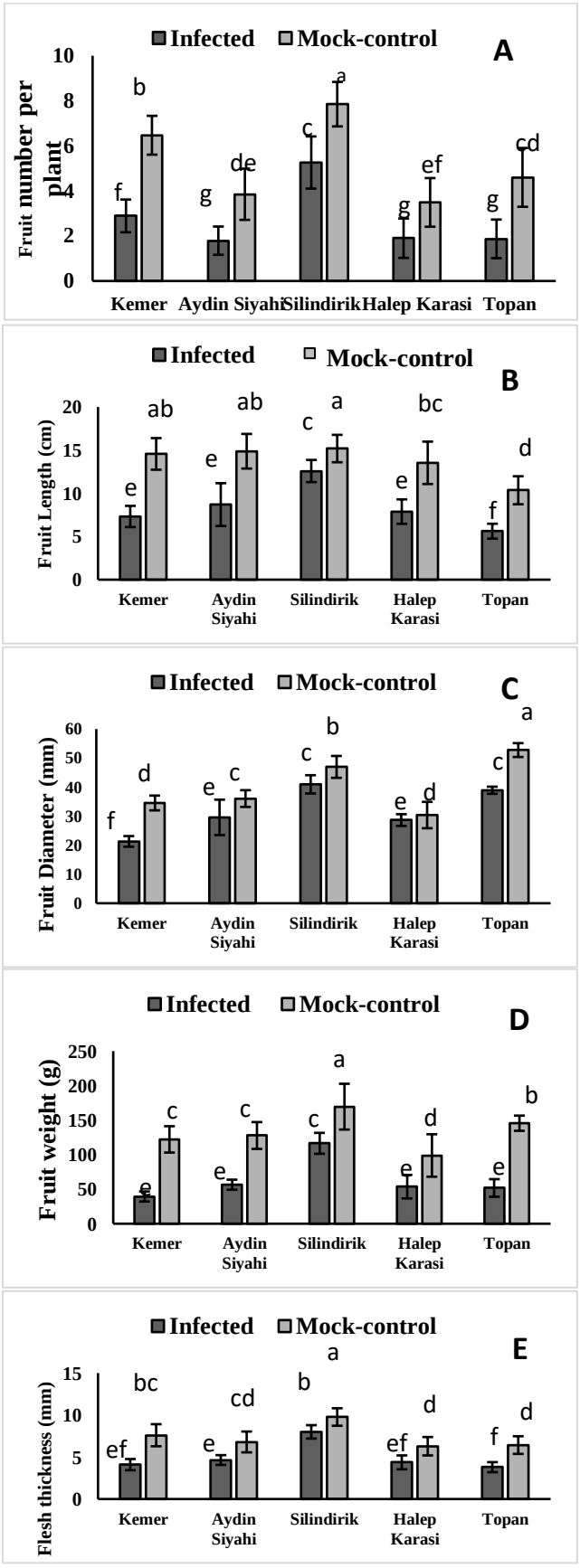


Figure 1. Tomato spotted wilt orthotospovirus (TSWV) symptoms on eggplant leaves, brown spots (A), concentric ringspot (B and G), chlorotic ringspot (D), necrosis (F) and necrotic ringspot (C, E, H, and I)


Infected
Mock-control
A
Fruit number per plant
Kemer
Aydin Siyahi
Silindirik
Halep Karasi
Topan
B
Fruit Length (cm)
C
Fruit Diameter (mm)
D
Fruit weight (g)
E
Flesh thickness (mm)

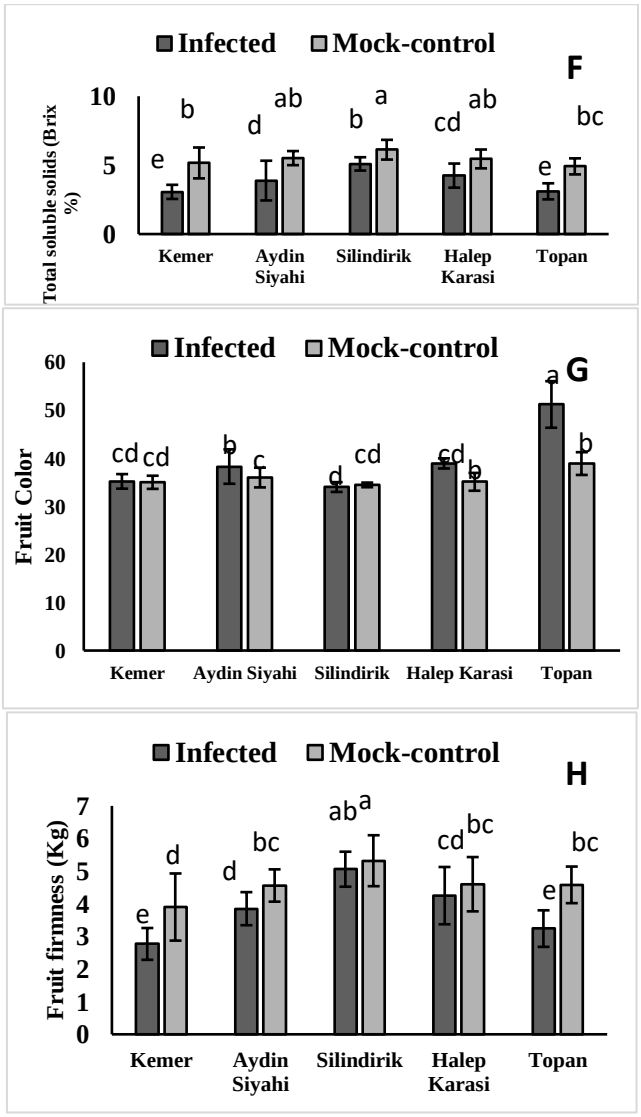


Figure 2. Fruit quality parameters of tested eggplant cultivars (both infected and mock-control plants). **A**: Number of fruits per plant, **B**: Fruit Length, **C:** Fruit Diameter. **D**: Fruit Weight, E: Fruit Thickness, **F:** Total Soluble Solids, **G**: Fruit Color, **H:** Fruit Firmness. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters show significant ($p \leq 0.05$) difference among cultivars.

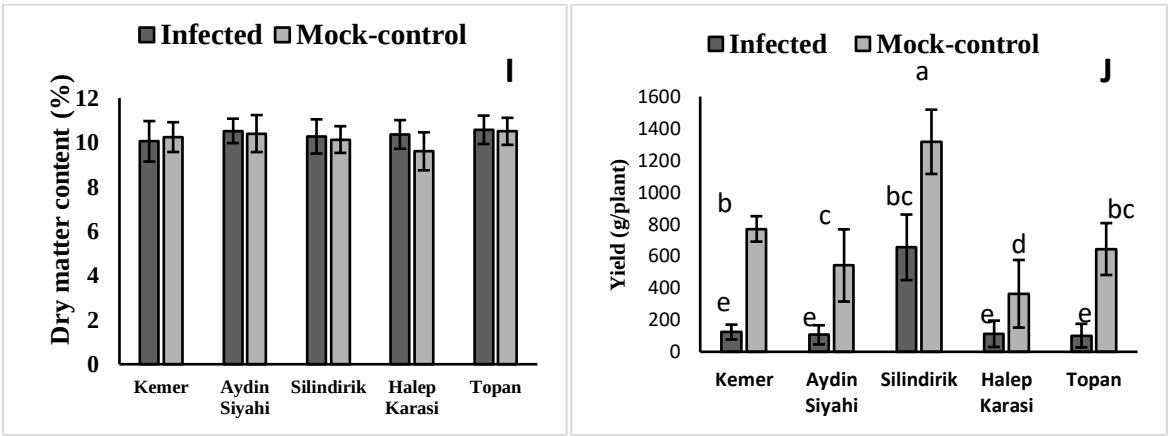


Figure 3. Fruit quality and yield parameters of tested eggplant cultivars (both infected and mock-control plants). **I:** Dry Matter Content, **J:** Yield. Bar shows mean value, vertical bar represents standard deviation, alphabets sharing same letter are statistically non-significant ($p \geq 0.05$) whereas different letters show significant ($p \leq 0.05$) difference among cultivars.

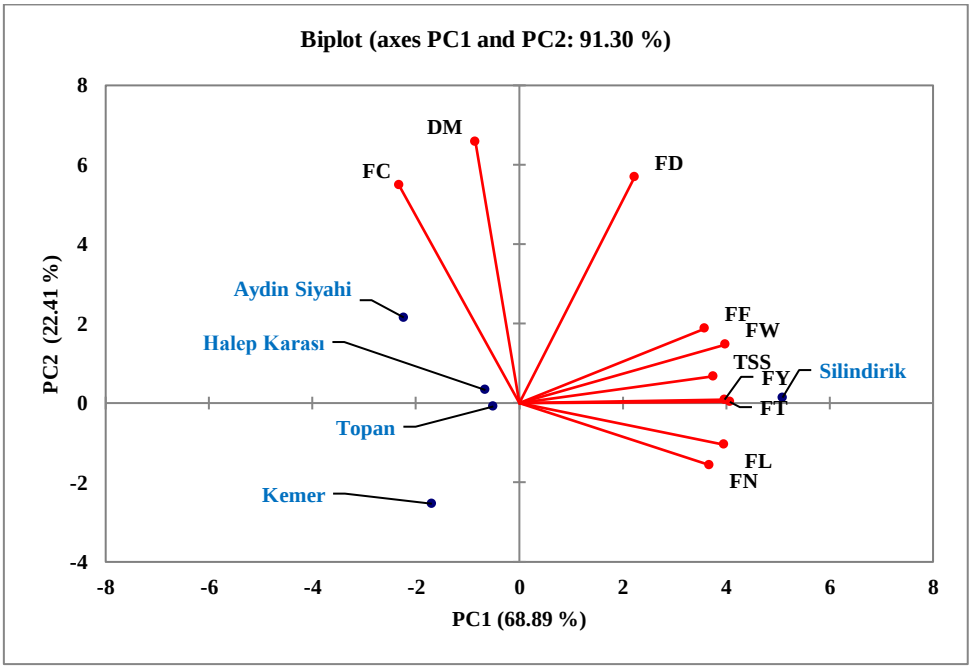


Figure 4. Principle component analysis (PCA) biplot for yield and quality variables of five eggplant cultivars infected by Tomato spotted wilt orthotospovirus (TSWV). PCA biplot is a combination of score plot of eggplant cultivars (represented in blue text) and loading plot of variables (represented by red vectors; black text). FD: fruit diameter, FF: fruit firmness, FW: fruit weight, TSS: total soluble solids, FY: fruit yield, FT: fruit thickness, FL: fruit length, FN: fruit numbers, DM: dry matter contents, FC: fruit color.

Table 1. Details of ELISA positive results and infection rate in eggplant cultivars.

Cultivars	Number of inoculated plants	Total number of tested samples	Number of TSWV infected samples	Infection rate
Kemer	25	17	10	58%
Aydın Siyahı	25	19	9	47%
Halep Karası	25	18	8	44%
Topan	25	19	10	52%
Silindirik	25	18	7	38%

Table 2. Pearson's correlation coefficients between the yield and quality traits of eggplant with the inoculation of Tomato spotted wilt orthotospovirus (TSWV)

	FN	FL	FD	FW	FT	TSS	FC	FF	DM	FY
FN	1	0.847*	0.421	0.866*	0.92*	0.659	-0.569	0.586	-0.493	0.961**
FL		1	0.369	0.904*	0.96**	0.905*	-0.719	0.840*	-0.268	0.903*
FD			1	0.718	0.56	0.476	0.376	0.587	0.532	0.604
FW				1	0.97**	0.871*	-0.374	0.866*	-0.035	0.970**
FT					1	0.873*	-0.550	0.833*	-0.209	0.983**
TSS						1	-0.536	0.985**	-0.026	0.799
FC							1	-0.384	0.717	-0.474
FF								1	0.131	0.762
DM									1	-0.259
FY										1

** Highly significant ($p<0.01$), *significant ($p<0.05$). FN, fruit number, FL, fruit length, FD, fruit diameter, FW, fruit weight, FT, flesh thickness, TSS, total soluble solids, FC, fruit color, FF, fruit firmness, DM, dry matter, FY, fruit yield.

Discussion

Current study showed characteristic symptoms of virus including ringspots and necrosis that started to appear 10 to 14 days after inoculation (Figure 1). Similar TSWV symptoms including ringspot, leaf yellowing and necrotic spots in tomato plants after 14 days of inoculation were reported earlier and that disease severity varied among the different cultivars (Ramkat et al., 2006). In eggplant, symptoms such as deformed leaves, necrosis of leaves, molted and stunted plants with the inoculation of TSWV have been observed (Kamberoglu et al., 2009). The infection rate of TSWV in this study was highest in cultivar Kemer (58%) followed by Topan (52%), whereas lowest infection rate was observed in Silindirik (38%), which performed best performance against TSWV compared to all tested cultivars (Table 1). Similar infection level ranging from 41% to 68% with similar symptoms was reported in different cultivars of tomato (Farooq and Akanda, 2007). The fruit number was significantly affected by TSWV in infected eggplants compared to mock-controls (Figure 2-A). The reduction percentage in number of fruits per plant ranged from 32.9 to 59.3% in all eggplant cultivars. Our results regarding reduction in fruit number were consistent with the findings that TSWV in tomato crop also had a significant effect on reduction of fruit number ranging from 50 to 72% (Farooq and Akanda, 2007). In another study the similar results in fruit number was reported by TSWV with a reduction of 20.18% in fruit numbers in pepper plant (Sevik and Sokmen, 2012). Furthermore, the results of this study were consistent with an earlier report of 90% decrease in fruit number of tomato crop by TSWV (Ramkat et al., 2006). The fruit length of mock-control plants was significantly higher than the infected plants (Figure 2-B). The reduction percentage of fruit length was 17.1 to 49.7% in all the eggplant cultivars. Our results are in accordance with the findings in which it was reported that impact of TSWV on reduction of fruit length was from 11 to 68% (Farooq and Akanda, 2007). Similar decrease in fruit length of tomato crop by 11.9% with the inoculation of TSWV was also reported by (Sevik and Sokmen, 2016). These studies showed that the infection of TSWV could reduce the fruit length.

In the current study the fruit diameter was also significantly affected by TSWV infection causing a reduction of 12.8 to 38.3% (Figure 2-C), which is in accordance with the findings of a reduction of 10.9% fruit diameter in tomato plant (Sevik and Sokmen, 2012). Fruit weight was significantly affected by TSWV resulting in reduction of 31.3 to 67.7% (Figure 2-D). The highest fruit weight of 169.4 g was measured from Silindirik mock-control plants whereas, Kemer infected by TSWV resulted in lowest fruit weight 39.4 g. The response of cultivars was different regarding fresh weight reduction against TSWV that might be due to their tolerance level. Viral diseases reduce yield, TSWV disease is being one of them (Ramkat et al., 2006). Significant decrease in fruit weight was reported ranging from 27% to 60% depending on the response of varieties to TSWV infection. In another study, it was revealed that TSWV affected the fresh fruit weight as well as plant weight (Díaz-Pérez et al., 2007). Kim et al. (2004) reported that TSWV caused weight and quality loss in pepper.

TSWV had a significant effect on fruit flesh thickness in the current study causing a reduction of 18.1 to 46.0% (Figure 2-E). The reduction rate varies among the cultivars due to several factors. For example, fruit size and shape also could be a reason for difference in flesh thickness. Several studies reported that TSWV had effect on fruit size, length and diameter; it might well be the decisive factor for reduction in flesh thickness. Based on the results, it was found that the infection of TSWV had reduced the total soluble solid (Brix) of fruit. The reduction rate was in the range of 16.8 to 40.6 percent among the cultivars (Figure 2-F). It might be due to reduction in water supply, decreased photosynthetic rate or poor assimilation of nutrient contents in infected plants. Our results are also similar to the results of a study that reported the decrease of soluble solids content in pepino by *Tomato mosaic virus* (Perez-Benlloch et al., 2001).

The results of current study indicated that TSWV influenced fruit color of eggplant as well. The changes in fruit color could also be due to different cultivars tested in the study. Nevertheless, in severely infected cultivars, the difference in fruit color was significantly different from their mock-control plants (Figure 2-G). The fruits from the infected plants indicated lighter color compared to healthy fruits. Similar results were obtained for color response in tomato against TSWV (Farooq and Akanda, 2007). Our results are also comparable to the study of that reported for the color variation of fruits due to TSWV infection (Swift, 2006). Fruit firmness traits attributes describes fruit texture and are vital in determining final fruit quality. In the current study, TSWV has influence on fruit firmness in a highly significant way (Figure 2-H). The firmness of vegetables is affected by different traits such as biochemical constituents, cellular organelles, cell wall composition and water content or turgor. Thus, TSWV affecting any of these traits could change the fruit firmness and could lead to changes in fruit quality. Examination of the dry matter content of eggplant fruits in the infected and mock-control plants demonstrated that the differences between the cultivars were not statistically significant (Figure 3-I). Dry matter content could be influenced by different parameters. However, it is reported that *Tobacco mosaic virus* infection has no effect on dry matter content (Elegba et al., 2013).

Eggplant yield is mostly associated with healthy vegetative growth of plants throughout the growing season. Results of this study revealed that TSWV damaged the plant growth which resulted in lower yield traits. Results indicated that the eggplant cultivars had different tolerance level as percent reduction in yield varies among the cultivars. Most

infected cultivar was Topan with highest percent fruit yield reduction of 84.2% while, the lowest infected one was Silindirik with 50.2% fruit yield reduction (Figure 3-J). These results were in agreement with the study of Ramkat et al., 2006 that reported the fruit yield loss of 37-90% due to TSWV in different tomato varieties. Farooq et al., 2017 found that under different tolerant conditions, TSWV resulted in 44.1- 55.6% reduction in fruit yield. Similarly, researchers determined that prior to harvesting TSWV reduced the crop yield by 2.1% to 2.3% for each day (Pérez-Benlloch et al., 2001). Previous reports showed that viruses caused different diseases in *Solanaceae* family, among them the effect of TSWV on yield varied with variety and stage of inoculation. Rapando et al., (2009) also found that TSWV disease caused 57% and 32% crop yield reduction in two different cultivars of tomato. Most of the fruits formed on the infected plants by TSWV exhibited abnormal coloration and the marketable yield of tomatoes was drastically reduced due to the abnormal ripening (Moriones et al., 1998).

Conclusion

Current study was conducted to evaluate the performance of five common eggplant cultivars with the infection of TSWV. Our results revealed a differential response of eggplant cultivars. It was concluded that cultivar Kemer showed the highest infection rate followed by the cultivar Topan and Aydın Siyahı. Infection of TSWV significantly reduced the yield and quality traits of eggplant such as fruit number, fruit length, fruit diameter, fruit weight, flesh thickness, total soluble solid, fruit color, fruit firmness and yield in infected plants as compared to their respective mock-control plants. The results showed that among the five cultivars, the performance of Kemer and Topan was poor, which suggested that these cultivars are highly sensitive to TSWV. Whereas, the performance Silindirik was good, this suggested that this cultivar might be tolerant to TSWV. Information presented here illustrated that TSWV had devastating effects on quality and yield parameters of eggplants. Therefore, strategies to prevent TSWV infection and control measures to avoid crop losses should be implemented. This study will help plant breeders to understand potential effects of TSWV on eggplant cultivars. Results of the study may also help breeders with the development of sustainable TSWV resistant varieties by using these tolerant cultivars to cope with TSWV infections in the field.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Data availability

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Consent for publication

Not applicable.

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Efficacy of S-metolachlor and flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) as pre-emergence herbicides in controlling weeds in maize at Chisumbanje Estate

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Abstract

Field trials were conducted during the 2019-2020 cropping season to assess the efficacy of S-metolachlor and flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) pre-emergence herbicides in controlling weeds in maize at Chisumbanje estate. The experiment was laid out as a randomised complete block design (RCBD) with three treatments and replicated thrice. Treatments used include hand weeding (control), S-metolachlor and flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) herbicide. The data collected was based on weed density, plant height, maize grain yield indicating significant differences ($p<0.05$) amongst the treatments. Flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) scored the least weed density per m^{-2} as compared to S-metolachlor showing that it is an effective pre-emergence herbicide (33, 27 and 22) on Mexican marigold (*Tagetes minuta*), shamva (*Rottboellia cochinchinensis*) grass and wild jute (*Corchorous tridens)* respectively. The control (hand weeding) scored the highest weed density per m^{-2}, indicating that the method was not effective as compared to S-metolachlor and flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) treatments. Flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) scored the highest yield of 10 tonnes/hectare whilst S-metolachlor and control scored 7.6 and 5.6t/ha respectively. Herbicides reduced the weed spectrum in maize resulting in realisation of higher yield in flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) experiment followed by S-metolachlor. Farmers are recommended to use flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) as a pre-emergence herbicide in controlling weeds in maize so as to realise higher yields and low weed density.

Keywords: Flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide), S-metolachlor, pre-emergence, herbicide

Introduction

Maize is ranked first in Zimbabwe and is the staple food not only for Zimbabwe but for many countries in Southern Africa (Tapiwa *et al*., 2020). Maize production has been declining in recent years due to drought and weed competition in some areas. Weeds continuously interfere with the normal growth of crops (Patel, 2013; Sakadzo *et al*., 2018). The knowledge in weed management is essential if enough food is to be produced at minimum costs to both the farmers and the consumer (Laizer *et al*., 2019).

Hand weeding is the predominant weed control practice in Sub Saharan Africa and about 50%-70%

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of the labour in crop production is spent on weeding (Chivinge, 1991; Mashingaidze, 2004). Maize requires 276 hours per hectare of hand weeding to get optimal yields. To weed one hectare a man or woman walks 10 kilometres in a stooped position. Maize is a widely spaced crop which gets infestated, resulting in reduced yield which varies from 18%-85%, depending on the type of weed flora, density and function of crop weed competition (Sunitha *et al*., 2012).

Integrated weed control was introduced around 1980s, despite all of these developments, the weeds are still one of the farmer's biggest problems (Saiz-Rubio *et al*., 2020). Mortensen *et al*. (2012) and Storkey *et al*. (2021) herbicides must be used judiciously in an integrated weed management framework because of the high cost and environmental concerns, Timeliness of weeding are often a problem among farmers hence the need to consider herbicide technology (Steckel *et al*., 2019). S-metolachlor (chloro-acetanilide) pre-emergence interferes with ezymes thereby inhibiting cell division and elongation (Lowry et al., 2013). Flumetsulam (triazolopyrimidine sulfonanilide inhibit acetolactate synthase which catalyses biosynthesis of amino acids. It has been used in controlling weeds in tobacco and has proven to be effective (Mazarura, 2013). Therefore the objectives were to assess the efficacy of S-metolachlor and flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) pre-emergence herbicides in maize production.

Materials and Methods

Study site

The trial was done at Chisumbanje small scale farmers' Section in Chipinge rural during the 2019/2020 cropping season. Experimental site is located within the following geographical coordinates with latitude 20º 48º 0˝ South and 32º 14º 0˝ East. It is along the Tanganda-Chiredzi highway, on the eastern bank of the save river, about 95 km south of Birchenough Bridge. It is a semi-arid area which lies in the Save valley receiving rainfall < 450 mm and is in natural region V (Mugandani *et al*., 2012). The precipitation is correspondingly lowand irrigation is essential throughout the year to supplement rain water (Nyagumbo *et al*., 2019; Tapiwa *et al*., 2020). The area is characterised with heavy clay soils in the classification of vertices which have good agronomic potential. Temperature ranges from 18-30 ºC with temperature above 40^0 C in the hottest months of October and November (Mugandani *et al*., 2012). The vegetation is dominated by Marula tree (*Sclerocarya birrea* (Caffra) and Mopane (*Colophospermum mopane*) with other tree species such as Baobab (*Adansonia digitata*), Acacia (*Acacia karroo)* and Mnondo (*Julbernadia globiflora*). Evaporation rates are high and can exceed 13mm/day.

Experimental design and treatments

The experiment was laid out as a randomised complete block design (RCBD) and replicated thrice. Weed control methods used includes hand hoeing (control) and use of herbicides. Two herbicides were used which include S-metolachlor (Dual magnum) and flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) (Bateleur gold) with application rates of 1 litre and 1.2 litres per hectare respectively (Table 1) as recommended by Syngenta Crop Protection (2020). This usually depends on clay content of the soil. The maize variety used was SC649 a hybrid maize medium variety. It was offered freely by the Seedco agronomist for use in demonstration plots so as to enhance their marketing. Field history indicated that the land has been under sugarcane for 5 years and was fallowed for a year. Predominant weed species included yellow nutsedge (*Cyperus esculentus*), Purple nutsedge (*Cyperus rotundus*) due to persistent flooding by irrigation water. Shamva grass, Wild jute, shamva and Mexican marigold were also prevalent. Common weed management practised was chemical greatly dominated by use of sulfonyurea herbicides. The plots were ploughed using an ox drawn plough during early November 2019. Maize was planted on plots measuring 7 m by 4.5 m where the treatments were randomly allocated. Row spacing of 0.9 m between rows and 0.3 m within row was used to achieve a plant population of 37037 plants/ha. This spacing was selected because it lies within the recommended row spacing for maize considered for household consumption.

Table 1. Herbicide Rates (l/ha)

Treatments	Herbicide rates (l/ha)
Control (hand hoeing)	Nil
S-metolachlor	1.0
Flumetsulam (triazolopyrimidine sulfonanilide) +S-metolachlor (chloro-acetanilide)	1.2

Plot management

This was done following procedures by Sakadzo *et al*. (2018). An application rate of 1.2 litres per hectare of flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) was sprayed to 100% or full cover and 1.0 litre per hectare of S-metolachlor was sprayed also to fully cover. Herbicides were applied using a hand operated knapsack sprayer with a flood jet nozzle. The Beaufort scale guide was used to assess the wind speed (Table 2). It is best to spray when there is a steady light breeze blowing (Force 2). Hand hoeing was done three weeks after planting and all weeds were removed from the field. This was also repeated when maize attained six weeks after planting. Ammonium nitrate (AN) was applied as a top dressing fertiliser three weeks after emergence at a

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rate of 350 kg AN/ha as the soils were depleted in nutrients due to sugarcane monocrop.

Table 2. Beaufort Scale

Beaufort scale	Description	Visible signs	Approx. air speed
Force 1	light air	the direction shown by smoke drift	up to 1.6 km/hr
Force 2	light breeze	leaves rustle wind felt on face	3.2 to 6.4 km/hr
Force 3	gentle breeze	leaves and small twigs in constant motion	6.4 to 9.7 km/hr
Force 4	moderate breeze	Small branches moved. Raises dust and loose paper	

Source: Nyanhete, 2004

Table 3. Modified European Weed Research Council Ratings

Category number	% kill of weed	Herbicide effectiveness on weeds
1	100	Complete kill
2	97.5 - 99.9	Excellent
3	95.0 – 97.5	Good
4	90.0 – 95.0	Adequate
5	85.0 – 90.0	Just adequate
6	75.0 – 80.0	Poor
7	65.0 – 70.0	Very poor
8	35.0 – 65.0	Useless
9	0 – 33.0	Almost no effect

Source: (WSSA, 2002)

Data collection

The weed density was determined by physically counting weeds in the quadrant ($1m^2$) and scoring on the European Weed Research Council scale (EWRC) (Table 3). Herbicide effectiveness was determined by scoring the level of phytotoxicity and recording percentage of killed weed species following the procedure by Sakadzo *et al.* (2018). Two weeks after application of the pre-emergence herbicides, weeds which were emerging were physically counted and recorded through the aid of a quadrat with an area of $1m^2$. Plant height of maize was determined from week one up to week 12 by using a metre rule from five maize plants randomly selected from each plot. Mean plant height was determined for each plot. Randomly selected plants were marked so that they were measured throughout the experiment. Maize was harvested using a Dickey, John moisture metre. Maize was harvested from a net plot measuring 4.5 m by 3.0 m. Yield was determined at harvesting by using a scale adjusted to 12.5% moisture and converted to tonnes per hectare (Sakadzo *et al.*, 2018).

Data analysis

Data analysis was done using the Genstat 14th version edition. Mean separation was done using Fischer's least significant difference (LSD) at the 5 % significance level.

Results

Effects of S-metolachlor and flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) herbicides on weed emergence density in maize crop

Herbicide effectiveness was determined by the EWRC scale of scoring phytotoxicity (Table 4). Results indicated that there were significant differences ($p<0.05$) across all the treatments on weed density. Control (hand hoeing) treatment scored the highest weed density of Mexican marigold (86.67 plants per m^{-2}), shamva (113.00 plants per m^{-2}) and wild jute (66.00 plants per m^{-2}) whilst flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) treatment had the least weed density of Mexican marigold (33.67 plants per m^{-2}), shamva (27.33 plants per m^{-2}) and wild jute (22.67 plants per m^{-2}) respectively as shown in Table 4 below.

Effects of S-metolachlor and flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) herbicides on plant height of maize

Results show that there were significant differences ($p<0.001$) across all the treatments in terms of plant height (Figure 1). Flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) treatment recorded the highest plant height as from week one up to week twelve, whilst control (hand hoeing) treatment had the least plant height (Fig 1).

Effects of S-metolachlor and flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) herbicides on final yield of maize

High maize yield was obtained from plots applied flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) with a high of 10 t/ha followed by plots applied S-metolachlor produced 7 t/ha and lastly the control treatments which had 5.7 t/ha (Figure 2). Results show that there was a significant ($p< 0.05$) difference on the effects of different weed control methods. Results also show that the use of flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) was better than the use of S-metolachlor and hand hoeing.

Table 4. Weed Density (plants/m^2) in Maize after Spraying Herbicides

	Weed density (plants/m^2)		
Treatments	**Mexican marigold**	**Shamva**	**Wild jute**
Flumetsulam(triazolopyrimidine sulfonanilide) +S-metolachlor (chloro-acetanilide)	33.67a	27.33a	22.67a
S-metolachlor	47.33b	95.67b	34.00a
Control (Hoeing)	86.67c	113.00c	66.00b
Grand mean	55.9	78.7	40.9
p-value	<.001	<.001	0.007
LSD	10.58	7.91	19.18
CV%	8.3	4.4	20.7

*Means followed by the same letters are not significantly different at 5% significance level.

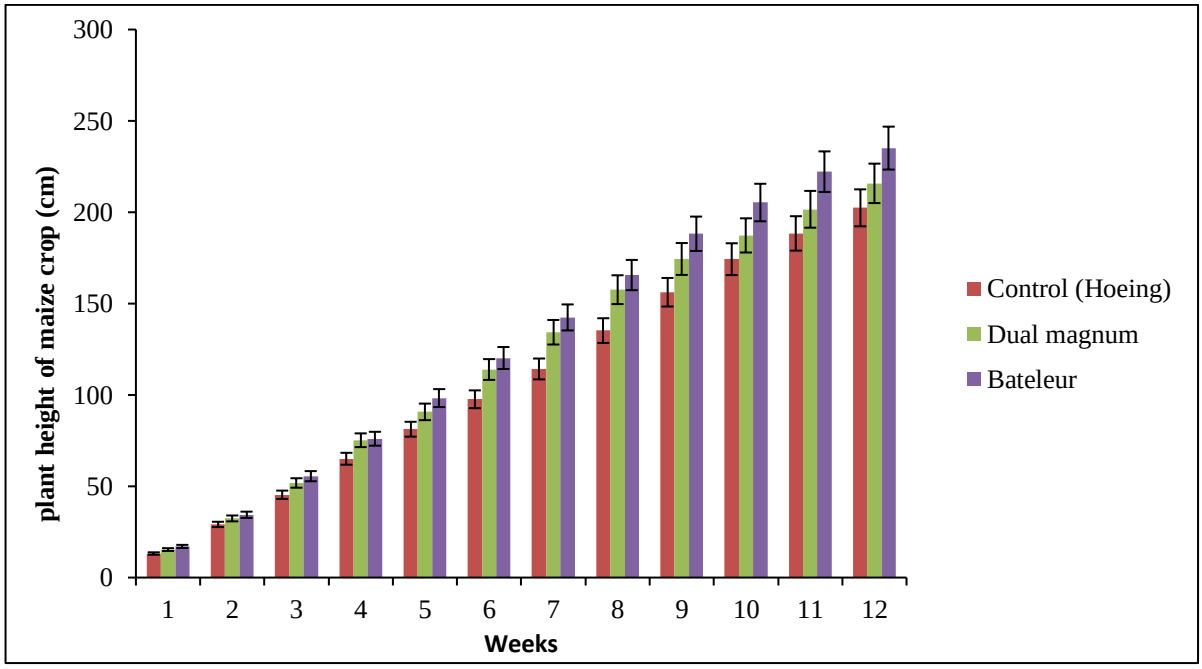

Figure 1. Plant height of maize from week one up to week 12.

Discussion

Effects of herbicides on weed density

Flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) and S-metolachlor had the least weed density as compared to the control. This proved that herbicides are effective in reducing the weed spectrum in maize fields. This is in agreement with results by Mazarura (2013) who reported that flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) was effective in controlling weeds on flue cured tobacco. On the second assessment done 14 days after the application of the pre-emergence herbicide, the flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) treatment scored a 98% on the EWRC scale in terms of weed density and S-metolachlor treatment scored 90%. Results also concurs with findings by Shinggu *et al*. (2009) who reported that more weed types and grasses were observed in the control treatments (hand weeding) than in the herbicide treated plots. Results from this study were also in agreement with findings by Rana *et al*. (2016) who reported minimum weed densities on herbicide treatments than in hand weeding treatment. Results might vary in relation to predominant weed species in the field as indicated in the experiment under study where shamva was prevalent.

Effects of S-metolachlor and Flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) herbicides on plant height of maize

Results showed that use of S-metolachlor and Flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) herbicides increased plant height of maize. This variation might have been as a result of elimination of resource competitors (weeds). Plant height reflects the efficiency of the plant for photosynthetic radiation interception and

vegetative growth character of crop plants in response of various applied inputs like fertilizer and herbicides. There were significant differences in plant height amongst the weed control treatments. The difference in height could be the varying effects of weed competition, duration of available resources offered by different weed densities in different weed control practices. These results are in line with those of Simic *et al*. (2020) who observed that plant height was significantly higher in the herbicide treatment plots.

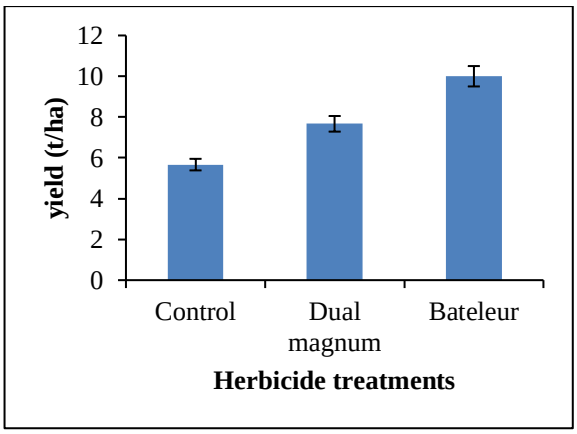

Figure 2. Effects of herbicides on yield of maize.

Effects of S-metolachlor and Flumetsulam (triazolopyrimidine sulfonanilide)+S-metolachlor (chloro-acetanilide) herbicides on maize yield (tonnes/hectare)

Results indicated that the use of herbicides significantly increased yield in maize. This might be an attribute of mode of action of herbicides on prevailing weeds. These findings are in line with those of Naveen *et al*. (2019). Competition for resources were reduced herbicide weed applied plots as compared to hand weeding. Results are also in agreement to those of Sakadzo *et al*. (2018) who concluded that grain yield of maize crop was increased with the use of herbicides. Herbicide application quickly suppresses the weed germination and ultimately provides a competitive free environment for the crop plant to get all the available resources alone. Hassan *et al*. (2010) also reported that herbicides are the most efficient and effective in controlling weeds in *Zea mays* and also increase grain yield, crop growth and canopy development.

Conclusion

Flumetsulam (triazolopyrimidine sulfonanilide)+ S-metolachlor (chloro-acetanilide) and S-metolachlor proved to be effective in reducing weed density of prevalent weeds (Mexican marigold, shamva and wild jute) as compared to manual weeding. Herbicides reduced the weed spectrum in maize resulting in realisation of higher yield in flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) experiment followed by S-metolachlor.

Recommendations

The researcher recommends farmers to use flumetsulam (triazolopyrimidine sulfonanilide) + S-metolachlor (chloro-acetanilide) and S-metolachlor in order to reduce weed density in maize, realise higher yield and less costs on weed control in maize fields. There is need to repeat the same research across a multiple environment to determine the effects of environment on mode of action of herbicides.

Areas for future research

Since the use of herbicides was not popular in most rural areas in Zimbabwe, there is need to evaluate the effectiveness of herbicides in controlling weeds and their effects on grain yield. There is need to evaluate this in various regions with different soil types because some herbicides persist in soils. There is also need to look on the effects of integrated nutrient management, rainwater harvesting and herbicides on maize productivity to see how herbicides affect nutrient uptake by crops and yields.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the aurhors to the present study is equal. All the authors verify that the text, figures, and tables are original. The authors read and approved the final manuscript.

Ethical approval

Not applicable

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Data availability

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Consent for publication

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Effects of the bio-fertilizers on potato mini tubers number and size produced from tissue culture plants

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Abstract

The present study aimed to increase mini tuber yield production of potato *in vitro* plants by decreasing mineral nutrients to 50% and applying biofertilizers micro-algae, bacteria, vermicompost, mycorrhizae and their combinations. The experiment was carried in controlled conditions in a growth chamber in pots with a capacity of 2L. The substrate was mixed soil with cocopeat (v/v). The evaluation of growth parameters and macro and micro elements was carried 30, 60, and 90 days after planting. Harvest was carried 120 days after planting and tuber numbers, size, and diameters were evaluated. The higher number of mini tubers obtained with 8.8, 8.2, and 7.6 per plant in control, algae, and the mixture of 4 biofertilizers, respectively. The higher tubers' diameter and weight values were 20.11 mm, 6.70 g, 18.65 mm, and 5.3 g in the plants treated with bacteria and vermicompost. For mini tuber seeds production, the number is important, yet the size and weight are the essential parameters to obtain high tuber yield. Thus, it is recommended that the seeds producers apply vermicompost and bacteria in their fertilizer's solution.

Keywords: In vitro plants, *Solanum tuberosum L*, tuber-size, tuber-yield

Introduction

Potato (*Solanum tuberosum L.*) is a versatile vegetable with almost 368 million tons overall worldwide production. More than 1 billion people worldwide consume potatoes; its prominence in agriculture follows cereals like rice, wheat, and maize (FAO, 2019). It belongs to the Solanaceae family comprising 26 genera and 2800 different species. Most potatoes' species have been native to the Andean highlands of South America, which produce underground stems in the form of a tuber (Fetena and Eshetu, 2016). It is considered one of the most economical crops due to its high yield and returns, but this yield is still insufficient to cover all the world's needs. The yield and quality of potato tubers are affected by many distinct factors such as genetics (cultivar properties), soil fertility, weather conditions, and chemical treatments (Torabian et al., 2021).

The potato is a highly heterozygous, tetraploid, and semi-perishable vegetable propagated by the tuber crop. It is also susceptible to many diseases and pests. The genetic nature, mode of spread, and vulnerability to disease/pests impose several inherent limitations in producing disease-free seeds. The production of pre-basic seeds is supported by tissue culture and generally requires in vitro culture techniques to rapidly multiply disease-free clonal plants and grow them under sanitary conditions (Tierno et al., 2013).

Plant tissue culture or micropropagation is a technique of maintaining in vitro parts of plants, cells, tissues, or organs on specified nutrient media under aseptic and controlled environmental conditions. It is based on the phenomenon of totipotence (Fazal Rehman et al., 2019). This technique has been used effectively to produce disease-free seed potatoes.

Current mini-tuber production only satisfies 1–2% of the production seed world; therefore, it is essential to improve the productivity and potential of this crop (Fazal Rehman et al., 2019).In Turkey, potato is one of the main crops with an average annual production of around 4.5 million tons (Çalışkan et al., 2010). Turkish potato production still depends on imported seeds, despite the efforts made in recent years to improve the output of mini tubers in terms of quantity and quality. Therefore, efficient

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mini-tuber production systems are needed to promote these efforts (Çalışkan et al., 2020).

Biofertilizers are preparations containing living or dormant cells, which benefit crop growth-promoting functions by producing phytohormones, macro, and micro-nutrient, improving soil's physical, chemical, and biological properties; thus, improve yield production (Kumar and Verma, 2018).

Generally, biofertilizers such as vermicompost, bacteria, algae, and mycorrhizae play a significant role in decomposing organic matter, which helps mineralize within the soil and increasing the availability of nutrients for crop yield (Rodríguez et al., 2006).

In recent research, biofertilizers have become essential practices for sustainable agricultural production and yield improvement through synthesizing phytohormones, metabolizing them, and acting on hormone biosynthesis in plants that affect plant growth, also by producing substances that work against soil-borne pathogens (Anelise Beneduzi, 2012).

Biofertilizers can be expected to promote the mini-tuber formation, increasing the number and size of mini tubers by some hormones, enzymes, and nutrients. Bio-fertilizers have biostimulant effects. Biofertilizers can increase the quantity and biodiversity of beneficial bacteria, such as plant growth-promoting rhizobacteria belonging to *Azotobacter*, *Bacillus*, *Burkholderia*, *Pantoea*, *Pseudomonas*, *Serratia*, and *Streptomyces* (Guo et al., 2019).

There is a beneficial interaction between biofertilizers and micro-organisms in the soil (Bulgarelli et al., 2015) that has advantageous effects on plants through direct or indirect pathways. Modern agriculture uses high amounts of chemical fertilizers to obtain actual product yields with improved cultivation efficiency. However, the excessive use of chemical fertilizers frequently causes severe environmental damage, such as water, soil, and atmosphere pollution (Savci, 2012). Furthermore, this excessive use leads to soil acidification and hardening, reducing the roots' respiration and vigour. This practice's population of beneficial micro-organisms is also decreased, resulting in a loss of soil fertility and a high incidence of root diseases (Chanda et al., 2019). Mahanty et al. (2017) recommended the application of biofertilizers in horticulture as an alternative to avoid the problems created by chemical fertilizers.

The objective of the present study, the effect of some biofertilizers; bacteria, micro-algae, vermicompost, and mycorrhiza on plant growth, plant nutrition and mini tuber yield production of in vitro plant Agria potato cultivar.

Materials and Methods

Plant material and experimental conditions

The present study was carried in the growth chamber at the University of Cukurova, Agriculture Faculty, Department of Horticulture in Adana/Turkey from May to August 2020. Potato cultivar Agria was used as plant material. The *in-vitro* plants were selected for the homogeneity of the plant height with approximately 12 cm height and transplanted in pots of 2 liters into a soil media (a cocopeat/soil mixture at 1:1 v/v). They were kept in controlled conditions for light at 16 h/8 h (day/night) and temperature at 24/16 °C (day/night).

The chemical and physical specifications of the experimental soil are given in Table 1.

Table 1. Chemical and physical characteristics of the experimental soil

Soil characteristics	Results
PH	7.70
EC (ds/m)	0.21
$CaCO_3$ (%)	20.33
Organic matter (%)	1.23
Phosphor (P_2O_5) (kg/da)	13.83
Potasium (K_2O) (kg/da)	72.52
Available Ca (mg/kg)	8132
Salinity (%)	0.01
Texture	Clay Loam
Available Zn (mg/kg)	0.603
Available Fe (mg/kg)	5.121
Available Mn (mg/kg)	2.124
Available Cu (mg/kg)	0.254
Water saturation (%)	59.40

Experimental design and management

The experiments were conducted under a Completely Randomized Design with 12 treatments, each with five replications. Each replication of treatment consisted of 5 pots and one plant in each pot. Bio-fertilizer treatments started after two weeks following plantation. Treatments details are given in Table 2.

Mineral nutrient solution composition and bio-fertilizers

The composition of the nutrient solution used for control treatment (mg L^{-1}) was as follows (Aydoner Coban et al., 2020) : Nitrogen (N) = 160, phosphorus (P) = 30, potassium (K) = 220, calcium (Ca) = 140, magnesium (Mg) = 40, iron (Fe) =2.5, manganese (Mn) = 0.25, zinc (Zn) = 0.25, boron (B) = 0.20, copper (Cu) = 0.02, and molybdenum (Mo) = 0.04. The nutrient solution concentrations used for control was decreased by 50% for all the nutrients and used for the bio-fertilizers. Mycorrhiza bio-fertilizer under the trade name "Endo Roots Soluble" (ERS) was used in the experiment. There were nine different mycorrhiza species as cocktail preparation: *Glomus intraradices, Glomus aggregatum, Glomus mosseae, Glomus clarum, Glomus monosporus, Glomus deserticola, Glomus brasilianum, Glomus etunicatum, Gigaspora margarita.* The commercial name of the Rhizofill was liquid bacteria bio-fertilizer used in the experiment. The bacteria

fertilizer contained three different bacteria species as *Basillus subtilis* ($1x10^9$), *Bacillus megaterium* ($1x10^9$) and *Pseudomonas fluorescens* ($1x10^9$). In vitro plants were inoculated with 1000 mycorrhizae spores during transplanting, and bacteria were applied every 7 days into the root by irrigation with 1ml Rhizofill in a 1 L nutrient solution. The commercial name of the Ekosolfarm was liquid vermicompost bio-fertilizer used in the experiment. The vermicompost composition were total organic matter 10%, total nitrogen 2%, organic nitrogen 2%, water-soluble phosphor pentaoxide (K_2O) 0.2%, free amino acids 10%. The vermicompost was applied every 7 days into the root by irrigation with 3 ml vermicompost in 1 L 50% nutrient solution. Eukaryotic green micro-algae *Chlorella vulgaris* produced in the Cukurova University Fishery Department has used $2x10^6$ microalgae in 1ml. This concentration was diluted 40 times with 50% nutrient solution (25 ml in 1L) during irrigation every 7 days.

Table 2. Treatments.

Treatment	Composition
C	Control :100% mineral nutrient solution
Myco	0.2 g Mycorrhizae for each plant
Bac	50% mineral nutrient + 50% Bacteria
Vermi	50% mineral nutrient + 50% Vermicompost
Alg	50% mineral nutrient + 50% Algae
Bac+Vermi	50% mineral nutrient + 25%Bacteria+25%Vermicompost
Bac+Alg	50% mineral nutrient + 25%Bacteria+25%Algae
Bac+Myco	50% mineral nutrient + 25%Bacteria+25% Mycorrhizae
Vermi+Myco	50% mineral nutrient + 25%Vermicompost+25% Mycorrhizae
Vermi+Alg	50% mineral nutrient + 25%Vermicompost+25% Algae
Alg+Myco	50% mineral nutrient + 25%Algae+25% Mycorrhizae
4 Bio	50% mineral nutrient + 12.5%Algae+12.5%Bacteria+12.5%Vermicompost +12.5% Mycorrhizae

Parameters determined

According to potatoes plants growth, three critical stages were fixed to evaluate growth parameters; plant length and diameter leaves number, leaves the area, chlorophyll content, and the dry matter and macro and micronutrients elements concentration in leaves.

Stage 1: Rooting and intensive growth of the above groundmass (30 days after planting)

Stage 2: Tuberization induction (60 days after planting)

Stage 3: Tuber growth (90 days after planting)

Chlorophyll measurements

A portable chlorophyll meter (SPAD-502, Minolta, Japan) was used to measure the leaf greenness of the fully matured leaves of all plants in each pot on the sampling day.

Leaves area measurement

In each development stage, and for each treatment, two leaves were taken from every plant. LI-3100C (Li-Cor) at a $1mm^2$ resolution area meter was used to determine the area of leaves cut from each plant in every treatment on the sampling day.

Leaf dry matter content

It was measured by weighing fresh material consisting of 2 mature and non-senescent leaves from each plant for every treatment. Leaves were weighed fresh, then dried for 24 h at 80 °C, and weighed again. According to the following formula, dry matter content was calculated: Leaf dry mass weight (g)/ Leaf fresh mass weight (g) x100

Macro and micro elements concentrations

Leaves were washed once with tap water and twice with deionized water. They were then dried in a forced-air oven at 65°C for 48 h and ground through a 40-mesh sieve for elemental analysis. The samples were dry-ashed in a muffle furnace at 550°C for 6 h. The ash was then dissolved in 0.1 M hydrochloric acid (HCl) solution. Concentrations of macro and micro elements (calcium, magnesium, potassium, copper, manganese, iron, zinc) were determined using an atomic absorption spectrophotometer (Jones, 2001). Nitrogen content in leaves was determined according to the Kjeldahl method.

Mini tuber potato harvest

The total number of potatoes per plant, weight, and diameter were determined 120 days after planting

Statistical Analysis

Data were subjected to ANOVA to determine the difference between the treatment means using JMP PRO14. The means were tested with the least significant difference (LSD) test, and the significance level was set at the 0.05 probability level.

Results and Discussion

Effect of the biofertilizers on growth parameters

Plant's length and diameter

The results obtained in Table 3, the effect of the different treatments growth parameters, showed a gradual increase in plants' height and diameter during the plant's development stages.

The highest heights were obtained in plants fertilized by bacteria and vermicompost. In fact, during the growth stage, plants' height rates increased about 40% and 30% for bacteria and vermicompost,

respectively. However, low's values were obtained for the treatment where it was mixing Bac+Myco in stage 3.

Regarding plants diameter, the highest value (6.0 mm) was observed in plants treated with bacteria, and the lowest (4.07 mm) was obtained in the mixture of Bac+Myco.

These results are in accordance with those of (Ali et al., 2020); in their work, the application of bacteria as a biofertilizer in potatoes plants increases the growth parameters and plants height with a rate of 15% compared to the control. Moreover, (Tuku, 2000) mentions that bacteria are significant micro-organisms that can improve vegetable yield growth and control pathogens through various mechanisms (Kang et al., 2021).

Leaves and branch number

The results obtained showed a significant effect of treatments and development stage of potato invitro plant. The maximum leaves number was obtained after 60 days of planting (stage2), in control and micro algae's treatments 15.0 and 15.4, respectively. In contrast, plants treated with Alg+Myco and Alg+Bac present the lowest leaves number at different development stages (Table 3). The vegetative development of tuber plants depends on the growth stage; it increases progressively to reach a high level in the tuber induction phase (Kolbe and Stephan-Beckmann, 1997).

Moreover, control and algae treatments developed more than one branch per plant. Pelealu et al. (2019) studies reported that the augmentation of branch number is related to the number of tubers produced.

Algae extracts contain phytohormones such as auxin (Romanenko, 2015; Stirk et al., 2013), an essential regulator of various plant developmental processes, such as cell division and elongation. Indole-3-acetic acid (IAA) and indole-3-butanoic acid (IBA), the two dominant types of auxins in microalgae, can both stimulate and inhibit the growth and metabolism of higher plants (Hashtroudi et al., 2013).

Chlorophyll SPAD readings and leaves area

The SPAD values and leaves area rates reflect the same evolution in function with the development stage; a significant increase during the second one followed by a decrease at the tuber stage growth (Table 3).

The control's both parameters' highest values were obtained, followed by micro-algae and mycorrhizae in the different development stages. In contrast, the mixture of the bio-fertilizers presents the lowest rates.

Algae are essential biofertilizers that promote plant growth and crop yield growth, the secretion of vitamins, and the enchanting of available nutrients such as nitrogen, phosphor, and potassium. Moreover, this nutrient improves cell growth, leaves expansion, transport between source and sink organs (Lee and Ryu, 2021). Additionally, NPK facilitates the diffusion of carbon dioxide (CO_2) through the leaf mesophyll, which plays a crucial role in photosynthesis and chlorophyll content (Torabian et al., 2021). Many research, reported the positive effect of mycorrhizal fungi in the agricultural system; it is a solution that improves the efficiency of phosphate used and contributes to the absorbance of potassium from soil (Sawers et al., 2010).

Potassium is very abundant in the soil, but its availability is deficient due to its strong mineral adsorption. Mycorrhizae play the role of mediator to accumulate and regulate this element and ensure its transport to the plant (Berruti et al., 2015). Thus, potassium increases the chlorophyll content in leaves and leaf areas (Torabian et al., 2021).

Dry matter

There is no statistical difference between treatments applied on dry matter content in leaves during the first and second stages (Table 3). On the contrary, there is a significant difference in the third stage with high values obtained in plants treated by biofertilizers and micro-algae, 10.7 and 10.6, respectively.

Dry matter content in leaves decreases parallel to the tuberization process in all the treatments with a dropping ratio varied from 3 to 18% in the tuber growth stage; explained by remobilization of minerals from leaves to mini tuber at the end of the vegetation period (Kolbe and Stephan-Beckmann, 1997).

According to Lee and Ryu (2021), using Eukaryotic green algae, *Chlorella vulgaris*, as biofertilizer, increased the fresh and dry weight, increasing the dry matter content in leaves.

Effect of the biofertilizers on mini tuber yield production

Applying the different treatments improves mini tubers' yield production. Tuku (2000) reveals that mini tubers produced per in vitro plant on soil media usually ranges from 2-5. The higher mini tuber number was obtained in the control, micro-algae, and Alg+Bac+Myco+Vermi, respectively 8.8,8.2 and 7.6. However, the lowest tubers numbers (3.4) were obtained in the mixture Alg+Myco and vermicompost (Table 4). Algae biofertilizers increase the number of mini tubers compared to the others; in fact, many researcher reported the beneficial fact of applying green algae "*Chlorella vulgaris*" in the yield production (Ergun et al., 2018; Farid et al., 2019; Tuku, 2000). Algae biofertilizers promote plant growth and crop yield and enhance plant robustness by a different process. Firstly, the production of phytohormones and regulators such as auxin and cytokinin increases plant growth and development and crop yield, secondly by producing macronutrients, vitamins, and insured nitrogen-fixing (Lee and Ryu, 2021). The control plants produce many tubers; however, their weight and size were lower than bacteria and vermicompost (Table 4). According to Altaf Hossain (2015), reported that mini tubers size classification: undersize ≤5 mm, pea-size (5-10 mm), small size (10-15 mm), medium size (15-20 mm), large size (20-25 mm) and extra-large ≥25

mm. Plants fertilized by bacteria and vermicompost produce a large-size mini tuber (20.11 and 18.65mm), respectively; Alg+Vermi, Mycorrhizae, and Myco+Vermi have a medium-size vary from 15.32 to 17.28 mm; the other application with control produces a small-size range from 12.49 to 14.96 mm. Many studies mention the effect of mini tuber size on seed production; seed tuber size is one of the significant factors affecting yield and quality in potatoes. Tuku (2000) revealed that larger mini tubers seeds give more vigorous plants than the small ones. Moreover, increasing the size of mini tubers seed is essential because size affects the duration of the dormancy, the plant's vigour, and the number of stems (Tuku, 2000), thus influencing the yield production and quality of the tuber obtained from the unit area (Ozkaynak, 2021). The weight of the protected mini tubers presents a highly significant difference in the treatments. The plants treated by bacteria and vermicompost produce the maximum tuber weight (6.7 g and 5.31 g), respectively.

Tuku (2000) and Özkaynak and Samanci (2006) mentioned that importance of mini tuber seeds weight in seed tuber production program; in fact, tuber number produced from mini tubers seeds with a high weight was significant (Table 3). Furthermore, the research of (Mahmoudpour, 2014), who studies the effect of different sizes of mini tubers on yield production of Agria potato variety, demonstrate the importance weight of mini tuber weight on yield potatoes production; and concluded that the range weight varies from 5 to 10 g is most suitable to obtain a high number and diameters of tubers production. In addition, Mahmoudpour (2014) mentions that the mini tubers lighter than 1g were unsuitable for planting.

The application of vermicompost increases the bioavailability of phosphorus in the soil, affecting plant growth in potato cropping and improving crop yield (Ansari, 2008). Bacteria's utilization in plant fertilization promotes the growth of plants with higher solubilization of tricalcium phosphate (TCP) by increasing nutrient uptake parameters and producing indole-3-acetic acid (IAA) and sierophores (Kang et al., 2021). The mixture Alg +Myco have the lowest tuber numbers, diameters, and weight values (Table 4). Generally, the mixture of biofertilizers can produce growth factors and exotoxins that promote or inhibit growth and development (Kang et al., 2021). In procedure research, some micro-organisms could not function with a high level of nutrients elements; the effect of mixtures of two biofertilizers or more simultaneously depends on the species, genotype, environmental conditions, and the concentration applied (Mujtaba and Lee, 2016). However, toxic relationships between the biofertilizers can inhibit the growth process by increasing the pH, dissolved oxygen concentration, soil temperature (Ribalet et al., 2008).

Table 4. Effect of different biofertilizers in mini tubers yield production

Treatments	Tuber/ Plant	Diameter/ Tuber (mm)	Weight/ Tuber (g)
C	8.80a	14.84c-e	2.51cd
Myco	5.60bc	15.80cd	3.23cd
Alg	8.20a	14.29de	2.31d
Bac	4.80c	20.11a	6.70a
Vermi	3.40c	18.65ab	5.31ab
4 Bio	7.60ab	12.49e	1.87d
Bac+Vermi	5.40bc	14.09de	2.31d
Myco+Vermi	4.40c	17.28b	4.01bc
Bac+Myco	4.60c	14.96c-e	2.76cd
Alg+Bac	4.60c	14.43de	2.44cd
Alg+Myco	3.40c	13.11de	1.83d
Alg+Vermi	4.40c	15.32cd	2.58cd

The differences between means shown with dissimilar characters in the same column is statistically important ($P < 0.05$). Differences between means shown with similar characters in the same column is not statistically important.

Effect of the biofertilizers on macro and micro nutrients in leaves

Nitrogen content

According to the results obtained in Table 5, a highly significant effect of the treatment and the development stage on nitrogen rate was noted. In fact, during the first stage, which corresponds to root and aboveground mass growth, the nitrogen level was higher, decreasing progressively during plant development. The most important values compared to the control were observed in micro algae treatment (4.28%) followed by bacteria (3.76%). During the second and the third stage, algae reduce the nitrogen content in leaves by about 36%, but Bacteria reduce it by 17%. The plants treated by the mixture Alg+Bac have the lowest nitrogen content in leaves. Microalgae *Chlorella vulgaris* can fix atmospheric nitrogen; also, this application to the plants improve nitrogen-fixing in the soil, thus increasing his availability and enhanced plant growth (Lee and Ryu, 2021). The application of bacteria in the soil increases the ratio of nitrogen uptake, thus improving the nutrient content in leaves and plant growth parameters (Adiloglu et al., 2021).

Macro elements

The calcium (Ca), magnesium (Mg), and potassium (K) contents show a significant difference for treatments and stages (Table 5). Their maximum values were recorded at the second development phase and decrease during the last stages. Araujo et al. (2019) mentioned that potato leaves assimilate a high level of nutrients during the vegetative growth phase; at the tuber growth stage, photoassimilates are translocation and remobilization stored in the aerial part of the plant to tubers. Compared to the control, the application of biofertilizers increases the Ca and K content rate in leaves. In addition, the regular content of Ca and K for potato leaves varies from 6 to 8% for K and 1.5 to 2.5% for Ca. Gondwe et al.

(2019) reported that algae application improves the rate of Ca and K content in leaves, a critical nutrient for the growth and development of potato tubers. Furthermore, Adiloglu et al. (2021) mentioned that the application of vermicompost, bacteria, and their combination increases Ca and K content in leaves. The effects of different biofertilizers on the Mg contents of potatoes leaves were not insignificant; we constate that the highest values it is obtained in the control. According to Adiloglu et al. (2021) and Chanda et al. (2019), the application of biofertilizers can stimulate plant growth and nutrient uptake; but this performance depends on the species of biofertilizers, soil parameters, and plant growth conditions (Çakmakçi et al., 2006).

Micro elements

Comparing to the control, the application of bio-fertilizers engenders a substantial effect on micro elements such as copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) by increasing their content in leaves (Table 6). Leaves acquire the most Cu, Mn, Fe, and Zn during vegetative growth; however, during tuber induction, these rates decrease significantly, which may be explained by the mobilization of nutritional elements from leaves to the tuber (Araujo et al., 2019; Kolbe and Stephan-Beckmann, 1997).

Table 3. Variation of the plant growth parameters affected by the different bio-fertilizers in leaves in the different growth stage of plant

Treatments	Plant height (cm)			Diam (mm)			Branch Number			Leaves Number		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
C	44.2a-c	56.8b-d	76.8bc	3.90cd	4.32de	4.75d	1.0a	1.8a	1.8a	8.0ab	15.0a	10.6b
Myco	48.8a	61.8a-c	83.2b	4.15bc	5.16b	5.66a-c	1.0a	1.0b	1.0c	7.4bc	12.6b	8.6de
Alg	48.0ab	53.6b-d	67.2ef	3.89cd	4.67cd	5.54a-c	1.0a	1.6a	1.6ab	9.0a	15.4a	13.6a
Bac	49.0a	66.0a	92.6a	4.35a	5.76a	6.00a	1.0a	1.0b	1.0c	8.4ab	11.4cd	9.4cd
Vermi	49.6a	65.6a	82.2bc	4.21ab	4.75c	5.18cd	1.0a	1.0b	1.0c	8.2ab	11.6c	9.6c
4 Bio	44.4a-c	52.6cd	69.0de	3.66de	4.39c-e	5.40bc	1.0a	1.0b	1.0c	7.4bc	10.2ef	8.6de
Bac+Vermi	39.4c	52.6cd	75.4d	3.48de	4.68cd	5.47a-c	1.0a	1.0b	1.0c	7.4bc	10.6de	8.6de
Myco+Vermi	42.2a-c	48.4d	61.4fg	3.55de	4.17ef	5.36bc	1.0a	1.0b	1.0c	8.6a	14.8a	13.4a
Bac+Myco	40.4bc	48.2d	55.0g	3.29de	3.21f	4.07e	1.0a	1.0b	1.0c	8.4ab	11.4cd	9.2c-e
Alg+Bac	40.2c	62.6ab	79.6bc	3.07e	3.91f	4.14e	1.0a	1.0b	1.0c	6.6cd	10.6de	8.6de
Alg+Myco	43.6a-c	58.4a-c	63.4ef	3.13e	4.34de	4.81d	1.0a	1.0b	1.0c	6.2d	9.4f	8.4e
Alg+Vermi	46.4a-c	60.4a-c	77.6bc	3.89cd	4.03ef	5.83ab	1.0a	1.2b	1.4b	8.4ab	11.4cd	9.4cd

Table 3. Variation of the plant growth parameters affected by the different bio-fertilizers in leaves in the different growth stage of plant (continuation).

Treatments	Chlorophyll (SPAD-values)			Leaves area (cm^2/two leaves)			Dry matter (%)		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
C	45.0a	50.8a	44.4a	101.8ab	232.1a	227.5a	10.3a	9.5a	9.2bc
Myco	43.3ab	46.1b	39.6bc	90.5c-e	188.8c	166.8c	10.0a	9.6a	9.2bc
Alg	42.7ab	48.9a	40.9b	99.8ab	221.9ab	201.4b	9.8a	9.9a	10.6ab
Bac	40.8bc	41.7cd	34.9d-f	87.2de	147.5ef	130.2ef	9.8a	9.3a	9.2a-c
Vermi	39.6cd	42.3cd	39.4bc	95.6b-d	157.8de	133.9d-f	9.7a	9.2a	9.4a-c
4 Bio	38.9c-e	42.8c	37.9cd	85.7e	138.1fg	105.6g	9.4a	9.6a	10.7a
Bac+Vermi	36.9ef	42.4cd	33.6ef	99.8ab	122.7gh	112.2fg	9.4a	8.9a	8.2c
Myco+Vermi	39.6cd	40.1df	37.3cd	104.1ab	166.6d	154.1cd	9.5a	10a	9.3a-c
Bac+Myco	37.2d-f	42.4cd	32.1f	107.3a	137.4fg	144.8c-e	9.8a	9.5a	8.6c
Alg+Bac	35.1fg	41.7cd	35.2de	97.8bc	208.4b	195.9b	10.4a	9.1a	8.6c
Alg+Myco	32.7g	37.3f	35.5de	100.7ab	117.9h	113.2fg	9.4a	9.0a	8.8c
Alg+Vermi	35.4f	39.2ef	37.9cd	100.8ab	160.3de	153.3cd	9.4a	10.1a	9.0c

The differences between means shown with dissimilar characters in the same column is statistically important ($P < 0.05$). Differences between means shown with similar characters in the same column is not statistically important.

Table 4.Variation of the macro-nutrients in leaves affected by the different biofertilizers in the different growth stage of plant (%)

Treatments	N (%)			Ca (%)			K (%)			Mg (%)		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
C	3.28c-e	2.60gh	2.43g	1.68f	1.98d	1.27f	5.22e	7.87bc	5.25b	2.57bc	4.85a	3.04a
Myco	2.92ef	2.46h	2.38g	1.52f	2.17cd	1.45ef	6.96a-c	7.92bc	8.70ab	2.42c	3.32ab	2.95a
Alg	4.28a	3.03de	2.76d	1.98e	2.75ab	1.54ef	5.63de	8.42bc	8.43ab	3.10bc	4.31ab	2.51ab
Bac	3.76b	3.39a	3.13a	2.05de	2.31b-d	1.75d-f	7.22ab	7.93bc	9.96a	2.98bc	3.33ab	2.31a-c
Vermi	3.15d-f	2.49h	2.76d	2.20d	2.25b-d	2.45bc	5.53e	7.89bc	10.25a	2.99bc	3.61ab	1.43bc
4 Bio	3.55bc	3.37ab	3.01b	1.97e	2.12d	1.72d-f	6.47b-d	6.70c	8.55ab	3.68ab	3.25ab	1.80bc
Bac+Vermi	3.41b-d	2.68g	2.52f	3.18a	2.34b-d	1.63ef	6.57a-c	7.59bc	7.25ab	3.37a-c	3.16b	2.05a-c
Myco+Vermi	2.63b	2.77a	2.39b-d	6.10c-e	8.54b	9.59a	3.30a-c	3.34ab	1.46bc	3.21c-e	3.05c-e	2.94bc
Bac+Myco	2.92ef	2.91ef	2.92c	2.42c	2.66a-c	2.09c-e	7.43a	10.76a	9.80a	3.23bc	3.20b	1.27c
Alg+Bac	2.80f	2.74fg	2.67e	2.70b	2.12d	2.58b	6.64a-c	9.22ab	8.85ab	4.45a	2.91b	1.38c
Alg+Myco	3.54bc	3.20bc	2.99bc	2.74b	1.90d	2.11c-e	6.51b-d	8.54b	8.76ab	4.47a	3.63ab	1.33c
Alg+Vermi	3.15d-f	3.09cd	2.77d	1.01g	2.73ab	3.44a	1.68f	8.32bc	9.67a	3.43a-c	3.21b	1.37c

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Table 5. Variation of the micro-nutrients in leaves affected by the different biofertilizers in the function of the growth stage of plant (ppm)

Treatments	cu (ppm)			mn (ppm)			fe (ppm)			zn (ppm)		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
C	88.5a-c	9.0c	14.0ab	84.0c-e	51.0e	31.0d	85.5ab	35.5a-c	40.0c	47.5b	26.5b	24.0c
Myco	107.0a	20.0ab	9.0b	85.5c-e	55.0de	35.5cd	82.5a-c	46.0ab	50.5bc	64.0a	26.5b	38.5a-c
Alg	29.5a-c	23.0a	12.0ab	86.0c-e	69.0bc	46.0a-c	67.5c-e	51.5a	50.5bc	25.5cd	27.5b	33.5bc
Bac	101.0ab	8.5c	15.5ab	80.5de	56.0de	60.0a	80.0b-d	39.5a-c	81.0a	41.5b	26.0bc	69.0ab
Vermi	26.5bc	12.5bc	13.5ab	88.5c-e	54.0de	46.0a-c	76.0b-d	44.5ab	68.5ab	28.0c	30.0b	74.5a
4 Bio	55.0a-c	5.0c	18.0a	88.5c-e	51.0e	42.0b-d	95.5a	26.5bc	68.5ab	44.0b	25.5bc	45.0a-c
Bac+Vermi	22.0c	22.0a	11.05ab	100a-c	69.0bc	33.0cd	81.5a-c	54.0a	45.0bc	39.0b	29.0b	29.0c
Myco+Vermi	12.0c	7.0c	9.0b	93.0b-d	79.5ab	37.0cd	72.5c-e	40.0a-c	31.0c	22.5cd	16.5c	26.0c
Bac+Myco	14.0c	4.0c	9.5b	75.0e	82.5a	42.5b-d	48.5f	40.0a-c	36.5c	19.5cd	26.0bc	30.5c
Alg+Bac	49.0a-c	5.0c	12.5ab	100.5ab	63.0c-e	33.5cd	65.5de	47.5a	35.0c	41.0b	34.0b	38.5a-c
Alg+Myco	17.5c	4.5c	10.0b	107.0a	66.05-d	46.0a-c	59.5ef	22.5c	69.5ab	22.0cd	44.5a	49.0a-c
Alg+Vermi	13.0c	7.0c	8.5b	39.0f	52.0e	55.5ab	22.5g	49.5a	58.0a-c	17.0d	34.0b	39.5a-c

The differences between means shown with dissimilar characters in the same column is statistically important (P <0.05). Differences between means shown with similar characters in the same column is not statistically important.

Conclusion

The results obtained showed a high effect of different biofertilizers and their mixture in mini tubers yield production.

The bacteria and vermicompost produce the highest tuber number per plant (4.8 and 3.4, respectively), tuber size (20.11 mm and 18.65 mm, respectively) and tuber weight (6.70 g and 5.31g, respectively). For mini tuber seeds production, the number is important, yet the size and weight are the essential parameters to obtain high tuber yield. Thus, it is recommended that the seeds producers apply vermicompost and bacteria in their fertilizer's solution.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this article, they have no actual, potential or perceived conflict of interest.

Author contribution

Hiba Boubaker performed the experiment in the growth chamber and laboratories and did data collection and manuscript writing. Hayriye Yildiz Dasgan contributes suggestions during the experiment and give ideas and reviewing the manuscript. Neji Tarchoun was read and revised the manuscript

Ethical approval

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GIS-Based Land Suitability Classification for Wheat Cultivation Using Fuzzy Set Model

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Abstract

In terms of food safety, it is important to use the lands correctly in agricultural production. In this study, potential crop suitability classes for wheat cultivation were created by using the fuzzy model and GIS together. Spatial and spectral factors considered as model inputs were separated four main groups, such as soil (drainage, depth, texture, CaCO3, stoniness, pH, organic matter, salinity, ESP), topography (slope), water availability (irrigation) and vegetation indices (NDVI). Criterion maps were standardized with the fuzzy membership model. Analytical Hierarchy Process was used to determine the weights of the factors. The vegetation change between years in the study area was determined by using NDVI values obtained from Landsat satellite images. In addition, the effect of temporal difference on land use and land suitability was evaluated. Land suitability index was created in GIS environment by weighted linear combination method and divided into four main suitability classes. The results with the Fuzzy method showed 9.7% (805 ha) of the study area as highly suitable for wheat, 46.5% (3868 ha) as medium suitable, 27.6% (2297 ha) as marginally suitable and 16.2% (1350 ha) as unsuitable. According to these classes, highly suitable and medium suitable classes are the areas that should be evaluated primarily in agricultural production. The Fuzzy model and GIS integration can be effectively used to identify priority areas for crop cultivation and sustainable land use management.

Keywords: Fuzzy set model, Analytical Hierarchy Process, Land suitability analysis, Geographic Information Systems, Wheat

Introduction

Today, there are restrictive threats to the conservation and sustainability of natural resources. Climate change is an important problem for today and for the future due to its negative effects on agricultural productivity and food safety in many regions of the world (IPCC, 2014). However, population growth adversely affects agricultural lands and natural resources. Accurate land use is critical for effective land use and agricultural sustainability. Identification of the physical and socio-economical potential of the land is necessary for sustainable planning and reducing negative effects. It is essential to determine and plan the potential crop pattern of the lands for agricultural planning. This evaluation process is related to spatial and temporal factors (Al Taani et al., 2021).

Land suitability analysis plays a fundamental role for the rational planning and use of lands. The assessment of the suitability of the land for the growth of a particular crop involves a process. According to this process, firstly, the ecological requirements of the product and the physical conditions of the land are compared (FAO, 1985; 1976). Land suitability analysis is performed to determine which area is suitable for a particular area in the correct management of natural resources (Bodaghabadi et al., 2015). In addition, a crop planning system can be created to increase land productivity for decision makers (Chen, 2014). In land suitability analysis, determining the limiting factors affecting the cultivation of a particular crop is a priority (Halder, 2013). The main feature of the land suitability assessment is that the land requirements are compared with land features such as soil, water availability, vegetation cover, climate and landforms (Dent and Young, 1981).

Land suitability analysis is defined as the Multi Criteria Evaluaition (MCE) approach since there are many criteria at the decision-making stage in the solution of a problem (Malczewski, 2006). Since transition values are available for most of the factors in the MCE process, it is difficult to express with an absolute value (Reshmidevi 2009). In nature, some

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objects can be defined as a homogeneous feature in terms of geographic area, while ecological characteristics such as soil and topography show continuity and variability. Therefore, boundaries between ecological features should be gradual rather than sharp boundaries (Van Ranst and Tang 1999; Burrough 1989). In addition, these properties can be expressed as different units and sizes. Standardizing and combining these characteristics on a common scale is important for land suitability assessment (Voogd, 1982).

Standardization and aggregation of criteria and modeling of vogue concepts are possible with the fuzzy sets approach (Jiang and Eastman, 2000). The fuzzy set theory facilitates the analysis of continuous structures and the membership degree is defined as an object class (Zadeh, 1965). Wang et al. (1990) proposed land suitability assessment with membership grading in fuzzy set theory instead of sharp boundaries such as true or false for suitable and unsuitable classes. The traditional approach tends to represent land features as discrete parts. However, there is a continuous structure in nature, except that a few elements are discrete. Fuzzy modeling is a suitable approach for defining continuous or uncertain structures (Burrough and Frank, 1996). Fuzzy logic-based land evaluation methods are widely used to determine agricultural land suitability (Garofalo, 2020; Zhang, 2015; Nurmiaty and Baja, 2014; Sicat et al., 2005; Baja, et al., 2002).

Analytical Hierarchy Process (AHP) is one of the most widely used multi-criteria assessment methods for land suitability analysis (Everest et al., 2020). AHP is widely used in agriculture as a decision support tool used to solve complex decision problems (Cengiz and Akbulak, 2009). In this case, AHP is a suitable method for determining weights by using pair-wise comparison (Saaty, 1980). In this process, the criteria in a hierarchical structure can be divided into groups and each group can have sub-criteria within itself. However, all criteria have not equal weight. Therefore, each criterion is weighted according to its importance. For this reason, a weight is assigned to the criteria for each level of the hierarchical structure. Criterion maps were created with the Geographical Information System (GIS) based fuzzy model (Yalew et al., 2016; Zabihi et al., 2015; Feizizadeh and Blaschke, 2013). Criteria maps were combined in GIS environment by using weighted linear combination (WLC) method for land suitability analysis. The total land suitability score is calculated by weighting the standardized criteria maps with the WLC approach (Tugac and Sefer, 2021; Tercan and Dereli, 2020; Tashayo, 2020; Herzberg, 2019; Malczewski, 2004).

In this study, land suitability classification was made by integrating GIS and fuzzy method in a multi-criteria evaluation approach according to land characteristics for wheat cultivation.

Material and Methods

Study area

The present study was performed at Bala Agricultural Enterprise. Bala is a district of Ankara Province in the Central Anatolia region of Turkey. It is geographically located between 33º 14’ 45’’ - 33º 21’ 20’’ E longitude and 39º 19’ 39’’ - 39º 30’ 58’’ N latitude. The elevation of the study area ranges from 750 to 980 m.a.s.l. The surface area covers approximately 8320 ha (Figure 1). This area has a semi-arid climate with cold and snowy winters and hot dry summers. In the region, the hottest month of the year is July, while the coldest month of the year is January. The mean annual total precipitation is 330 mm. The annual average, average minimum and average maximum temperatures are 11,7ºC, -4ºC, and 30ºC, respectively.

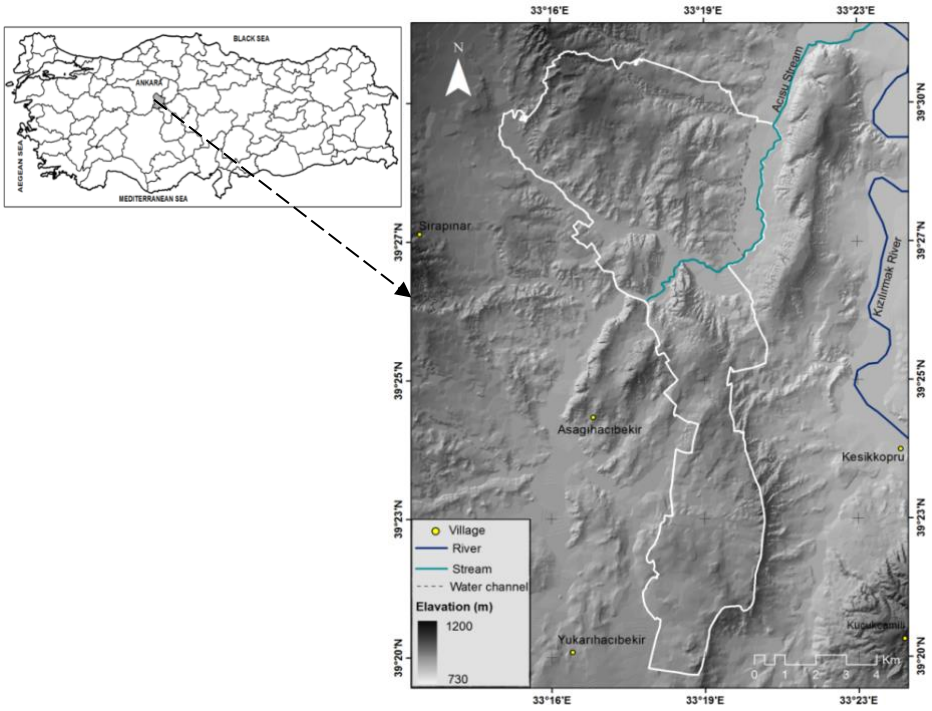

Figure 1. Study area

The area consists of four different physiographic structures: alluvial plain, undulating, sloping and hilly. The parent materials in the study area are limestone, alluvium, marl, gypsum and gravel. The study area includes entisols, aridisols soil orders and 23 different soil series (Soil Survey Staff, 1987). The alluvial soils formed by the river are the most productive soils occupying the middle of the study area. The existing land use in the study area consists of rain-fed agriculture, irrigated agriculture, pasture, degraded land and natural vegetation. Bala agricultural enterprise, which continues its activities under the General Directorate of Agricultural Enterprises, was leased to a private sector subsidiary in 2008.

Data Sources

In this study, different databases including maps and images such as soil, topography, land use, vegetation development were used for land suitability analysis. In determining the soil structure of the area, physical and chemical soil properties obtained from the detailed soil study map with a scale of 1: 16.000 were used (Arcak, 1992). Soil physical features (soil depth, texture, surface stoniness, drainage, erosion), which were digitized in vector-based databases, were converted into the raster-based features. Thematic maps of soil chemical properties such as pH, salinity, lime, organic matter, ESP were created using the Inverse Distance Weighted (IDW) interpolation method in ArcGIS software. Climate data was obtained from meteorological station, which belong to Turkish State Meteorological Service. Topographic criteria, slope produced from digital elevation model (DEM) which was obtained 1 / 5.000 scale topographic maps.

Land use maps provide information on land use types, irrigation areas, parcel borders, water bodies, roads, rocky places, service areas. These maps were defined and digitized from the ariel photograph and Sentinel-2A image. Landsat 5, 7 and 8 satellite images were used to obtain Normalized Difference Vegetation Index (NDVI) data. In order to determine the vegetation activity in the field during the year, 16-day and cloudless NDVI data were transformed into maximum composite data. NDVI is derived from the red and near infrared band reflectance values (Tucker, 1979).

Model input maps were prepared in raster data format using the ArcGIS 10.4 (ESRI, 2015) program in the Geographic Information System (GIS) environment. The crop suitability map was created at 10 m cellular resolution.

With this study is to generate wheat (*Triticum aestivum L*) suitability classes using the GIS-based fuzzy set model and AHP (Fig.3). The general evaluation procedure followed in this study can be divided into four main parts: (1) Selection of criteria and definition of hierarchical structure.: (1) Selection of criteria and definition of hierarchical structure. (2) Determining membership function and applications of fuzzy model. (3) Obtaining weight for the criteria by using the AHP method (4) Creation of agricultural land suitability classes and map. The flowchart of the land suitability analysis for wheat is shown in Figure 2.

Hierarchical structure

The hierarchical structure of the model can be separated into three main parts. The first level is the definition of the goal that implies land suitability index for wheat. The second level, the agricultural land suitability assessment is to determine the relevant ecological variables. The criteria selected for the assessment of land suitability are divided into four main groups: (1) soil, (2) topography, (3) vegetation indices, and (4) water availability factors. The third level is the determination of sub-criteria related to the main group. At this stage, thirteen factors such as soil depth, texture, surface stoniness, drainage, erosion, organic matter, $CaCO_3$, pH, ESP, salinity, slope, NDVI and irrigation zone were selected. Depending on the purpose of the project, the systematic classification of components reveals a relative hierarchy and a model tree structure is created (Figure 3).

Fuzzy membership model

Fuzzy set theory creates a system for defining uncertain data and assigning membership degrees (Mendel, 1995). The fuzzy set is widely used in nature to classify continuous ecological data where class values are not sharp. For a class with permanent membership, each object is assigned a value ranging from zero to one, and the higher the membership value, the higher the suitability class value (Zadeh, 1965).

In traditional set theory, the membership value of a set is expressed as 1 (full membership) or 0 (non-full membership) (Tang et al., 1997). Fuzzy set models are used to classify membership features whose attributes are uncertain (McBratney and Odeh, 1997). A fuzzy set *X* is a supposed finite set of attributes. A fuzzy set (A) can be expressed as follows (Burrough and McDonnell, 1998).

$$A = \{x, \mu_A(x)\} \text{ for each } x \in X$$

Where, $x \in X$ is a finite set of points and $\mu A(x)$ is a membership model, which describes the degree of membership of x in A. For all A, μA (x) a value in the unit interval [0, 1]. In this context, $\mu A = 0$ indicates that the value of x does not belong to A and $\mu A = 1$ indicates that the value belongs entirely to A. On the other hand, if, $0 < \mu A (x) < 1$, it is defined as A for a certain degree.

There are some models to create the fuzzy membership (FM) function. The FM model functions applied in grading the land features are based on the Semantic Import (SI) model (Elaalem et al., 2011; Braimoh et al., 2004; Baja, et al., 2007; Burrough and Frank 1996; Davidson et al., 1994; Burrough 1989). This application consists of two basic functions: symmetrical and asymmetrical (Figure 4). The first model, also referred optimum interval, is divided into two parts: one uses a single ideal point (Model 1), the other uses an interval of ideal points (Model 2). The second model, an asymmetric model, is used when

only the upper and lower boundary of a feature is important. This model can be divided into two parts: asymmetric left (Model 3) and asymmetric right (Model 4) (Burrough and McDonnell, 1998). Membership functions are given below.

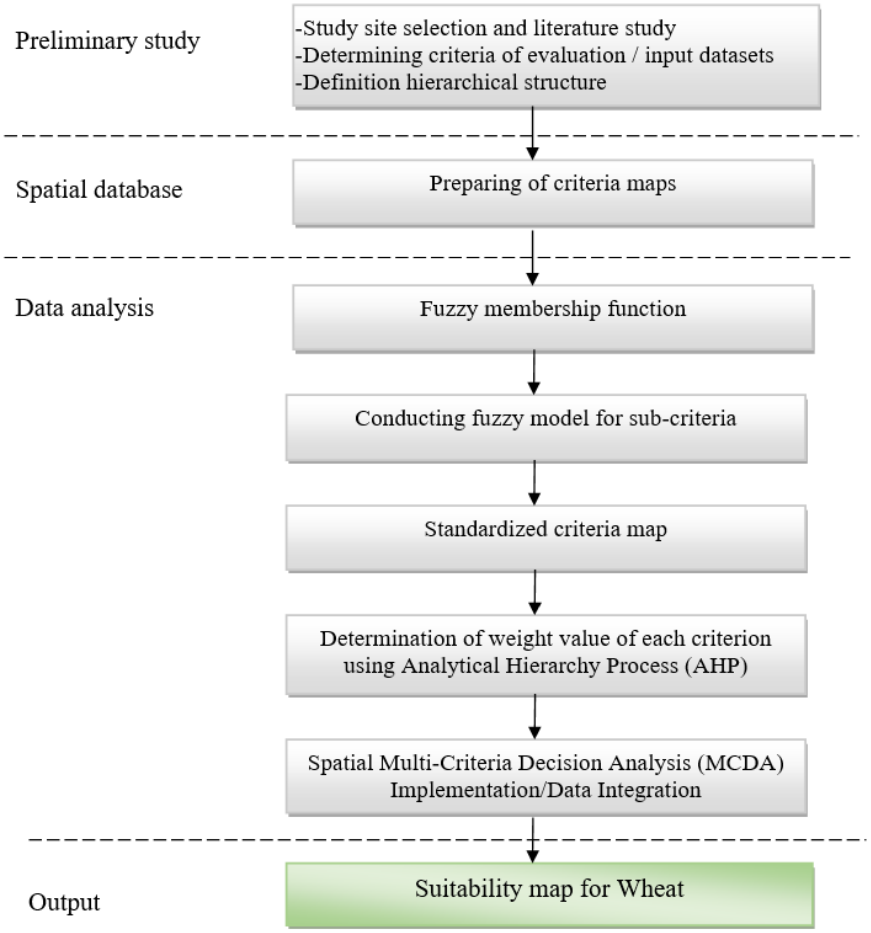

Figure 2. Schematic diagram of suitability assessment for wheat

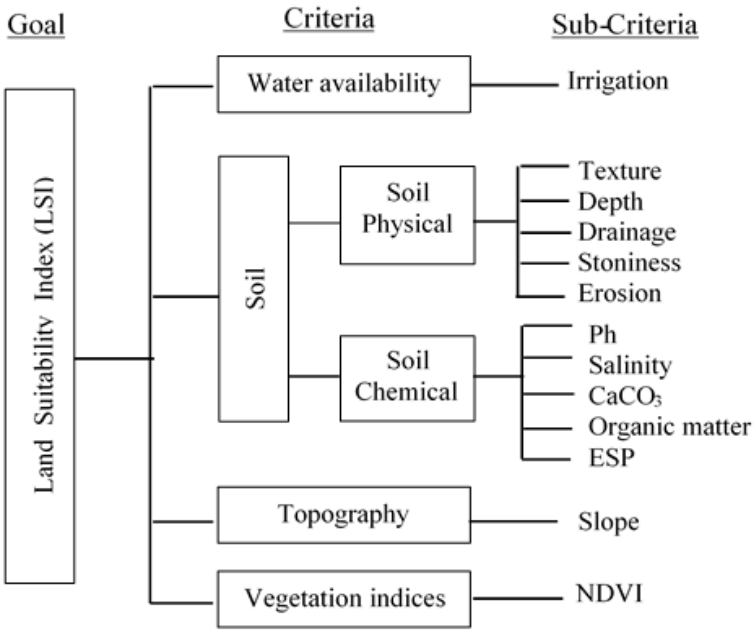

Figure 3. The hierarchical structure for the suitability assesment

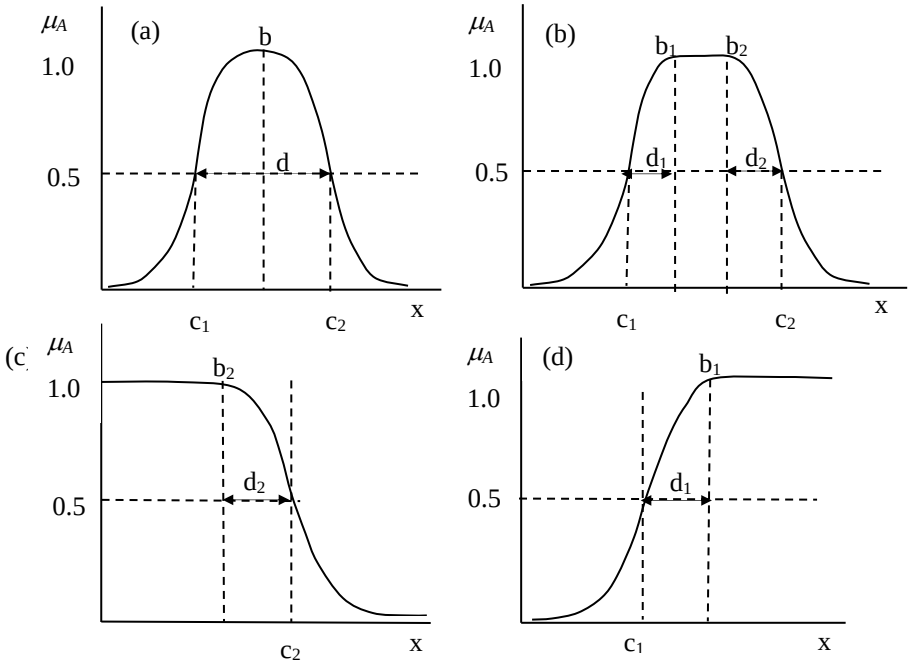

Figure 4. symmetric model (a,b), asymmetric right (c), asymmetric left (d)

$\mu_A(x_i) = [\ 1/\ (1 + \{(x_i - b) / d\}^2)]$ if $0 \leq xi \leq 1$ (Model 1)

$\mu_A(x_i) = 1$ if $(c_1 + d_1) \leq xi \leq (c_2 - d_2)$ (Model 2):

$\mu_A(x_i) = [1/(1 + \{(x_i - c_1 - d_1)/d_1\}^2)]$ if $xi < (c_1 + d_1)$ (Model 3):

$\mu_A(x_i) = [1/(1 + \{(xi - c_2 + d_2)/d_2\}^2)]$ if $xi > (c_2 - d_2)$ (Model 4):

Where $\mu_A\ (x_i)$ shows MF values for cell *i* of land characteristic *x* in a raster layer, b is the value of land attribute *x* at the ideal point, d is the width of the transition zone, c_1 and c_2 are LCP and UCP respectively.

The crossover point can be defined as the lower crossover point (LCP) or the upper crossover point (UCP) according to the criteria. Asymmetric models only have LCP or UCP values while both LCP and UCP are available in symmetric models. In this study, asymmetric right decreasing (ARFM) and asymmetric left (ALFM) increasing membership functions were applied. Increasing values such as slope, CaCO3, salinity and ESP indicates a decreasing suitability value. On the other hand, increasing soil organic matter and NDVI values indicates increasing suitability value. Therefore, ARFM and ALFM models were applied for this factors, respectively. Also, a symmetric membership function (SFM) was used for soil pH for the model. The class values of land features such as soil texture, drainage, surface stoniness and irrigation have been converted into fuzzy numbers. While assigning membership values to classes for discrete structures, layer class values are normalized as follows (Voogd, 1982):

$\mu_A(x_i) = x_i - x_{min} / x_{max} - x_{min}$

where $\mu_A\ (x_i)$ is the membership value for cell *i* of land characteristic *x* in a raster layer; x_i is the raw rank value; x_{min} is the minimum value of the criteria; and x_{max} is the maximum value of the criteria.

The FM was used to create the factor maps. In this context, the lowest and highest suitability level values were determined. The threshold values for wheat suitability analysis were determined according to literature information (Nwer, 2005; Sys et al., 1993; Van Diepen et al. 1991; FAO, 1985) and expert opinions. (Table 1).

Land suitability analysis

The land suitability index (LSI) was calculated using the ArcGIS program, taking into account the factor scores and weights. AHP technique was used to weigh the criteria according to their importance. Factor priorities are determined according to expert opinion. In a multi-criteria analysis, factor weights were applied to a pairwise comparison approach to determine the relative preference between factors (Saaty, 1980). The suitability score was obtained by integrating the standardized layers with the 'weighted overlay analysis' technique (Eastman, 2012). This model combines multiple variables on a linear basis for the main purpose. Weighted criterion maps are combined to obtain the land suitability score. LSI is calculated using the WLC method with the equation given below:

$$LSI = \sum_{i=1}^{k} w_{i*}\ \mu_A(x_i) \quad (i=1, 2, 3, ..., k;\ \sum w_i = 1;\ w_i > 0)$$

Where LSI is the Land Suitability Index of overall suitability for all variables,
$A_1,\ldots, A_k$ are fuzzy subclasses of the defined universe of objects X,
$\mu_A(x_i)$ is the membership value for land characteristic $x_{i,}$
$w_1,\ldots, w_k$ are the weights of the membership values.

The total suitability index of the land for wheat was created according to the fuzzy classification approach. Both, weight and membership grade values are between 0 and 1. The index value of the suitability map produced using the fuzzy model varies between 0 and 1. Where a value of 0 indicates completely unsuitable, and a value of 1 indicates completely suitable. The Suitability Index map is divided into four classes according to the FAO framework approach (FAO, 1976). In this classification, LSI values were classified as highly suitable (1–0.85), moderately suitable (0.85–0.6), marginally suitable (0.6–0.4), and unsuitable (0.4–0).

Table.1. Fuzzy membership limit degrees of criteria for wheat

Sub-Criteria	Complete membership (suitable)	Nonmembership (unsuitable)	Data type	Fuzzy membership function
Slope (%)	<2	>12	Continuous	ARFM,$[r_{(0.5,R)},d_{(R)}]$= [7,5]
Soil Depth (cm)	>90	<25	Thematic	[deep(1),medium(0.65),shallow (0.40),very shallow(0)]
Textur (class)	L,ZL,Z,CL, ZCL, SCL	S, LS	Thematic	[L, SiL, SiCL, CL (1), SCL, C<%45 (0.75), SiC, SC, C>%45 (0.6), SL (0.4), LS (0.3)]
Soil stoniness (class)	Absent	Severe	Thematic	[absent (1), low(0.75), medium(0.45), severe(0)]
Drainage (class)	Well drained	poorly	Thematic	[well drained (1),moderately (0.70),imperfectly (0.4),poorly (0.1)]
Erosion (class)	Absent	Severe	Thematic	[absent (1), low(0.85), medium(0.55), severe(0.1)]
Organic matter (%)	> 3	< 0.5	Continuous	ALFM,$[r_{(0.5,L)},d_{(L)}]$= [1, 2]
$CaCO_3$ (%)	< 10	> 30	Continuous	ARFM,$[r_{(0.5,R)},d_{(R)}]$= [20,10]
pH	6.5-7.5	>8.5 , <5.5	Continuous	SFM,$[r_{(0.5,R)},d_{(R),}\ r_{(0.5,L)},d_{(L)}]$= [8.2,0.7,5.8,0.7]
ESP (%)	< 10	> 25	Continuous	ARFM,$[r_{(0.5,R)},d_{(R)}]$= [18, 8]
Salinity(dS m^{-1})	< 2	> 16	Continuous	ARFM,$[r_{(0.5,R)},d_{(R)}]$= [9,7]
NDVI	>0.65	< 0.3	Continuous	ALFM,$[r_{(0.5,L)},d_{(L)}]$= [0.45,0.2]
Irrigation (class)	irrigated	non irrigated	Thematic	[irrigated area(1), rainfed area (0.65)]

C: Clay, CL: Clay loam, L: Loam, LS: Loamy sand, S: Sand, SC: Sandy clay, SCL: Sandy clay, L: loam, Si: Silt, SiC: Silty clay, SiCL: Silty clay loam, SiL: Silt loam

Results and Discussions

The main goal of the case study is to determine the priority areas of the land for wheat cultivation by using the GIS based fuzzy set model. In this context, determining the ecological criteria that affect the cultivation of the crop is a priority. In agricultural areas, topographic structure, soil and irrigation facilities are determining factors due to the soil fertility of the land and the sensitivity of the soil to degradation. Although rainfed agriculture is common in the region, the existence of irrigated lands was also important for constructing the model and selection of the criteria. The factors that affect the determination of the suitability of agricultural areas have different levels of importance. Factors taken into consideration; water availability (irrigation), soil (texture, depth, drainage, surface stoniness, pH, salinity, $CaCO_3$, organic matter), topography (slope) and vegetation index (NDVI). Soil and irrigation are the highest weighted factors in terms of wheat cultivation and productivity. The weights of these factors were determined as 0.374 and 0.324, respectively. Soil properties include soil nutrients and water availability for plant growth. The soil factor was evaluated physically and chemically. Among the physical factors, texture (0.332) and soil depth (0.290) are the most important factors. However, among the chemical factors, pH (0.383) and salinity (0.242) were the determining factors. The topographical factor, with a weight of 0.201, is another factor. The slope is related to the movement of soil particles and soil erosion; consequently, it

affects the soil quality. NDVI with a weight value of 0.101 has a lower importance than other main factors (Table 2).

In the multi-criteria approach, as the number of criteria increases, it becomes difficult to determine the weight values. While it is important to determine the relative priorities of the criteria with respect to each other, it also requires experience (Keshavarzi and Sarmadian, 2009). Criterion weight values may vary according to land conditions and ecological requirements of the crops.

Table 2. Main criterion and sub criterion weight values

Goal	Criteria	Weight	Sub-Criteria	Weight
Land Suitability Index (LSI)	Soil	0.374	Soil Physical	
			Texture	0.332
			Depth	0.290
			Erosion	0.166
			Drainage	0.131
			Soil stoniness	0.081
			Soil Chemical	
			Ph	0.383
			Salinity	0.242
			$CaCO_3$	0.194
			Organic matter	0.118
			ESP	0.064
	Irrigation	0.324		
	Topography	0.201		
	NDVI	0.101		

The main land uses in the area are rainfed agriculture (5100 ha), irrigated agriculture (1053 ha), pasture (1022 ha), degraded land (918 ha), orchard (95 ha), natural vegetation (92 ha) and service area (40 ha). The proportions of these areas in the total area are 61.3%, 12.7%, 12.3%, 11.0%, 1.14%, 1.11% and 0.48%, respectively. In dry and irrigated farming areas; wheat, barley, sainfoin, vetch, sunflower, chickpea, corn and alfalfa are grown. Rangeland includes both natural meadowlands and artificial lands. Natural vegetation land is characterized by shrub, pine, wooded. Most of the pasture and natural plant areas are within marginal suitable areas. Degraded areas contain rock and eroded lands. The service area consists of accommodation, livestock facilities and stores.

Bala agricultural enterprise, which continues its activities under the General Directorate of Agricultural Enterprises, was leased to a private sector subsidiary in 2008. With the investments made in the enterprise such as irrigation and facilities, there have been changes in the field use related to crop production, fruit orchard and animal husbandry. Irrigated agricultural areas were increased as a result of irrigation investments in 2012.

Satellite images are used extensively to detect temporal and spatial changes and determine crop yields in agricultural production areas. NDVI data is the most widely used plant growth index for monitoring vegetation in remote sensing technology (Basso et al., 2013). NDVI defines the chlorophyll concentration of plants and varies between -1 and +1 values. Increasing positive values of the index indicate healthy and high plant density. The temporal variation of precipitation has a great effect on crop development, biomass and yield. The correlation between NDVI and vegetation increases during the growing period (Labus et al., 2002). In this study, the differences in vegetation were determined from the long-term averages of the maximum composite data obtained for each year with Landsat 5, 7 and 8 images. NDVI data was taken as a criterion to determine the change in vegetation density between years in dry and irrigated areas. In particular, the change in irrigated farming areas was clearly observed after the irrigation investments. In this context, NDVI data between 2001-2011 and 2012-2020 were evaluated. While the rate of areas with high vegetation density (NDVI > 0.65) was 3% (211 ha) until 2011, it was observed that this rate increased to 11% (932 ha) with the increase in irrigated areas (Figure 5).

The parcel map of the area was digitized and the obtained parcel database was updated over the satellite image data, and land use classes belonging to two different periods were created. Land-use changes in two different periods for 2011 and 2020 were determined in the area (Table 3). The highest change was in rainfed agricultural areas with a decrease of 7.2%, while the highest increase was 6.0% and 1.1% in irrigated agriculture and fruit orchard, respectively. The effects of these changes over the years on land suitability have also been determined.

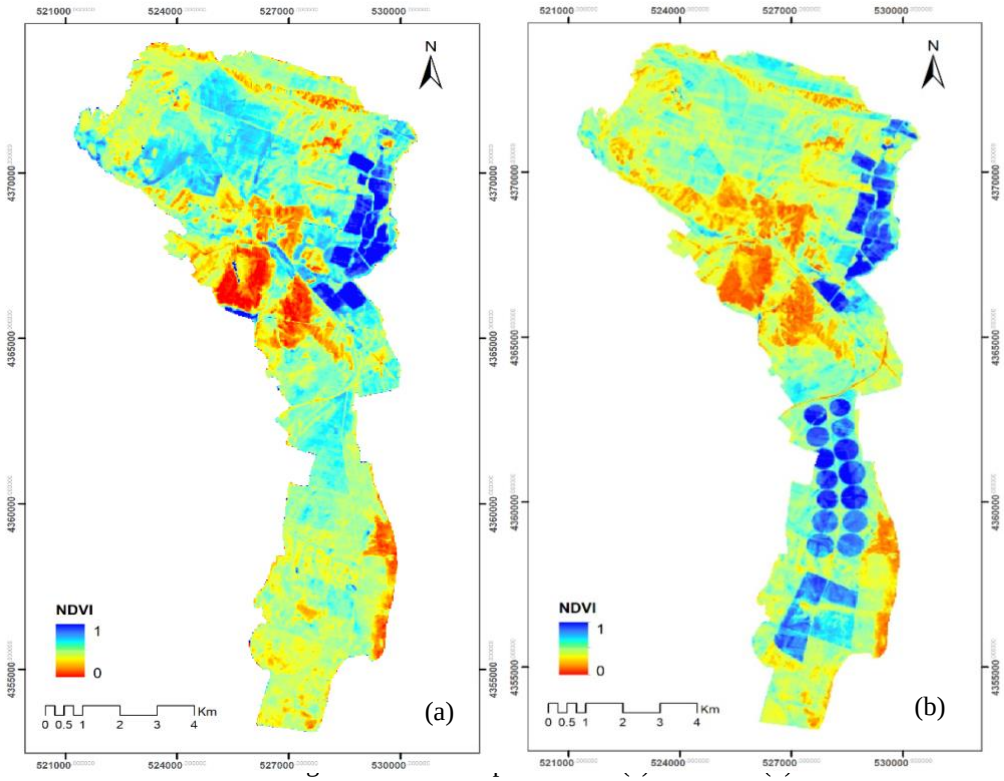

Figure 5. NDVI maps for 2011 (a) and 2020 (b)

Table 3. Distribution of land use classes for 2011 and 2020

Landuse classes	2011		2020		Difference	
	Area (ha)	%	Area (ha)	%	Area (ha)	%
Rainfed agriculture	5702	68.50	5100	61.30	-602	-7.2
Irrigated agriculture	556	6.70	1053	12.70	497	+6.0
Rangeland	1015	12.2	1022	12.30	7	+0.1
Dagraded areas	918	11.0	918	11.0	-	-
Fruit orchard	6	0.07	95	1.14	89	+1.07
Natural vegetation	89	1.10	92	1.11	3	+0.01
Service area	34	0.42	40	0.48	6	+0.06

The effect of the investments made on the land was investigated for two different periods, as the study area was rented out with a private partnership (Figure 6). While the highly suitable area was 4.8% (403 ha) in 2011, 9.7 % (805 ha) of the total area was found as highly suitable for wheat in 2020. Irrigated agricultural areas have great potential in terms of productivity. With the increase in irrigated agricultural lands, the areas with suitable land class increased by 4.9% (403 ha). Between the years, the moderately suitable areas with the majority of dry farming areas decreased by 3.7% (310 ha). According to the current land use, 46.5% (3868 ha) of the area is in the medium suitable class, while 27.6% (2297 ha) is in the less suitable class. However, 16.2% (1350 ha) of the total area is unsuitable for agriculture (Table 4). As expected, rainfed farming and irrigated farming areas are among the highly suitable and moderately suitable areas within the current land use. In the study area, the highly suitable areas for wheat cultivation can be generally characterized by flat, deep soil, soil pH level between 7.7 and 7.9, lime content of 10-20%, high water-holding capacities and humidity. In these areas, there are partially low salinity and drainage problems. In moderately suitable lands, medium depth soils are common and some areas have stoniness, liminess and erosion problems. On the other hand, there are drainage and

salinity problems in irrigated lands. In irrigated agricultural lands, there are negative impacts on a part of the land due to the low quality of irrigation water and salinity. Salinity negatively affects product development, nutrient intake and yield (Munns and Gilliham, 2015). For this reason, some areas in irrigated agricultural lands are in a lower class. In marginally suitable areas, low soil depth, rugged areas, low water-holding capacities and erosion are limiting factors. In addition, there are problems with high salinity and liminess in some areas. Areas that are not suitable for agriculture are especially rocky areas with steep slopes and very shallow soil depth. There is a very severe erosion problem for these areas.

Spatial matching was made by comparing the crop suitability classes with the existing land use map. 76.4% (805 ha) of irrigated agricultural lands are very suitable for wheat production. In irrigated agricultural lands, 23.6 % (248) ha is in the middle class due to restrictive land features such as drainage and salinity. In rainfed areas, 68 % (3471 ha) is in the medium suitable class, while 31.5 % (1606 ha) is in the marginally suitable class. On the other hand, 96.1% of the medium suitable class was found in rain-fed agriculture and irrigated agriculture. For the medium suitable class, 1.8% of the class was found in the rangeland. 69.9% of the marginally suitable class is rain-fed areas, and in these areas, land deficiencies are sighted. In addition, 21.3% of this class is rangeland (Table 5).

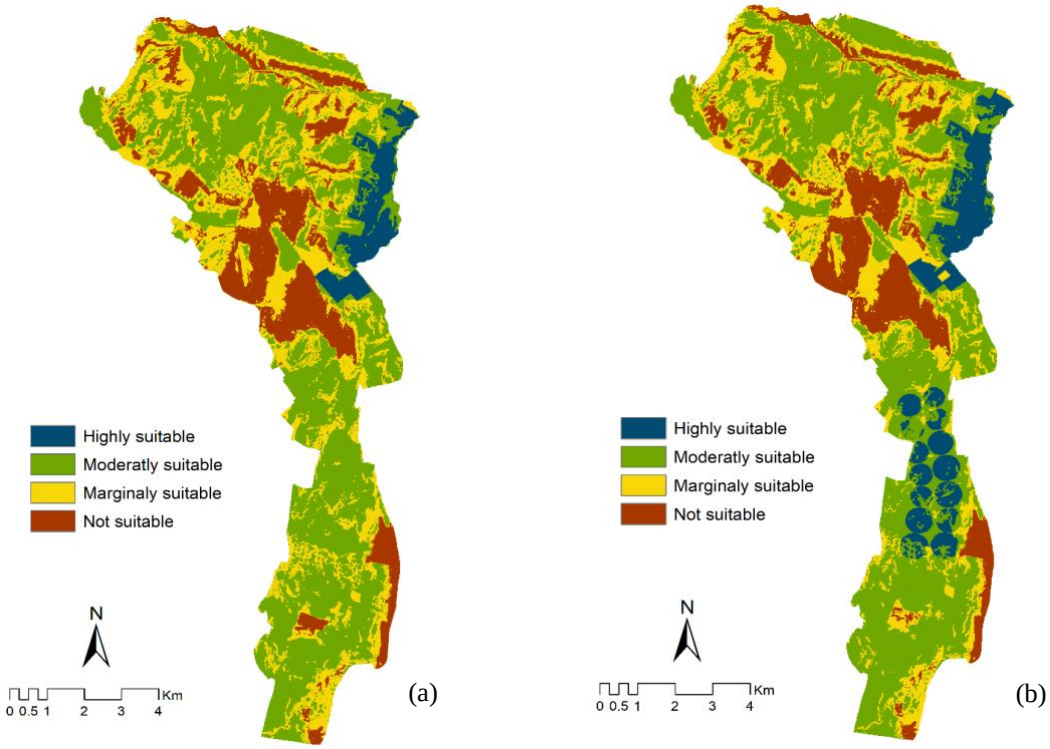

Figure 6. Land suitability maps of wheat for 2012 (a) and 2020 (b)

Table 4. Distribution of suitability classes for 2011 and 2020

Suitability level	2011		2020		Difference	
	Area (ha)	%	Area (ha)	%	Area (ha)	%
Highly suitable	402	4.8	805	9.7	+403	+4.9
Moderately suitable	4178	50.2	3868	46.5	-310	-3.7
Marginally suitable	2390	28.8	2297	27.6	-93	-1.2
Unsuitable	1350	16.2	1350	16.2	-	-

Table 5. Comparison of land use and suitability classes in the study area

Land use	Suitability level							
	Highly suitable		Moderately suitable		Marginally suitable		Unsuitable	
	ha	%	ha	%	ha	%	ha	%
Rainfed farming			3471	89.7	1606	69.9	23	1.7
Irrigated farming	805	100	248	6.4				
Rangeland			70	1.8	490	21.3	462	34.2
Natural vegetation					62	2.7	30	2.2
Degraded areas					90	3.9	828	61.4
Fruit orchard			67	1.7	28	1.2		
Service area			12	0.3	21	0.9	7	0.5

In this study, the potential suitability of dry and irrigated agricultural lands for wheat cultivation was determined. In the land suitability analysis, the Fuzzy model, AHP and GIS were used together. There are critical stages in the modeling process. By applying the fuzzy set model, the criterion values were transformed into membership degrees. Thus, different land features were converted into a standard index. Model inputs were determined and their weights were calculated. With the applied model approach, it is aimed to create a sustainable land management.

Conclusion

In this study, fuzzy set model, GIS and MCE techniques were applied in land evaluation analysis for wheat cultivation. Thirteen factors were selected, including soil, topographic and water availability and their grade of membership functions were calculated by the fuzzy set model. Suitability analysis was performed by integrating GIS-based spatial data with MCE. AHP technique is used in the relative weighting of the criteria. Although a large number of model inputs, the field was evaluated quickly and accurately with the hierarchical structure of the model.

Most of the model inputs include continuous data structures such as slope (0-20 %). Sometimes, in cases where there are data structures of different sizes, it may be difficult to correlate these data with land suitability. In this case, the Fuzzy set is standardized in the 0-1 range by converting all data into membership functions. A value of 1 indicates that the land is suitable for 100 % wheat cultivation. While integration of MCE and GIS is very useful for land assessment, the selection of assessment factors, factor boundary values, and weight ratios have a direct effect on outcomes.

A comprehensive definition of the land characteristics within the natural and continuous structure of the land was made by applying the FM approach. Therefore, the suitability map shows a more accurate result (Burrough 1989). The Fuzzy method is useful in grading the criteria in which the land characteristics show continuity and variation. Moreover, the FM model was effective to create standardized criteria maps. Therefore, the final map provides a more realistic result as the ecological conditions are taken into consideration. The accuracy of the results mainly depends on the weight assignments by selecting the correct land features. The critical point of the fuzzy methodology in crop suitability analysis is the determination of the class centers, transition values and weight values of membership functions. The weakest part of the fuzzy set method used for land suitability classification is the determination of the membership functions and the crossover point value. On the other hand, in the assignment of criterion weights, attention should be paid to determining the effective and restrictive criteria according to the crop growing requirements.

Remote sensing data were useful in spatial analysis between potential suitability areas and the existing land use type, and in determining the land use changes among the years. This information ensures optimum use of the land and the correct future land use preferences. The land suitability index map provides basic data to decision-makers by revealing the physical analysis of the area. In this study, vegetation change between 2001-2011 and 2012-2020 was determined with NDVI values obtained from Landsat satellite images. In addition, the effect of temporal difference on land use and land suitability has been evaluated.

The quality of the investment in the land, such as irrigation investments, necessary financial support and facilities, will also have a positive impact on the increase in land suitability potential. In this context, irrigation availability is very important in semi-arid climatic conditions, it should be evaluated with soil and topographic conditions.

The land suitability index is useful for revealing the variability in yield of the crop depending on the land characteristics (Dedeoglu and Dengiz 2019; Sharififar et al. 2016; Braimoh et al. 2004). With this approach, it can be ensured that optimal land use planning is made by determining the places where the land is advantageous to reach the highest yield. However, the high correlation between crop yield and Fuzzy method (Tang et al., 1992; Van Ranst and

Tang, 1999; Braimoh et al., 2004; Meleki et al., 2010; Keshavarzi and Sarmadian, 2009, Mohammadrezae et al. 2014) will provide a more accurate estimation of yield in the field.

With the model approach in this study, it is possible to grade the land characteristics in a way that reflects the land conditions more accurately and to reduce the uncertainties. The obtained suitability index map can serve a basis for the decision support tool in the sustainability, optimum use and planning of the land.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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The effect of biological periods and number of individuals on the damage amount of *Leptinotarsa decemlineata* Say (Coleopteta: Chrysomelidae)

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Abstract

In this study, the relationships between the amount of damage and the phenology of the potato plant, and biological periods and the number of individuals of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) were investigated. For the relationship between the different biological stages of the insect and the amount of damage, individuals were allowed to be fed for 24 hours. The most fed in biological period on the potato plant was the third larval stage of *L. decemlineata* while the least period was recorded as adult individuals. For the relationship between the phenological period of the plant and the amount of damage, potato plants with a length of about 20 and 40 cm were used. Adult individuals of *L. decemlineata* averaged 0.46 g in leafy branch pieces of a 20 cm potato plant, and 0.36 g on average in 20 cm leafy branch pieces from the tip of a 40 cm potato plant. It was determined that adult individuals of *L. decemlineata* feed more on leaves of young potato plant. For the relationship between the number of individuals and the amount of damage, experiments were set up with adults in groups of 1, 2, 3, 4 and 5, respectively. While a single adult individual of *L. decemlineata* cause an average of 0.15 g feeding damage on the leafy branch parts of the potato plant, it was recorded as 0.13 g for two individuals, 0.24 g for three individuals, 0.21 g for four individuals, and 0.33 g for five individuals, respectively.

Keywords: *Leptinotarsa decemlineata*, Behavior, Potato, Damage amount

Introduction

Leptinotarsa decemlineata Say (Coleoptera: Chrysomelidae) causes significant damage to many plants from the Solanaceae family in the world and in Turkey (Atak, 1973). Although the best host of this pest is potato, it can also cause damage on tomato, pepper and some weeds from this family (Atak, 1973; Has, 1992; Hare, 1990). Adults and larvae of potato beetle cause significant damage by feeding on all green parts of the potato plant. It has been noted that it causes significant damage to the plants, especially in the early development stages when the high population level is reached with the pest. In addition to the damage caused by direct feeding, this pest is also effective in the spread of potato brown rot, potato ring rot and spindle tuber viroid, which are important diseases of potatoes (Yuceer, 2011; Anonymous, 2017). If there is any struggle against this pest, the damage ratio can reach 100% (Has, 1992; Alyokhin et al., 2008; Cam et al., 2012).

In this study, the relationships between the phenological period of the potato plant, the biological period of *L. decemlineata*, the number of individuals and the amount of damage of the pest were separately investigated. For this purpose, it was presented that biological period of *L. decemlineata* caused more damage, phenological period the potato plant was fed more and how the increase in the number of individuals of *L. decemlineata* affected the change in the amount of damage.

Material and Method

Plants and pest culture

Potato (*Solanum tuberosum* L.) tubers belonging to the Agria potato variety were planted in 1.5 l pots with a 1:1 soil: peat mixture. Daily care and irrigation were carried out when needed. During the growing of plant, no chemical control was made against disease, pest or fertilization. Adult individuals of *L. decemlineata* (Coleoptera: Chrysomelidae) were collected from potato growing experiment area in Isparta University of Applied

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Sciences, Faculty of Agriculture in May 2020. Collected adult individuals were brought to the laboratory in plastic culture containers and placed on 30x50x60 cm sized potato plants in a plexiglass cage covered with tulle on the sides and top. The eggs laid by the adults fed on these potato plants were taken from the cage together with the leaf on which they were laid, and placed in a 5x8x12 cm plastic culture container with blotting paper at the bottom. Larvae hatched from these eggs were infested on potato plants in another plexiglass cage. In order to increase the population of the pest and to ensure mass production, potato plants used as food were renewed when necessary.

Establishment of Trials

The Relationship Between the Biological Period and the Amount of Damage

Experiments were carried out according to the randomized plot design with 10 replications. All larval and adult individuals of *L. decemlineata* taken from cages where the insect is mass-produced were used in the applications. Insects of different biological periods taken from the mass-produced cages were transferred into a 5x8x12 cm plastic culture container with blotting paper at the bottom as one for each according to their biological periods. Then, weight measurements of leafy branch pieces taken from the potato plant were made with an electronic precision scales (Kern ABC 220-4 m, max.=220 mg, min.=10 mg, e=1 mg, d=0.1 mg). The cut parts of the plants were placed in 5 ml eppendorf tubes containing water and fixed in eppendorf with the assistance of cotton.

The prepared leafy twigs were placed in plastic culture containers containing different biological stages of the insect, as food. After the different biological stages of the insect were allowed to feed for 24 hours, the re-weights of the leafy branches given as food were measured with precision balances and recorded.

The Relationship Between the Plant Phenology and the Amount of Damage

Experiments were carried out according to the randomized plot design with 10 replications. Potato plants grown in 1.5 l pots with a 1:1 soil: peat mixture was carried out with plants reaching a length of approximately 20 cm and 40 cm. The weights of leafy branch pieces taken separately from plants with 20 cm and 40 cm lengths were made and the cut parts of the plants were placed in 5 ml eppendorf tubes containing water and fixed with the help of cotton so that they do not dry out. These tubes, in which plant parts were placed, were placed in a 5x8x12 cm plastic culture container with blotting paper at the bottom according to the different phenological periods of the plant parts taken from the plants. Then, the adult individuals taken from the insect mass production cages were transferred to the culture containers, one for each. After the pests were allowed to feed for 24 hours, the weights of the leafy branches given as food were recorded with precision scales again.

The Relationship Between the Number of Individuals and the Amount of Damage

Experiments were carried out in a randomized plot design with 5 replications. In the applications, the weight measurements of the leafy branch pieces taken from the potato plant were made. Then, the cut parts of the plants were placed in eppendorf tubes containing water and fixed with the help of cotton so that the leafy branch pieces taken from the potato plant did not dry out. These tubes were placed in a 5x8x12 cm plastic culture dish with blotting paper at the bottom. Adult individuals taken from cages where the insect was mass produced were placed in groups of 1, 2, 3, 4 and 5 in culture pots containing one leafy branch piece, respectively. After the pests were allowed to feed for 24 hours, the re-weights of the leafy twigs given as food were measured and recorded.

All experiments with plant and pest production were carried out in a climate room with 25°C temperature, 60±5% relative humidity and 16 hours of light and 8 hours of darkness.

Data analysis

After the analysis of variance (ANOVA) conducted on data which obtained from this trial, Tukey's HSD multiple comparison test was applied ($P \leq 0.05$). Two-way t-test (Paired-samples t-test) was applied to the data to determine the relationship between plant phenology and amount of damage ($P \leq 0.05$). In addition, correlation and regression analysis ($P \leq 0.01$) between the number of individuals and the amount of feeding obtained in grams were performed. Statistical analyses were performed by IBM® SPSS® Statistics (Version 20.0, August 2011, SPSS Inc., Chicago, Illinois, USA) package program.

Findings and Discussion

The Relationship Between the Biological Period and the Amount of Damage

The feeding amount of *L. decemlineata* on the potato plant was recorded as 0.10 g for the first larval stage, 0.12 g for the second larval stage, 0.30 g for the third larval stage, 0.14 g for the fourth larval stage and on average as 0.08 g. Third larval stage had the highest amount of feeding on the leafy branch parts of the potato plant and a statistically significant difference was found between the other periods. There was no significant difference between the first larval stage of *L. decemlineata* and the adult stages in terms of the amount of feeding on the potato plant.

The feeding rates of the second larval stage and the fourth larval stage of *L. decemlineata* were found to be close to each other on the potato plant. The feeding amount of the third instar larvae of *L. decemlineata* on leafy stem parts of the potato plant is higher than the first larval stage and adult stages. Likewise, the feeding amount of the third larval stage is higher than the feeding amount of the second instar and fourth larval stage, and the difference between the feeding amounts is important. In this study, it was determined that the second and fourth larval stage of *L. decemlineata* fed more than the adult and first

instar larvae. While the third larval stage of *L. decemlineata* were the most fed biological period on the potato plant, the least fed period was recorded as the adult individuals (Figure 1).

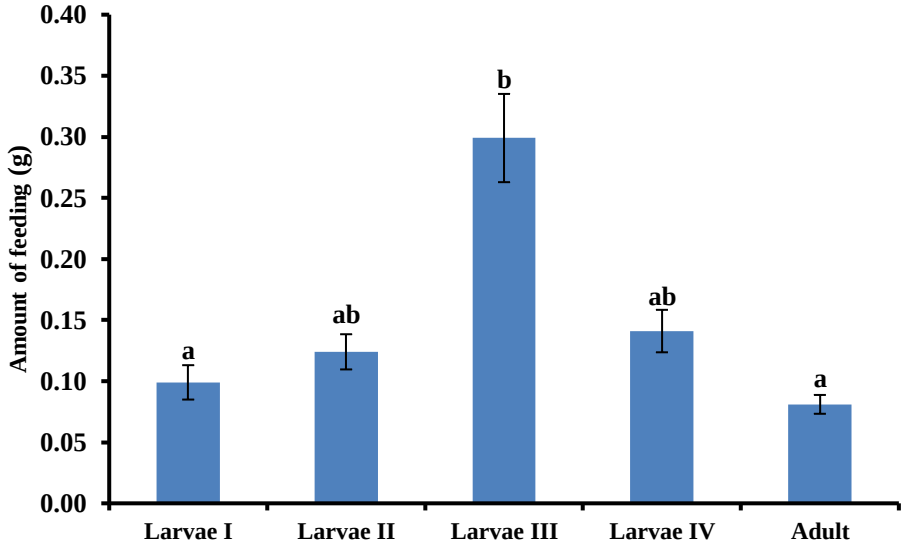

Figure 1. Feeding amounts of different biological stages of *Leptinotarsa decemlineata* fed on potato plant in 24 hours (The difference between the means (±standard error) represented by columns with the same letter is statistically insignificant (Tukey's HSD test P≤0.05; F = 3.660; sd= 4.45; P = 0.012).

Yaglıkcı and Karsavuran (2011) recorded the highest average live weight of *L. decemlineata* fed on potato plants in the fourth larval stage as 155.91 mg. This period was followed by the third instar larvae with 79.12 mg, the second larval stage with 27.25 mg and the first larval stage with 10.50 mg. The area consumed by *L. decemlineata* on potato leaves in different biological stages was recorded in the fourth larval stage with a maximum of 328.39 mm^2. This period was followed by preoviposition with 317.54 mm^2, oviposition with 281.75 mm^2, young male with 280.41 mm^2 and third larval stage with 119.02 mm^2 (Ozsarı, 2018). Noronha et al., (2002) noted in their field study that the damage potential of *L. decemlineata* increased during the third-fourth larval stage and the first adult emergence. In another study, the consumption amount of the second larval stage of *L. decemlineata* at 25°C was recorded as 1,313.32 mg. The consumption amounts of the third and fourth larval stage of the pest were determined as 1.097.72 and 711.22 mg. It was determined that the adults in the adult period are in the same statistical group with the consumption amount of 850.52 mg of the fourth larval stage (Cınar, 2020).

The Relationship Between the Plant Phenology and the Amount of Damage

The relationship between plant phenology and damage amount of *L. decemlineata* fed on potato plant is given in Table 1.

Table 1. Feeding amounts of *Leptinotarsa decemlineata* fed on potato plants in different phenological periods in 24 hours (g)*

	Amount of feeding ± SE	t	sd	P
Plants 0-20 cm tall	0.46±0.085	1.108	9	0.297
Plants 20-40 cm tall	0.36±0.0230			

**Two-way t test (Paired-samples t test) (P≤0.05) was used.

Adult individuals of *L. decemlineata* fed on potato plant in different phenological periods average was recorded as 0.46 g on average in leafy branch pieces of a potato plant with a size of about 20 cm, and the amount of feeding in leafy branch pieces 20 cm from the tip of a 40 cm potato plant as 0.36 g. It was determined that the adult individuals of *L. decemlineata* were fed more on the leaves of the young potato plant and there was no statistically significant difference between the feeding amount on the leaves of the partially older potato plant. In a study, it was determined that young leaves of *L. decemlineata* preferred more than old leaves, respectively, 55.00% for the first larval stage, 64.22% for the second larval stage, 81.67% for the third larval stage, 54.17% for the fourth larval stage and 65.00% for individuals (Telli, 2012).However, it was determined that feeding on young leaves was high with weight gains between the larvae transitions of the potato beetle (Cibula et al., 1967). In females

of *L. decemlineata* fed with old potato leaves, symptoms such as immobility and reproductive arrest were recorded (de Wilde et al., 1969).

The Relationship Between the Number of Individuals and the Amount of Damage

While a single adult individual of *L. decemlineata* causes an average of 0.15 g feeding damage on leafy branch parts of the potato plant, the average feeding damage was recorded as 0.13 g for of two individuals, 0.24 g for three individuals, 0.21 g for four individuals, and 0.33 g for five individuals. According to the results of variance analysis on amount of damage to the adult individuals of *L. decemlineata* fed on the potato plant. The relationship between the number of individuals and the amount of damage variables is significant at the P<0.01 level. The functional expression of this relationship is $F(1, 18)=312.556$; P<0.01.As a result of the correlation analysis, it was found that the correlation coefficient between the number of individuals and the amount of damage was $R_{(\text{Number of individuals, Amount of damage})}= 0.883$. According to this correlation coefficient, the relationship between the number of individuals and the amount of damage is accepted as a strong and positive relationship. Accordingly, it can be said that the increase in the number of individuals fed on the potato plant may cause a significant increase in the amount of damage. According to the coefficient of determination obtained as a result of the correlation analysis, approximately 78% of the damage amount varies depending on the number of individuals. The regression equation obtained as a result of the regression analysis; Damage amount=0.08+0.04*Number of individuals. As a result of the study, it has been determined that there is a strong relationship in terms of the number of individuals and the amount of damage and this relationship is positive. In addition, it has been observed that the amount of damage will increase as the number of individuals increases up to a certain level. It was seen that there is a correlation between the number of individuals and the amount of damage caused by *L. decemlineata* (Figure 2).

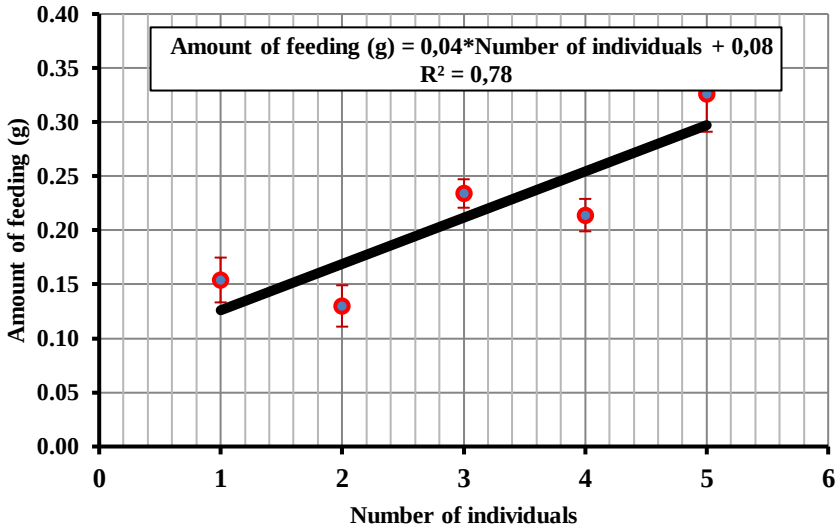

Figure 2. Linear regression of feeding amounts of *Leptinotarsa decemlineata* in different numbers of individuals fed on a potato plant in 24 hours and the correlation between the number of individuals and the amount of feeding (P≤0.01).

Results

In the study, it was founded that the feeding amounts of different biological periods of *L. decemlineata* on the potato plant differed. In this experiment, it was seen that while the third larval stage of *L. decemlineata* were fed the most, there was no significant difference between the feeding amounts of the other periods. It was observed that there was no statistical difference in terms of feeding amounts on leafy branch pieces taken from approximately 20 cm and 40 cm potato plants of *L. decemlineata* in the experiment using different plant phenologies. In the experiment conducted on the relationship between the number of individuals and the amount of damage of *L. decemlineata*, it was determined that there was a relationship between the number of individuals and the amount of feeding ($R^2_{(\text{Number of individuals, amount of damage})}= 0.78$). It was observed that there was a linear increase in the amount of feeding as the number of individuals increased.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Force and Energy Requirement for Cutting of Corn Stalk and Cob

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Abstract

In this study, the cutting force and energy requirement of corn (Zea mays L.) stalk at different internodes (diameter), from the bottom to the top internode positions for Pionner 2105, Pioner 1570, KWS and MAY 75 varieties, were determined. The mean diameter of internodes varied between 11.28-19.00 mm from top to bottom. Also, cob breakout force and energy requirements were determined for these varieties. According to test results, the cutting force requirement of the varieties were found statistically different. While the highest cutting force requirement was found in MAY75 variaty as 504.79 N and Pioneer 2105 as 537.80 N, the lowest values were obtained in the KWS variety as 409.50 N. Similar results were found for cutting energy values. While, there were no found difference between MAY 75 and Pioneer 2105 varieties, cutting energy requirements were found statistically different ($p<0.01$) among the other varieties. The highest cutting energy requirements has been found in Pioneer 2105 and MAY75 varieties, followed by Pioneer 1570 and KWS variaty, respectively. The lowest value was obtained in the KWS variety as 3.83 J. The difference between internodes was found statistically significant. The cutting force values varied between 806.00 N and 203.00 N, the cutting energy varied between 7.91 Joules and 1.56 Joules depend on internodes. The highest cutting force and cutting energy values were obtained at the first node as 806.00 N and 7.91 Joules, these values decreased as the diameter decreased from the bottom to the top. Tukey test results showed that there were no significant differences among the varieties in terms of both breakout force and breakout energy. However, the highest values were obtained as 382.7 N and 15.50 J in Pioneer1570 variety, while the lowest values were obtained as 319.0 N and 9.830 J in Pionner 2105 variety.

Keywords: Corn stalk, Cob breakout force, Cutting force, Cutting energy

Introduction

Corn (Zea mays L.) has a great importance in human and animal nutrition and it is the second most cultivated and produced in the world because of high consumption, quality, and food value after wheat and barley. Also, one of the most important agricultural residues is corn stalk. Corn stalk is produced huge quantities worldwide in relative the other crops. Because, it is the richest regenerative resource and offers huge potential as a renewable and domestic feedstock for bio-energy and fiber production and it is consists of rind, a high content of lignin, pith, leaf, etc., and the chemical components of the different parts vary greatly (Klingenfeld, 2008; Zhang et al.,2016). However, it is difficult to digest for the animals such as ruminants. So, rich corn stalk resources have not yet been effectively utilized. Plenty of them are burned off in farmland, and not only are the resources wasted, but the fires also leave the natural environment damaged. Therefore, for the most effective utilization of the different parts of corn stover resources, each part of corn stalk requires effective separation. The cutting characteristics of corn stalk are important parameters in the process of the separation of rind and pith (Zhang et al.,2016; Zhang et al.,2017). Information on plant properties and the power or energy requirement of equipment has been very valuable for selecting design and operational parameters of the equipment (Persson, 1987). Such information is needed for the design of corn harvesters and chopper, assuring appropriate machine functions and an efficient use of energy. So, knowing the stem cutting force energy required for cutting plant stalks and corn cob breakout force and energy are important parametrs for both machine design and the parting of the stem. Crop stem cutting

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is an inevitable process in harvesting, and it is a process that can result in mechanical failure. Although it depends on the structure and strength of the plant material, the epidermis is the outermost protective tissue of stem structure. As the stem is mostly fibrous matter, the length of harvest period affects the fiber hardness and moisture content of the crop. The fiber fracture is an important part of the shear failure process (Persson, 1987; Lien and Liu., 2015).

Until now, several studies have been carried out to investigate mechanical properties of plant materials by different researchers in the worldwide for various plants and purposes such as designing a new harvest machines, designing of biomass processing equipment, chopping of plant stalk and studying how to utilize fibers of plants.

Recently, many research was directly conducted on mechanical properties with the corn plant stalk. Corn stalk is a complex material from the point of view of mechanical strength. For example, mechanical properties such as shearing resistance of corn stalk were investigated by Mani et al. (2006) and chopping of corn stalk by Igathinathane et al. (2011). Kaliyan and Morey (2009) studied the densification process of maize stover grinds by using a non-linear elasto-visco-plastic model under uniaxial compression conditions. The mechanical properties of the rind of corn stalk were studied by Chen et al. (2012), whose purpose was to maintain the rind integrity during the separation of corn stalk rind and pith. Reddy and Yang (2005) measured the tensile properties of maize stalk for determine to suitable for producing various textile products. Prasad and Gupta (1975) measured the cutting force and energy for cutting corn stalks for determine to suitable knife type at different loading combinations. Taghijarah et al. (2011) studied on shearing characteristics of sugar cane stalks as a function of the rate of the applied force. Igathinathane et al. (2010, 2011) tested corn stalks using a linear knife grid size reduction device to determine ultimate shear stress and cutting energy at different moisture content. Chen and Qu (2017) and Zhang et al. (2017) studied on physical and mechanical properties of corn stalk for to provided the data for optimizing the corresponding mechanical parameters. According to Wright et al. (2005), for successfully a designing and developing new systems requires knowledge of the mechanical properties of maize stover. A similar expression is made by Kovács and Kerényi (2019). According to this reserchers, in order to optimize the design and working parameters of agricultural machinery related to harvesting, knowledge about the physical properties and mechanical behaviour of harvest-ready maize is required. Womac et al (2005) studied on shearing characteristics of biomass for size reduction. They used A Warner-Bratzler shearing device in a universal test machine for evaluate of corn stems and cob characteristics. They were studied with different knife bevel angles (30° and 45°) at a fixed cutting speed of 254 mm/min. Biomass cutting energy was determined on a stem cross-sectional area basis (specific cutting energy, kN/m). Mean specific cutting energies for corn stover were found 28 and 34 KN/m for 30° and 45° knife bevel angles, respectively. Thus, the shallower 30° bevel angle required less cutting energy.

As can be seen from previous studies, size reduction of agricultural stalk also is an important prerequisite to produce forage and biomass energy. Size reduction/grinding is considered to be one of the most energy-intensive or energy in efficient operations (Mohsenin, 1986; The efficiency of the size reduction has typically been assessed through the amount of cutting force and energy required (Igathinathane et al.,2010; Azadbakht et al., 2015; Allameh and Alizadeh.,2016; Vu et al., 2020). It has been found that equipment using shear mode for size reduction may hold promise for improved energy efficiency (Igathinathane et al.,2010). So, there is need to improve harvesting, processing and chopping of corn stalks. In general, the cutting knife of a harvesting machine cut the plant material and separates it into different parts by external force. For this, some cutting properties need to be known. Because varieties of plants can show different characteristics each other. Therefore, plants energy needs can be also different according to variety (Sessiz et al.,2013).

Turkey is one of the leading corn producers in the world with terms of climate, soil and environmental conditions. Southeastern part of Turkey is among the important corn producers. Espicially, corn is producued in Şanlıurfa, Diyarbakır and Mardin provinces and corn harvesting is performed with combine- harvester. During harvesting by a combine-harvester, maize is mainly processed by the maize header; only maize ears are threshed inside the machine. The whole plant is cut and pulled down to gather the maize ears that are conveyed into the machine, while the rest of the maize plant (stalk, leaves, husk, tassel) is chopped. The wet mass of the stalks is more significant than the wet mass of the leaves, husk and tassel (Igathinathane et al., 2010; Kovács and Kerényi., 2019).

The objective of this study was to determine cutting force and cutting energy requirements to cut corn stalk as a function of internodes of stalks along bottom to top regions. Also, breakout force and energy of the cob from stalk was to determine for four different corn varieties. The purpose is to provide a scientific basis for designing a corn and chopper machine with high efficiency and low power consumption.

Materials and Methods

Corn Stalks and Cobs

Samples of whole corn plants used in the cutting tests were obtained from farmers corn fields in the same location (location: 38.066 ° N, 40.2715 ° E) in Çınar district of Diyarbakır province. Four corn varieties, Pionner 2105, Pioner 1570, KWS and MAY75 were selected for stalk cutting and cob breakout force tests. The samples were taken during

the harvesting season on 20 October 2020. In order to prevent moisture content, the samples were bagged according to variety and stored in nylon sack for 2 months in the Laboratory of Agricultural Machinery and Technologies Engineering Department until the time of tests. Before started to tests, 10 plants for each variety were selected and seperated according to stem diameters and tested for cutting force and energy (Figure 1). It has been considered that the plant stem diameters are the same as possible. To determine cob breakout force and energy, the same plan's cob was used. The corn cob used in the research had a uniform shape and size and had a mass of 293-390 g, a maximum diameter of 49-53 mm and a length of 207-295 mm.

Preparation of test material

The mechanical testes were conducted two stages. In the first stage, the stalk cutting force and energy were determinded depend on nodes. The second stage, breakout force the cob of corn from stalk were determined. The corn stalks with cobs for the mechanical tests were obtained from whole plants randomly selected and harvested by hand cutting at ground-level for each varieties during the harvesting season and then transported to laboratory for tests. Before tests, seven nodes, different diameter (cross-sectional) sizes, were prepared from the bottom sections of the stalks to top for each varieties, respectively. The diameter of the stalk decreases towards the top of the plant due to different physical properties at different heights due to cross-sectional heterogeneity. Internodes were labeled from IN1 to IN7, respectively (Figure 1). It has been taken to ensure that stem diameters and moisture contents are the same as possible. Tests were made in 9 repetitions. Nine diameter measurements were taken for each sample after which their average was calculated. Digital caliper with an accuracy of 0.01 mm was used to measure the diameter values. Average diameter values measured for each variety from the first node to the seventh node are given in the Table 2. Cutting force and cutting energy were determined based on these diameter values of stalks. The mean diameter varied between 11.28-19.00 mm from top to bottom.

After testing, the plants samples were weighed, oven dried, and reweighed to obtain moisture content. Moisture content of the samples was determined according to ASABE Standards by the oven -drying method 50 g of each sample at 105 ° C for 24 hours (ASABE Standards, Sec. 358.2, 2008). The four varieties were tested. The corn stalk moisture contents during the tests varied between 56.40% and 59.30%. It was measured as 59.30% for Pionner1570 variety, 58.00% for KWS variety, 56.40% for MAY75 variety and 57.45% for Pionner 2105 (Igathinathane et al., 2010).

The cutting and cob detachment tests

An Instron universal (Llyod LRX plus) testing machine was used to measure the cutting force, cutting energy and force-displacement. The testing frame is also shown in Figure 2. The maximum cutting speed of the machine, 5 mm s-1, was used for all tests. In the experiments, a knife with a straight cutting edge was used for all varieties. Trials were performed at 90° blade cutting angle. In the tests, the stalks were placed under the cutting platform and loaded at both ends, keeping them fixe (Figure 2). As you shown in Figure 2, The same device was used to determine the breakout force and energy of corn cobs (Figure 3).

Figure 1. The samples of corn stalk and cobs are used for experiment.

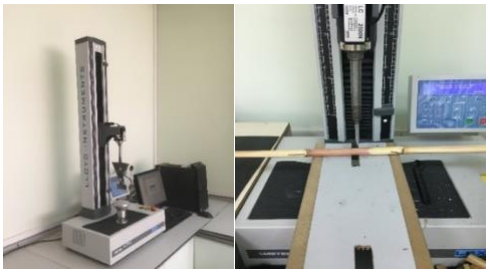

Figure 2. Universal testing device used in cutting experiments.

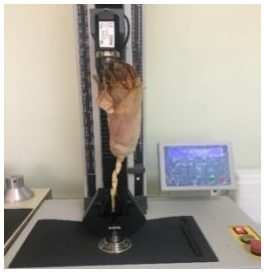

Figure 3. Universal test device used in cob breakout force experiments.

The stalk cutting energy and cob detachment energy was calculated by measuring the surface area under the force-deformation curve via material testing machine by using a Nexygen data analysis computer program (Yore et al., 2002; Chen et al., 2004; Kocabıyık and Kayışoğlu, 2004; Nazari et al., 2008; Ekinci et al., 2010; Hassan-Beygi et al., 2010; Zareiforoush et al., 2010; Heidar and Chegini, 2011; Sessiz et., 2013; Ozdemir et al., 2015; Ozdemir and Sessiz, 2018; Nowakowski, 2016; Pekitkan et al., 2018; Oztürk et al., 2017 Sessiz et al.,. 2018). A computer data acquisition system recorded all force-displacement curves during the cutting process.

Experimental design and data analysis

Statistical procedure of this study was planned as a completely randomized block design. Independent variables were selected internode number and crop

variety. Dependent variables were peak stalk cutting force, cob detachment force. Cutting force and energy properties were determined with nine replications and nine internodes of stalks and cob detechman force from stalks for four varieties. Analysis of variance (ANOVA) was performed to examine the main effects of experimental factors and their interactions. The means were compared at the 1 and 5 % levels of significance using the Tukey multiple range tests in JMP software, version 11.

Results and Discussion

Corn stalks cutting force-deformation characteristics

Cutting energy of a plant stem can be estimated from the relationships between the force of cutting the stem and the displacement of the knife (force–displacement curves) (Chen et al., 2004). Typical force-time characteristics of corn stalks (left side) and cop detachment force from plant (right side) in this study are shown in Figure 4. The force-time curves (force–displacement curves) is shown that, at the beginning, cutting force and breakout force increased from zero at the moment of initial contact between the knife and the stem, and then decreased due to the failure in stem structure (collapse of the hollow core). The compression continued along with cutting as the knife moved. When the force reached to peak point, cutting operation took placed. Then, the force dropped as the cutting was completed. That is, the first peak correspond to the biological yield point at which stalk damage was initiated. The second peak (upper yield) corresponds to maximum force (Figure 4). After reaching the upper yield, the force suddenly decreases with the displacement increasing. After, the second low peak, cutting or detachment has occurred.

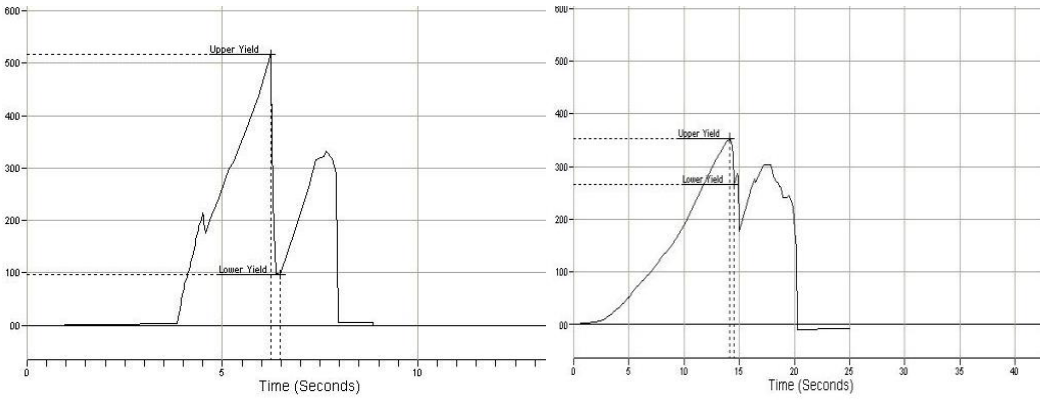

Figure 4. Typical force-deformation characteristics of corn stalks (left side) at various nodes and cop break force (right side).

Stalk cutting force and energy depending on the variety

Measured mean cutting force and energy data for each varieties are given in Table 1. As can be seen from the table, the cutting force requirement of the varieties was statistically different. While, no difference was found between MAY 75 and Pioneer 2105 varieties, cutting force were found statistically different ($p < 0.01$) among the other varieties. The highest cutting force requirements were found in MAY75 as 504.79 N and Pioneer 2105 as 537.80 N, followed by Pioneer 1570 as 464.60 N and KWS as 409.50 N varieties, respectively. The lowest value was obtained in the KWS variety. Similar results were found for cutting energy values. While, there were no found difference between MAY 75 and Pioneer 2105 varieties, cutting energy requirements were found statistically different ($p < 0.01$) among the other varieties. The highest cutting energy requirements were found in Pioneer 2105 and MAY75 varieties, followed by Pioneer 1570 and KWS variants, respectively. The lowest value was obtained in the KWS variety as 3.83 J.

Table 1. Mean cutting force and cutting energy

Varieties	Cutting Force* (N)	Cutting Energy Nm (Joule)
MAY 75	504.79 a	5.25 a
Pioneer 2105	537.80 a	5.15 a
Pioneer 1570	464.60 b	3.89 b
KWS	409.50 c	3.83 b
LSD	19.00	0.231

*means followed by the same letter in each column are not significantly different by Tukey multiple range tests at the 5% level.

Stalk cutting force and energy depending on the internodes (diameter)

The cutting force and energy values depending on the internodes (diameter) obtained from the average values for all varieties are given in the Table 2. As can be seen from the Table, there is no difference between the diameters of internodes of the varieties. The diameters of the internodes were almost the same. Therefore, only the average values of all varieties have been taken into account.

Table 2. Mean diameter values for each variety depending on the number of internodes.

Internodes	Diameter of nodes			
	Pioneer 2105	Pioneer 1570	KWS	MAY75
N1	18.70	19.66	19.06	18.59
N2	17.76	18.40	18.44	18.33
N3	16.83	17.47	17.85	17.87
N4	16.16	17.00	17.10	17.26
N5	14.88	15.26	16.63	16.32
N6	13.78	12.86	12.95	13.30
N7	11.25	10.74	11.23	11.90

As can be seen from the Table 3, as the internodes thickness (diameter) decreased from the bottom to the top, the cutting force and cutting energy requirement were decreased from bottom to top internode for all varieties. While the highest cutting force values varied between 806.00 N and 203.00 N, the cutting energy varied between 7.91 Joules and 1.56 Joules depend on internodes. The difference between internodes was found statistically significant. While the highest cutting force and cutting energy values were obtained at the first node, which is the lowest node, 806.00 N and 7.91 Joules, these values decreased as the diameter decreased from the bottom to the top. This effect is in agreement with a previous study on maize stalks, in which both the cutting energy and maximum cutting force were directly proportional to cross-sectional area (Prasad and Gupta, 1975). The effect of stem diameter on the maximum cutting force and cutting energy is consistent with Chen et al. (2004), who reported that both the cutting energy and maximum cutting force are directly proportional to the cross-sectional area of hemp stalk. The results have shown that the cutting strength and cutting energy related to plant physical and mechanical properties (Igathinathane et al., 2010). Similar results were reported by Yore at al. (2002) for rice straw, by Chen et al. (2004), Kronsberg et al. (2011) for hemp stalk, by Alizadeh et al. (2011) for rice stem, and by Ghahraei et al. (2011) for kenaf stems, by Sessiz at al. (2013) for olive sucker, by Ozdemir at al. (2015) for grape sucker, by Sessiz at al. (2015) for cane of some different grape variety, by Öztürk et al. (2017) for soybean stem. These results also are in agreement with Aydin and Arslan (2018) who determined shearing force and energy for shoot of cotton plant at different height of plant. Also, similar results were observed for cotton stalk by Pekitkan et al. (2018). Proper equipment design to accomplish the cutting will maintain the quality of the harvested product while minimizing the force and energy needed to accomplish the task (Srivastava et al., 2006; Sessiz et al.,2019).

Cob breakout force and energy depend on the varieties

The change in the force and energy of breakout the cob from the stalk depend on the varieties are given in the Table 4. As can be seen from the Table, Tukey test results showed that there were no significant differences among the varieties in terms of both breakout force and breakout energy. However, the highest values were obtained as 382.7 N and 15.50 J in Pioneer1570 variety, while the lowest values were obtained as 319.0 N and 9.830 J in Pionner 2105 variety.

Table 3. Cutting force and energy requirements depending on the stalk internodes

Internode	Diameter (mm)	Cutting force* (N)	Energy consumption of stalk (J)
1	19.00	806.00 a	7.91 a
2	18.23	722.00 b	6.67 b
3	17.50	610.00 c	5.87 c
4	16.88	450.00 d	4.37 d
5	15.77	354.00 e	3.14 e
6	13.22	270.00 f	2.21 f
7	11.28	203.00 g	1.56 g

*means followed by the same letter in each column are not significantly different by Tukey multiple range tests at the 5% level.

Table 4. Cob breakout force and energy depend on the varieties

Variety	Cob breakout force* (N)	Cob breakout energy (J)
Pioneer 1570	382.70 a	13.50 a
KWS	352.00 a	11.33 a
MAY 75	332.00 a	10.93 a
Pioneer 2105	319.00 a	9.830 a
LSD	3.95	2.07

*means followed by the same letter in each column are not significantly different by Tukey multiple range tests at the 5% level.

Conclusion

In this study, cutting force and energy requirement of corn stalk at different internode and cob breakout force and energy were examined for Pionner 2105, Pioner 1570, KWS and MAY 75 corn varieties. According to test results, as the internodes thickness (diameter) decreased from the bottom to the top, the cutting force and cutting energy requirement were decreased from bottom to top internode for all varieties. The cutting force requirement were found statistically different among varieties. While the highest cutting force requirement was found in MAY75 variety as 504.79 N and Pioneer 2105 as 537.80 N, the lowest values were obtained in the KWS variety as 409.50 N. Similar results were found for cutting energy values. While, there were no found difference between MAY 75 and Pioneer 2105 varieties, cutting energy requirements were found statistically different ($p<0.001$) among the other varieties. The highest cutting energy requirements has been found in Pioneer 2105 and MAY75 varieties, followed by Pioneer 1570 and KWS variety, respectively. The lowest value was obtained in the KWS variety as 3.83 J.

The difference between internodes was found statistically significant. The highest cutting force and cutting energy values were obtained at the first node as 806.00 N and 7.91 Joules, these values decreased as the diameter decreased from the bottom to the top. The cutting force values varied between 806.00 N and 203.00 N, the cutting energy varied between 7.91 Joules and 1.56 Joules depend on internodes.

Tukey test results showed that there were no significant differences among the varieties in terms of both breakout force and breakout energy. However, the highest values were obtained as 382.7 N and 15.50 J in Pioneer1570 variety, while the lowest values were obtained as 319.0 N and 9.830 J in Pioneer 2105 variety.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Analysis of bioactive compounds and antioxidant activities of cultivated garlic (*Allium sativum* L.) and red onion (*Allium cepa* L.) in Algeria

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Abstract

In all times, humankind has used several species of the genus *Allium* as food, spice, or herbal remedy. Some of these species have been cultivated, such as garlic (*Allium sativum*) or onion (*Allium cepa*). Today, their value for human health care is one of the most important aims of research. Up to now, many applications of *Allium* species are known for the use of phyto-pharmaceutical preparations. Therefore, the present study aimed to determine the phytochemical profile of cultivated garlic (*Allium sativum*), and red onion (*Allium cepa*) in Algeria, both quantitatively (total phenolic, total flavonoids, condensed and hydrolysable tannins contents) and qualitatively (phytochemical screening), to characterize the phenolic compounds using HPLC method and to evaluate the antioxidant properties using DPPH assay. Red onion gave the higher amounts of total phenolic compounds (86±1.00mg GAE/100g DM), flavonoids (43.33±0.57mg QE/100 g DM), condensed tannins (4.4±0.52 mg CE/100g DM) and hydrolyzable tannins (0.22±0.04mg TAE/100g DM) compared to garlic (45±1.00mg GAE/100g DM, 34.66±0.57mg QE/100g DM, 6.8±0.34mg CE/100g DM and 0.05±0.01mg TAE/100g DM) respectively. Five compounds were found in red onion extract and one compound in garlic extract after chromatographic analysis of the samples. Furthermore, red onion possessed the higher antioxidant activity (IC_{50}= 420.9±5.00 µg/ml) as compared to garlic (919.87±4.43 µg/ml). These findings provide ample evidence of the existence of bioactive compounds in garlic and red onion, both of which are rich in phenolics primarily flavonoids and tannins, have strong antioxidant activity, and can be further consumed directly or as food products.

Keywords: *Allium sativum* L., *Allium cepa* L., Phytochemistry, HPLC, DPPH, Algeria.

Introduction

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube et al., 2008). Garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) are the most important species of the Amarylidaceae family and, for thousands of years, have been used for their characteristic flavour as spices or food, or for their medicinal properties (Takahashi and Shibamoto, 2008).

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During the last years, *Allium* spices were among the most studied vegetables and aroused great interest for food industries. These interests appear from the encouraging results of the antioxidant capacity of some of their compounds, which have been to be comparable to and sometimes higher than that of synthetic antioxidants used in food industry particularly BHA (butylated hydroxyanisole,) and BHT (butylated hydroxytoluene) (Barlow, 1990).

According to previous studies, both vegetables play a role in lowering the risk of chronic diseases like cardiovascular disease, cancer and aging-related disorders in which reactive oxygen species are involved (Moreno et al., 2014). Their beneficial effect on health was attributed to high contents of biologically active phytomolecules, such as phenolic compounds, especially flavonoids, and several organosulfur compounds (Goldman et al., 1996).

Garlic and red onion are especially common in Algeria with a total production of 103.627 and 1.526.339 tons respectively (Bouhenni et al., 2019). However, few studies were carried out concerning these important plants, in this concept, the objective of this paper was to determine and quantify the phenolic contents (flavonoids and tannins) of cultivated garlic (*Allium sativum*), and red onion (*Allium cepa*) in Algeria using HPLC method, as well as investigate their antioxidant activity. The originality of this study consists in the fact that it is the first report on polyphenolic composition of methanolic extract of cultivated garlic and red onion in Algeria.

Materials and Methods

Chemical Reagents

Methanol, Folin Ciocalteu reagent, quercitin, aluminium chloride, catechin, sulfuric acid, tannic acid, hydrochloric acid, chloroform, chlorhydric alcohol, isoamyl alcohol, acetic anhydride, glacial acetic acid and DPPH were purchased from Sigma-Aldrich, U.S.A. Gallic acid and vanillin were obtained from Merck, Germany, sodium carbonate was from Acros Organics, Belgium, and ferric chloride was purchased from Alfa Aesar, Germany.

In HPLC analysis, Fortis column was obtained from Fortis Technologies Ltd, UK. The used solvents (acetonitrile and formic acid) were purchased from Merck, Germany. Concerning the standards; caffeic acid, chlorogenic acid, ferulic acid, trans- p-coumaric acid, gallic acid, rosmarinic acid, salicin, apigenin, quercetin, quercitrin, isoquercitrin, hyperoside, luteolin -7-O-glucoside, luteolin, kaempferol were from Phytolab, Germany and ellagic acid, salicylic acid, chicoric acid, naringenin, chrysin, myricetin were purchased from Merck, Germany.

Determination of total phenolic content

According to the procedure defined by Singleton and Rossi, (1965), the method of Folin-Ciocalteu reagent has been used to estimate the total phenolic content. 0.5 ml of varying concentrations of each used extract and 2.5 ml of Folin-Ciocalteu (1/10 dilution in water) were mixed with 1ml of sodium carbonate (20%). This mixture was incubated in the dark at room temperature for 30 min. The absorbance of the solution was measured at 765 nm using UV-Vis spectrophotometer HITACHI (Ratio Beam U-V 5100). A calibration curve was established using gallic acid as standard. The results were expressed as milligram of Gallic acid equivalent (GAE) per 100 g of Dry Matter.

Determination of total flavonoids content

The total flavonoids content of both extracts was determined using the aluminium chloride method as described by Zou et al., (2004). 1.5 ml of various concentrations of both extracts was mixed with 75µl of aluminium chloride solution and 0.5 ml of sodium acetate solution, the mixture was completed with distilled water until a volume of 2.5 ml. After an incubation period of 30 min at room temperature in the dark, the absorbance of the solution was measured at 415 nm using UV-Vis spectrophotometer. The results were expressed as milligram of Quercitin equivalent (QE) per 100 g of Dry Matter.

Determination of condensed tannins content

The analysis of condensed tannins was carried out according to Price et al., (1978). 1ml of each extract was mixed with 2.5 ml of 4% methanol vanillin solution and 2.5 ml of H_2SO_4. After 15 min, the absorbance was measured at 500 nm. Condensed tannin contents were expressed as milligram of Catechin equivalent (CE) per 100 g of Dry Matter.

Determination of hydrolysable tannins content

Hydrolysable tannins were estimated using method of Waterman, (1987). 500 µl of the extract was added to 3.5 ml of the ferric chloride solution. The contents were then quickly mixed and the absorbance read at 660 nm, 15 secs after the addition of the extract solution. Hydrolysable tannins content was expressed as milligram of Tannic acid equivalent (TAE) per 100 g of Dry Matter.

Phytochemical screening

Qualitative tests were realized to detect the presence of some secondary metabolites in plants extracts according to Trease and Evans, (1989); Sofowora (1993) (Table 1).

The analytical method used is high-performance liquid chromatography (HPLC), the identification of substances was performed according to their polarity in the solvents, the model of HPLC used for analytical control was: Shimadzu Nexera-I HPLC with autosampler and quaternary pump. Each extract was dissolved in methanol in a ratio of 1 part extract to 5 parts solvents. The extracts were analysed as such by injection into HPLC. The operating conditions are as follows: Column: silica gel-C18 type Fortis C18, 150 x 2.1 mm x 3 µm, Eluent: A = water, B = 0.1% formic acid, aqueous solution with pH = 2.5, and C = acetonitrile, Flow rate: 1 ml / min, Injected volume: 5 µl, Detector: DAD, spectrophotometric 220-400 nm, with chromatograms recorded at 254, 326 and 360 nm. The evaluation was based on a comparison of retention times and absorption maxima in the UV-Vis spectra. The resulting chromatographic profile is compared to standards (standard pure of

phytochemical molecules) injected into the same operating conditions as that of the sample. Retention time (Rt) of each component is determined by the integrator giving a peak on the chromatogram (Vlase et al., 2014).

Table 1. Phytochemical screening of garlic and red onion

Metabolites	Added reagent	Expected result
Flavonoïdes	KOH (50%)	Yellow color
Tannins	$Fecl_3$ (1%)	Blue coloration
Alcaloids	HCl 2%+ Wagner reagent	Brown precipitate
Sterols and triterpenes	Anhydride acetic + H_2SO_4 (98%)	Red color (surface) + Greenish fluorescence
Terpenoids	Chloroform + H_2SO_4	Reddish brown coloration
Saponosides	Distilled water	Formation of foam
Anthocyanins	Chlorhydric alcohol+ isoamyl alcohol	Reddish brown Coloration
Cardiac glycosides	Glacial acetic acid + $Fecl_3$(5%)+ H_2SO_4 (98%)	Brown ring
Reducing compounds	Fehlings (A+B)	Brownish-red precipitate

Determination of phenolic content by High Performance Liquid Chromatography (HPLC) analysis

Antioxidant activity

The antioxidant activity of extracts was measured with the DPPH method describing by Shimada et al., (1992). A solution of DPPH (0.1 mM) was freshly prepared by dissolving 4 mg DPPH in 100 ml methanol. Mother solution (1 mg/ml) was prepared and followed by serial dilution in order to obtain all increasing concentration needed (0.1, 0.2, 0.3, 0.4, 0.5 mg/ml), from each extract 1 ml of each prepared diluted extract was added to 1 ml of DPPH (0.1 mM). The solutions were then incubated for 30 min at room temperature in the dark, and the absorbance was measured at 570 nm. The antioxidant activity was calculated according to the following formula:

% inhibition= [(Acontrol - Asample) / Acontrol] × 100, where A control is the absorbance of DPPH solution without extract and A sample is the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%.

Statistical analysis

The data from phytochemical composition and antioxidant activity were analyzed with a statistical software program (SPSS version 20). Differences between plants were compared at $P < 0.05$ with One-Way ANOVA followed by Tukey's post hoc test in order to find the statistically significant differences. The assays were carried out with three repetitions and the results were expressed as mean values and standard deviation.

Results and Discussion

Phytochemical analysis

The results of extraction yield, total phenolic, total flavonoids, condensed and hydrolysable tannins content of garlic and red onion extracts were summarized in Table 2.

The extraction yield (mass of extract/mass of dry matter) was used as an indicator of the effects of the extraction conditions. According to the findings, the extract yield of garlic using maceration method and methanol 70% as solvent was higher (62.87±0.50 %) than red onion (57.38±0.56%).

In the present study, the results showed that red onion extract had a higher phenolic content (86±1.00 GAE/100g DM) than garlic extract (45±1.00mg GAE/100g DM). In addition, red onion extract had the highest total flavonoid content (43.33 ±0.57mg QE/100 g DM) compared to garlic extract (34.66 ±0.57mg QE/100g DM). In contrast to red onion (4.4 ±0.52 mg CE/100g DM), garlic has a higher value of condensed tannins (6.8 ±0.34mg CE /100g DM).

Table 2. Results of phytochemical analysis of garlic and red onion

Analysis	Garlic extract	Red onion extract
Extract yield (%)	62.51^{b}±0.50	57.35^{a}±0.56***
TPC (mg GAE /100g DM)	45^{a}±1.00	86^{b}±1.00***
TFC (mg QE /100g DM)	34.66^{a} ±0.57	43.33^{b} ±0.57***
CTC (mg CE /100g DM)	6.8^{b} ±0.34	4.4^{a} ±0.52**
HTC (mg TAE /100g DM)	0.05^{a} ±0.01	0.22^{b} ±0.04**

TPC: Total Phenolic Content TFC: Total Flavonoids Content CTC: Condensed Tannins Content HTC: Hydrolysable Tannins Content DM: Dry Matter

**** Significant at 0.001 or 0.1%*

*** Significant at 0.01 or 1%*

a, b corresponds to the homogeneous groups obtained by the post-hoc Tukey test for each parameter.

Our percentage yield of garlic extract was higher than previous studies findings (Park and Chin, 2010), (Ali and Mohsen Sabri, 2014) and (Bhanot and Shri, 2010) which were 2.46%, 6% and 7% respectively. According to Kallel et al., (2014), aqueous garlic extract has a higher percentage of extract yield (26.5%) than ethanolic and methanolic garlic extracts, which were 4% and 7% respectively.

Although, Park and Chin, (2010) reported a percentage yield of 52.38% for red onion extract, (Bhanot and Shri, 2010) reported a much lower percentage yield of 6.8%. Statistically, there was a significant difference between garlic and red onion (p=0.000), however, this difference can be due to variety diversity, growing conditions, ripening degree and climate (Kaoru et al, 2006). Also, the particle size and shape of samples in extraction

process are important factors that affect the yield extraction, another factor that may have affected differences in yield between garlic and red onion is sample pre-treatment (Ali and Mohsen Sabri, 2014). The highest extraction yield with aqueous solutions can be attributed to the addition of water, which increases the polarity of the solvents (Kim et al., 2004).

The total phenolic compounds content of garlic was approximately comparable to that found in many studies (Chekki et al., 2014) and (Jastrzebski et al., 2007); with 43.6 mg GAE/100g and 49.3 mg GAE/100g respectively, while the present result was significantly higher than that reported by (Nuutila et al., 2003) and (Sarafa et al., 2016) with values of 11.5 mg GAE/100g and 0.42±0.02 mg GAE/100g respectively. However, the results found in the studies of Lenkova et al., (2016), Park et al., (2009), Chekki et al., (2014) and Kallel et al., (2014) were significantly higher with 105.1±18.09 mg GAE /100g, 562.6±1.93 mg GAE/100g, 500-4360 mg GAE/100g and 2283±1.69 mg GAE/100g respectively.

Nuutila et al., (2003) found that Giant onion had a total phenolic content of 84.5 mg GAE /100g, which was close to the current result. Petropoulos et al., (2015) result was lower in the range of 8.05-10.8 mg GAE/100g. Although several studies have been carried out to estimate the amount of total phenolic contents present in red onion; Sarafa et al., (2016), Lu et al., (2011), Cheng et al., (2013), Skerget et al., (2009) and Singh et al., (2009) found a higher result than our result, with amounts of 103±0.00 mg GAE/100g, 428 mg GAE /100g, 571±0.20 mg GAE /100g, 6362±2.03 mg GAE /100g and 38470±5.0 mg GAE/100g respectively. The high total phenolic content of red onion compared to garlic (p= 0.000) may be due to differences in the method of sample extraction (e.g., solvent used), wherever, these contradictory results are most likely due to differences in the methodology and the experimental conditions used in the different studies (Nuutila et al., 2003).

In general, red onion had higher phenolic content than garlic; variations found between these two plants may be due to differences in their genetic composition and growing conditions, which have a strong influence on the levels of phenolic compounds (Soto et al., 2016).

Total flavonoids analysis revealed that garlic contains significantly more total flavonoids content than that reported by Soto et al., (2016) which was in the range of 7±0.007 - 11±0.02 mg QE/100 g. On the other hand, it was approximately similar to the findings of Chekki et al., (2014) and Shuxia chen et al., (2013), which were in the range of 0.42-59.5 mg QE/100 g and 7.5-67.5 mg QE/100 g, respectively. Kallel et al., (2014), Sarafa et al., (2016) and Moumen et al., (2016) found an increased amount; 60 mg QE/100 g, 113±0.01 mg QE/100 g, and 1521±0.93 mg QE/100 g respectively.

Significant variations in total flavonoids content were also found in red onion compared to previous studies of Soto et al., (2016) and Abuga (2014) with values of 8±0.008-18±0.033 mg QE/100 g, and 10±0.69 mg QE/100 g, respectively. Other researchers, Cheng et al., (2013), Sarafa et al., (2016), Skerget et al., (2009) and Singh et al., (2009) found higher contents; 165.8±0.41 mg QE/100 g, 366±0.01 mg QE/100 g, 1376±0.41 mg QE/100 g and 16520±3.2 mg QE/100 g, respectively.

TPC and TFC variability in garlic can be due to numerous cultivar characteristics, but clove size must be taken into account because it has an indirect effect on the final concentration of phenolic compounds (Lu et al., 2011). Different garlic cultivars had different phenolic contents, according to previous study (Chen et al., 2013). The present data revealed a highly significant difference in total flavonoids between the two plants (p=0.000), which can be explained by several factors, including experimental parameters and natural qualitative and quantitative variability in the raw material (Chen et al., 2013).

The presence of condensed tannins in garlic agreed with the report of Nwinuka et al., (2005) and Sarafa et al., (2016) with significant differences; 0.01±0.0mg CE/100g, 0.82±0.01mg CE/100g respectively. Moumen et al., (2016) observed that garlic methanolic extract showed the highest number of condensed tannins 3.01±0.39 mg CE/100g compared to aqueous and ethanolic extract; 1.35±0.5mg CE/100g and 0.69±0.2 mg CE/100g respectively.

Furthermore, a lower condensed tannins content was recorded in red onion in comparison with garlic (p=0.003), the present result was similar to Abuga (2014) result; 4.99±0.06 mg CE/100g, higher to Nwinuka et al., (2005) result; 0.01±0.01 mg CE/100g and lower to Sarafa et al., (2016) result; 9.82±0.02 mg CE/100g. This may be attributed to genetic and climatic factors rather than storage time, processing and extraction methods (Sarafa et al., 2016). Condensed tannins are water-soluble phenolic metabolites commonly found in almost all plants parts (Kunyanga et al., 2014).

For hydrolysable tannins contents, there was a significant difference between these two plants (p=0.002), these findings suggest that the level of hydrolysable tannins is greatly influenced by tissue type, solvents (different polarities), and extraction conditions (Saleha, 2019).

The results of the qualitative assay of samples were shown in Table 3. They revealed the presence of flavonoids, tannins, terpenoids in garlic, as well as anthocyanins and cardiac glycosides in red onion. While, alkaloids, sterols, triterpenes, saponosides and reducing compounds were absent in both extracts.

Results of the phytochemical screening of methanolic extracts of the samples did not concur with Gazuwa et al., (2013) data, who reported the absence of tannins, saponins and phenolics in red onion and garlic. The presence of flavonoids and tannins in garlic and red onion agreed with the report

of Nwinuka et al., (2005), but contradicted the results of Green et al., (1997). This implied that the studied spices are potential sources of phytochemicals, many of which have been confirmed to have medicinal activity as well as physiological activity (De and James, 2002). However, the presence of these vital chemical substances supported the observation of Pandey (1980) that plants have some vital chemical substances (alkaloids, carbon compounds, glycosides, tannins and others).

Table 3. Results of phytochemical screening of garlic and red onion

Analysis	Garlic extract	Red onion extract
Flavonoids	+	++
Tannins	+	+++
Alkaloids	-	-
Sterols and triterpenes	-	-
Terpenoids	++	+++
Saponosides	-	-
Anthocyanins	-	++
Cardiac glycosides	-	+
Reducing compounds	-	-

(-): absent ;(+): low presence; (++): medium presence; (+++); high presence

Determination of phenolic content by HPLC analysis

The molecular separation of garlic and red onion methanolic extracts using HPLC was realized in three different wave lengths 254nm, 326nm and 360nm. The chromatograms with peaks and retention time of each molecule are shown in Figure 1- 4.

HPLC results revealed the presence of five components in red onion extract (Fig. 1-3) and one component in garlic extract (Fig. 4). The identification of molecules found in the samples is based on comparing their retention times (Rt) with that of pure standards under the same experimental conditions. Table 4 lists the compounds identified in methanolic extracts of garlic and red onion.

Chromatographic analysis of the samples identified five phytochemical molecules for red onion extract namely: Gallic acid, Quercitin, Rutin, Hyperoside and Karempferol and one molecule for garlic extract which is Gallic acid. The rest of the compounds that appeared on the chromatograms could not be identified.

Table 4. The polyphenolic compounds of garlic and red onion analysed by HPLC

Extract	Compounds	Retention time (min)
Red onion	Gallic acid	3.137
	Unknown	3.687
	Quercitin	10.728
	Rutin	14.734
	Hyperoside	15.490
	Unknown	16.451
	Karempferol	17.967
Garlic	Gallic acid	5.904

The polyphenols separated from the red onion extract at retention times of 3.137 min and 3.687 min are of the tannin class, probably Gallic acid derivatives, according to the spectra and absorption maxima. Flavonoids are isolated from the same extract at retention times of over 10 min, with the ones from 14.734; 16.451; and 17.967 min being probably Quercetol derivatives with maximum absorption at over 350 nm. Among the majority flavonoids in the red onion extract, the flavonoid from the minute 14.734 represents 48.7%. The flavonoid from minute 10.728 represents 26.5%, with the rest being in the proportion of less than 10%. There are not many polyphenols in the garlic extract. The only observable component of minute 5.904 is in very low concentration.

Under the same experimental conditions, a comparison of the retention times (Rt) of molecules found in the samples with those of pure standards identified five compounds in the methanolic extracts of red onion (Gallic acid, Quercitin, Rutin, Hyperoside, and Karempferol), as well as one compound in garlic (Gallic acid) and two other compounds that could not be identified.

Previous study concerning characterization of secondary metabolites in red onion observed the presence of Quercetin, Protocatechuic acid, Spiraeoside, Tyrosine, Vanillic acid and Hydroxybenzoic acid (Lachman et al., 1997). Afterwards, Lachman et al., (2002) found that phytochemical characterization of different cultivars of onion (red, yellow and white) revealed the presence of six phenolic compounds with Spiraeoside, Rutin and Quercetin as major constituents, as well as three other unidentified compounds. Different onion varieties (Nirvana, DPS 1032, Yellow 2025, King-Midas, and SBO 133) are one of the highly rich sources of main flavonols, Quercetin (Sellappan and Akoh, 2002). In contrast to other vegetables, onions have a 5–10 times higher overall Quercetin content (347 mg/kg). The most common flavonol, Quercetin, is present in both bound and free forms (Leighton et al., 1992).

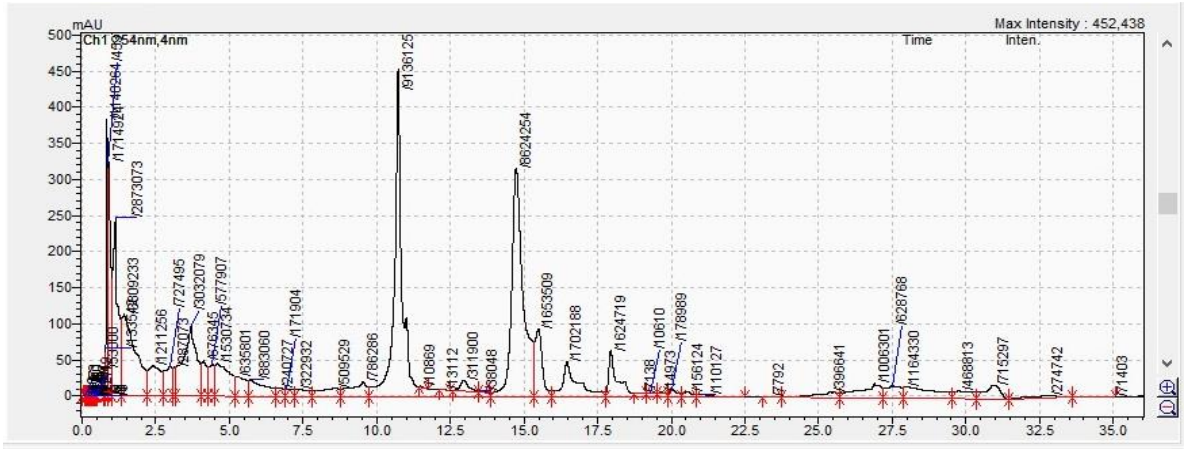

Figure 1. HPLC chromatogram of red onion dry extract at 254 nm

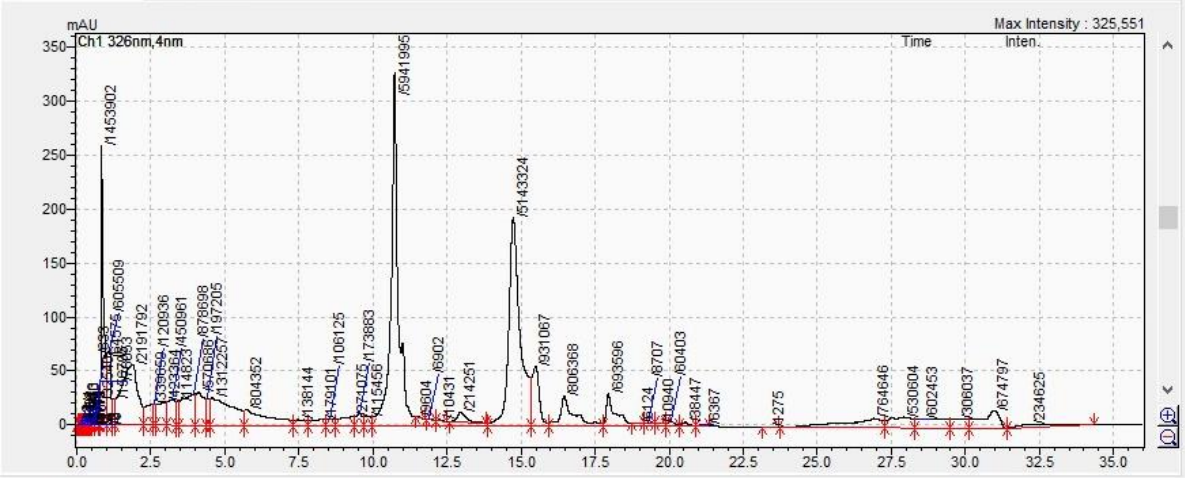

Figure 2. HPLC chromatogram of red onion dry extract at 326 nm

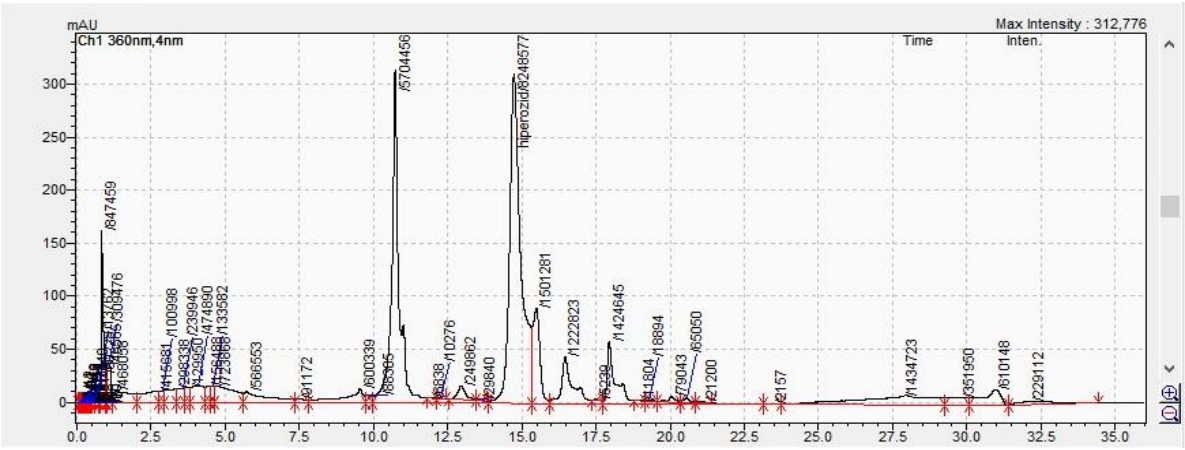

Figure 3. HPLC chromatogram of red onion dry extract at 360 nm

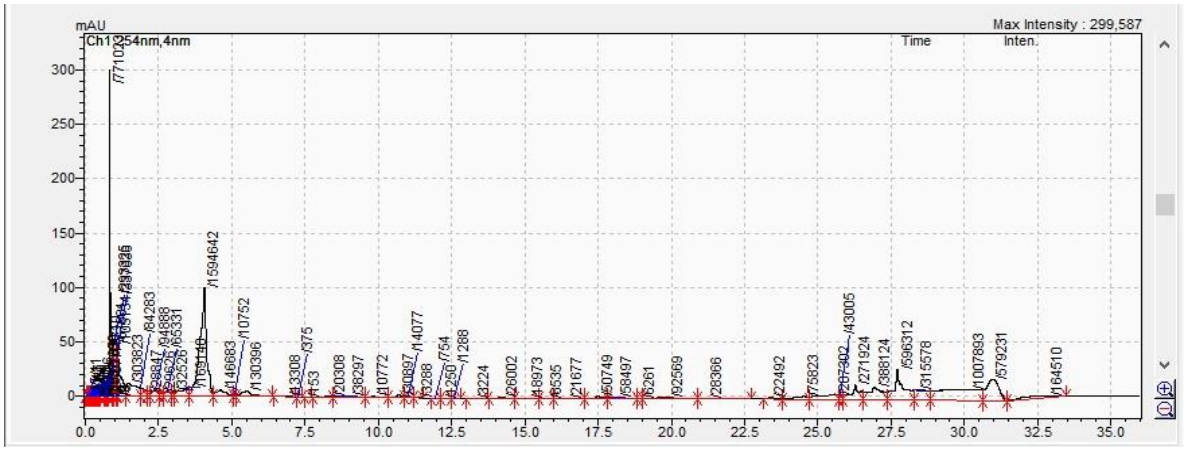

Figure 4. HPLC chromatogram of garlic dry extract at 254 nm

Quercetin-monoglycosides spiraeoside (4´-O-β-D-glucoside), 3-O-β-D-glucoside, 3´-O-β-D-glucoside, and 7-O-β-D-glucoside are very highly manifested (Ioku et al., 2001). There are also kaempferol-glycosides present at minor amounts 3,4´-O-β-D-diglucoside, 7,4´-O-β-D-diglucoside, 3-O-sophoroside-7-O-β-D-glucuronide, 4´-O-β-Dglucoside. Another type of flavonols – isorhamnetin – is present only in yellow and red cultivars of onion in both free and bound form in glycosides as: 3,4´-O-β-D-diglucoside, 4´-O-β-D-glucoside and 3-O-β-D-glucoside (Park and Lee, 1996).

Eleven major phenol compounds were identified in peel and skin of some onion cultivars (Donna, Barito and Hy Park): Quercetin-3,7,4-triglucoside, p-hydroxybenzoic acid, Quercetin-7,4-diglucoside, Vanillic acid, Quercetin-3,4-diglucoside, Quercetin-3-O-glucoside, Kaempferol-3-O-glucoside, Isorhamnetin-3-O-glucoside, Quercetin-4-O-glucoside, Quercetin and Kaempferol (Burri et al., 2017).

Many studies on the phenolic profile of different onion cultivars reported that the only phenolic compound found in detectable quantities was Quercetin (Hertog et al., 1992; Miean and Mohamed (2001); Sultana and Anwar, 2008; and Zill-e-Huma et al., (2011). Quercetin and Gallic acid were the two phenol compounds found in the hydrolyzed extract of garlic and onion (Soto et al., 2016). Our results obtained for garlic cultivars were close to those of Sultana and Anwar (2008), who found no detectable amounts of Quercetin and Kaempferol.

The significant difference in the phenolic profile obtained by HPLC between garlic and red onion confirmed the previous results of total phenolic and flavonoids content. These differences may be due to many factors including genotype, maturity stage, growing and climate conditions, harvest period and even post-harvest conditions (Chun et al., 2006). Furthermore, the results of the characterization by HPLC depend on column's separating strength, flow velocity, and mobile phase composition (Johnson et al., 2011).

Antioxidant activity

The results of the antioxidant activity of plants extracts carried out by DPPH radical scavenging activities were summarized in Table 5. They showed that methanolic extract of red onion had the strongest radical-scavenging effect compared to garlic methanolic extract.

The results of our study showed that free radical scavenging activity of garlic was lower than red onion. In terms of IC_{50}, red onion had the lowest value (420.9±0.01 µg /ml), followed by garlic (919.87±0.01 µg/ml). However, these findings clearly show that red onion has more capacity to scavenge the free radicals compared to garlic (p=0.000).

Che et al., (2011) found that garlic extract has a similar IC_{50} to the current result with an amount of 0.95±0.01 mg/ml, however other researchers revealed a lower radical scavenging activity (Nuutila et al., 2003; IC_{50}=1000 mg/ml), (Moumen et al., 2016: IC_{50}=8.36mg/ml), (Lenkova et al., 2016: 17.17%±0.634) and (Fredotović et al. ,2017: IC50 82.64 mg/ml), while Kallel et al., (2014) study showed a higher radical scavenging activity of garlic: (IC_{50} 0.64 mg/ml). Regarding red onion, previous studies showed that its radical scavenging activity was higher (Nuutila et al., 2003: IC_{50}=67 mg/ml) and (Fredotović et al., 2017: IC_{50}=77.13mg/ml).

Table 5. Results of evaluation of antioxidant activity of garlic and red onion

Extract	Extract concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)
Garlic	1000	54.85	919.87^{b}±4.43 ***
	500	25.31	
	250	14.76	
	125	02.10	
Red onion	1000	80.79	420.9^{a}±5.00
	500	68.31	
	250	40.19	
	125	24.35	

********* *Significant at 0.001 or 0.1%*
******** *Significant at 0.01 or 1%*
a, b *corresponds to the* homogeneous groups obtained *by the post-hoc* Tukey *test for each parameter*

Similarly, according to Benkeblia (2005), garlic has higher free radical scavenging activity than red onion. Similar research conducted in other plants and fruits have shown that high radical scavenging activities are commonly associated with high TPC. For instance, Lim et al., (2006) reported that high phenolic content in extracts led to high radical scavenging activity. Several other studies have shown that phenolic compounds contribute to high radical scavenging activity. Mohd et al., (2006) suggested that free radical scavenging activity is not due to the phenolics only.

In contrast to our results, Miller et al., (2000) found that garlic has a six-fold higher antioxidant activity than onion. The difference is probably at least partially due to the different methods used. Miller et al., (2000) extracted the fresh vegetables using 50% methanol whereas, in our study, 70 % methanol was used for extraction. The high antioxidant activity of *Alliums* and especially high DPPH radical scavenger of garlic were reported by numerous investigators (Velioglu et al., 1998; Yin and Cheng, 1998). However, DPPH radical scavenger activity depended on both phenolics and sufur compounds of *Alliums*. On the other hand, Nuutila et al., (2003) reported that the lowest antioxidant activity was detected in garlic. According to Benkeblia (2005) garlic extract reacted faster than other extracts and was the most effective DPPH radical scavenger, followed by purple, red and yellow onion extracts, while green onion extract showed the lowest DPPH radical scavenger. Previous study has suggested that garlic contains phenol, flavonoid, and various sulfur compounds such as disulfide (hydrophobic), and S-ally-(L)-cysteine (SAC, hydrophilic), this latter has high radical scavenging activities (Colin-Gonzalez et al., 2012). The number of phenolic compounds and flavonoids has positive correlation with DPPH radical scavenging activities, which is due to hydrogen and electron donation from hydroxyl groups of these compounds' compounds (Rice-Evans et al., 1996).

Conclusion

The polyphenolic profile and the antioxidant activity for cultivated garlic (*Allium sativum*) and red onion (*Allium cepa*) in Algeria were evaluated in order to complete scientific data related to previous studies about proximate composition of these two plants. The phytochemical screening showed significant differences between these two species, both qualitatively and quantitatively. Red onion was rich in polyphenols, flavonoids and tannins and possessed the higher antioxidant activity as compared to garlic which could be related to its high content of Quercitol derivatives. This variation is explained by difference in the genetic background of the plant material tested, rather than by differences in environmental conditions. The present study provides valuable information on phytochemical composition and functional activity of cultivated garlic and red onion species, which could be further used for their direct consumption or in the formulation of food products for human health. Future research should concentrate on the relationship between chemical structure and activity (SAR), as well as clinical trials to assess the potential effects both of the crude extracts and of the total extracts isolated compounds in human health.

Compliance with Ethical Standards

Conflicts of Interest: The authors declare no conflict of interest.

Author Contributions: Hasna Bouhenni, Koula Doukani / conception of the work; contribution in phytochemical analysis; writing the manuscript; Daniela Hanganu, Neli-Kinga Olah / contribution in HPLC analysis; analysis and interpretation of data; Nazım Sekeroglu, Sevgi Gezici contribution in phytochemical analysis; analysis and interpretation of data. All authors prepared and revised data, read and approved the manuscript.

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Functional Response of *Chrysoperla carnea* on Two Different Aphid Species (*Aphis fabae* and *Acyrthosiphon pisum*)

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Abstract

In this study, it was tried to determine the amount of prey consumed by *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) at varying prey densities and its potency in reducing the population of the pest. Aphids used as nutrients [*Aphis fabae* Scopoli and *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae)] were given to each larval stage (InstarI, InstarII, InstarIII) of the predator in certain numbers (5, 10, 20, 40, 80, 160). According to the data obtained, an increase in the amount of food consumed by *C. carnea* was observed depending on the food density given. The attack coefficient (a) and handling time (Th) were also calculated separately for each larval period. These values were 1.12 and 23.62 min, respectively, when the third stage larvae of the predator were fed with *A. fabae*; while these values were found to be 1.11 and 21.89 min, respectively, when fed with *A. pisum*.

Keywords: *Chrysoperla carnea*, *Aphis fabae*, *Acyrthosiphon pisum*, functional response, biological control

Introduction

Agricultural production has been significantly effective in both nutrition and development since the beginning of humanity (Tunçer and Günay, 2017). The most important of these problems are the diseases and pests that cause economic losses in plants. Chemical products have been preferred for many years in the control of them and a new one has been released day by day. However, organisms that are harmful in plant production develop different resistance to the applied chemicals, which means that the producer uses more chemicals. In recent years, researchers have started to look for alternative methods due to the negative effects of chemicals on the environment and human health (Lacey et al., 2001).Although chemical control of pests is highly preferred by the manufacturers due to the short-term solution, this causes undesirable residues on the products produced. In recent years, efforts have been continued to develop methods of controlling agricultural pests without harming the environment in order to prevent this. There is continuity in biological control in these studies and environmental pollution does not occur (DeBach, 1969; Uygun et al., 1987).

When aphids fed on the plant, growth in the plant stops and even deaths in plants are observed to a great extent. This also leads to yield and quality loses in production. In addition, the sweet substances produced during feeding of these pests cover the plant surface and the development of fumagine is observed. These pests also indirectly cause harm because they secrete toxic substances and being vectors for virus diseases (Lodos, 1982; Catherall et al., 1987; Kovalev et al., 1991; Elmalı and Toros, 1994).

Chrysopidae species prefer aphids, mites, thrips and white flies in their feeding and spread throughout the world The intense presence of this family in the natural ecosystem, ease of production for scientific studies, high search and consumption power increases the importance of this family in all and biological control studies (Tauber et al., 2000; Pappas et al., 2011). *Chrysoperla carnea* (Neuroptera: Chrysopidae) is a very common polyphagous species observed in agricultural production areas (Jokar and Zarabi, 2012). In terms of biological control, it is known that this species plays an important role as a biological control agent in greenhouses and open production areas (Venkatesan et al., 1997). The larvae begin to feed as soon as they hatch and feed on a very wide range. Their foods include lepidopter larvae, mites, mealybugs, crustaceans, thrips, aphids and adult

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and nymphs of white flies (Syed et al., 2005; Sattar et al., 2007; Sattar, 2010; Jokar and Zarabi, 2012; Batool et al., 2014). This beneficial insect can catch up to 80% of plant pests, feed on a wide range of nutrients and survive in different environmental conditions (Jokar and Zarabi, 2012).

Knowing functional and numerical responses of predator insects to prediction of the effect power of them against their prey is very important in biological control studies (Davis et al., 1976; Trexler et al., 1988). Functional response is indispensable for predator-prey models in the predator-prey relationship (Jeschke et al., 2002) and one of the key components in selecting agents for biological control (Lester and Harmsen, 2002). Some factors may affect the predatory efficiency of the predator insects both abiotic factors such as temperature (McCaffrey and Horsburgh, 1986; Mohaghegh et al., 2001; Skirvin and Fenlon, 2003), relative humidity (Svendsen et al., 1999) and prey or host species (Donnelly and Phillips 2001; Hoddle 2003; Allahyari et al., 2004; Faria et al. 2004), presence of alternative nutrients (Wei and Walde, 1997), sex of predator (Parajulee et al., 1994), age of predator and nutrition (Castagnoli and Simoni, 1999; Eveleigh and Chant, 1981). In addition, the effects of the host plant on prey have an indirect effect on the functional response (Price et al., 1980; Van Haren et al., 1987; Messina and Hanks, 1998; Sabelis et al., 1999).

The functional response of a predator shows the rate of prey consumed by the predator at varying prey densities and its power to prevent pest population (Murdoch and Oaten, 1975). The main factor in the relationship between predator and prey is the functional responses of predators to their prey when the prey population increases (Hassell, 1978). In this study, it was tried to determine the amount of prey consumed by C. carnea at varying prey densities and the potency of decreasing the population of pests.

Materials and Methods

Production of Broad Bean (*Vicia faba*)

The broad beans used as host plant in the experiment were grown in the production room of Yozgat Bozok University, Faculty of Agriculture, and Department of Plant Protection. For this purpose, bean seeds were planted to the small plastic and paper cups (in 1:1:1 ratio of soil:peat:perlite mixture) and seedlings were left under timed light (16L:8D) after they started to grow. When the height of the cultivated plants became suitable for aphid production, they were used in the experiment. This process was repeated periodically as long as the experiments continued, and weekly maintenance of the plants was carried out. All plant productions were carried out in production room set on 26±1 ºC and 60±5% humidity and long day illuminated.

Production of Aphids (*Aphis fabae* and *Acyrtosiphon pisum*)

In this study, *A. fabae* and *A. pisum* individuals produced as food for predators were obtained from Isparta University of Applied Sciences, Faculty of Agriculture, Biological Control Research and Application Center and mass production was carried out. Individuals from mass production were infected to clean plants and aphid samples used in the experiment were obtained from this production. All aphid production were carried out in the cages in production room set on 26±1 ºC and 60±5% humidity and long day illuminated (16D:8L).

Production of *Chrysoperla carnea*

The adult individuals of *C. carnea* used in the study were collected from clover fields around Isparta and Yozgat in Turkey with the help of netting and mouth aspirator. The collected individuals were brought to the laboratory and then placed in plastic containers covered with tulle. Yeast extract + honey + water mixture was placed in the plastic containers (Kişmir and Şengonca, 1981; Tireng et al., 1999) and tulles were left in strips for laying the eggs of the adult females. In order to adapt to the laboratory environment and the nutrients of the adults, individuals were used in the experiment after giving a generation. The newly hatched larvae were fed separately with the aphids used in the experiments. All productions were carried out in the cages in production room set on 26±1 ºC and 60±5% humidity and long day illuminated.

Functional Response Trials

Eggs belonging to *C. carnea*, which were mass produced for the experiments, were expected to be hatched. The larvae were separately taken into petri dishes as soon as they emerged. After larvae were famished for 24 hours, the aphids (2nd and 3rd nymph) were separately given to the predator larvae and in certain numbers (5, 10, 20, 40, 80, and 160). The number of aphids consumed by the larvae 24 hours was recorded after this procedure. These processes were performed separately for the *C. carnea* larval periods. Functional response trials were performed as 50 replications for each larval period (Mean 50 rep x 6 different densities of *A. fabae* x 3 *C. carnea* instars = 900 petri dishes. Mean 50 rep x 6 for *A. pisum* x 3 larval period of *C. carnea* = 900 petri dishes). All experiments were conducted at 26±1 ºC and 60±5% humidity and long day illumination conditions.

Statistical Analyzes

The functional response of the *C. carnea* was calculated by the formula used by Holling (1959). The hunting rate, catch time and standard errors of the hunter in the formulas were calculated according to Holling (1959)'s disk equation.

$Na = TPaN/(1+aThN)$ (Holling, 1959).

(Na:the number of prey eaten, T:the total time available for the predator, P:the number of predator,

N:the number of prey offered, a:the searching efficiency, Th:the handling time)

The parameters that were obtained according to this curve is calculated using SPSS (ver. 17), MS Excel (ver. 2010) and Minitab (ver. 16).

Results and Discussion

In this study, two different aphids (*A. fabae*, *A. pisum*, different densities) were given to different larval stages of *C. carnea*. Accordingly, the number of aphids consumed by different larval stages of the predator was determined (Table 1 and Figure 1, 2).

According to the results, when 5 aphids were given to predator, consumption amounts in the second and third larval periods were similar (P>0.05); while there was a statistical difference between the amount of food consumed in the first larval periods (P<0.05). When 10 aphids were given to *C. carnea*, it was observed that individuals in the third larval period consume all aphids (P>0.05); while there was a difference between the amount of food consumed in the first and second larval stages (P<0.05). It was determined that there was a difference between the amount of food consumed in all three larvae periods when 20 prey were given to predator (P<0.05). When 40, 80 and 160 nutrients were given to predator, it was also determined that there was a difference between the amount of food consumed in all three larval periods (P<0.05).

In this study, after calculating the average number of aphids consumed by different larval stages of *C. carnea*, the attack coefficient (a) and handling time (Th) values of the predator were also calculated. Accordingly, for both nutrients, as the larval stages of the predator develop, the attack coefficient increased; while handling time was found to be shortened. In larvae fed with *A. fabae* and *A. pisum*, the highest attack rate was observed in the third period larvae; while the lowest value was seen in the first period larvae. The highest handling time was also observed in the first period larvae; while the lowest value was seen in the third period larvae. When two nutrients were compared, the attack rate values were the highest in the third period larvae fed with *A. fabae*; while the lowest catch time was determined in the third period larvae fed with *A. pisum* (Table 2).

According to the obtained data, it was determined that the type of functional response (depending on the aphid density and type) was Type-II (for three larval periods). The graphics of 1/H, 1/Ha were given Figure 3, 4, 5, 6, 7, 8 (H:Prey density, Ha: Prey consumed). Amount of nutrients consumed depending on nutrient density, hunting rate values, attack coefficient and handling times were calculated using these graphics and the disk equality of Holling (1959).

In this study, the functional response of hunter insect *C. carnea* on two different aphids was determined. In recent studies, it has been stated that especially fourth stage larvae of coccinellids consume a lot of nutrients (Moura et al., 2006; Omkar and Pervez, 2004; Bayoumy, 2011; Lee and Kang, 2014). This situation is due to the high energy need for development and the weight need for the pupal period (Hodek and Honěk, 1996). A similar situation has observed in the third stage larvae of *C. carnea* (Hassanpour et al., 2011). Many factors are effective on hunting efficiency of predator insects such as benefit from prey (Matter et al., 2011), species of prey (Sarmento et al., 2007), age of prey (Koch et al., 2003), temperature (Skirvin et al., 1997), leaf morphology (Bayoumy et al., 2014), cannibalism and intraguild predation (Burgio et al., 2002), larvae parasitism (Bayoumy, 2011; Bayoumy and Michaud, 2012).

Khan and Zaki (2008) identified the functional and numerical response of *C. carnea*'s third-term larvae on *Aphis fabae solanella* Theobald in their study. According to the data obtained, it was reported that the functional response resulting from nutrient density is Type-II and the attack rate value increases with increasing aphid density. Attack coefficient and handling time values were calculated as 0.54 and 2.17, respectively. Montoya-Alvarez et al. (2010) determined functional response of *Chrysoperla nipponensis* (Okamoto) and *C. carnea* (Stephens) (Neuroptera: Chrysopidae) fed on seven different densities of *Aphis gossypii* (Glover) (Homoptera: Aphididae) at 20 °C on laboratory conditions. It has been reported that both predator have Type-II on cotton aphid. The maximum amount of aphids consumed of *C. carnea* has been more than *C. nipponensis*. According to the data obtained, the handling time of both species decreased due to the increasing nutrient density; while this time of *C. nipponensis* was found to be higher than that of *C. carnea*. When the attack rate values were examined, it was found that the attack coefficient of *C. nipponensis* was slightly higher than those of *C. carnea*. Hassanpour et al. (2011) determined the functional response of *C. carnea*'s three larval stages on egg and first larval stages of the cotton bollworm *Helicoverpa armigera* Hübner. According to the data obtained, the first and second larval stages of *C. carnea* showed Type-II functional response on both nutrients. However, third larval period of *C. carnea* had Type-II functional response when fed on first larval stage of *H. armigera*, had Type-III when fed with eggs of cotton bollworm. According to the results, the highest hunting rate was observed in the third period predator larvae feeding on the eggs of *H. armigera*. It was also concluded that the larvae of *C. carnea* (especially the third period) had good hunting potential under the control of the larvae and eggs of *H. armigera*. Batool et al. (2014) carried out studies on biology and functional response of *C. carnea* (Stephens) (Neuroptera: Chrysopidae) under laboratory conditions. They gave eggs of *Sitotroga cerealella* (Lepidoptera: Gelechiidae) (different densities: 20, 30, 40, 50, 60, 70, 80, 90 and 100) to the *C. carnea* larvae in petri

dishes. According to the results, it was observed that consuming density had a significant effect on *C. carnea*'s positive consumption rate, growth rate and fertility. It has been observed that hunting potential increases as food density increases in all trials. The daily catching rate of *C. carnea* increased gradually during the first two larval stages; reached the highest point in the third larval period. Memon et al. (2015) determined functional response of third larval stage of *C. nipponensis* (Neuroptera: Chrysopidae) on different preys [artificial food and *Corcyra cephalonica* eggs and cowpea aphid (*Aphis craccivora*), papaya mealybug (*Paracoccus marginatus*) and whitefly (*Bemisia tabaci*)] on different densities. According to the results, the larvae of *C. carnea* showed Type-II functional response on all foods. Saljoqi et al. (2016) determined functional response of larvae of C. carnea on *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae) in their studies. According to the data obtained, the amount of aphid consumed increased according to the larval periods and the prey density. The maximum catching rate was calculated in the third larval stage; while this value decreased in other larval periods. The lowest handling time was observed in the third period larvae, while this value increased in the other two larvae periods when the handling times were examined. In addition, the type of functional response of the *C. carnea* on *B. brassicae* was also reported to be Type-II. Alhamawandy (2017) determined functional response of *C. carnea* on A. fabae on laboratory conditions. According to the results, *C. carnea* showed a second type (Type-II) functional response and it was reported that the functional response of the predator increased due to the increasing prey density. The highest attack rate (a) was seen in the third larval period of *C. carnea* with 0.976; the lowest attack rate was 0.635 in the first larval period. In addition, the lowest handling time was calculated in the third larval period of *C. carnea* with 5.33 minutes; the highest handling time was 21.6 minutes in the first larval period. Rana et al. (2017) gave frozen eggs of Corcyra and seven different aphids to the *C. carnea*, and then observed the development of predator. The hunting effect was calculated by recording the amount of food consumed by the predator insect every day. According to the results, it was reported that the hunting effect increased from the first larval period to the third larval period and *C. carnea*, a potential biological control agent, was found to be highly effective against different aphid species. However, in order to obtain better data, it was concluded that trials should also be conducted in field conditions simultaneously with this study. Bayoumy and Awadalla (2018) investigated effects of two different preys [*Myzus persicae* Sulzer ve *A. craccivora* Koch (Hemiptera: Aphididae)] of different densities on third larval stage of *C. carnea* Stephens (Chrysopidae: Neuroptera) and forth larval stage of *Coccinella septempunctata* L. and *Hippodamia variegata* Goeze (Coccinellidae: Coleoptera). According to the data obtained, the species of prey and predator, prey density and their relations with each other have a significant effect on aphids consumption, but the functional response type has not been changed (Type-II). Ail-Catzim et al. (2019) determined Type-III functional response of third larval stage of *C. carnea* on forth nymph of Myzus persicae. According to the regression analysis, they also determined the handling time and attack rate of the hunter. According to the results obtained, they found that there was an increase in the amount of aphids consumed by the predator in the third larval period depending on increased prey density. It is thought that this difference arises from the fact that the food age given to predators (N4) is different from the food age given in our study (N2-N3). Mahzoum et al. (2019) determined functional response of larvae of *C. carnea* on *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) in their study. The consumed prey amounts were recorded depending on prey densities (3, 5, 10, 15, 25 and 40). According to the results, it was found that the functional response of *C. carnea* on *S. oleae* of all larval stages was Type-II and the amount of consumption increased depending on the prey density. Costa et al. (2019) examined developmental biology and functional response of *Leucochrysa* (*Nodita*) *azevedoi* (Neuroptera: Chrysopidae) on different preys. According to the data obtained, it has been reported that the larvae of the predator insect showed functional type II response and has a potential in the biological control of these preys.

Conclusion

When this study is compared with other studies, similarities (especially functional response type: Type-II) are observed. In addition, an increase in the amount of nutrients consumed by *C. carnea* depending on the nutrient density was also observed in our study. Looking at the data we obtained, it is thought that *C. carnea* larvae have an important place in the integrated pest management programs in terms of control of *A. fabae* and *A. pisum*. However, it is thought that predator-prey relations should be investigated in natural terrain conditions in order to better reveal the potential of the predator.

Table 1. Amount of consumed aphids by different periods of *Chrysoperla carnea* larvae

		Number of Aphids (*A. fabae* / *A. pisum*) consumed		
	n	L1	L2	L3
Aphis fabae	5	3,96±0,1 a^B	4,52±0,07 a^A	5,00±0,00 a^A
Acyrthosiphon pisum	5	4,42±0,06 a^A	4,64±0,07 a^A	5,00±0,00 a^A
Aphis fabae	10	5,90±0,15 b^B	6,82±0,15 b^B	10,00±0,00 b^A
Acyrthosiphon pisum	10	6,44±0,10 b^A	7,42±0,11 b^A	10,00±0,00 b^A
Aphis fabae	20	11,80±0,17 c^B	14,42±0,13 c^B	19,02±0,13 c^B
Acyrthosiphon pisum	20	12,34±0,16 c^A	15,32±0,20 c^A	19,42±0,12 c^A
Aphis fabae	40	22,54±0,21 d^B	24,16±0,26 d^B	27,44±0,20 d^B
Acyrthosiphon pisum	40	23,46±0,21 d^A	25,64±0,18 d^A	28,74±0,17 d^A
Aphis fabae	80	24,14±0,22 e^B	25,78±0,23 e^B	31,52±0,23 e^B
Acyrthosiphon pisum	80	25,04±0,17 e^A	27,00±0,23 e^A	32,78±0,18 e^A
Aphis fabae	160	25,74±0,23 f^B	27,22±0,22 f^B	33,82±0,27 f^B
Acyrthosiphon pisum	160	26,54±0,25 f^A	28,50±0,21 f^A	34,50±0,21 f^A

Values bearing the lowercase letters in the same column represent the nutrient concentrations given to the predator insect; the values given in uppercase letters compare the consumption amounts of different foods. Different uppercase letters indicate a statistical difference between nutrients (one-way ANOVA, Tukey Test, α=0.05) (L1: 1st Instar, L2: 2ndInstar, L3: 3rd Instar) (n: Prey Density).

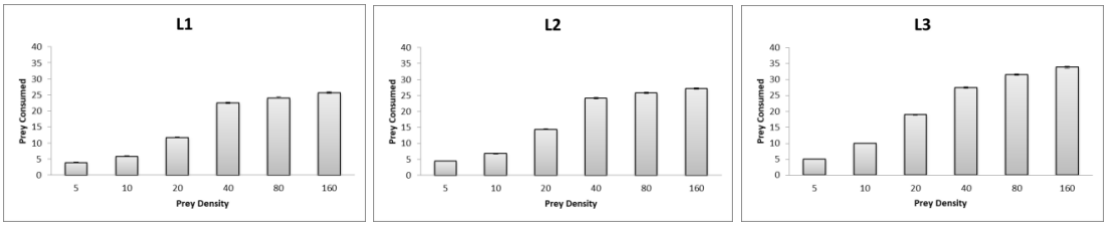

Figure 1. Amounts of *Aphis fabae* consumed by different periods of *Chrysoperla carnea* larvae

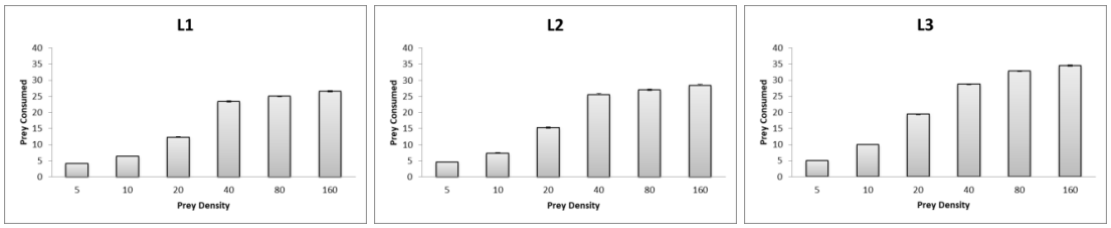

Figure 2. Amounts of *Acyrthosiphon pisum* consumed by different periods of *Chrysoperla carnea* larvae

Table 2. The functional response values [Attack coefficients (*a*), Handling times (*Th*)] of the larvae of *Chrysoperla carnea* on *Aphis fabae* and *Acyrthosiphon pisum*

		L1	L2	L3
Aphis fabae	Attack coefficient (*a*)	0,85543	0,98981	1,11844
	Handling time (*Th*)(min)	41,04	37,584	23,616
		L1	L2	L3
Acyrthosiphon pisum	Attack coefficient (*a*)	0,91676	1,0202	1,11025
	Handling time (*Th*)(min)	39,6	33,84	21,888

(L1: First instar, L2: Second instar, L3: Third instar)

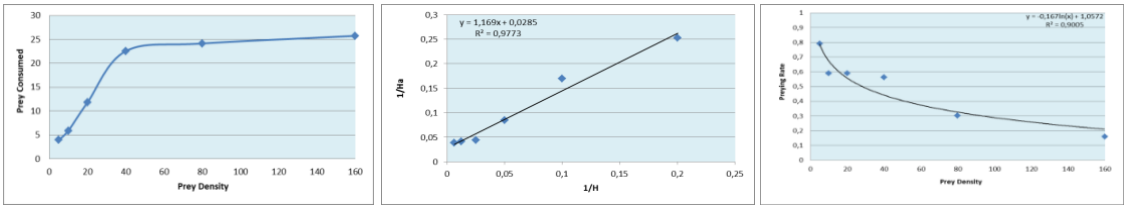

Figure 3. The functional response of first instar of *Chrysoperla carnea* on *Aphis fabae*

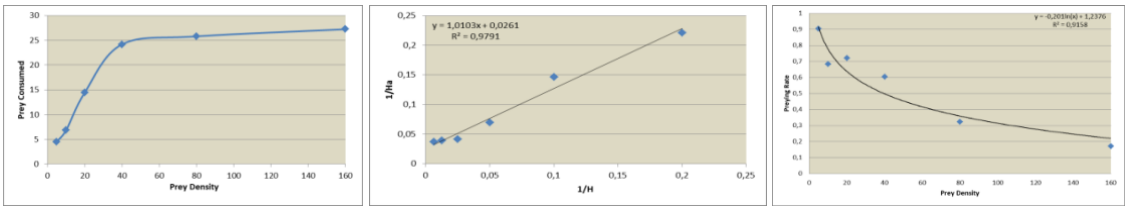

Figure 4. The functional response of second instar of *Chrysoperla carnea* on *Aphis fabae*

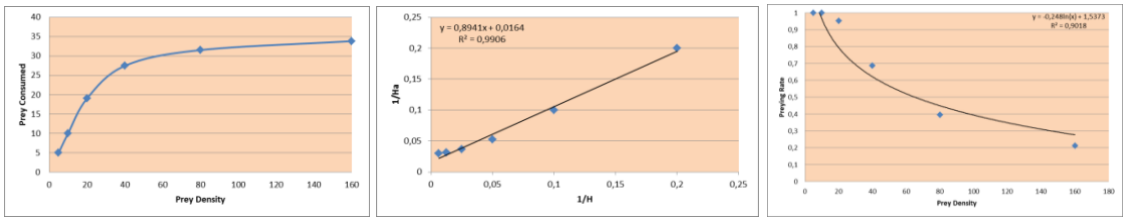

Figure 5. The functional response of third instar of *Chrysoperla carnea* on *Aphis fabae*

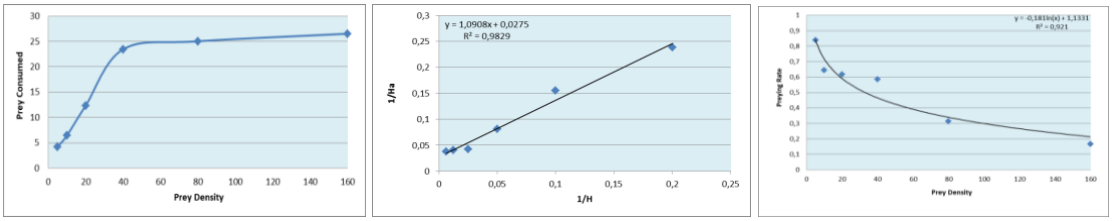

Figure 6. The functional response of first instar of *Chrysoperla carnea* on *Acyrthosiphon pisum*

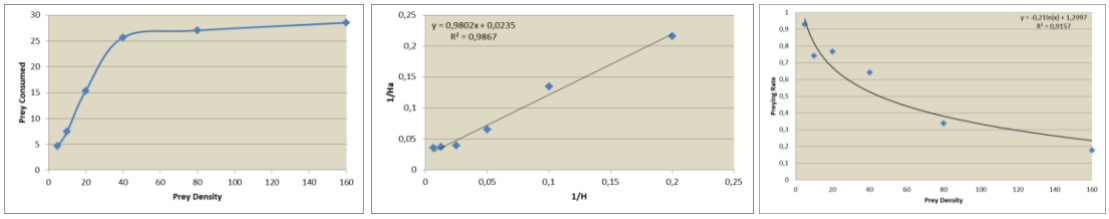

Figure 7. The functional response of second instar of *Chrysoperla carnea* on *Acyrthosiphon pisum*

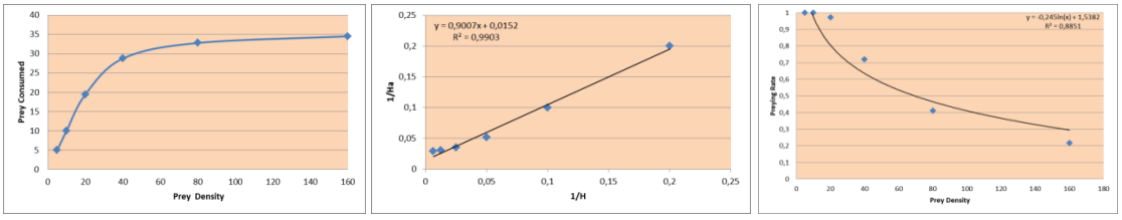

Figure 8. The functional response of third instar of *Chrysoperla carnea* on *Acyrthosiphon*

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Impact of Contract Farming on Productivity and Food Security Status of Smallholder Maize Farmer's Households in Kano and Kaduna States, Nigeria

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Abstract

This study analyzed impact of contract farming on productivity and food security status of smallholder maize farmer's household in Kano and Kaduna States, Nigeria. A multistage sampling technique was used to collect data from 466 smallholder maize farmers with the use of a-structured questionnaire. Descriptive statistics, household dietary diversity scores (HDDS), and propensity score matching were used to achieve objectives of the study. Result of descriptive statistics shown that, average age of contract participants was 39years; with farming experience of 20years and had an average farm size of 2.39 hectare. On the other hand, non-contract participants had an average age of 37years and average farming experience of 18years with a farm size average of 2.34hectare. HDDS result revealed households participating in contract farming to have mildly better food security status with an average dietary diversity score of 5.16, against non-contract participating farmers that have 3.15 household dietary diversity score average. PSM result for the impact revealed that contract farming had positively ($P<0.01$) impacted on maize yield (ATT=1.7ton/ha), and food security status of the participating household (ATT=0.893). Therefore participation in maize contract farming increases productivity and reduces food insecurity status of smallholder maize farmers; it can therefore be recommended that contract farming can be used as an instrument to reduce food insecurity and poverty among rural farming household.

Keywords: Contract farming, Propensity Score Matching, Food Security

Introduction

Maize (*Zea mays*) is a staple food for a large part of the population around the Globe and is of great socio-economic importance in the Sub-Saharan Africa (FAO, 2013). It is one of the most heavily cultivated cereal crop globally, and one of the main cereals crops of west Africa and the most important cereal food in Nigeria (Onuk, Ogara, Yahaya & Nannim, 2010). Maize is grown in many parts of Nigeria but the northern part dominates all other regions. Murphy, (2010) indicated that growing maize by smallholder farmers can overcome food insecurity in their households. These smallholder farmers make up to 80% of farmers in Nigeria, they produced substantial percentage of food consumed by Nigerians particularly maize crop, however these farmers are producing below their capacity that result to food insecurity among their households because of numerous challenges they experience such as limited access to modern agricultural production technology; inadequate agricultural credit; lack of access to extension service; small land holding and poor access to market (Mgbenka, Mbah & Ezeano, 2015).

Product supply chain for agricultural goods have become increasingly globalized, as a result greater number of smallholder farmers in Sub-Saharan Africa (SSA) are now participating in the chain, which is mostly through contract farming (Armah, Schneider and Gugerty, 2010). These make it to become one of the first steps in the transition from subsistence to commercial agriculture as an

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intermediate sector between the agricultural and manufacturing sector. It is also basically an arrangement that establish agreement between processing/marketing firms and smallholder farmers for production and supply of food and commercial crops base on predetermine future quality and price (Bellamere and Novak, 2017). Models of contract farming play major role on welfare of smallholder farmers by increasing their crop productivity through delivering better technology, coordinating producer's and consumer's market along with strong grass-root linkages (Iro, 2016).

Contract farming in Africa and Asia is mainly promoted by private sector with little support from public institution. In Nigeria there are few emerging evidences of contract farming (Miet and Katrein 2017; Fawale and Thomas 2014; Iro 2016; Oluesegun, 2016). The existing once are mostly owned by the private companies/individuals as an out grower schemes and few by the Government such as Anchor borrowers Scheme; that is aim at giving input credit to facilitate the production of staple and cash crop in the country. The most notable out-grower schemes for maize in Nigeria especially northwest axis were Bunkasaman, Manomalinks, Olarm, WACOT, Babbagona and Afex-Agra among others. These firms operate using various contract farming models that are usually in the form of centralized, nuclear estate, multipartite, informal and intermediary models. Each of these models provide services to the farmers that include access to credit; extension service, agricultural production inputs; training on good agronomic practices, farm supervision, storage facilities and ready markets for harvested crop.

Several studies conducted world-wide has shown positive impacts on indicators of farmers' welfare; others do not find such effect, most of the studies conducted in developing countries on contract farming impact revealed increasing income of the farmers with the exception of few studies like that by Ragasa, lambrach and Kufoalar in (2017) that revealed decreasing income of the farmers. Studies specifically conducted on contract farming impact on food security in developing countries are limiting with the exception of recently conducted studies that includes one by Bellamere and Novak, in 2017 that analyzed the impact of contract farming on food security using Period of hunger as proxy in Madagascar; which is a subjective assumption of food security and the other by Adebisi *et al* in 2019, that studied the impact of contract farming on the households' food security of farmers using calorie intake as proxy to food security, this gives an avenue to researchers in developing countries to explore contract farming impact on food security at other food security dimension such as food consumption score, household dietary diversity score and body mass index, etc.

Methodology

Study area

The study was conducted in Kano and Kaduna States Nigeria where maize production is dominant and there exists evidence of contract farming operation. The local government in the states where there is evidence of contract farming in Kaduna state include Soba, Kubau, Furu, Lere and Igabi local Government while in Kano state the local governments includes Rano, Bunkure , Garunmalam, Warawa, kura Karaye Rogo and Shanono local government.

Kaduna state is located between latitudes 11^0 32`` and $09^0$ 02``N and longitudes 80^0 50`` and $06^0$ 15``E. There are two marked seasons in the State: the rainy (wet) season and the dry windy season. The wet season is usually from May to October with great variations in different areas of the state from 600mm to 1500mm.On the average, the State enjoys a rainy season of about five months. The length of the growing periods varies from 100 to 200days. The dry season starts from November to April Temperature in the state ranges between 28^0C and 34^0C. Farming is the main occupation of the people, with emphasis on the crops grown which include maize, sorghum, rice, millet, wheat, cotton, yam, cassava, pigeon pea, cowpea, soya bean and groundnut. They also grow vegetable crops like tomato, pepper, onion and carrot. Livestock is also important in the economy of the state and the livestock kept include cattle, sheep, goats and poultry.

Kano state is located between latitudes 10^o 3`` and $12^o$ 37`` N and longitudes 7^o 3`` and $9^0$ 5`` E (Ogungbile *et al*., 1999). Kano State is the commercial nerve centre of Northern Nigeria. It has a total land area of 20, 760 square kilometres with 1,754,200 hectares of fertile agricultural land, of which 86,500 is exclusively Fadama land. About 75,000 hectares is made up of grazing lands (Olofin *et al*., 2008). The dry season is usually from October to April, while the rainy season begins from April to September with an annual rainfall of 134.4mm Kano. Farming is the main occupation of the people, with emphasis on the crops grown which include maize, sorghum. They also grow vegetable crops like tomato, pepper, onion and carrot. Livestock is also important in the economy of the state and the livestock kept include cattle, sheep, goats and poultry

Sampling procedure

Multi-stage sampling technique was employed for the study; it involve identification of Local Governments Areas (LGAs) where there are evidences of contract farming participation by smallholder maize farmers, first stage was random selection of communities with evidence of contract farming systematically. Second stage was selection of two communities from the list of contract farming participating communities through balloting; in the third stage raosoft sample size formula was used to determine sample size from sample frame of maize farmer's population of each community selected consisting of participating and non-participating maize contract farmers. Finally, in the fourth stage 233 contract farmers and 233 non-contract farmers were randomly selected systematically from the

sampling frame; making 466 respondents for the study as shown in Table 1.

Table 1. Sampling Summary of Maize Farmers in Kano and Kaduna State

State	LGA	Communities	Selected Communities	CPF Sample Frame	CPF Sample Size	NCPF Sample Frame-	NCPF Sample Size
	Ikara	10	Saulawa	52	20	42	16
			Kurmin Kogi	54	21	54	21
	Makarfi	8	Mayere	35	14	40	15
Kaduna			Dorayi	42	17	40	15
	Soba	12	Gimba	67	27	77	27
			Awai	70	28	70	25
	Bebeji	7	Alkalawa	38	15	38	15
			Damau	59	23	59	24
Kano	Rano	10	Yalwa	37	15	47	19
			Doka	47	19	49	20
	Bunkure	9	Danhassan	40	16	28	13
			Barge	46	18	46	19
Total	6	56	12	587	233	590	233

Note: CPF= contract participating farmers, NCPF= non-contract participating farmers
Note: DDS stand for dietary diversity score.

Method of data collection

Primary data were used for the study; the data were collected through the use of questionnaire administered to respondents by the researcher with the aid of trained enumerators. The data collected includes information on farmer's socio-economic characteristics, maize production data for 2018 cropping season, household food consumption pattern and challenges faced by the farmers participating in maize contract farming.

Data analysis

Descriptive statistics was used to analyze data for socioeconomic characteristics of the farmers, Household dietary diversity score (HDDS) for food security measurement and propensity score matching for evaluating the impact of contract farming on food security of farming household and maize productivity

Household dietary diversity score (HDDS)

Household dietary diversity score was used to measure food security status of contract and non-contract smallholder maize farmer's households following International Food Production Research Institute (2006). This type of metric captures the number of different kinds of food or food groups that people eat and the frequency with which they eat them the score represents the diversity of intake; the scores have been shown to be significantly correlated with caloric adequacy measures (IFPRI, 2006). Coates *et al.* (2007) also recommended to use the mean score or distribution of scores for analytical purposes and to set program targets or goals.

Procedure for calculating HDDS

i. For each food group create a new binominal variable that has two possible values: 1=Yes**:** the household / individual consumed that specific food group and 0 = No if they did not consume that food.
ii. Sum all the binominal variables in order to create HDDS;
iii. The new variable will have a range from 0 through the maximum number of food groups collected (7)
iv. IFPRI proposes to use the following thresholds:
6+: High = Good dietary diversity
4.5 – 6: Medium dietary diversity
<4.5: Low dietary diversity

Table 2. Food groups for household dietary diversity score (HDDS) Measurement

Food groups used	**Food Groups used for HDDS**
Cereals and grain, roots and tubers	1. Cereals, roots, and tubers
Legumes / nuts	2. Pulses and legumes
Orange vegetables (vegetables rich in Vitamin A)	3. Vegetables
Green leafy vegetables, Other vegetables Orange fruits (Fruits rich in Vitamin A), Other Fruits	4. Fruits
Meat Liver, kidney, heart and / or other organ meats Fish / Shellfish Eggs	5. Meats, fish and seafood, and eggs
Milk and other dairy products	6. Dairy products
Oil / fat / butter	7. Oils and fats
Sugar, or sweet	Not considered
Condiments / Spices	Not considered

Propensity Score Matching (PSM)

Propensity Score matching was used to evaluate the impact of participation in contract farming on food security (household dietary diversity score proxy), profitability (Return on investment proxy) and variable production cost, this technique is a non-parametric approach that involves constructing a statistical comparison group by modeling the probability of participating in contract farming on the basis of practical features that are unpretentious by the contract farming. The underlying principle of PSM is that the predicted probabilities (propensity scores) from an estimated Probit model is used to find matches for farmers participating in contract farming (participants). The estimation of average treated effect on the treated (ATT) is specified as follows.

$$ATT = \left(\sum \frac{H1}{D} = 1\right) - \left(\sum \frac{Ho}{D} = 1\right) \ldots\ldots\ldots\ldots 1$$

The problem with estimation of the equation (1) is that it is not observable. However, it is probable to appraise equation (1) by replacing $\sum \frac{H1}{D} = 1$ with $\sum \frac{Ho}{D} = 0$as follow

$$ATT = \left(\sum \frac{H1}{D} = 1\right) - \left(\sum \frac{Ho}{D} = 0\right) \ldots\ldots\ldots\ldots 2$$

Valuation of equation (2) is a biased estimate of the causal effect of membership in contract farming. This leads to the modeling of a more reliable estimation by controlling observable characteristics to ensure that participation in maize contract farming is random and not connected with the outcome variables i.e. restricted independence hypothesis is satisfied

$$ATT = \left(\sum \frac{H1}{D} = 1\right) - \left(\sum \frac{Ho}{D} = 1\right) \ldots\ldots\ldots\ldots 3$$

$$P(z) = \Pr\left(D = \frac{1}{z}\right) = \sum\left(\frac{D}{Z}\right) \ldots\ldots\ldots\ldots 4$$

$$ATT = \left(\sum \frac{H1-H0}{D} = 1\right) \ldots\ldots\ldots\ldots 5$$

$$ATT = \sum\left(\sum \frac{H1-H0}{D} = 1, P(Z)\right) \ldots\ldots\ldots 6$$

$$ATT = \sum\left\{\sum\left\{\sum \frac{H1}{D} = 1, P(Z)\right\} - \sum\left\{\sum \frac{H0}{D} = 0, P(Z)\right\}\right\} \ldots\ldots\ldots\ldots 7$$

Where, H1= value of the outcome for participants in maize contract farming, Ho = value of the outcome for non-participation in contract farming, D= Participation (1 for participants in maize contract farming and 0 otherwise), Z= socioeconomic characteristics of the farmers. The study employed three matching techniques (Nearest Neighbor Matching, Radius Matching, and Kernel Based Matching) in which one with more robust outcome was selected to determine the impact of farmers' involvement in maize contract farming.

Results and Discussion

Socio-economic characteristics of smallholder maize farmers

The result in Table 3 and Figure1 indicate socioeconomic characteristics of contract and non-contract maize farmers in the study locations, the socio-economics characteristics were; Age, household size, faming experience, farm size, road accessibility, access to extension service, access to credit and cooperative membership.

The average age of contract farmers was found to be 39years while that of non-contract farmers was 37years. Farming experience was 20 years for maize contract farmers while non-contract maize farmers had average farming experience of 18years in maize production The t-values of their mean difference was 2.47 at (P<0.1). This implies more experience of maize production among contract farmers than their counterpart. The average farming experience of maize farmer is similar to that of Ragasa *et al*, (2018) that found 21years as average farming experience in study of maize out-grower scheme in the upper west Ghana and that of Yakubu (2016) that studied technical efficiencies of maize production Kaduna State Nigeria.

Majority(85%) of contract farmers had road accessibility to their farms that is more than that of non-contract participation farmers as only (36%) of them had accessible road to their farms as shown in figure 1. The difference in terms of accessibility have implication with regard to participation contract farming, this is due to the fact that maize contracting firms in the study area prepared and select the farmers farm that is close to main road. may be because road accessibility ease transportation of harvested maize to firm location and also facilitate supervision by the firms extension officers.

Farmer's access to extension services result shows that majority (83%) of the contract maize farmers had access to extension service while non-contract farmers had only 48% of them that have access to extension services, this implies more access to extension services among contract participating farmers, this is due to the fact that one of the important services of contracting firms is the extension service delivery.

All (100%) of the contract participating farmers have access to credit while; non-contract participating farmers have 33% of them with access to credit. This implies that participating in contract farming ensures farmers access to Agricultural credit. Therefore maize production contracting firm delivered their services of improving farmers' access to credit facilities for increased production.

Contract participating farmers, all (100%) of them belong to a particular cooperative group while non-contract farmers have only 36% of them belonging to cooperative group. This implies that for a farmer to participate in contract he has to belong to particular cooperative group may be because formal signing of contract is between the farmer group and contracting firm, also cooperative groups helps to facilitate farmers control, management and supervision by the contracting firm, the finding is similar to that found by Geoffrey (2016) on "performance of cotton smallholder farmers under contract farming in Bariadi district.

Table 3. Socio-economic characteristics of maize farmers

Variables	Contract Maize farmers					Non-Contract Maize farmers					t-value
	Freq(%)	Min	Max	X	SD	Freq(%)	Min	Max	X	SD	
Age(Years)		18	65	39	9		18	70	37	11	1.79***
18-29	29(12)					58(25)					
30-41	127(55)					98(42)					
42-53	55(24)					55(24)					
54-65	22(9)					13(11)					
66-77						5(2)					
Household Size		1	30	8	5		1	33	8	6	0.85
1-7	118(51)					127(55)					
8-15	101(43)					85(36)					
16-23	11 (5)					16(7)					
24-31	3(1)					4(2)					
32-38						1(1)					
Experience		4	45	20	8.45		1	50	18	9	2.47*
1-10	37(16)					63(27)					
11-21	116(50)					116(50)					
22-32	62(27)					46(20)					
33-43	17(6.6)					14(6)					
44-54	1(0.4)					5(2)					
Farm Size (ha)		0.5	10	2.4	1.63		0.5	8	2.3	1.6	1.72
0.5-2.5	175(75)					172(74)					
2.6-4.6	40(17)					35(15)					
4.7-6.7	9(2)					25(11)					
6.8-8.8	4(2)					1(0.5)					
9.9-10.9	5(2)										
Total	233(100)					233(100)					

Source: Field survey 2019; X= Mean; *, **, *** donates significant at 10%, 5% and 1% respectively

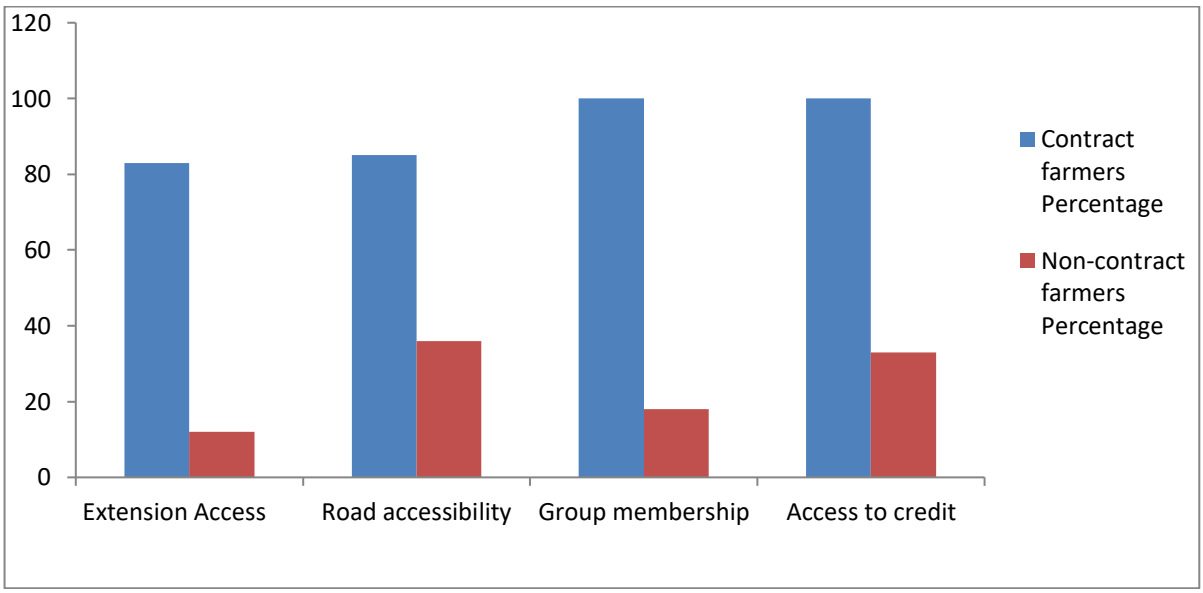

Figure 1. Socio-economic characteristics of the farmers (Source: Field Survey, 2019).

Food security status of contract and non-contract farmers household

The result of food dietary diversity score (DD) of smallholder farmers households was presented in Table 4. The result show that contract participating households in the study area had average DD score of 5.36 and 85% of their households had medium to high dietary diversity score. On the other hand, non-contract farmers in the study area also had DD average of 3.15 and 72% of their households had good to medium dietary diversity. The result implies that contract participating farmer's households have mildly better food security than the non-contract farmer's households in the study locations given

their higher dietary score average. The findings is consistence with that of Bellamere and Novak (2017) that studied the food security status of contract participating farmers in Madagascar, and also in line with that of Adebisi *et al* (2019) who study the impact of contract farming on the households' food security of poultry farmers.

Table 4. Estimated Food Dietary Diversity Score (HDDS) of farmers Household

Variables	Contract farmers Household		Non-Contract farmers Household	
Dietary Score	Frequency	Percentage	Frequency	Percentage
Good DDS (6+)	78	33.48	22	10
Medium DDS (4 – 6)	120	51.50	145	62
Low DDS (<4)	35	15.02	66	28
Total	233	100	233	100
Mean	5.36		3.15	
Standard deviation	1.42		1.39	

Note: DDS stand for dietary diversity score.
Source**:** Field Survey, 2019.

Covariate balancing and matching quality test

The overall balancing test was presented in table 5. The high total bias reduction, the significant p-values of likelihood ratio test after matching, low pseudo-R^2, and significant reduction in the mean standardized bias are indicative of successful balancing of the distribution of covariates between participants and non-participants groups. The result revealed that standardized mean difference for all covariates used in the PSM is reduced from 23.9% to 3.7% post-matching; result also show the matching reduction bias by 97.2%. In addition, the joint significant of covariates post-matching was also rejected (p-value=0.972). In addition to that, propensity score histogram in Figure 2 also revealed the quality distribution of the matching.

Table 5. Covariate Balancing and Matching Quality Test

Sample	Ps R2	LR chi2	p>chi2	MeanBias	MedBias
Unmatched	0.067	35.35	0.000	23.9	22.8
Matched	0.005	2.79	0.972	3.7	3.7

Source: Field Survey, 2019

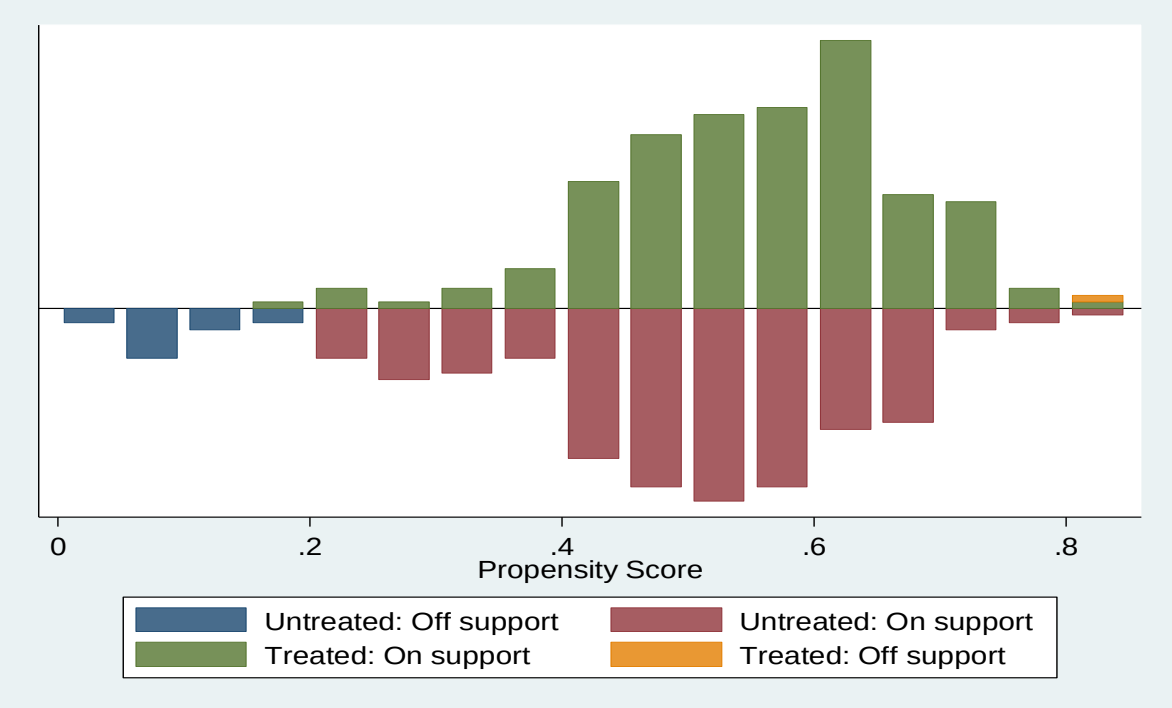

Figure 2. Matching histogram, for the contract and non-contract maize farmers **(**Source: Field Survey, 2019).

Impact of Contract Farming on Maize Yield per hectare

Impact of contract farming on maize yield per hectare was presented in table 6, result revealed that contract farming had a positive and significant effect on maize yield of smallholder maize farmers considered in the study area, the average treatment effect on treated(ATT) was NGN1742.98Kg/ha of maize produced by farmers. Average treatment effect on the entire farmers population (ATE), that is picking any farmer at random was also 1742.98Kg/ha. This implies that participation in contract farming will result to yield increase by about 1.7tonne/ha. For the effect on untreated category ATU value was 1732.98Kg/Ha, implying that, categories of respondents if assume they were treated their maize yield will also increase by 1732.98Kg/ha

Table 6. Impact of Contract Farming Participation on Maize Yield per Hectare

Outcome	Sample	Treated	Control	Difference	t-test
Yield per hectare	Unmatched	3628.65241	1895.67298	1732.97943	7.06***
	ATT	3628.22826	1885.24375	1742.9845	7.66***
	ATU	1885.24375	3628.22826	1742.9845	
	ATE			1742.9845	

Note:*, **, *** donates significant at 10%, 5% and 1% respectively
Source: field survey, 2019

Impact of Contract Farming on Food Security Status

Impact on food security result was presented in table 7; result revealed that participation in maize contract farming had positive and significant influence at 1% level of confidence on the food Security status of smallholder farmers, the average treatment effect on treated (ATT) was 0.8933. This implies 0.9 increase in food security of participating households. Result further show ATU of 1.136 for non-participants, this also implies 1.136 DDS increase in food security of non-contract household had it been they participated in the contract farming. Increase in nutritional food security status may be because contract participating farmers obtained higher yield and premium price of maize, which enables them to have more income to cater for household food expenditure than the non-contract maize farmers. The result is consistence with findings of Bellamere and Novak (2016) that studied the impact contact participation on food insecurity in Madagascar, in which they used the length of time household members go without eating three meals per day as proxy to food insecurity that revealed significant impact of contract participation in reducing the period of hunger. It's also in line with that of Adebisi L.O et al (2019) that analyzed impact of contract farming on the households' food security of the poultry farmers, in which their findings revealed a calorie intake increased on the average by 1047 kCal/AE/day as a result of participating in contract farming.

Table 7. Impact of Contract Farming Participation on Food Security Status

Outcome	Sample	Treated	Control	difference	t-test
Food security status	Unmatched	5.337755	4.28804348	1.04971162	6.46***
	ATT	5.32923077	4.43589744	0.8933	4.81***
	ATU	4.33529412	5.47152941	1.13623529	
	ATE			1.00646575	

Note:*, **, *** donates significant at 10%, 5% and 1% respectively
Source: field survey, 2019

Challenges Faced by Maize Contract Farmers

Result for challenges faced by farmers participating in maize contract production was presented in table 8. result shows that excessive control on pricing by contracting firm and inadequate insurance provision were. the 1st and 2nd major challenges faced by the farmers; the excessive control on pricing by contracting firm was due to the larger quantity of harvested maize that is collected by the firm and their dominance on price decision and inadequate insurance provision was as a result of contracting firms forcing the farmers to provide or pay for the required quantity even in the case of crop failure as a result of pest and disease or drought incidence and the farmers have no insurance to protect them. lower pricing of harvested maize by contracting firm was ranked as 3rd and delay in payment of farmers benefits as 4th challenge, the lower pricing was stated by the farmers as because the firm always possess highest power in deciding the price to be paid per bag of harvested maize and is mostly below market price, while the delay in payment of farmers benefits was due to the fact that after harvesting the farmers are not given their profit after company deducted their services fees and credit in time. Low quality fertilizer and herbicide was ranks 5th and high transaction cost as 6th, the low inputs quality was related to the quality of production inputs supplied to the farmers and the likely production inputs diversion by the farmers and yield they produced less than expected. While the high transaction cost were realized by the farmer

as a result of small amount of money they received as final payment from company and the number of bags given per hectare to contract firm as signed initially in the contract. limited farm monitoring by contracting firm agent was ranked 7th this was stated by the farmers as because the firms staffs number of visit to their farm is limited to only time of input supply and the harvesting periods.

Table 8. Challenges Faced by Maize Contract Participating Farmers

Challenges	Frequency	Percentages	Rank
Excessive pricing control by contracting firm	186	79.83	1st
Inadequate insurance provision	182	78.11	2nd
Lower pricing by contacting firm	176	75.54	3rd
Delay in payment of farmers benefits	172	73.82	4th
Low quality fertilizer and herbicide	168	72.10	5th
High transaction cost	162	69.53	6th
Poor farm monitoring by contracting firm agent	86	36.91	7th
Total	233	100	

Source: Field Survey 2019.

Conclusion

Participation in maize contract farming increases per hectare productivity of smallholder maize farmers and also reduces food insecurity status of their households. This suggested that contract farming can be used as an instrument to reduce food insecurity and poverty among rural farming household.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Ethics committee approval is not required.

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Data availability

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Consent for publication

Not applicable.

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Edible Film Production from Effluents of Potato Industry Incorporated with *Origanum onites* Volatile Oils and Changes Its Textural Behaviors under High Hydrostatic Pressure

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Abstract

Development and characterization of edible film incorporated with *Origanum onites* volatile oil from the effluents of potato industry, determination of changes on its textural properties of force and elongation at break (EAB) under high hydrostatic pressure (HHP) in addition to its antimicrobial effect against *Escherichia coli* O157:H7 and *Salmonella* Enteritis were prompted. The optimum operational conditions under HHP for maximum force and EAB were achieved with 350 MPa pressure, 8 min operational time, and addition of 45 µL *O. onites* volatile oil concentration (VOC). Inhibition zones for *S.* Enteritis and *E. coli* O157:H7 at the optimum conditions were 1.7 ± 0.109 and 2.386 ± 0.07 cm, respectively. Textural properties of force and EAB of the HHP-processed films ranged from 2.27 ± 0.52 to 5.23 ± 0.79 N, and from 7.47 ± 1.68 to 15.71 ± 0.65 mm, respectively. Thermal transition of the edible film was observed at 86.77°C for 7.19 min. The microscopic observation of the film surfaces shoowed homogenous and translucent structure. The improved textural properties with HHP and VOC revealed that it carries a potential to be used as a food packaging material.

Keywords: Edible film, High hydrostatic pressure, Potato industry effluent, Antimicrobial activity, Textural properties

Introduction

Recent interests have intensified the search for biodegradable food packaging and edible polymers (films) to replace plastics or synthetic polymers due to the growing environmental concerns (Borah et al., 2017; Lopez-Rubio et al., 2017). The high cost of biopolymers, on the other hand, makes agricultural by-products a viable feedstock to produce edible films or coatings. Different edible films developed from polysaccharide, protein, and lipid biopolymers have potential to be considered as food packaging or coating to enhance food quality and safety (Nandane and Jain, 2018; Park et al., 2017). Because it carries advantages of having lower cost, being available, higher thermoplasticity, and inherent biodegradability; starch, as the carbohydrate-based polymers, is one of the most suitable and promising material for biodegradable, and edible packaging (Ehiyet et al., 2011).

According to data released by FAOSTAT in 2019, Turkey ranked in the 14th place, with its 4.8 million tons of potato production (Anonymous, 2019). Potato industry produced about 0.16 tons of solid waste per ton of processed potato such as pulp, potato wastewater, and peel (Pathak et al., 2018). In general, waste from potato industry has been utilized as components of microbial media (Kot et al., 2020) as well as production of edible film from potato peel waste (Othman et al., 2017).

Active food packaging is a rapidly developing food packaging technology actively allowing to eliminate contamination of foods (De Kruijf et al., 2002). Active packages are mostly incorporated with antioxidants, vitamins, antimicrobial agents, or flavoring agents as biologically active compounds to increase its functionality (Quintavalla and Vicini, 2002), and thus antimicrobial agents containing packaging can be as an excellent packaging alternative to improve the safety of food products.

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Essential plant oils of origanum species as antimicrobial agents are getting more interests because of their potential antimicrobial properties and consumer demand for less synthetic and artificial additives in foods (Ehivet et al., 2011; De Kruijf et al., 2002; Quintavalla and Vicini, 2002; Burt, 2004; Min and Oh, 2009).

High hydrostatic pressure (HHP) processing involves application of high pressures from 100 to 800 MPa to liquid and solid foods at ambient, lower, and higher than the ambient temperature (Farkas and Hoover, 2000; Torres and Velazquez, 2005). HHP is mainly used for food pasteurization, but studies related to effect of HHP on the buckwheat, tapioca starch film, and modified amaranth proteins preparation and its effect on starch gelatinization as well as film properties were reported (Condés et al., 2015; Kim et al., 2018). Even though development of edible films and measurement of their textural and mechanical properties are reported in the literature, information regarding the use of food industry by-products in the development of potential food-packaging materials, measurement of its properties, and determination of changes in its properties under food processing technologies such as HHP when used as food packaging material is not reported in the literature. Thus, the objective of this study was to produce an edible film from effluents of potato production industry having potential to be food packaging incorporated with *Origanum onites* volatile oil to carry antimicrobial properties and determine its textural behaviors under HHP.

Materials and Method

Effluents of potato production treatment plant

Effluents were sampled from a potato processing plant based in Bolu, Turkey (Köksal Patates Sanayi ve Ticaret A.Ş.). The samples were taken at the same day when discharge was started.

Extraction and determination of major constituents of *Origanum onites* volatile oil

Dried leaves of *Origanum onites* were purchased from local stores (Bolu, Turkey). Leaves (30 g) were placed in a 2 L-volume Clevenger type apparatus and mixed with 500 mL water before extraction. Temperature of the heater was gradually increased up to 250 ºC for 2 h for extraction. Temperature of the extracted oil was lowered to room temperature with cooling coils connected to tap water. Obtained oil was kept in an amber-colored bottle at 4 ºC until its further use (Dadalioglu and Evrendilek, 2004).

In order to determine the major constituents, 30 µL of the *O. onites* volatile oil were mixed with 1.5 mL hexane before injecting to a gas chromatography mass spectroscopy (GC-MS) (Shimadzu 2010 QP 2010 Plus GC-MS Kyoto, Japan) coupled with HP-5 MS capillary column (30 m x 0.25 µm x 0.25 µm) with 250 ºC injection block temperature. A maximum column temperature of 325 ºC was used starting at 50 ºC for 5 min, with a 2 ºC/min increase to 90 ºC, then a 5 ºC/min increase to 210 ºC for 5 min. Helium as a carrier gas at a flow-rate of 1.5 mL/min. Mass spectrum of each compound was taken over the 35–350 *m/z* range with the electron ionization mode of 70 eV. Volatile oil components (VOC) were identified with comparison of their retention indices (RIs) and mass spectra to Wiley (275) library (Dadalioglu and Evrendilek, 2004).

Preparation of the edible film

Effluent samples contained pieces of solid potato particles and peels, thus liquid phase was removed at room temperature after the samples were settled for three hours. After removal of macro particles, the fraction contained starch was centrifuged at 0.45 x *g* for 5 min. Pellets from the effluent were dried at 37 ºC for 24 h, and grinded with an 8-mm sieve. About 300 g dry residue were obtained from 5 L effluent.

Twenty-five g of dried residue mixed with 250 mL water (1:10 w/v ratio) and 5 % (v/v) plasticizer (glycerol). The magnetic hot plate (Wisestir, Germany) at 240 rpm was set to 150 ºC, and the mixture at 85-90 ºC was heated for at least 75 min. pH was adjusted to 2.6 with 0.1M HCl (Sigma Aldrich, Steinheim, Germany). Food grade green colorant (0.2 mL) and *O. onites* volatile oil at three different concentrations of 30, 45, and 60 µL/mL were added to edible film mixtures (Table 1). The mixture solution was stirred until colorant and *O. onites* oil are homogenously mixed. The final solution was cooled at room temperature and poured into an aluminum casting tray (26 x 17 cm) placed into a natural convection oven at 40 ºC for 10 hours to dry the film samples.

Bacterial cultures

Activation of *Escherichia coli* O157:H7 (ATCC 35218) and *Salmonella* Enteritis (OSU 799) cultures (The Ohio State University, Columbus, OH) was realized by transferring both cultures to Tryptic Soya Broth (TSB, Fluka, Seelze, Germany). Both cultures were incubated at 35 ± 2 ºC overnight. After transferring grown cells into sterile tubes; the samples were centrifuged at 0.49 x *g* for 15 min, separately, and the supernatants of the liquids were removed. Obtained pellets were resuspended with 10 mL pre-sterilized phosphate buffered saline (Sigma Chemical Co., Stockholm, Sweden). Cell resuspensions were washed with PBS in several times to remove TSB. Number of viable cells as log cfu/mL were determined by preparing a 10-fold serial dilutions of 0.1 mL aliquot plated on TSA plates. Prepared plated were incubated at 35 ± 2 ºC for 24 h.

High hydrostatic pressure processing

A 2-L capacity pilot-scale HHP equipment (Avure, Middletown, OH, USA) with water used as pressured fluid was used to process the film samples. The pouches were put into the flexible pouches composed of a multilayer polymer/aluminum/polymer film (polyethylene–aluminum–polypropylene) (APACK Packaging Technologies, Istanbul, Turkey). After heat sealing of the pouches, the samples were processed by the pressures changing from 200 to 500 MPa, treatment time from 1 to 15 min, *O. onites* volatile oil volumes (VOC) from 30 to 60 µL (Table 1), and the maximum processing temperature of 29 ºC.

Table 1. (Un)coded variables of Box-Behnken design for textural properties of biopolymer with *Origanum onites* volatile oil.

Run Order	HHP pressure (*P*, MPa) X_1	Volatile oil concentration (*VOC*, µL) X_2	Time (*T*, min) X_3	Force (*N*)	Elongation at break (mm)
Control	-	-	-	3.60 ± 0.23^{bc}	8.83 ± 0.14^{d}
1	500	45	1	4.57 ± 0.07^{a}	11.78 ± 1.35^{b}
2	500	60	8	4.90 ± 0.60^{a}	15.71 ± 0.65^{a}
3	350	30	1	5.23 ± 0.79^{a}	12.53 ± 0.62^{b}
4	200	60	8	4.64 ± 0.51^{a}	10.25 ± 1.09^{c}
5	500	45	15	3.79 ± 0.26^{bc}	12.79 ± 1.49^{b}
6	200	30	8	4.70 ± 0.39^{a}	12.71 ± 2.72^{bc}
7	350	45	8	4.43 ± 0.28^{ab}	9.82 ± 1.20^{c}
8	200	45	15	2.27 ± 0.52^{d}	7.47 ± 1.68^{e}
9	500	30	8	4.68 ± 0.16^{a}	10.78 ± 1.13^{c}
10	350	30	15	3.95 ± 0.08^{c}	12.67 ± 1.33^{bc}
11	350	60	1	3.92 ± 0.54^{b}	12.49 ± 1.65^{bc}
12	200	45	1	2.68 ± 0.01	9.04 ± 0.00^{d}
13	350	60	15	3.85 ± 0.39	12.30 ± 2.46^{b}

*Data with different superscript letters in the same response column show a significant difference at $p \leq 0.05$.
**Data is presented as average ± standard deviation

Properties of the effluent

pH of the effluents was measured at room temperature with 10 mL of the samples (Orion perpHectlogR meter, Inolab WTW, Germany).

Conductivity (mS/cm) measurement was performed by conductivity meter (Sension 5 model, HACH, CO, USA) with 50 mL of the samples at room temperature.

Twenty-five mL of the effluent samples at room temperature was used for turbidity measurement. Turbidity (NTU) was conducted by the turbidimeter (Micro TPI, HF Scientific, FL, USA).

Fifty mL of the samples after filtration through Whatman 42 filter paper dried at 105 °C for 30-40 min. Suspended solid matter (SSM) was calculated by taking into account the initial weight (g) of the paper after drying (A) and the weight (g) of the paper after filtration (B) (Rice et al., 2005).

$$SSM\left(\frac{mg}{L}\right) = \frac{(B-A)}{V} x100 \quad (1)$$

Spectroquant cell tests (DIN ISO 15705, Merck, Germany) were used to determine chemical oxygen demand (COD). The effluent samples were homogenized, and then diluted with distilled water at 1:50 ratio. Three mL of the diluted samples were taken and placed into the COD tubes heated at 148 °C in thermoreactor for 120 min. When the tubes were cooled to room temperature, the measurement was performed using the photometer.

Initial microbial load of effluent

Appropriate dilutions of effluent samples prepared with 0.1 % peptone were plated onto potato dextrose agar (PDA, Fluka, Seelze, Germany) to count total mold and yeast count (TMY); and plate count agar (PCA, Fluka, Seelze, Germany) to count total aerobic mesophilic bacteria (TAMB), respectively. PCA plates were incubated at 35 ± 2 °C for 24 to 48 h, while PDA plates were incubated at 22 ± 2 °C for 3 to 5 days. Results were expressed in log cfu/mL.

Properties of the edible film

Weight and thickness of edible films cut in an 8.64-mm diameter using a mold were measured using a balance with 0.001 g sensitivity and micrometer (Mitutoyo, Japan) with 0.0001 mm sensitivity, respectively (Seydim and Sarikus, 2006).

Edible films were cut at 40 x 40 mm^2 dimensions, and each piece was transferred into beaker containing 50 mL of water for the determination of water solubility. Each beaker was sealed with parafilm and they were transferred into shaking water bath at 25 °C for 24 h. Insoluble fraction of the film samples was dried at 70 °C for 10 min until a constant weight was reached. Results were calculated as follows (Razavi et al., 2015):

$$\%\ water\ solubility = \frac{W_0 - W_{24}}{W_0} * 100 \quad (2)$$

where W_0 is the initial dry weight of the film before drying (g), while W_{24} is the final dry weight (g) of the insoluble film after drying.

L^*, a^*, and b^* color parameters of the edible film were measured using a colorimeter (Konica Minolta CR-400, Osaka, Japan) Total color difference (ΔE) was calculated as follows;

$$\Delta E = \sqrt{(L_0 - L*)^2 + (a_0 - a*)^2 + (b_0 - b*)^2} \quad (3)$$

Both the control and the HHP treated films samples having *O. onites* volatile oil were cut in 80 x 25 mm dimensions using a standard mold at 25 °C and 53 ± 2% relative humidity for 48 h before textural measurements. Elongation at break (EAB) and force of the film samples were measured using a TA-XT2 model texture analyzer (Stable Micro Systems, Surrey, England) with a tensile grip at 1 mm/s speed, and calculated by the software (Texture

Expert Exceed 2.3, Stable Micro System, Survey, England) (Chaichi et al., 2017).

Thermal properties of edible film

Differential scanning calorimeter (DSC, TA Q20 Instruments, Shimadzu, Japan) was used in order to determine the thermal properties of biopolymers. Calibration was performed with the indium standard (156.6 ºC melting temperature and 28.5 J/g melting enthalpy) under the nitrogen atmosphere with 30 mL/min flow rate. Thermograms were obtained with 2-3 mg of the samples weighed into sampling cups, and scanning was made between 20 and 100 ºC at an interval of 10 ºC/min.

Surface properties of biopolymer was observed using a binocular microscopy (BK 5000 L modal, Soif Optical Instruments, China) at 4x magnification. X-ray diffraction (XRD) patterns of the films were measured using a MRD X-ray diffractometer (Rigaku, Neu-Isenburg, Germany) with Coka radiation. The samples were prepared by placing the square shape of each film (2 cm x 2 cm) on a glass side. A nickel monochromator filtering wave was used at 36 kV and 26 mA with scanning at $2\Theta = 5^0$- 80^0 at a rate of 2.20^0/min and with a step size of 0.02^0.

Antimicrobial activity of edible film

Antimicrobial activity of the edible film was tested against *S.* Enteritis and *E. coli* O157:H7, separately. Both cultures at the 10^6 cfu/mL cell count were plated onto PCA agar plates, and the plates were incubated for 48 h at 35 ± 2 ºC for surface growth. An edible film disc of 8.64 mm with 45 µL *O. onites* volatile oil (concentration at optimum point) added during film preparation was placed onto surface of PCA agar. The plates were incubated for additional 24 h at 35 ± 2 ºC, and zone of inhibition was measured using a micrometer. Control samples were prepared the same but without addition of *O. onites* volatile oil.

Data analyses

The force and EAB responses of the edible film production were optimized as a function of pressure (200 to 500 MPa), volatile oil concentration (30 to 60 µL), and processing time (1 to 15 min) using the Box-Behnken design (BBD) with a quadratic model. The levels of these settings were determined by initial experiments. The overall BBD configuration with its (un)coded predictors is presented in Table 1. Data analyses were conducted by MINITAB 17.0 (Minitab Inc. State College, PA, USA). A quadratic regression model was used to best-fit the experimental data:

$$Y_n = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (4)$$

where Y_n is the response variable of force and EAB; b_0 to b_{33} are slope coefficients; and X_1, X_2 and X_3 are pressure (MPa), VOC (µL), and processing time (sec), respectively. Additional experiments, to validate the models, were carried out under the optimal conditions. Analysis of variance (ANOVA) and regression models were applied to determine the significant terms of the predictive model ($p < 0.05$). Tukey's test was further utilized to determine the multiple comparisons. Verification of the predicted model was conducted by the coefficient of variation (CV, %) as follows:

$$CV = \frac{\sigma}{\overline{X}} 100 \quad (5)$$

where σ is sample standard deviation, and $\overline{C}$ is sample mean.

Results and Discussion

Properties of the effluent and the edible film

The effluent samples had pH of 6.16 ± 0.14, conductivity of 1774 ± 38 mS/cm, and turbidity of 1170.00 ± 57.01 NTU at the temperature of 12.49 ± 0.34 ºC. The mean COD was determined as 50093.75 ± 3361.41 mg/L. The amount of SSM was 0.65 ± 0.13 mg/mL. The initial TMAB and TMY loads were > 9 log cfu/mL.

Average weight and the thickness of the film samples were 0.0177 ± 0.0004 g and 0.370 ± 0.005 mm, consequently. Water solubility of the edible film was estimated at 32.69 ± 4.34%. The color of the edible film changed from cream to green with the addition of green coloring agent. The natural color of the edible film before addition of green color was measured as 79.55 ± 0.64, -0.0011 ± 0.01, and 4.19 ± 0.18 for *L**, *a**, and *b**; whereas they were measured as 76.8 ± 1.84, -21.70 ± 4.92, and 28.94 ± 4.77 after addition of green color, respectively. ΔE of the edible film samples were recorded as 84.89 ± 11.36.

The initial force and EAB values of the film were 3.60 ± 0.23 N and 8.83 ± 0.14 mm, respectively. With HHP treatment, the force ranged from 2.27 ± 0.52 to 5.23 ± 0.79 N. Except for run 8 (200 MPa pressure, 45 µL volatile oil, and 15 min treatment time) and run 12 (200 MPa pressure, 45 µL volatile oil, and 11 min treatment time), HHP treatments regardless of the processing time and VOC, led to higher force values than that of the control samples ($p \leq 0.05$). The highest VOC (60 µL) and pressure (500 MPa) under the moderate processing time (8 min) provided the highest force to film (5.23 ± 0.79 N) on run 3 ($p \leq 0.05$) (Table 1).

EAB of the HHP-treated samples ranged from 7.47 ± 1.68 to 15.71 ± 0.65 mm. Except for run 8 with (200 MPa pressure, 45 µL volatile oil, and 15 min treatment time), all the other HHP processes revealed higher EAB value than that of the control samples. Again, the highest VOC (60 µL) and the pressure (500 MPa) under the moderate processing time (8 min) provided the highest EAB (15.71 ± 0.65 mm) on run 2 ($p \leq 0.05$). The increased force did not linearly increase with the VOC and processing time, but with the pressure and the combination of pressure and VOC (Table 1).

Inhibition zone diameters of *S.* Enteritis and *E. coli* O157:H7 with the addition of 45 mL volatile *O. onites* oil were 1.70 ± 0.109 and 2.39 ± 0.07 cm with the calculated inhibition zone area of 2.15 ± 0.383 and 4.95 ± 0.341 cm^2, respectively. No zone of

inhibition was observed for the edible film samples with no *O. onites* volatile oil.

GC-MS analyses of *O. onites* volatile oil revealed 76 different compounds among which 5-methyl-2-propan-2-ylphenol (thymol, 34.67%), 2-methyl-5-(propan-2-yl) phenol (carvacrol, 28.32%), 3-methylpentane (5.65%), 3,7-dimethylocta-1,6-dien-3-ol (linalool 5.06%), 1-methyl-4-(propan-2-yl) benzene (*p*-cymene, 3.48%), and 2-methylpentane (2.22%), were the highest. High antibacterial activity of *O. onites* volatile oil correlated with the important percentages of thymol (34.67%) and carvacrol (28.32%). Both constituents were also found higher in *O. onites* samples by 5.97 and 71.22% (*23*) and 1.51 and 37.08% (Sevindik et al., 2019). The ability of origanum species to inactivate bacterial strains in synthetic media as well as in food system were related to higher amount of both thymol and carvacrol (Lambert et al., 2001; Knowles et al., 2005; Valero and Francés, 2006). It is also reported that not only the components present in high proportions are responsible for the antimicrobial activity, but also the less abundant ones, like p-cymene should also be considered. Aqueous extract of *O. onites* displayed MIC value of 25 mg/mL against methicillin susceptible *Staphylococcus aureus* (MSSA), methicillin resistant *S. aureus* (MRSA), *Klebsiella pneumoniae*, *Pseuodomonas aeruginosa*, *Bacillus cereus*, and *Enterococcus faecalis*, and 6.25 mg/mL against *E. coli* (*28*). Indeed, it was also presented that carvacrol, thymol and p-cymene of *O. compactum* essential oil have strong antibacterial affect against different bacteria (Andrade-Ochoa et al., 2015; Bouyahya et al., 2017; Chauhan and Kang, 2014; Li and Liu, 2009; Xu et al., 2008).

Starch based active films and food packaging with the addition of plasticizers is a promising alternative due to their low cost, availability and functionality (Castillo et al., 2017). Antimicrobial properties of these films make them suitable for food packaging providing significant inactivation of food borne pathogens and food spoilage microorganisms. For instance, tapioca starch-glycerol edible films incorporated with potassium sorbate controlled growth of *Zygosaccharomycess bailii* contamination in semisolid product (Flores et al., 2007). Starch-clay nanocomposite films contained potassium sorbate presented antimicrobial property against *Aspergillus niger* (Barzegar et al., 2014). Starch film having different concentrations of chitosan and lauric acid exert an inhibitory effect on *Bacillus subtilis* and *E. coli* growth (Salleh et al., 2014).

Due to the antimicrobial properties of Maillard compounds, 1-2 log inactivation was observed on *Listeria innocua* and *E. coli* inoculated in starch based edible film from corn formulated with different oxidation degree and addition of bovine gelatin, glycerol, as a plasticizer, and ethyl lauroyl arginate (LAE) as antimicrobial compound (Moreno et al., 2017). Inhibition efficiency of the starch films incorporated with tea polyphenol (TP) ranged approximately from 60 to 90% for *E. coli* and from 90 to 100% for *S. aureus* revealing good antimicrobial properties (Feng et al., 2018).

It is desired for edible films to have high water resistance because they can be used as protective layers on high as well as intermediate moisture foods (Gontard et al., 1992). In order to prevent the complete dissolution of the film, corporation of antimicrobial agents into edible films are also desired. Antimicrobial edible films having poor water resistance is dissolved very quickly, thus its antimicrobial properties protected (Castillo et al., 2017; Ozdemir and Floros, 2008). Water solubility of the buckwheat starch film prepared using thermal processing (90 ^{o}C for 20 min) and HHP (600 MPa 20 ^{o}C for 20 min) were 19.85 and 11.67%, respectively. It was observed that the water solubility of both films decreased with the application of HHP in spite of the higher moisture content (Kim et al., 2018). The water solubility of the edible film developed in the present study was comparable to those cited in the literature (Kim et al., 2018).

Thermal properties of the edible film

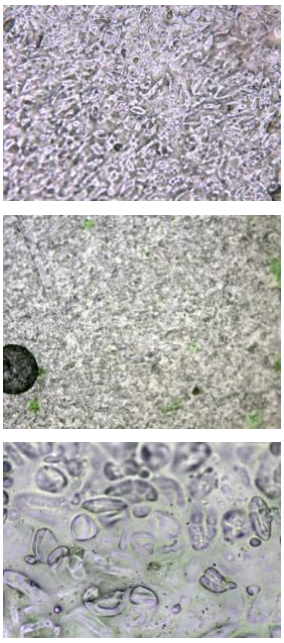
Thermogram results showed that the thermal transition of the edible film was at 86.77 °C for 7.19 min. No activity recovered from the samples after thermal transition temperature indicated that the phase transition was irreversible.

Thermal degradation behavior and stability of starch based materials is important property because it determines the behavior of the film during processing (Liu et al., 2013). Thermo-gravimetric analysis has been widely used to determine the thermal stability and decomposition of starch based film formulated using different sources (Aggarwal et al., 1996; Guinesi et al., 2006; Soares et al., 2005). Both dehydration and decomposition are taken into consideration with the degradation mechanisms of starch (Liu et al., 2009; Liu et al., 2013). These values give information to predict how these materials behave under more realistic atmospheric conditions (Acar et al., 2008; Peterson et al., 2001).

Thermal properties of the film change depending on the base materials and type of the plasticizers used. For example, sage seed gum (SSG) edible films with glycerol and sorbitol presented two exothermic peaks with T_g at about 59.7 and 277.7 °C, which the latter may be related to the thermal decomposition of SSG. T_g of SSG film shifted to 52.2 and 179.1 °C with the presence of glycerol, while sorbitol reduced it to 47.2 and 245.3 °C, respectively. In the glycerol plasticized sample, two additional peaks with T_g of about 286.4 and 340.8 °C were appeared (Razavi et al., 2015).

The microscopic observation of the film surfaces showed that the film was homogeneous and translucent. Even though film formation occurred due to the solubility of starch molecules, some starch molecules were still intact and not completely soluble (Figure 1a). Water-based green color was dissolved in the film and distributed in the continuous starch matrix. HHP processing caused swelling of starch molecules and led to the even distribution of green color (Figure 1b). Starch

molecules grew in size and were dissolved better after the HHP processing (Figure 1c).



a) Control film samples (4X magnification)

b) *O. onites* volatile oil added film samples (4X magnification)

c) *Origanum onites* volatile oil added and HHP processed (200MPa 1 min) film samples (4X magnification)

Figure 1. Microscopic observations of the film samples

Pressure application provides gelatinization of starch in two steps: At first stage, hydration of the amorphous parts of the starch granules occur, and then crystalline region starts to swell (Grossi et al. 2012; Simonin et al., 2011). Applied pressure level, model of pressure application (continuous or cycle), pressure time, processing temperature, as well as the constituent and phase state of the food affect the resistance of starch microstructure to pressure (Simonin et al., 2011). These structural changes after the HHP treatment helped to understand the changes in the textural properties of the film. Depending on the applied HHP processing and VOC concentrations, the texture of the film changed, thus affecting the force and elongation properties

It is reported that HHP caused a decrease in molecular order revealing higher amylopectin amorphous layer (ρ_a) (unlikely), or induced changes in both crystalline and amorphous regions leading to ρ_a decrease (Lopez-Rubio et al., 2017). Applied magnitude of pressure plays an important role in starch structure depending on the starch type. For example, pressure at the magnitude of 200 MPa does not cause a noticeable shifts or changes in the main peaks, but pressure application at 600 MPa for 30 min significantly reduced the peak intensity at 18 °. Dual peaks observed at 17° and 18° were merged into a single peak at 17°, characteristic of B-type starches after the application of 600 MPa at cycle mode. Due to their scattered and flexible amylopectin branching structure, A-type starches are generally believed to be more sensitive to pressure than B- and C-type ones (Deng et al., 2014).

Table 2. ANOVA and regression model results for two functional responses of biopolymer film with *Origanum onites* volatile oil.

	Force (g) (Y_1)		Elongation (mm) (Y_2)	
Term	Coeff	*p*	Coeff	*p*
Linear				
P (X_1)	55.20	0.000	1.45	0.000
VOC (X_2)				
T (X_3)				
Square				
P^2				
VOC^2	43.70	0.003	2.35	0.000
T^2	-79.50	0.000		
Interaction				
*P*VOC*	41.70	0.003	1.85	0.000
*P*T*	-26.50	0.049		
*VOC*T*				
Lack-of-fit		**0.249**		**0.616**
Intercept	442.7	0.000	10.076	0.000
R^2_{adj}	0.77		0.68	

Table 3. Summary of experimental versus predicted values for textural properties of biopolymer film according to BBD.

	Force (Y_1)	Elongation (Y_2)
Predicted values	366.87	12.83
Experimental values	368.06 ± 7.31	12.85 ± 0.08
Coefficient variation (*CV*, %)	0.16	0.07

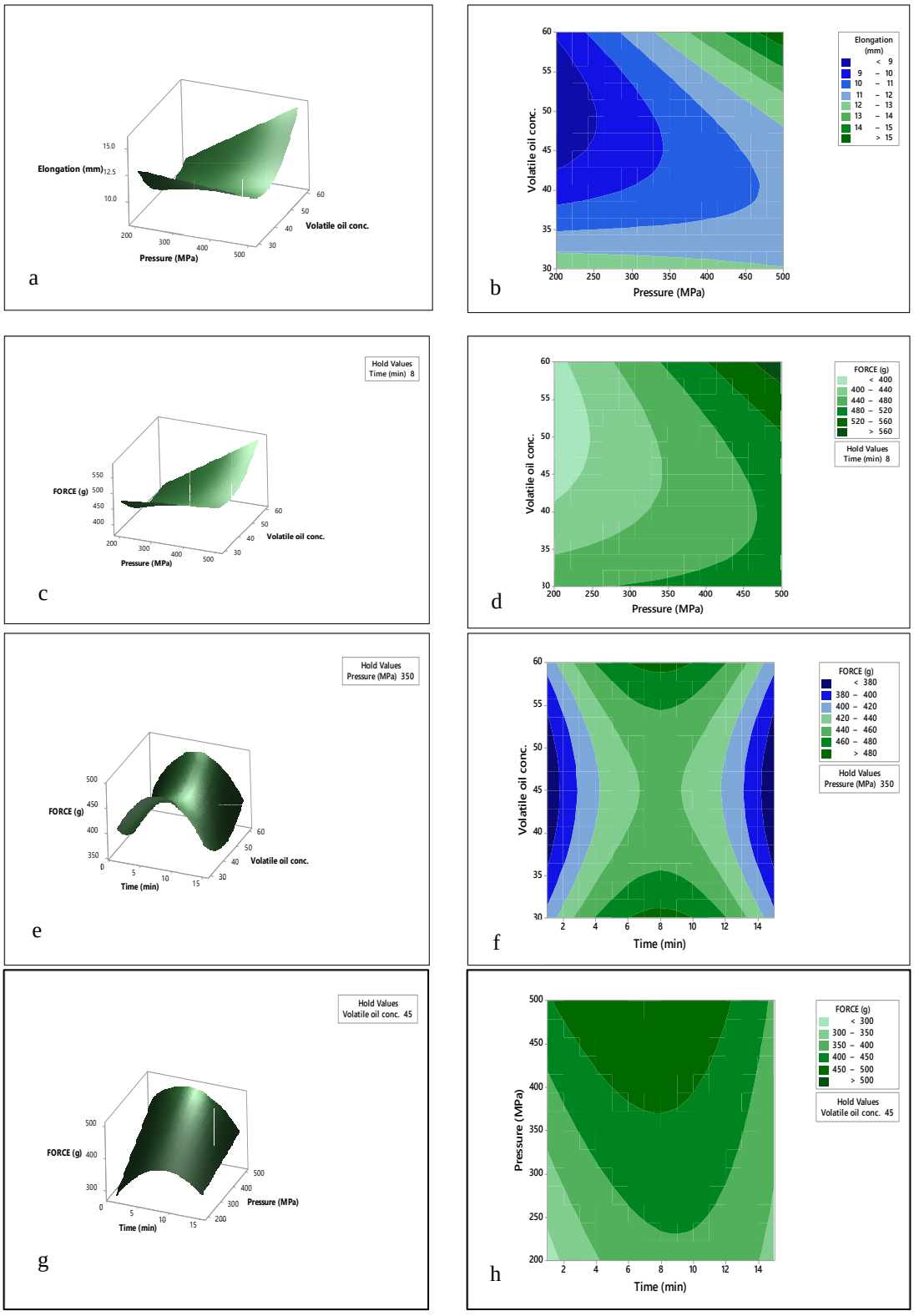


Figure 2. Interaction effects of pressure-volatile oil concentration with treatment time as constant value on elongation for textural properties of biodegradable film showing a) response surface and b) contour plots; interaction effects of pressure-volatile oil concentration on force for textural properties of biodegradable film showing c) response surface and d) contour plots; interaction effects of time-volatile oil concentration on force for textural properties of biodegradable film showing e) response surface and f) contour plots; interaction effects of pressure-time on force for textural properties of biodegradable film showing g) response surface and h) contour plots

Modelling of the textural properties of the edible film

Among the three explanatory variables, only HHP significantly affected ($p \leq 0.05$) both force and EAB of the edible film. According to the best-fit regression models, there existed a positive correlation between pressure and VOC ($p \leq 0.001$) for both textural properties (Table 2).

The significant quadratic terms were found for time ($p = 0.000$) and VOC ($p = 0.003$) with a positive effect on force and VOC ($p = 0.000$) for EAB of the film with a negative effect (Table 2). There was a significant interaction between VOC and pressure ($p \leq 0.05$) with a positive effect, and between time and pressure ($p \leq 0.05$) with a negative effect on force. A significant interaction between pressure and VOC ($p \leq 0.05$) with a positive effect on EAB of the film was also observed (Table 2). The degree of influence of the operational conditions on the force and elongation responses can be inferred from comparing the magnitudes of the coefficients of the quadratic regression models. HHP was the most important factor for both force (55.20) and EAB (1.45) (Table 2). The goodness-of-fit (R^2_{adj}) of the models showed 0.77 and 0.68 % of variations in force and EAB, respectively (Table 2). The insignificant lack-of-fit values for the two models also indicated that the model fitted the experimental data well (Table 2).

According to ANOVA results, the insignificant terms were excluded from the models of the textural properties of the film. The best-fit regression models explained 81 and 71% of variations in force and EAB, respectively. The EAB estimates of the film as a function of HHP, time and VOC are illustrated in Figure 2a. EAB increased with the increased VOC under the highest pressure at an increasing rate (Figure 2a). The highest VOC maximized EAB at the highest HHP of 500 MPa (Figure 2a). Force decreased with the increased VOC under the lowest pressure at a decreasing rate (Figure 2c). The lowest VOC values minimized force at the constant pressure of 350 MPa (Figure 2e). The lowest time minimized force at the lowest pressure (200 MPa) (Figure 2g). Three-dimensional (3D) response contour plots were used to observe the interaction effect of variables in pairs on both EAB and force (Figures 2a, 2c, 2e, 2g).

While circular contour plots indicate a negligible interaction, elliptical or saddle contour plots indicate a significant interaction between the corresponding variables (Murthy et al., 2000). In both figure 2b and 2d, the contour plot showed a significant interaction between VOC and HHP. In figures 2f and 2h, the circular shape of the contour plots indicated a negative time-by-HHP and a negligible time-by-VOC interaction for the force of the film.

Joint optimization and model validations

The overlaid contour plots were used to visualize how the operational settings simultaneously influenced the force and EAB of the film (Figure 3). The solid contour line is the lower bound and the dotted contour is the upper bound (Figure 3). The contours of each response are displayed in a different color. The white area of the plots (feasible region) shows the range of pressure, VOC, and time where the criteria for three response variables are satisfied whereas the shaded area shows regions do not fit the optimization conditions (Figure 3). Figure 3a shows that VOC at 45µL, the HHP range of 200 to 400 MPa with 350 MPa of optimum pressure and 1.7 min for processing time were the optimum setting. With 8 min, both HHP and VOC were maintained in a very narrow range as shown in figure 3b. The two feasible zones (Figure 3c) were obtained with the optimum settings of both time and VOC.

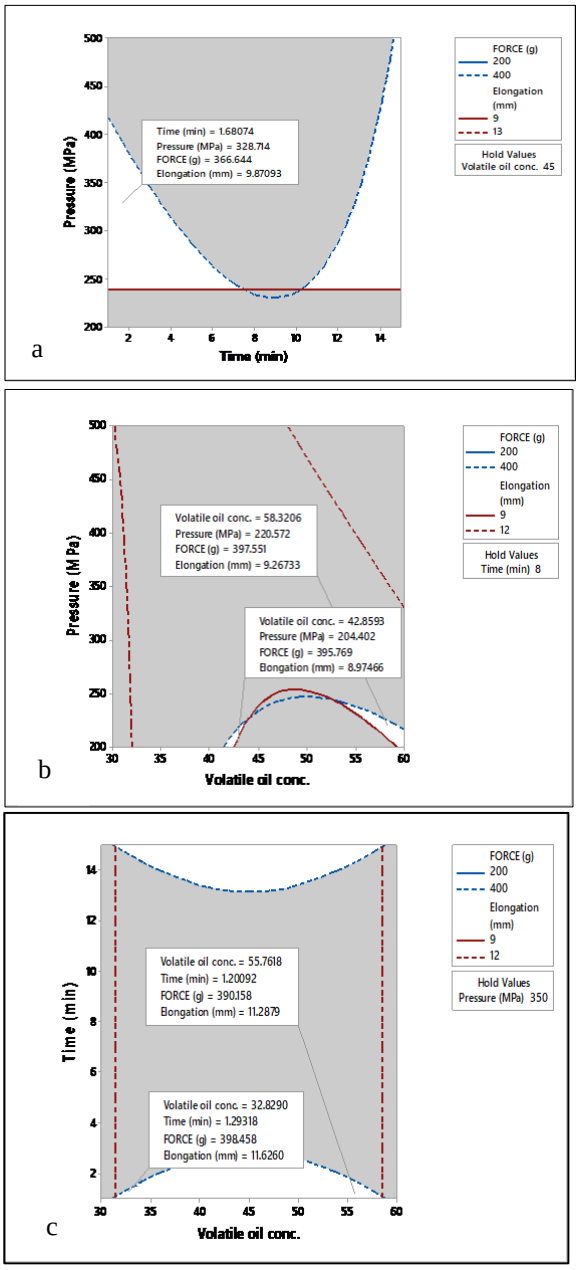


Figure 3. Overlaid contour plots showing feasible operational regions to maintain textural properties of biodegradable film under the constant values of (a) VOC of 45µL, (b) time of 8 min and (c) HHP pressure of 350 MPa

The operational settings for force and EAB of the film production revealed the best solution for the multiple response optimization with 350 MPa pressure, 45 μL VOC, and 8 min operational time. The minimum force (366.87 g) and maximum EAB (12.83 mm) values were obtained with the optimum operational conditions. These conditions were experimentally tested to validate the predictive power of the models. The resultant force and EAB values of 368.06 ± 7.3 g and 12.85 ± 0.08 mm, respectively, indicated no significant difference between the measured and predicted values (Table 3). The smaller CV values showed the better reproducibility of the model.

Conclusions

Although different food industry wastes were used to produce edible films, the production of an edible film from the effluents of potato industry carrying antimicrobial properties with addition of *O. onites*, measurement of its physical properties, and determination of the changes in mechanical properties under HHP are limited. The edible film produced from the effluents of a potato industry was HHP-processed (up to 500 MPa) with the addition of volatile *O. onites* oil. The combination of *O. onites* and HHP-processing provided antimicrobial activity against both *E. coli* O157:H7 and *S.* Enteritis with 2.386 ± 0.07 and 1.7 ± 0.109 cm of inhibition zones and 4.95 ± 0.341 and 2.153 ± 0.383 cm^2 of the calculated inhibition zone areas, respectively. HHP significantly affected both force and EAB of the edible film with a positive correlation between pressure and VOC for both textural properties. The optimum operational conditions obtained for the multiple response optimization were achieved with 350 MPa pressure, 45 μL VOC, and 8 min operational time.

This study focused on the production of the edible film from the effluents of potato industry, and its textural properties and antimicrobial activity. Its flexible and durable structure showed that this film may have potential to be applied as a food packaging, but food-safety, barrier properties, and migration test must be performed in order to evaluate its potential as food packaging. Thus, future studies remain to be conducted to determine the effectiveness of the film on the shelf life extension of different foods.

Compliance with ethical standards

Conflict of interest

The authors have no conflict of interest to declare

Author contribution

Gulsun Akdemir Evrendilek: Supervision, conceptualization, methodology, writing manuscript & editing, data analyses, project administration, funding acquisition; Nurullah Bulut: Data curation, experimental; Sibel Uzuner: Writing manuscript & editing, data analyses, editing.

All the authors read and approved the final manuscript. Text, figures, and tables are approved by all thr authors and they have not been published before.

Ethical approval

Not applicable.

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Data availability

Data will be available upon request.

Consent for publication

Not applicable

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In vitro antioxidant activity and carbonic anhydrase inhibitory features of *Ferula communis* extracts

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Abstract

Carbonic anhydrases (CAs; EC 4.2.1.1) are essential family of metalloenzymes which catalyze the interconversion between carbon dioxide (CO_2) and bicarbonate (HCO_3^-) in all organisms of three-domains of life. Huge amounts of attempts related to catalytic activity of CAs have been widely expanded to treat many clinical diseases. This study aimed to determine *in-vitro* antioxidant activities and human CA I (hCA I) and II (hCA II) inhibitory properties of *Ferula communis* extracts. Among all extracts of *F. communis*, the hexane extract has showed the best inhibitory profile on hCA I and II with IC_{50} values 8.68 µg/mL and 28 µg/mL and K_i values 2.026 µg/mL and 11.6 µg/mL, respectively. All extracts showed mild to moderate antioxidant activity. According to the results of DPPH assay, ethanol-water extract showed the highest activity with IC_{50}: 0.1128±0.0066 mg/mL value. Chloroform extract showed the highest activity on CUPRAC assay with the value of 1.305±0.037 mM Trolox equivalent/mg extract. However, further analytical, *in-vivo* and clinical studies are needed to confirm the activities of *F. communis*.

Keywords: Antioxidant activity, Carbonic anhydrase, *Ferula communis*, Inhibition

Introduction

Belonging to Apiaceae family, genus *Ferula* consist of 180-185 species, which makes it the third largest genus in the family. Plants from genus *Ferula* possess plenty of compounds which are biologically active. These include disulfide compounds coumarin derivatives, sesquiterpene compounds, aromatic lactones, daucane esters (Salehi et al., 2019). Plants belonging to this genus are also used in various diseases for therapeutic purposes such as antipyretic, smooth-muscles relaxant, contraceptive and aphrodisiac. In addition to this, plant compounds obtained from this genus are used as folk medicine for the treatment of numerous problems such as headache, digestive and neurological disorders, arthritis, rheumatism and dysentery (Iranshahi et al., 2018).

Ferula communis has been used to treat hysteria, skin infections and dysentery in folk medicine and has a long history of medical use in practice. Additionally, it has been used as analgesic, diuretic and anti-helmintic. *Ferula communis* has two chemotypes, poisonous and non-poisonous ones. Non-poisonous chemotype possesses sesquiterpene daucane esters while poisonous chemotype has mostly prenylated coumarins (Akaberi et al., 2015). These two chemotypes cannot be separated according to their morphological features. *Ferula communis* has been traditionally used as phytohormone and reported as a potential source of phytoestrogens. This effect is associated with isoflavones and ferutinin which is an aromatic ester of a daucane alcohol (Arnoldi et al., 2004; Nguir et al., 2016).

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Free radicals are highly reactive molecules and produced in the body as part of the physiological process. Free radicals cause oxidative stress, resulting in cell damage or even cell death (Amir Aslani and Ghobadi 2016). Diseases like cancer, atherosclerosis and diabetes mellitus are caused by oxidative stress (Ceylan et al., 2019). Plant extracts have been reported to show biological activities and are known to have phenolic compounds. Phenolic compounds delay lipid peroxidation caused by free radicals. Antioxidant activities of plant extracts have been widely studied and various extract and their compounds have been determined as antioxidants. Therefore, plant extracts and their components have been investigated for their antioxidant activities thoroughly (Kähkönen et al., 1999; Pernin et al., 2018).

Enzymes which are protein-based biomolecules regulate many metabolically important reactions in all living beings (Nagao et al., 2014; Çetinkaya et al., 2014). Carbonic anhydrases (CAs; EC 4.2.1.1) are a superb family of metalloenzymes catalyzing the interconversion between carbon dioxide (CO_2) and bicarbonate (HCO_3^-) (Aggarwal et al., 2013; Mete et al., 2016; Özensoy Güler et al., 2016). They are expressed in all organisms of three-domains of life (Uygun et al., 2014) with encoded eight unrelated gene families (α, β, γ, δ, ζ, η, ɵ ve ι) (Ozensoy Güler et al., 2020). In mammalian organisms, α-gene family expresses 16 isoforms of the enzyme which differ from each other in terms of their location (Nar et al., 2013; Orhan et al., 2016) their molecular characteristics, organization, and kinetics towards inhibitors/ligands (Nocentini et al., 2016). Some isoenzymes are present in cytosol (hCA I, II, III, VII and XIII), five isoenzymes are in the form of membrane-bound (hCA IV, IX, XII, XIV and XV), hCA VA and VB are found in mitochondria, and hCA VI is secretory isoform (Almajan et al., 2008). The other three isoforms (hCA VIII, X and XI) are described as non-catalytic protein forms (Abdel-Aziz et al., 2014; Ekinci et al., 2010).

Common practices related to catalytic activity of CAs have been largely expanded to treat many clinical diseases (Akbaba et al., 2013). Modulation of the catalytic activity of CAs with carbonic anhydrase activators (CAAs) or carbonic anhydrase inhibitors (CAIs) are crucial for prevention and/or treatment of many diseases/syndromes (Scozzafava et al., 2004). CAIs which are classified according to their affinities and inhibitory characteristics for the CA isoforms (Maslanka, 2015) have been used in essential clinical applications as pharmaceutical agents for ophthalmology, obesity, cancer, osteoporosis and neurological disorders (Supuran, 2008). Up to date, heavy metal ions (Kuzu et al., 2018), anions (De Simone and Supuran, 2012), bromophenols (Balaydın et al., 2012; Taslimi et al., 2016), chalcones (Gençer et al., 2013), coumarins (Karatas et al., 2014), pyrazoles (Mert et al., 2016), sulfonamides (Ceruso et al., 2014; Le Darz et al., 2015; Arslan et al., 2016; Carta et al., 2016), thiosemicarbazone and its metal complexes (Ucar et al., 2020), thiourea derivatives (Korkmaz et al., 2015), uracil derivatives (Güney et al., 2015; Türkoğlu et al., 2017) have been used as CAIs. These types of compounds have been synthesized in complex procedures and showed considerable side effects. However, natural products have gained popularity and also performed in the inhibition studies of CAs (Akkemik et al., 2019).

Keeping in view of the medical and therapeutic importance of the enzyme and natural product, this study was designed to evaluate the extracts of *F. communis* for total phenolic content and antioxidant features and also investigate the inhibitory characteristics on hCA I and II for the first time.

Materials and Methods

Plant material

Ferula comminus was obtained from Zeytinburnu Medicinal Plant Botanic Gardens in İstanbul, Turkey in 2020 and authenticated by Dr. Zeki Severoğlu from Biology Department of Marmara University with the number of 5087 for future reference.

Chemicals and instruments

Human carbonic anhydrase I and II enzymes, trizma base and 4-nitrophenylacetate as substrate were commercially obtained from Sigma-Aldrich (Saint Louis, MO, USA). N-hexane, chloroform, ethanol, acetone, sulfuric acid and dimethyl sulfoxide were purchased from Isolab (Turkey). The laboratory blender (8011 EG, Waring Commercial, USA) was used for fine powder. Water for buffers and experimental processes was supplied from Direct Q®3 UV water purification system (Millipore Corp., France). ZX3 Advanced Vortex Mixer (Velp Scientifica, Usmate, Italy) and IKA RT10 magnetic stirrer (IKA-Werke GmbH & Co KG, Germany) were used for stirring and mixing purposes. Accurate weighing measurements of all chemicals and extracts were obtained from Ohaus PA224C (Ohaus Corp., USA) with the readability up to 0.1 mg. pH values were analyzed with Mettler Toledo Seven Compact pH-meter (Greifensee, Zürich, Switzerland). Organic solvents were evaporated by Hei-VAP Core rotary evaporator (Heidolph Instruments, Germany). Bioactivities of plant extract on the enzymes were examined by V-730 UV–Visible Spectrophotometer (JASCO International Co., Tokyo, Japan) with the resolution of 1 nm. All liquid in the experiment was transferred with Eppendorf Research Plus single channel pipettes (Eppendorf AG, Hamburg, Germany).

Preparation of the extracts

The aerial parts of *Ferula communis* were dried in the shade and powdered. The powdered aerial parts (40 g) were macerated with 200 mL of hexane, chloroform, ethanol, ethanol:water (50:50), respectively. Procedure was repeated at a 24-hour cycle until colorless solvent was obtained. The extracts were filtered and evaporated with a rotary evaporator. Extracts were kept at +4°C until the experiment day.

Determination of total phenolic contents

Extracts were prepared at the concentration of 5

mg/mL. 0.1 mL of extract were taken into a tube then 4.5 mL water was added. Diluted with distilled water (1/3 ratio with distilled water), 0,1 mL Folin-Ciocalteu reagent was taken and added to the mixture. After adding 0.3 mL of 2% sodium carbonate solution, mixture was kept at room temperature for 2 hours. Absorbance was measured at 760 nm against the reference. The total phenolic contents in the extracts were given as mg gallic acid equivalents/mg extract (Taşkın et al., 2018).

***In vitro* evaluation of antioxidant assays**

2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay

DPPH• solution was prepared as 0.10 mM using MeOH. 3.90 mL of the solution was added to 0.1 mL of the extracts which were prepared at different concentrations (0.5-5 mg/mL). Absorbance was measured at 517 nm 30 min later. IC_{50} values were determined by percentage inhibition-concentration curve. Ascorbic acid was used as reference (Fu et al., 2010).

Cupric reducing antioxidant capacity (CUPRAC) assay

60 µL ethanolic solution of neocuproine ($7.3.10^{-3}$ M), 1 M NH_4Ac buffer solution (pH 7) and Cu(II) (1.10^{-2} M), were mixed in 96-well microplate. Afterwards, 10 µL ethanol was added to the mixture. Lastly, extracts were prepared in the concentration of 1 mg/mL and 60 µL of the extracts were added to the plate. Mixture was kept at room temperatures for 1 h and then absorbance was measured at 450 nm against the blank. CUPRAC values of the plant extracts were given as mM trolox/mg extract (Taşkın et al., 2019).

Esterase activity assay of human carbonic anhydrase isoenzymes

Esterase activity assay of hCA I and hCA II was assayed with the method of (Verpoorte et al. 1967). Absorbance changes of 4-nitrophenyl acetate (NPA) to 4-nitrophenylate ion under certain conditions (348 nm, 3 min, 25°C) was recorded in this spectrophotometry-based detection (Ağgül et al., 2020; Balaydin et al., 2012). The reference measurement without enzymatic solution was investigated before the kinetic studies and then the effects of extracts were tested. The extract for each concentration in the assay were examined in triplicate. Activity of control cuvette in case of no inhibitor was accepted as 100%. First hCA I and II, inhibition (%) studies were performed with all extracts. Then activity (%) - [Inhibitor] graph was drawn for the extract which possesses best inhibition (%) profile for the hCA isoenzymes. K_i values of the extract was calculated from Cheng–Prusoff equation (Kuzu et al., 2016).

Results and Discussion

Total phenolic content was analyzed with Folin-Ciocalteu method and the results are shown in Table 1. In the results, the highest phenolic content was observed in ethanol:water (50:50) extract (0.031±0.001 mg gallic acid equivalent/mg extract).

Hexane, chloroform, ethanol and ethanol:water (50:50) extracts have been tested for their 2,2-diphenyl-1-picryl-hydrazyl scavenging activity and cupric reducing antioxidant capacity. The results are given in Table 2. According to the results of the experiments, all extracts have mild to moderate antioxidant properties. DPPH scavenging activity of the extracts from highest to lowest was in the following order: Ethanol:water (IC_{50}: 0.1128±0.0066 mg/mL), hexane (IC_{50}: 0.2058±0.0114 mg/mL), ethanol (IC_{50}: 0.2121±0.0170 mg/mL), chloroform (IC_{50}: 1.0451±0.0550 mg/mL). None of the extracts showed stronger activity than ascorbic acid (IC_{50}: 0.0028±0.0004 mg/mL) which was used as standard.

Cupric reducing antioxidant capacity of the extracts obtained from *Ferula communis* was evaluated. According to the results, the highest activity was determined in chloroform extract (1.305±0.037 mM Trolox equivalent/mg extract). It was followed by ethanol:water extract (1.127±0.030 mM Trolox equivalent/mg extract), ethanol extract (0.712±0.028 mM Trolox equivalent/mg extract) and hexane extract (0.702±0.024 mM Trolox equivalent/mg extract). Extracts showed lower activity than ascorbic acid (5.700±0.020 mM Trolox equivalent/mg extract).

There are some studies related to the antioxidant activities of *Ferula communis* in the literature. Aerial parts of *Ferula communis* were tested for its antioxidant properties. In the study, methanol extracts of different parts of the plant (stem, fruit and flower) were evaluated separately. According to the results, highest DPPH radical scavenging activity was obtained from flower extract (IC_{50}: 24.07 µg/mL) (Rahali et al., 2018). In another study, essential oil from *F. communis* was evaluated for its antioxidant properties. The essential oil was obtained from four different organs as roots, stems, flowers and leaves. Essential oil from stems exhibited the best results on DPPH assay (IC_{50}: 0.03±0.001 mg/mL) (Nguir et al., 2016).

In vitro activity of seed extracts of *Cassia absus* L. on carbonic anhydrase enzyme have been reported by Ahmad et al. (2019). In this study crude ethanol extract and the fractions of *n*-butanol, chloroform, *n*-hexane and water obtained from ethanol extract were examined as *in vitro* carbonic anhydrase bioactivity. In this study, ethanol extract of *Cassia absus* L. showed the best potent inhibitory activity (inhibition (%): 54.1±0.06; IC_{50}: 1875 ± 0.9 µg/mL) on carbonic anhydrase enzyme.

Table 1. Total phenolic contents of the extracts of *Ferula communis*

Extract	Total Phenolic Content (mg gallic acid equivalent/mg extract)
Hexane	0.030±0.003
Chloroform	0.027±0.001
Ethanol	0.025±0.001
Ethanol:water (50:50)	0.031±0.001

Table 2. *In vitro* antioxidant activity assay of the extracts of *Ferula communis*

Extract	DPPH (IC_{50} mg/mL)	CUPRAC (mM Trolox equivalent/mg extract)
Hexane	0.2058±0.0114	0.702±0.024
Chloroform	1.0451±0.0550	1.305±0.037
Ethanol	0.2121±0.0170	0.712±0.028
Ethanol:water (50:50)	0.1128±0.0066	1.127±0.030
Ascorbic acid*	0.0028±0.0004	5.700±0.020

* : Standard

Another study has focused on the effects of the extracts of five different plants (*Alcea rosea*, *Foeniculum vulgare*, *Elettaria cardamomum*, *Laurus azorica* and *Lavandula stoechas*) on hCA I and II. According to the results, the methanol extract of *Elettaria cardamomum* has showed the highest inhibitory potential (0.032 mg/mL) on hCA I and the highest inhibition for hCA II (0.054 mg/mL) has been obtained from the methanol extract of *Lavandula stoechas* (Kaya et al., 2019).

Akkemik et al. (2019) has investigated the inhibition characteristics of *Cucumis melo* L. seed extracts on hCA I and II. While oil and methanol extracts of the seeds activates the hCA I isoenzyme activity, these extracts have also inhibited the hCA II isoenzyme. IC_{50} and K_i values of oil extract on hCA II are 0.497 ng/mL and 0.369±0.166 ng/mL, respectively and 10.98 µg/mL and 7.25±0.400 µg/mL for hCA II have been obtained from the methanol extracts.

To the best of our knowledge, the inhibitory potential of the extracts of *Ferula communis* on hCA I and II have been investigated for the first time. The hexane extract has showed the best inhibitory feature on hCA I. It was followed by chloroform extract which exhibits good inhibition for hCA I. However, other two extracts, ethanol and ethanol-water (50:50), have demonstrated weak inhibition for hCA I. The inhibition studies on hCA II isoenzyme have been performed with the extracts of *Ferula communis*. The hexane extract has demonstrated the best inhibitory effect on hCA II and other three extracts have had no meaningful inhibition on the isoenzyme. Inhibition (%) studies of the extracts of *Ferula communis* on hCA I and hCA II are illustrated in Table 3. According to inhibition (%) studies, hexane extract of *Ferula communis* has showed the most potent inhibition against hCA I and hCA II isoenzymes.

The hexane extract has exhibited the highest inhibitory activity on hCA I and hCA II among all tested extracts. Therefore, the hexane extract has been determined as the best inhibitor of the evaluated extracts for hCA I and II with IC_{50} values 8.68 µg/mL and 28 µg/mL and K_i values 2.026 µg/mL and 11.6 µg/mL, respectively (Table 4).

Table 3. Inhibition (%) studies of the extracts of *Ferula communis* on hCA I and II

Extract	Inhibition (%) for hCA I	Inhibition (%) for hCA II
Hexane	61.236±1.685	18.595±1.894
Chloroform	59.551±2.919	2.479±0.716
Ethanol	25.281±0.973	-
Ethanol-water (50:50)	16.854±0.973	-

- : No activity

Table 4. Effect of *Ferula communis* n-hexane extract on hCA I and II

	n-hexane extract of *F. communis*		Standard (acetazolamide)	
Sample Enzyme	hCA I	hCA II	hCA I (Taslimi et al., 2016)	hCA II
IC_{50}	8.68 µg/mL	28 µg/mL	6.07 nM	8.549 ng/mL
R^2	0.9251	0.9510	0.9154	0.9891
K_i	2.026 µg/mL	11.6 µg/mL	6.76±2.55 nM	5.813 ng/mL

The antioxidant and carbonic anhydrase inhibitory activities of *F. communis* have been reported in this study and to best of our knowledge, it has been the first study conducted on hCAI and hCAII inhibitory effects of *Ferula communis* extracts. These discoveries about the bioactivities of natural products are essential for pharmaceutical and nutritional sciences. According to the results, the study can be advanced by further analytical studies. Thus, novel active natural compounds might be illuminated by determining the extract content.

Conclusion

Carbonic anhydrase activity of hexane extract of *Ferula communis* has showed the most inhibitory potential among the other extracts. Inhibition profiles of the hexane extract of *Ferula communis* on hCA I and II have been determined as µg/mL. IC_{50} and K_i values of acetazolamide were determined in the range of ng/mL and nM. Further purification studies for the extracts and clinical studies will be

needed to determine the active natural products and their potential for pharmaceutical and nutritional sciences.

Compliance with Ethical Standards

Conflict of interest

The authors declare that the study was performed in the absence of any commercial and/or financial relationships that could be perceived as a conflict of interest.

Author contribution

F.G.A. obtained the bioactivity measurements, analyzed the data of antioxidant activity and wrote the manuscript. Z.A.K. obtained the enzymatic activity and performed in all laboratory tasks. E.A.T. operated methodology, collaboration, analysis, validation, supervision and writing of the study. M.K. has contributed on the analysis and validation of the bioactivity data for the study. Z.S. identified the plant material. The authors have verified that all data in the manuscript have not been published before and have given approval for the final version of manuscript.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Responses of *Allium cepa* L. exposed to silver nanoparticles

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Abstract

The study was aimed to determine the gallic acid, rutin and quercetin contents and yield of Narli onion genotype (*Allium cepa* L.,) exposed to four different doses (0, 25, 50, 75, 100 mg L^{-1}) of silver nanoparticles (AgNPs)for30 days, after planting the onion bulbs, attwo-week intervals. Quercetin, rutinand gallic acid contents in the leaves and bulbs of onion plants were determined.While the quercetin content was the highest in 25 mg L^{-1}ofAgNPs treatment (575.0 ± 10.39 µg g^{-1})in the bulb parts, gallic acid content reachedtothe highest rate in 50 mg L^{-1} of AgNPs(3605.8 ± 90.96µg g^{-1}), inthe onion bulb, compared to the control (2819.3 ± 65.72µg g^{-1}).The content of rutinwere enhanced in 25 (19.72 ± 0.28µg g^{-1}), 50 (21.66 ± 0.57µg g^{-1}) and 75 mg L^{-1}(31.08 ± 0.53 µg g^{-1}) of AgNPs treatments, but it was significantly close to the control (7.15 ± 0.93µg g^{-1}), in100 mg L^{-1}(10.92 ± 0.38 µg g^{-1}), in bulb parts.Chlorophyll content showed reducesin all doses, except for25 mg L^{-1} of AgNPs treatment. Total yield enhanced in treatments of AgNPs, but the highest increase was obtained in treatment of 50 mg L^{-1} of AgNPs (97.49 ± 0.92 µg g^{-1}). The analysis of quercetin, rutin and gallic acid contents were performed by high performance liquid chromatography (HPLC), and Chlorophyll was determined by SPAD.

Keywords: Chlorophyll, Onion, Quercetin, Rutin, Silver Nanoparticle

Introduction

Onion (*Allium cepa*L.), a member of *Liliaceae* family, is consumed asdry, fresh and cooked. The onion contains A,B and C vitamins and rich in sulfurand antibiotic; thus,itis considered a medicinal plant(Morimitsuet al.,1992).China was the largest producer of onion in the world in 2017 with 1056139 tons fresh onion. The production of fresh onion in Turkey was138993 ton in the same year (FAO, 2020). Nanotechnology has been developing rapidly. Nanoparticles differ from the bulk products due to their physical and chemical characteristics. Different responses have been reported regarding the commercial and scientific applications of nanoparticles (Oberdorsteret al., 2005). The word nanooriginatesfromGreek, meaningvery small and indicates one billion of any physical size (Tegart, 2003). Silver is used in different stages of plant production due to the nanomaterial antibacterial characteristics (Kimetal., 2007). Nanotechnology is a field of applied science deals with biological or non-biological particles less than 100 nm diameters (Cıracıet al., 2005). Nanotechnology is the use and examining of materials at the atomic and molecular sizes (a scale ranging from 10 to 100 nm) (Kaphleet al.,2018). Nanoparticles are defined as organic and inorganic materials. The organic nanoparticles contain carbon and inorganic nanoparticles contain titanium, zinc, silver, gold and copper (Xuet al., 2006).

Significant effects of nanoparticle applications at various doses on morphological and biochemical growth and development of plants have been reported (Maetal.,2010). The silver nanoparticles are the most commonly used commercialized nanoparticles among the different nanoparticles (Ahmed et al., 2008), which have been used in biological synthesis due to rapid disintegration, low cost and potential of compatibility (Vermaet al.,2014). Babu et al. (2008) reported that applications of silver nanoparticle at various concentrations (10, 20, 40 and 5 mg L^{-1}) caused significant decrease in mitosis index and structural deviation in chromosome *Allium* plants. Similarly, the cytotoxic and geotoxic effects of silver nanoparticles were reported in exposure todifferent

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silver doses (20, 25, 75, 100 mg L^{-1}) *Allium* plants (Mamta Kumariand Mukherjee, 2009). Theresearchers indicated no irregularity in the control plants, while the mean mitotic index was 60% in the silver treatments. Onion contains different flavonoids such as quercetin. Kamferol, quercetin, rutin, resveratrol, isorhamnetin and myelicinare the examples of important flavonoids (Sefer, 2000).The flavonoids are generally found in shells of different onion genotypes along with red, purple and brown anthocyanins(Griffiths et al.,2002). Buckwheat plants contain high amounts of protein, vitamins, minerals and also rich in important phenolic components such asrutin and quercetin. Similar to the other germinated plants, buckwheat shoots were rich in lysine, minerals, raw fiber, phenolic substances, vitamin C, etc. compared to the usual seeds (Hsu et al.,2008; Kim et al., 2004). The rutin is the only flavonoid which is capable of chelating metal ions such as iron and causes the formation of oxygen radicals with its high antioxidant activities. Park and Cha (2008) reported the presence of 19 flavonoids (6 quercetin derivatives, 6 isorhamnetine derivatives and 7 camherol derivatives) in the leaves of 30 different grape varieties and the amounts of phenolic compounds varied between the grape varieties. The aim of this study was to determine the effects of different silver nanoparticleapplication doses on yield and quality traits of onion plants.

Materials and Methods

The study was carried out in Şırnak University Faculty of Agriculture, Department of Horticulture, at research laboratory. Narli onion genotypewas used as the plant material in theexperiment. Four different silver nanoparticle doses (0, 25, 50, 75, 100 mg L^{-1}) in addition to 0 mg $L^{-1}$of pure water were used as the treatments of the experiment. The silver nanoparticles, purchased from Gute Chemie-abcrGmbh, Deutschland, were applied between 8-10 hours intervals during the experiment. Silver nanoparticles used in the experimentwereof80 nm in size, 99.995% purity and metal basis. pHand EC values of the nutrient solution onion cultivation was set at 5.5-6.1 and 1.9 dS m^{-1}, respectively. In the onion growing stage (in mg L^{-1}): N (200), P (60), K (300), Ca (170), Mg (60), Fe (3.0), Mn (0.8 -1.0), Cu (0.1), Zn (0.3), B (0.3) and Mo (0.05) nutrient solutions were used.Plant height, chlorophyll (SPAD), stem diameter and plant width were measured on November 20 and December 14, 2018. The plants were harveste don January 14, 2019.

Calibration curves of quercetin, rutin and gallic acid compounds

The standard solutions of quercetin, rutin and gallic acid were prepared at four different concentrations (0.25, 0.5, 1.0, 2.5, 5.0 µg mL^{-1}) by dissolvingtheir stock solutions in dimethyl formamide (DMF, 1 mg mL^{-1}). Each standard solution was injected in triplicate to obtain the calibration curves (Figure 1). The concentrations of quercetin, rutin and gallic acid in the extracts were calculated using the calibration curves of the compounds.

Extraction of quercetin, rutin and gallic acid

The leaves and bulbs of onion plants were dried in an oven and ground into powder using a blender. Then, 0.2 mg of powder sample was accurately weighed, extracted using10 mL of DMFkeptat overnight. The final volume of extracts was adjusted to 80 mL with DMF. The solutions were filtered through a 0.45 mm nylon filter membrane prior to analysis.

QuercetinandrutinanalysisinHigh Performance Liquid Chromatography (HPLC)

The quercetin, rutin and gallic acid contentsin plant samples were determined by using a High Performance Liquid Chromatography (HPLC) system (Shimadzu Corporation, Japan). The HPLC system was equipped with an Inertsil ODS-3 C18 column (5µm x 4.6 mm x 250 mm), LC-20AT pump, DGU 20A5R degasser, SIL 20A-HT auto sampler and SPD M-20A PDA detector. The mobile phase consisted of 60% water and 40% acetonitrile with 0.1% TFA for quercetin and gallic acid and 80% water and 20% acetonitrile for rutin. The mobile phase was filtered through a 0.45 mm filter and then degassed by ultrasonification. An isocratic elution profile was used for both compounds, and the column temperature and flow rate wereadjusted to 30 ^{0}C and 0.6 mL min^{-1}. Separation process was carried out at room temperature. Quercetin, rutin and gallic acid contents were detected at wavelengths of 320, 256 and 266 nm, respectively. Retention times of quercetin, rutin and gallic acid were 13.4, 10.6 and 6.5 min, respectively. Injection volume was set to20 µl. The correlation coefficients (R) of the standards were 0.998 for quercetin, 0.997 for gallic acid, and 0.9997 for rutin. Quantification of the three compounds was performed by comparing the retention time of the samples with the standards.

Chlorophyll measurements

Chlorophyll measurements were carried outon3 leaves at outside of the plant in a sunny weather and around 10 a.m. SPAD meter (502 Minolta brand) was used in the chlorophyll measurements.

Yield

The plants were harvested on January 14, 2019. Onion roots were weighed after cutting from the leaves.

Results and Discussion

Quercetin, rutin and gallic acid contentsin onion bulbs

Quercetin, rutin and gallic acid contents of onion bulbs exposed to AgNPs are shown in table 1and HPLC chromatogram of quercetin, rutin and gallic acid compounds on different silver nanoparticle doses were presented in figure 2. a-b.While the quercetin content enhanced in 25 and 50 mg L^{-1} AgNPs treatments, it was reduced in 75 and 100 mg L^{-1} AgNPs, in onion bulbs.The

highestquercetin was obtainedinbulbs treated with 25 mg L^{-1} AgNPs treatments (575.0 ± 10.39 µg g^{-1}), comparedtothecontrol (285.3 ± 3.86µg g^{-1}). Rutin content enhanced in increasing doses of AgNPs, but it was the lowest in 100 mg L^{-1} (10.92 ± 0.38µg g^{-1}).The content of rutin was the highest in bulb treated with75 mg L^{-1} AgNPs (31.08 ± 0.35 µg g^{-1}). Gallic acid content was the highest in 50 mg L^{-1} of AgNPs (3605.8 ± 90.96 µg g^{-1}), but in the other treatments, it was reduced or closetothe control group (2819.3 ± 65.72 µg g^{-1}) (Table 1).

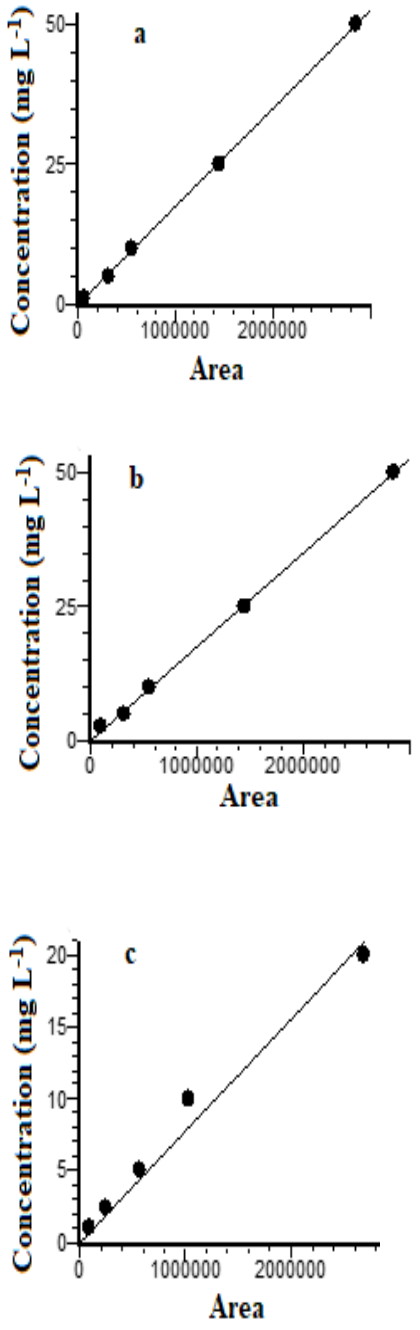

Fig1. Calibration curves, (a) quercetin, (b) gallic acid, (c) rutin

Similar to our findings, several authors stated an increase in some flavonoids and phenolic compounds (rosmarinic and salvanolic acids) intreatment with a certain doses of silver nanoparticles (GeandWu 2005; Xing et al.,2015; Zhang et al.,2004). Chung et al. (2018) was reported that it was found an enhancement in Total Phenolic and flavonoid content contents in hairy bulb cultures of *cucumisanguria*treated with the AgNPs and $AgNO_3$. In another study, it was found thatthe Total phenolic content was increased in the tissue culture of *Vanilla planifolia* Jacks. Ex Andrews, with the application of 25 and 50 mg L^{-1}AgNPs (Spinoso-Castilloet al.,2017).The silver nanoparticles induced some phytochemical production of in the bulb parts of *Cucumisanguria*(Chung et al.,2018). Zhang et al. (2013) reported that treatment with Ag-SiO_2core-shell nanoparticle increased artemisin content in *Artemisia annua*plant.

Quercetin, rutin and gallic acid contents in onion leaves

The quercetin, rutin and gallic acid contents in the leavesof onion exposed to AgNPswere significantly different (Table 2). The highest quercetin (10.99±0.12µg g^{-1}) and gallic acid contents (3562.05±112.3µg g^{-1}) in leaf samples was recorded in 50 mg L^{-1}AgNPsapplication. The highest rutincontent (16.31±0.78) was detectedin 75 mg L^{-1} application, compared to the control plants (6.55 ± 0.81µg g^{-1})(Table 2). Similar changes in quercetin content of onion have been reported by Patil et al.(1995).The oxidant and anti-oxidant contents in arabidopsis plants exposed to silvernanoparticleswere higher compared to the control (Qian et al., 2013). Silver nitrate accumulation in the cell walls of *Cucumissativus* (Zhang et al.,2012)and pumpkin(Corredoret al.,2009) have been reported absorptionby plants and accumulated in leaf tissues.In this study, phenolic acid contents were significantly affected byapplication of silver ions. The nanoparticle may increase activation of enzymatic pathways; thus may contribute to the production of metabolites. Xinget al. (2015) reported significant increases of rosmarinic acid (Ra), cafeic acid and ferulic acid in in *Salvia miltiorrhiza*, while salvanolic acid, danshensu and cinnamics significantly decreased with the application of silver nanoparticles.

Chlorophyll Content, Yield and Leaf Dry Matter Ratio

The effects of different silver nanoparticle doses on yield and leaf dry weight of onion were significantly important (P≤0.05). The onion yield in control (74.64g $plant^{-1}$) insignificantly increased in 25 mg L^{-1}(75.44 g $plant^{-1}$) application; however, application of50 mg L^{-1} dose significantly increased the onion yield (97.49 g $plant^{-1}$). Further increase in silver nanoparticle doses caused a significant decrease in onion yield which was 85.42 g $plant^{-1}$in

75 mg $L^{-1}$and 73.18 g $plant^{-1}$ in 100 mg L^{-1} doses (Table 3).

While thechlorophyllcontentin 25 mg L^{-1} AgNPs application (63.50 ± 0.10) was close to the control (62.25 ± 0.22), treated with 50, 75 and 100mg L^{-1} AgNPsreducedchlorophyll content in the onion leaves. Qian et al. (2013) also reported a decrease in chlorophyll content of*Arabidopsis*treated with AgNPs. The inhibitor effect of AgNPsonphotosynthesis pigment contents reported of *Spirodelapolyrhiza*and *Dunaliellatertiolecta*, in the study ofJiang et al.(2012).

Table 1. Quercetin, rutin and gallic acid contents in onion bulb (µgg^{-1})

AgNPs Doses	Quercetin	Rutin	Gallic acid
0 mg L^{-1}(Control)	285.3 ± 3.86 ^{c}	18.27 ± 0.49 ^{d}	2819.3 ± 65.72^{b}
25 mg L^{-1}	575.0 ± 10.39 ^{a}	19.72 ± 0.28 ^{c}	2411.5 ± 60.51^{c}
50 mg L^{-1}	477.0 ± 68.97^{b}	21.66 ± 0.57 ^{b}	3605.8 ± 90.96^{a}
75 mg L^{-1}	245.0 ± 7.43^{c}	31.08 ± 0.53 ^{a}	2821.6 ± 72.22^{b}
100 mg L^{-1}	179.3 ± 5.73^{d}	10.92 ± 0.38 ^{e}	2310.2 ± 53.62^{c}
Mean	352.3	20.33	2793.68
$LSD_{0.05}$	47.51	0.70	105.17

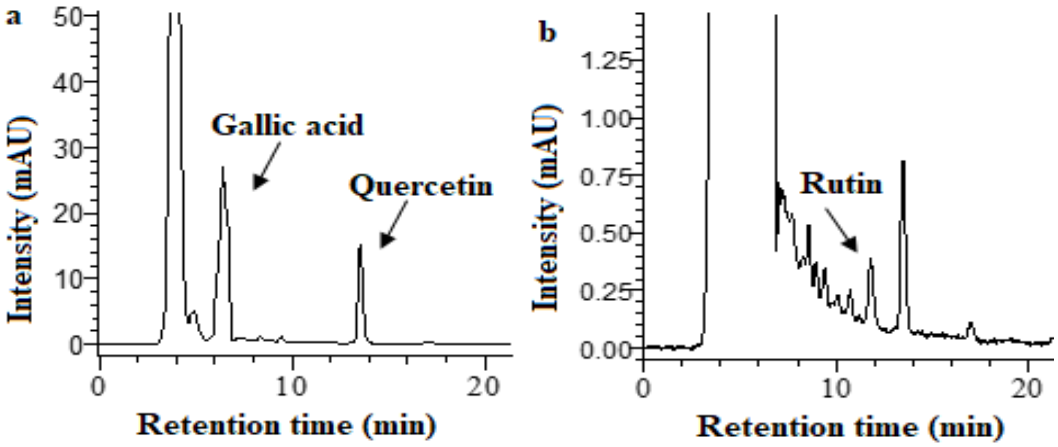

Fig 2. HPLC chromatograms of the onion extracts (a) gallic acid and quercetin (b) rutin

Table 2. The quercetin, rutin and gallic acid contents in onion leaves (µg g^{-1})

AgNPs Doses	Quercetin	Rutin	Gallic acid
0 mg L^{-1}(control)	3.19 ± 0.12^{d}	6.55 ± 0.81^{d}	2432.86 ± 58.06^{d}
25 mg L^{-1}	9.44 ± 0.07^{b}	8.07 ± 0.29^{c}	2793.57 ± 101.31^{c}
50 mg L^{-1}	10.99 ± 0.12^{a}	11.80 ± 0.22 ^{b}	3562.05 ± 112.3^{a}
75 mg L^{-1}	5.92 ± 0.30^{c}	16.31 ± 0.78^{a}	3343.47 ± 95.84^{c}
100 mg L^{-1}	6.08 ± 0.39^{c}	7.15 ± 0.93^{cd}	2292.86 ± 35.03^{e}
Mean	7.12	9.97	2884.96
$LSD_{0.05}$	0.35	1.01	129.07

Table 3. Yield, Chlorophyll and Leaf dry matter ratio of onions

Silver Np Doses	Total yield (g $plant^{-1}$)	Chlorophyll (SPAD)	Leaf dry matter (%)
0 mg L^{-1}(Control)	74.64 ± 0.35^{cd}	62.25 ± 0.22^{a}	8.78 ± 0.95^{d}
25 mg L^{-1}	75.44 ± 0.17^{c}	63.50 ± 0.10^{a}	9.03 ± 1.82^{c}
50 mg L^{-1}	97.49 ± 0.92^{a}	55.75 ± 0.1^{bc}	9.78 ± 2.38^{a}
75 mg L^{-1}	85.42 ± 2.95^{b}	57.00 ± 0.02 ^{b}	9.33 ± 1.70^{b}
100 mg L^{-1}	73.18 ± 0.99^{d}	54.00 ± 0.06^{c}	8.67 ± 0.81^{d}
Mean	81.23	58.50	9.11
$LSD_{0.05}$	2.21	2.47	0.18

Conclusion

This study revealed that the interaction of onion with silver can be optimized by promoting the yield and chemical compounds testedin the onion without damaging the plant. Accordingly, the use of silver nanoparticles in different plant species will be commercially successful in future studies. In Statistically significant difference were obtained between different silver nanoparticle doses. The results indicated that the best useful doses of AgNPs on onion are25 and 50 mgL^{-1}.

Compliance with Ethical Standards

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

Author contribution

The contribution of the authors is equal. All the authors' readand approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before

Ethics committee approval

This article does not contain any studies with human or animal subjects. Ethics committee approval is not required.

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Data availability

Not applicable

Consent for publication

Not applicable.

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Impacts of Lavandula angustifolia Mill. and Thymbra spicata L. essential oils on postharvest gray mold of strawberries

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Abstract

Antifungal activities of two essential oils (EOs), derived from the *Lavandula angustifolia* L. and *Thymbra spicata* L. plant leaves were tested in current study against two isolates (M1-5 and M3-5) of *Botrytis cinerea* in Potato Dextrose Agar (PDA). These studies were performed *in vitro* and a further *in vivo* test with vapor contact application of the EOs was performed with strawberry fruits to confirm the antifungal activities in postharvest storage. *In vitro* studies were conducted with four different application doses (0.25, 0.50, 1.00 and 2.00 mL L-1) of both EOs with poisoned food technique. The highest dose (2.00 mL L-1) of *L. angustifolia* had a 92.50% mycelial growth inhibition on M1-5, where the same dose of same oil had 0.00% mycelial growth inhibition on M3-5. On the other hand, the highest dose (2.00 mL L-1) of *T. spicata* had 16.76% and 51.18% of mycelial growth inhibition on M1-5 and M3-5, respectively. The lower doses had less or no antifungal activity, thus only the highest doses were tested in the consecutive *in vivo* studies. Results suggested that both of the EOs had moderate impact on the prevention of disease severity at strawberry cv. Camarosa fruits, inoculated with M1-5 and M3-5 isolates. The EOs were also noted to have a significant influence on the prevention of the weight loss and loss of soluble solids concentration. Results suggested that the vapor contact application of *L. angustifolia* and *T. spicata* essential oils have potential to be alternative to synthetic fungicides for controlling gray mold in strawberry fruits caused by *B. cinerea*.

Keywords: Alternative control, *Botrytis cinerea*, Fruit storability, Poisoned food technique, Vapor contact

Introduction

Berry fruits, including mainly strawberry, blackberry, blueberry, cranberry and raspberry, are rich in a wide variety of nutrients and phytochemicals (Skrovankova et al., 2015; Usanmaz, 2019). The high and diverse contents phenolics, flavonoids and alkaloids gives significant antioxidant capacity to the berries and help to fight with various diseases including cardiovascular diseases and cancer (Mukherjee et al., 2020). Among the berries, strawberry (*Fragaria* x *ananassa* Duch.) fruits are the most popular because of their flavors and adaptability to different environmental conditions. However, strawberry fruits are very sensitive to postharvest storage and have a very short storage life (2-5) which obstruct its marketability (Parvez and Wani, 2018). The high respiration rate and sensitive fruit skin makes the strawberries susceptible to mechanical injuries and pathogen infections (Caleb et al., 2016). The main causes of the pathogenic decay on the strawberries is the gray mold caused by *Botrytis cinerea* (Parvez and Wani, 2018). *B. cinerea* causes soft rots and water-soaked paranchyma tissues on the fruits, which is followed by the growth of gray conidia, decayed fruits and rendered marketability (Williamson et al., 2007). Fungicide application is the most widely used technique for fungus control (Adaskaveg et al., 2021), but its acceptability by the consumers is decreasing due to the scientifically confirmed hazards on human health and nature (Koch

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et al., 2017). Besides to that, under excessive- and mis-use of fungicides have been reported to cause the development of resistant genotypes and reduce their efficacy (Hao et al., 2011). Therefore, safely and healthy alternatives for fungicides have become very important in the last decades (Huang et al., 2021).

Use of plant derived products, including proteins, lipids, polysaccharides and secondary metabolites, as a protective coating material or different means of treatment have been reported to provide success in improving storability of fruits and vegetables (Wan et al., 2021). The most important advantage of these materials is that they are biodegradable (Nor and Ding, 2020) while their correct use have no known negative impact on human health. Biodegradable materials lower petroleum consumption, help to reduce carbon dioxide levels in atmosphere, reduce wastes and require less energy during production. Application of plant natural products as edible coatings helps to reduce respiration and transpiration of harvested products and maintain storage quality (Wan et al., 2021). Several plant essential oils have been reported to control *B. cinerea* infections at strawberry fruits, including lemongrass oil, lemon oil and orange oils (Kahramanoğlu, 2019). The different biochemical compounds of the essential oils are known to provide antifungal activity to the oils. Some examples to these compounds are eucalyptol, linalool, carvacrol and γ-terpinene (Kordali et al., 2016; Moazeni et al., 2021). *Lavandula angustifolia* Mill. and *Thymbra spicata* L. are rich in these biochemical compounds. *L. angustifolia* contains eucalyptol (15.10%) and linalool (11.98%) (Karadağ et al., 2021), while *T. spicata* contains carvacrol (56.03%) and γ-terpinene (6.87%) (Sengun et al., 2021). Several studies reported antifungal activity for the essential oils of these plant species, i.e. *L. angustifolia* against *Aspergillus niger* (Stupar et al., 2014) and *T. spicata* against *Aspergillus parasiticus* (Gumus 2010). However, according to authors' knowledge, no information available about the postharvest efficacy of *L. angustifolia* and *T. spicata* essential oils against *B. cinerea*. Therefore, present study was conducted to determine the *in vitro* antifungal activity of *L. angustifolia* and *T. spicata* against *B. cinerea* and test their further impacts on fruit quality in *in vivo* studies on strawberry fruits cv. Camarosa.

Materials and Methods

Plant material and essential oils (EOs) production

The leaves of the *Lavandula angustifolia* Mill. and *Thymbra spicata* L. plants, both belonging to the Lamiaceae family, were gathered during their flowering stages (between 6th and 8th months in 2020) in Turkey. Leaves were air dried and ground. Then, a total of 500 g dried sample was subjected to hydro distillation with Clevenger-type apparatus to obtain oils. The *L. angustifolia* and *T. spicata* leaves resulted 2.00% and 1.47% (w w-1, dry weight basis) oil, respectively. The EOs of both plants were kept under 4 °C temperature until being used in the studies.

In vitro antifungal studies

Two different isolates of Botrytis cinerea, namely M1-5 and M3-5, which were taken from the collection of Iğdır University, Department of Plant Protection and Phytopathology Laboratory, were used in current study. The poisoned food technique (Euloge et al., 2012) was used in current research to investigate the antifungal activity of the EOs of *L. angustifolia* (LA) and *T. spicata* (TS). The EOs were tested in four different doses, which are: 0.25 mL L^{-1}, 0.50 mL L^{-1}, 1.00 mL L^{-1} and 2.00 mL L^{-1}. The EOs were dissolved in 70% ethanol in a ratio of 1:2 (v v^{-1}) and mixed into molten and cooled PDA at 45 °C. In each petri dish (9 cm in diameter), 20 mL solution was added and solidified at room temperature for 1 hour. Next, an agar disc of mycelia (with 5-mm diameter) which were cut from actively growing 7-days-old isolates (M1-5 and M3-5) of *B. cinerea* were placed in the mid-point of each petri dish. Besides to these 4 doses of EOs, three different controls were included into the current study. First control was prepared by following the same method without oil and the second control was prepared with the 70% ethanol. The third and final control was prepared with a fungicide (the treatment dose was: 100 mL 100mL-1 of 500 g L^{-1} Fenhexamid (Teldor® SC 500)). Four plates (replications) were repeated for each of the seven treatments and they were incubated at 25 °C for 7 days. During this 7 days of incubation, the colony diameter in petri plates were measured regularly on day 3, day 5 and day 7. Next, the mycelial growth inhibition (%) ability of the treatments was calculated by following the formula of Thomidis and Filotheou (2016): mycelial growth inhibition (%) = {[(dc − dt)/dc] × 100}. In this formula, the dc represents the mean radial diameter of the *B. cinerea* in control sample, and dt is the mean radial diameter of the *B. cinerea* in treated sample.

In vivo antifungal and postharvest studies

Strawberry (*Fragaria x ananassa* Duch. cv. Camarosa) fruits were used in the second part of this study to reveal the antifungal activity of EOs and to determine their impacts on fruit quality. Fruits were collected from a commercial business located in Iğdır, Turkey. These studies had been carried after evaluating the *in vitro* results, which suggested that only the highest doses can be successful. Thus, the 2.00 mL L^{-1} dose was used for both EOs. First of all, strawberry fruits were selected to eliminate any damaged fruits and then the remaining healthy fruits were disinfected in 2.0% sodium hypochlorite for 5 minutes. After that, fruits were washed 3 times under pure water and dried on sterile papers for 30 minutes. Then, 1 mm wide and 1 mm deep wound was opened on each fruit with a sterile scalpel (10 µL). Next, 10 µL conidial suspension of *B. cinerea* (1.0 × 106 conidia mL^{-1}) was infected into the wounds.

This experiment was composed of five different treatments. First two treatments of present study are

the EOs. The other three treatments are the three separate controls. One control was designed with no any treatment after artificial infection of the fruits (Control-1) and another control was designed with no artificial infection and left natural (Control-2). Final control composed of artificial infection and with the same fungicide as described in *in vitro* studies. Fungicide treatment was done by direct spraying onto the fruits. In this study, three replication (each with four fruits) was used for each treatment. The studies were designed to continue for 21 days and the measurements were done with 3 days interval. Therefore, 21 different replications were prepared on the first day and 3 replications were used in each (totally 7) measurement point. After inoculation of the *B. cinerea* isolates, the four fruits of each replication were placed in PVC boxes. The EOs application in these studies were done by contact vapor application. The application doses (2.00 mL L-1 of air) of EOs were prepared according to the air space (~500 mL) of the PVC boxes and the determined oil concentrations were soaked onto a sterile paper plate (20 mm2). Then, these oil containing plates were put onto the inner part of the box cover. Hereafter, the plastic PVC boxes were sealed with parafilm (9 mic) to prevent the loss of volatile compounds. The fruits were then stored at 3.5 °C ± 0.5 °C (Mohammadi et al., 2015a) for 21 days to determine the effects of EOs under storage conditions. Before postharvest storage, fruits were kept at 25 °C for 2 h to initiate infection (Xu et al., 2021).

As mentioned above, fruit quality characteristics (weight loss, soluble solids concentration-SSC, pH and ascorbic acid-AA) and disease severity were measured with 3 days interval. The 0-5 scale was used to assess disease severity (DS) of each fruit (Huang et al., 2011). In this scale, 0 means no infection, where 1, 2, 3, 4 and 5 means < 20%, 20.1% to 40%, 40.1% to 60%, 60.1% to 80% and > 80.1% rotted area, respectively. Weight loss (%) was determined for each replication by measuring the initial and final weights and calculation the loss with the standard ratio method. SSC of each fruit was then measured with a hand refractomer as % Brix. A pH meter was then used to determine the pH of each fruit. Lastly, the AA (mg 100 g-1) of each fruit was determined by titrating the fruit juice with iodine solution (Skinner 1997).

Data analysis

The data of each mycelial growth inhibition (%), disease severity and fruit quality characteristic were all summed in Microsoft Excel by calculating the means and standard deviation of each treatment. These descriptive statistics were used to prepare the figures and tables for better presentation of the data. Statistical comparison of the means was then carried with Tukey's test after ANOVA at 5% significance level. The SPSS 22.0 software was used for statistical analysis.

Results and Discussion

Antifungal activity of essential oils

The influence of the tested treatments on the colony formations (diameter-cm) of two isolates of *B. cinerea* are given in Table 1. It is clear from the results that the colony diameter increased during the incubation period and reached to maximum in the 7th day of incubation.

Table 1. Influence of *L. angustifolia* and *T. spicata* essential oils, incorporated into the PDA media, on the colony diameter (cm) of mycelial growth of *B. cinerea* isolates (M1-5 and M3-5) during 7 days of incubation

Treatments	3 days	5 days	7 days	3 days	5 days	7 days
	***L. angustifolia* on M1-5 isolate**			***T. spicata* on M1-5 isolate**		
EO (0.25 mL L^{-1})	3.99 b	6.69 c	8.03 b	5.08 a	8.34 a	8.44 a
EO (0.50 mL L^{-1})	2.99 c	6.14 c	7.46 c	4.04 b	7.71 bc	8.50 a
EO (1.00 mL L^{-1})	0.40 d	2.89 d	6.68 c	3.93 b	6.86 d	7.63 b
EO (2.00 mL L^{-1})	0.49 d	0.50 e	0.64 d	1.24 c	4.01 e	7.08 c
Control-1 (sterile water)	4.49 a	7.33 b	8.50 a	4.49 ab	7.33 c	8.50 a
Control-2 (70% ethanol)	4.56 a	7.99 a	8.50 a	4.56 ab	7.99 ab	8.50 a
Control-3 (fungicide)	0.50 d	0.50 e	0.50 d	0.50 c	0.50 e	0.50 d
	***L. angustifolia* on M3-5 isolate**			***T. spicata* on M3-5 isolate**		
EO (0.25 mL L^{-1})	4.63 c	8.26 a	8.50 a	5.49 b	8.50 a	8.50 a
EO (0.50 mL L^{-1})	4.65 c	8.50 a	8.50 a	2.98 d	7.89 b	8.50 a
EO (1.00 mL L^{-1})	2.89 d	7.45 b	8.50 a	4.03 c	8.00 b	8.26 b
EO (2.00 mL L^{-1})	1.55 e	4.48 c	8.50 a	1.18 e	2.80 c	4.15 c
Control-1 (sterile water)	5.68 b	8.50 a	8.50 a	5.68 b	8.50 a	8.50 a
Control-2 (70% ethanol)	6.10 a	8.50 a	8.50 a	6.10 a	8.50 a	8.50 a
Control-3 (fungicide)	0.50 f	0.50 d	0.50 b	0.50 f	0.50 c	0.50 d

Different letters next to the values at each incubation day for different oils and isolates, indicates significant difference according to Tukey's HSD test ($p \leq 0.05$).

The fungicide treatment was noted to have very high influence on the prevention of the colony formation in both isolates. The EOs were then observed to have varying significant influence on the colony formation. The EO of *L. angustifolia* reduced the colony formation of M1-5 isolate only at its high concentrations, while the same concentration had no effect on the other isolate, M3-5. On the other hand, the EO of *T. spicata* had very low influence on the M1-5 isolate, while its highest dose was noted to have significant influence on the prevention of the M3-5 isolate. The formation of the colonies and their visual appearance was also showed in Figure 1.

The results about the mycelial growth inhibition (%) of the treatments are presented in Figure 2. The results clearly presented that the low to high doses of *L. angustifolia* essential oil had low to high influence on the mycelial growth inhibition of the M1-5 isolate, but no effect on M3-5. The highest dose of *L. angustifolia* (2.00 mL L^{-1}) was found to have more than 90% of mycelial growth inhibition on M1-5. The results for *T. spicata* essential oil were found to be quite different. This EO had higher influence on the M3-5 isolate and lower on the M1-5 isolate. The highest concentration of *T. spicata* (2.00 mL L^{-1}) provided more than 50% of mycelial growth inhibition on the M3-5 isolate.

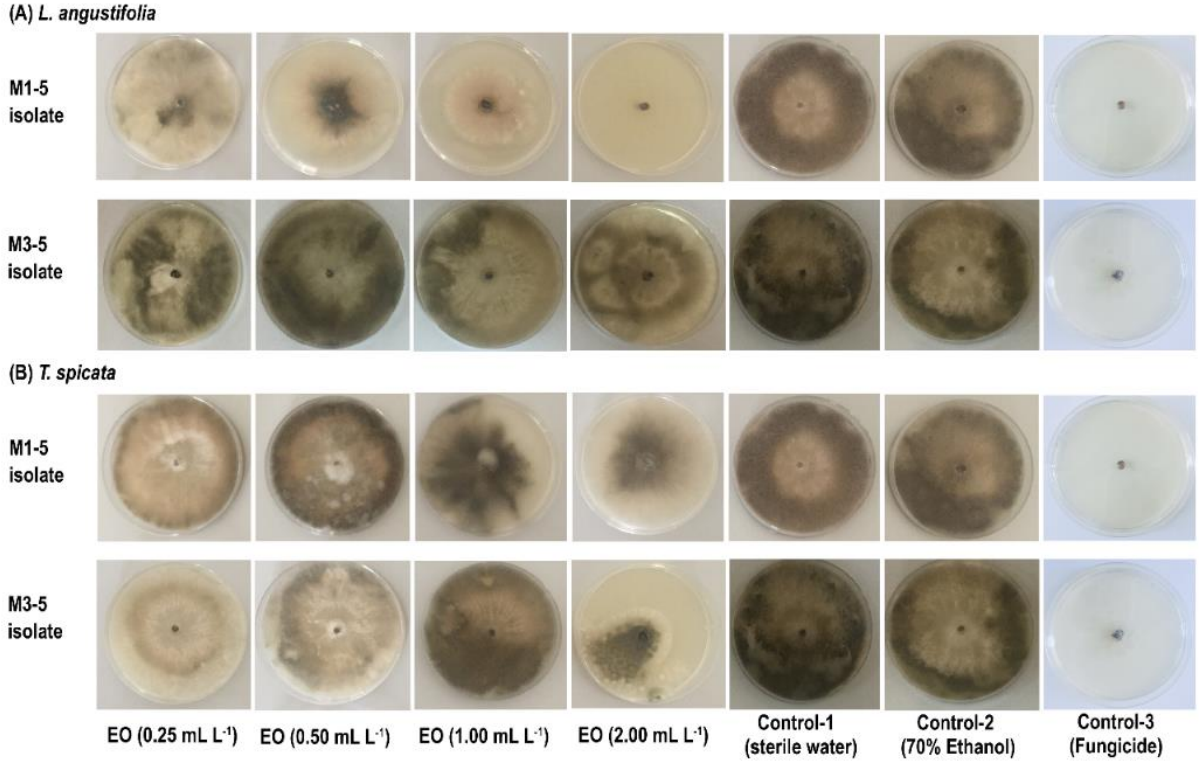

Figure 1. Visual appearance of mycelial growth of two isolates of *B. cinerea* on PDA growing media, as affected by different application doses of (A) *L. angustifolia* and (B) *T. spicata* essential oils, after 7 days of incubation.

Results of present study were found to be in accordance with several studies which reported high influence of essential oils on the mycelial growth of different fungi (Gumus, 2010; Stupar et al., 2014; Kordali et al., 2016; Kahramanoğlu, 2019). The success of these EOs can be associated with their diverse chemical compositions, i.e. eucalyptol and linalool for *L. angustifolia* (Karadağ et al., 2021) and carvacrol and γ-terpinene for *T. spicata* (Sengun et al., 2021). In a similar study, *L. angustifolia* with a high concentrations (37.61%) of linalool was noted to have significant influence on the growth of *Aspergillus niger*, *Aspergillus ochraceus*, *Penicillium* sp., *Trichoderma viride* and *Bipolaris spicifera*. The antifungal activity of *L. angustifolia* was noted to vary among concentrations and isolates and was noted to have similar effects with the EOs of *Origanum vulgare* and *Rosmarinus officinalis* (Stupar et al., 2014). The antifungal activity of *T. spicata* was also previously noted for different fungi. Gumus (2010) reported that the EO of *T. spicata* reduce the activity of *A. parasiticus* (NRRL 465 and NRRL 2999).

Impacts of volatile essential oils on disease severity

Figure 3 shows the effects of vapor contact application of *L. angustifolia* and *T. spicata* EOs on the disease severity, caused by two isolates of *B. cinerea*, on cv. Camarosa strawberry fruits. Results clearly showed that both EOs have significant influence on the prevention of disease severity, where this impact is higher than un-treated controls, but lower than the fungicide application.

Although the impacts are lower than the fungicide treatment, the disease severity of two isolates treated with EOs, especially with *T. spicata*, was noted to be acceptable in 12 days of storage. The *T. spicata* EO treatment reduced the disease severity score of M1-5 isolate from 4.1 to 1.5 at the 12th day of storage, which at the same time provided better control of disease severity of M3-5 isolate (reduced from 4.2 to 1.2). Slightly lower impact was observed from the *L. angustifolia* which provided a score of 1.8 on M1-5 and 1.4 on M3-5. Several studies suggested that the disease severity or decay at strawberry fruits generally starts after 4-6 days of storage at 4 °C (Mohammadi et al., 2015b; Kahramanoğlu, 2019).

Camarosa is a highly perishable strawberry cultivar and 12 days is a very good success in storage without or with acceptable level of disease severity (Kahramanoğlu 2019). Current results are in agreement with some previous studies, which reported moderate-to-high antifungal activity for *L. angustifolia* (Stupar et al., 2014) and *T. spicata* against (Gumus 2010) different fungi. Several studies (Fadli et al., 2012) recommended that the antifungal activity of essential oils can be due to their ability to disrupt cell membrane of the fungi, ability to cause a loss of integrity in cell wall and ability to prevent the respiration (Fadli et al., 2012).

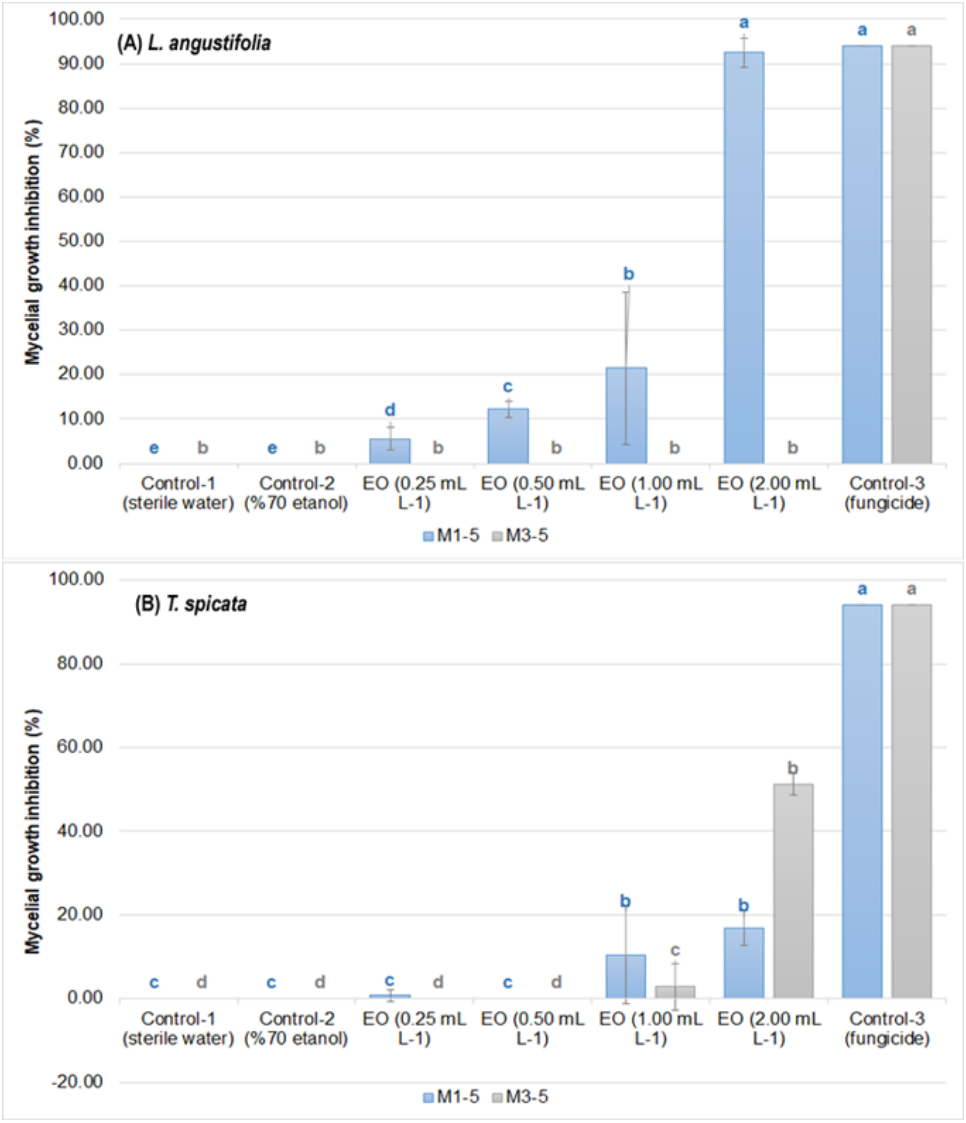

Figure 2. Mycelial growth inhibition of *B. cinerea* isolates (M1-5 and M3-5) after 7 days of incubation, caused by the (A) *L. angustifolia* and (B) *T. spicata* essential oils added on PDA. Different letters above the columns of each isolates separately indicates significant difference among the treatments according to Tukey's HSD test ($p \leq 0.05$).

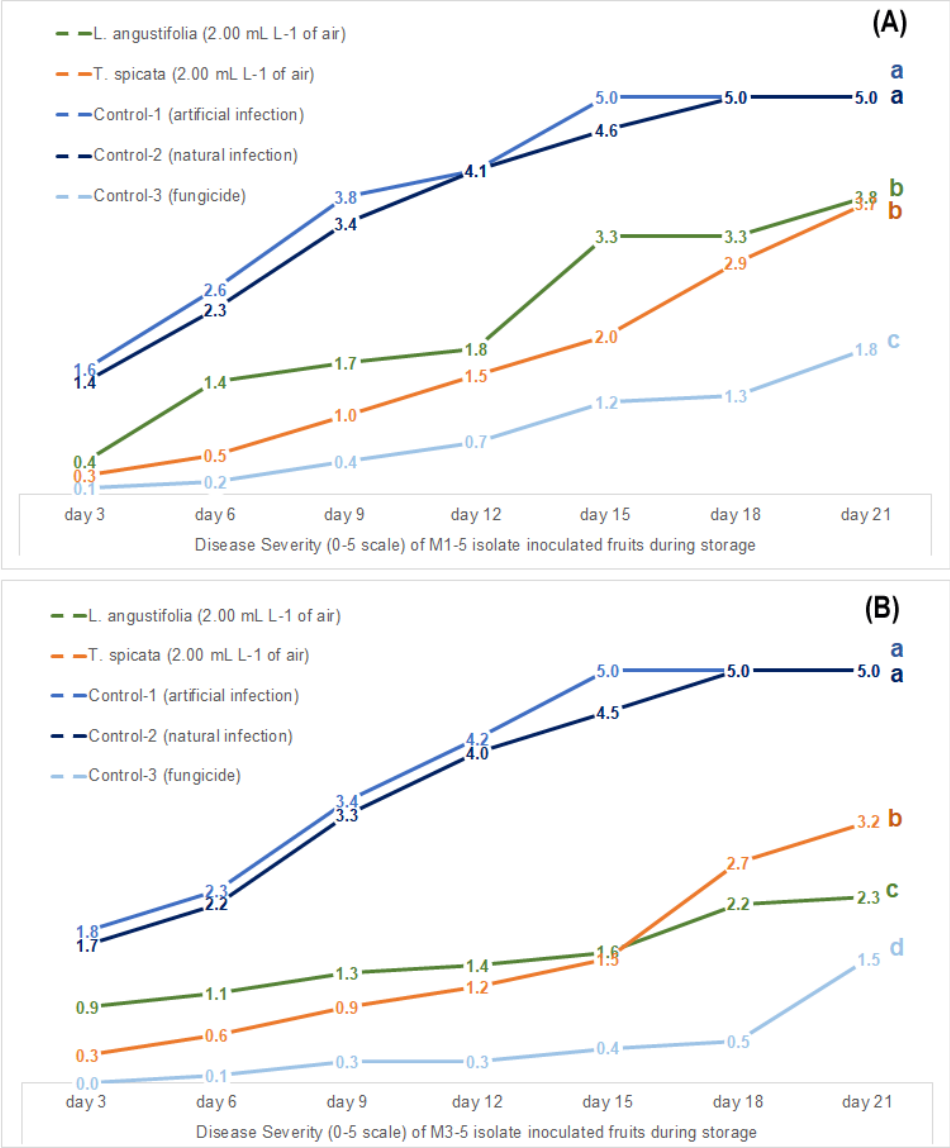

Figure 3. Disease severity of strawberry fruits during 21 days of storage as affected by vapor application of *L. angustifolia* and *T. spicata* essential oils after inoculation with two isolates (A) M1-5 and (B) M3-5 of *B. cinerea*. Different letters on the right side of lines indicates significant difference (by comparing the final day) among the treatments according to Tukey's HSD test ($p \leq 0.05$).

Results of present study are also valuable in terms of their application type. Essential oils have high ability to volatilize and are not soluble in water. Thus, the application of EOs as coating or film is difficult in practice and these characteristics may reduce their activities (Carvalho et al., 2016). In present study, the EOs, during the *in vivo* studies, had been applied as vapor contact and found to have significant influence on the mycelial growth of *B. cinerea*. Most of the available studies in the published literature (Pavinatto et al., 2020) suggest the contact application or incorporated application of EOs with coatings or films; therefore current results are important in terms of their characteristics of application. The vaporized application of EOs had also been reported to have less or no impact on the sensory quality of fruits, which make it as an important alternative (Velázquez-Nuñez et al., 2013). Current results are not novel for the science (in terms of their application method) which was tested and recommended by several studies for controlling different fungi (Velázquez-Nuñez et al., 2013; Paris et al., 2020), but novel for the EOs of *L. angustifolia* and *T. spicata* against *B. cinerea*. A closely related study by Mpho et al., (2013) tested the combined effects of vapor of lemongrass oil (100 µL) and modified atmosphere packaging (MAP) in avocado fruits and suggested better performance in the combined treatment for controlling the *C. gloeosporioides*. MAP is a very important technique for improving postharvest quality and nutritional parameters of fruits and vegetables (Kurubas et al., 2019). Discussion of current results made it possible to conclude that the combination of vapor application of EOs with packaging techniques (i.e. MAP), could be more effective in controlling fungi and improving the storability of fruits, including strawberry.

Impacts of volatile essential oils on fruit quality

The impacts of vapor application of the *L. angustifolia* and *T. spicata* essential oils on different fruit quality parameters (weight loss, AA, SSC and pH) were also tested in current study. The results are presented in Figure 4.

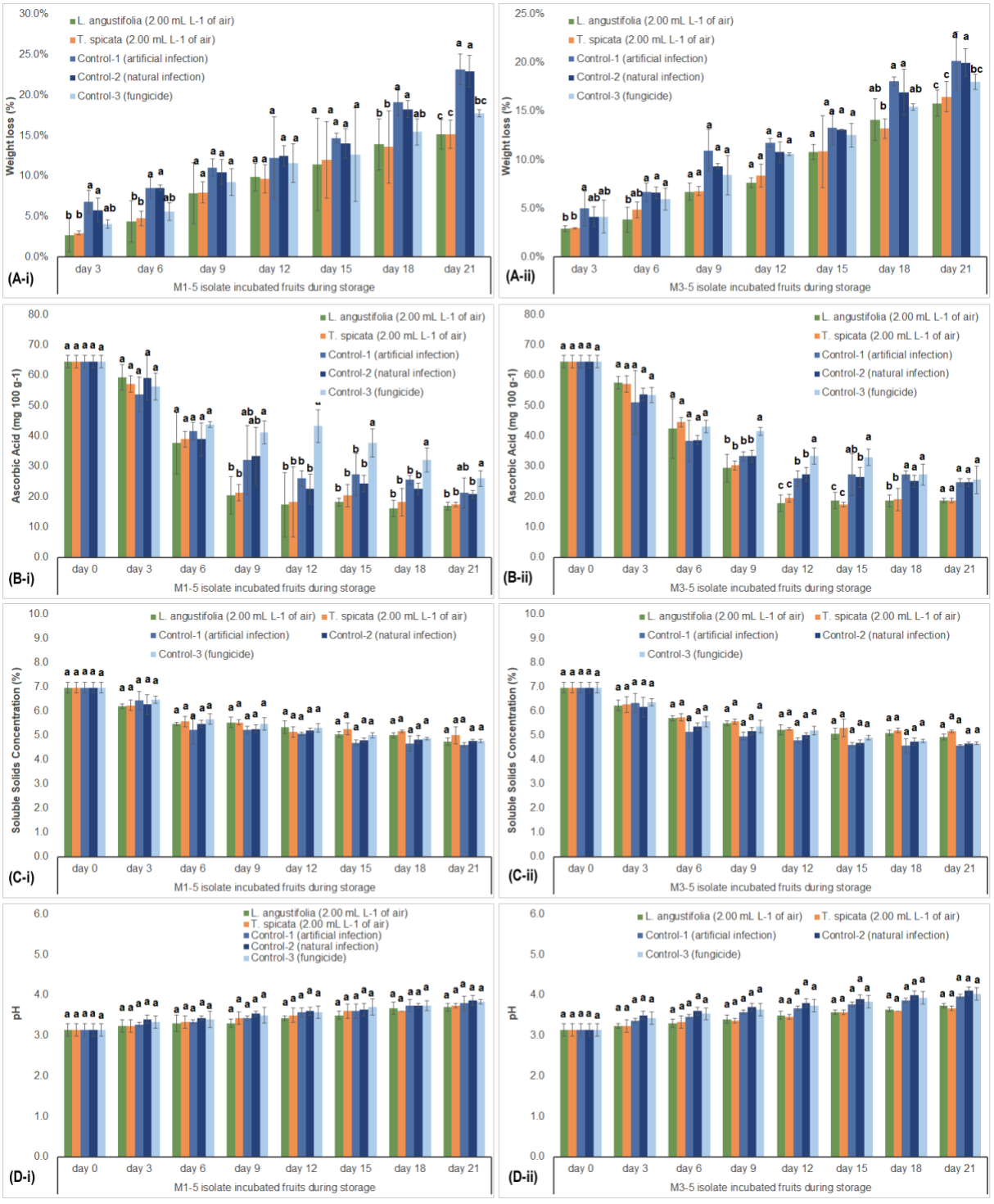

Figure 4. (A) Weight loss, (B) AA, (C) SSC and (D) pH of strawberry fruits during 21 days of storage as affected by vapor application of *L. angustifolia* and *T. spicata* essential oils after inoculation with two isolates (i) M1-5 and (ii) M3-5 of *B. cinerea*. Different letters above the columns at each measurement point indicates significant difference among the treatments according to Tukey's HSD test ($p \leq 0.05$).

It was observed that the reduction disease severity and weight loss of the fruits have a significant relationship. The treatments which were noted to provide better performance in controlling the disease severity were also found to reduce the weight loss of fruits. Similar impacts were noted on both isolates of *B. cinerea* (Figure 4A). In a similar work, it was noted that another EO, belonging to the lemongrass,

provides good performance in maintaining the weight of strawberry fruits when it is combined with chitosan edible coating (Khalifa et al., 2016). The ascorbic acid (AA) content of the fruits decreased during storage. This result is in accordance with the reports of Atress et al., (2010) and Kahramanoğlu (2009). The treatments were noted to have no significant impact on the AA content of the fruits (Figure 4B). The results for SSC was noted to be similar with the AA contents of the fruits. It was decreased during the storage and no impact was noted for the treatments (Figure 4C). Contrary to SSC and TA values, the fruit pH was noted to have an increasing trend during storage. The treatments were again noted to have no significant influence on the pH (Figure 4D). Even though the EOs had very minor impact on the fruit quality, the prevention of the weight loss is an important result for the study. The direct application of EO had been noted to provide similar positive impacts on the fruit quality which was associated with some physiological changes in fruits, such as inducing the synthesis of several enzymes (PPO, SOD, CAT and POD) (Kahramanoğlu et al., 2020; Wan et al., 2021).

Conclusion

The essential oils of *L. angustifolia* and *T. spicata* had been found to have significant influence on the prevention of mycelial growth of *B. cinerea*. The results are novel in terms of the direct vapor contact application of the oils into fruit packaging. Besides to that, it is well-known that packaging of fruits with special materials allowing modification of the inner atmosphere (reducing oxygen and increasing carbon dioxide) is highly beneficial for improving storability of the fruits. Therefore, it is thought that the combination of such materials together with direct vapor application of essential oils would have better performance. However, further studies are required to clarify their effects, especially in combination with different fruit packaging materials.

Compliance with Ethical Standards

Conflict of interest

Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

İ.K.: Conceptualization, Methodology, Data curation, Writing - original draft; **T.G.K.**: Conceptualization, Methodology, Resources, Investigation; **A.U.B.**: Methodology, Resources, Investigation; **R.G.**: Conceptualization, Methodology, Resources, Investigation, Writing - review & editing; **H.A.**: Investigation.

Ethical approval

Authors declare no requirements for any ethical approval.

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Data availability

All data were summed and presenten in the paper. The complete list of raw data is available upon request.

Consent for publication

We, as the authors of present paper, give our consent for the publication of this paper in the International Journal of Agriculture Environment and Food Sciences.

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Determination of morphological responses and plant nutrient preferences of some vine rootstocks grown under *in vitro* salt stress conditions

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Abstract

The study was performed to determine mineral nutrition preferences and the morphological response against the salt stress of the rootstocks used in Turkey. 41B, 5BB, 140Ru, Salt Creek, and SO4 were used as rootstocks, and NaCl at concentrations of 0 (control), 0.75, and 1.5 g L^{-1} were applied to the plantlets grown in MS medium. The values of all shoot and root properties examined in this experiment decreased with increasing NaCl concentrations compared to control plants. The highest damage degree was seen on 41B, while there was no damage on Salt Creek plantlets. Shoot and root tolerance ratios of Salt Creek rootstock were found to be the best among the rootstock. These ratios were higher in 0.75 g L^{-1} than 1.5 g L^{-1} concentration. Leaf chlorophyll and nutrient content were negatively affected by the increasing NaCl doses. It has been found that all nutrient elements are positively affected by each other's uptake. The highest N, K, Ca, and Mg levels were detected in Salt Creek, while the lowest level was detected in 41B rootstock. Considering all the parameters examined, rootstocks are ranged from the most sensitive to the most resistant to salinity conditions; 41B, SO4, 5BB, 140Ru, and Salt Creek.

Keywords: Chlorophyll content, Grapevine, NaCl, Plant nutrition, Tissue culture

Introduction

Vine (*Vitis vinifera* L.) is grown latitude between 11-53º in the northern hemisphere and 20-40º in the southern hemisphere (Çelik, 2011). There are some biotic and abiotic factors affecting grapevine cultivation in Turkey, where is among the ideal growing area for viticulture (Mahajan and Tuteja, 2005; Kacar et al., 2006).

Salinity is the most important abiotic stress factor, especially in arid and semi-arid ecologies (Boscaiu et al., 2008; Edriss et al., 2016; Mohammadkhani and Abbaspour, 2018; Haider et al., 2019; Lo'ay and El-Ezz, 2021). The salinity problem in Turkey, as well as in many countries, is growing day by day. It is stated that this is caused mainly by improper irrigation and excessive fertilization, and lack of drainage (Zhani et al., 2012; Patil et al., 2020). Salt stress prevents growth depending on tolerance and can lead to chlorosis and necrotic spots. In addition, weight loss, stunting in both root and stem, and decreasing plant stem and root length can be seen (Fozouni et al., 2012b; Dag et al., 2015).

Salt is an important factor limiting growth in grapevines, as in all plants (Upadhyay et al., 2018; Barakat et al., 2019). Vine can absorb 1-6% of the salt in the soil (Storey et al., 2003; Munns, 2005). Vine development and yield decreased in a salt concentration above 2.5 ds m^{-1} (1.6 g L^{-1}), and when it reaches EC 6.7 ds m^{-1} (4.29 g L^{-1}), deaths can be seen in the vine (Battany, 2004; Bakır, 2012). The sensitivity of American grapevine rootstocks used for combat pests such as phylloxera and nematodes is higher. It is known that rootstocks are more susceptible to adverse soil conditions such as drought and calcareous besides salinity than *V. vinifera* (Patil et al., 2020). The role of grapevine rootstocks in nutrient uptake is also important, and their effects on the growth and yield of cuttings are diverse (Tangolar and Ergenoğlu, 1989).

Various studies have shown differences in salt tolerance between American species and *V. vinifera* varieties (Müftüoğlu et al., 2006). It is stated that some of the rootstocks are tolerant to salinity due to their ability to prevent Na and /or Cl uptake (Troncoso et al., 1999; Storey et al., 2003). There

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are some studies (Desmukh et al., 2003; Xiucai et al., 2004) and *in vitro* (Sivritepe and Eriş, 1999; Troncoso et al., 1999; Hamrouni et al., 2008; Popescu et al., 2015; Barakat et al., 2019; Hao et al., 2021) conducted *in vivo* conditions to determine the physiological and morphological responses of *V. vinifera* varieties and American vine rootstocks against soil salinity. In these studies, stress mechanisms have been studied, and it has been determined that the mechanisms developed by plants within the same species against salt stress are different (Sivritepe and Eriş, 1999). Because the studies carried out under *in vivo* conditions require a long time and cost, *in vitro* studies have started to be shortened of this period, albeit in a limited number, recently. In the study of Fisarakis et al. (2005), salinity increased the phosphorus (P) concentration of the leaf blade, petiole, and shoots; decreased NO_3-N and K concentrations. They reported that Ca and Mg concentrations in the shoots, P and Mg concentrations in the stem, and P, Ca, and Mg concentrations in the root were not affected by salt amount. In certain studies, it has been reported that salinity has a negative effect on mineral element uptake (Singh et al., 2000; Hepaksoy et al., 2006). The way salinity affects plant growth has still not been fully understood. However, it has been reported that the salinity tolerance of plants can be changed by mineral nutrition (Fisarakis et al., 2005).

There are limited studies about the mineral nutrient preferences of vine rootstocks under *in vitro* salt stress conditions. In recent years, how to feed rootstocks in viticulture against abiotic stress conditions has become an important agenda issue. Therefore, this study was planned to determine the morphological responses of some American grape rootstocks grown under different salt (NaCl) stress conditions *in vitro* and determine their mineral nutrition preferences. In addition to this subject, a protocol will be created for early screening of salinity tolerance in breeding new rootstocks using tissue culture technique.

Materials and Methods

Materials

This study was carried out in 2018 at the Department of Horticulture, Faculty of Agriculture, Cukurova University, Adana, Turkey. In the study, 41B, 5BB, 140Ru, and SO4 rootstocks, which are widely used in Turkey against phylloxera, and Salt Creek rootstock used for nematode problems, were used as plant materials. These materials were obtained from the Viticulture Research and Application area of Cukurova University.

Methods

In the active growth period (April-May), the nodal explants containing a single bud prepared from the 10 cm shoot tip of the rootstocks were disinfected with 5% commercial sodium hypochlorite solution containing few drops Tween 20 for 15 minutes in a sterile laminar flow cabinet. Then, the explants were rinsed three times with sterile distilled water (Meşe and Tangolar, 2019). After surface sterilization, the explants were planted into 25 mm x 150 mm sized test tubes containing 10 mL of MS (Murashige and Skoog, 1962) medium supplemented with 1 mg L^{-1} BAP, 30 g L^{-1} sucrose, and 8 g L^{-1} agar. Explants were cultured in tubes for four weeks. When the shoots had 2-3 leaves, they were cut and transferred to MS medium containing 1 mg L^{-1} IBA for rooting. After 5-6 weeks, the upper parts of the shoots containing three leaves were cut from the plantlets and used for salt applications. To create salt stress, 0, 0.75, and 1.5 g L^{-1} concentrations of NaCl were added to the MS medium containing 1 mg L^{-1} IBA. All the explants cultured in this study were incubated in a growth chamber with a temperature of 25±1 ^oC, a photoperiod of 16 hours, and exposure of 3000-4000 lux (11000-15000 watt. m^{-2}) for 45 days. Lighting was provided by Cool daylight type TLD 36 w/54 fluorescent lamps.

Investigated Characteristics

The plantlets were removed from the tubes at the end of 45 days in the growth room, and the roots were cleaned from the nutrient medium. After that, plant damage degree (0: No salt stress sign, 1: slowing in growth, local yellowing of leaves, 2: yellowing of leaves and 25% necrotic spotting, 3: 25-50% necrotic spots on leaves, 4: 50-75% necrosis on leaves and stems and death, 5: 75-100% severe necrosis on leaves and stems and total death), according to Kıran et al. (2015) were determined. Average shoot length (cm $plant^{-1}$), node number (n $plant^{-1}$), shoot fresh and dry weight (g $plant^{-1}$), root length (cm $plant^{-1}$), root number (n $plant^{-1}$), root fresh and dry weight (g $plant^{-1}$), rooting rate (%) and leaf chlorophyll content (SPAD readings, SPAD-502, Konica Minolta Sensing, Inc., Tokyo, Japan) were detected according to Meşe and Tangolar (2019). Plant vitality rate (%) was determined according to Edriss et al. (2016) and Uyar (2016). In the plant viability calculation, according to the 0-5 scale value taken into account in the plant damage degree, the plants that got 0 and 1 were considered alive, and the others were considered as dead. In addition, shoot and root tolerance rate (TO) and shoot and root tolerance index (TI) were calculated (Dardeniz et al., 2006; Uyar, 2016) as follows:

TO = shoot and root tolerance ratio

$TO = T_x / T_o$

T_x = shoot and root dry weight of the NaCl treated plant (g)

T_o = shoot and root dry weight of the plant without NaCl application (g)

TI = Shoot and root tolerance index

$TI = 100 + \sum^n [x (T_x / T_o)100]$

n = 3 (Number of applications)

x = 0; 0.075 and 0.150 g NaCl 100 mL^{-1}

For mineral nutrient analysis, the shooting part of the plantlets, which were dried for 24 hours at 65 ^oC in the oven, was ground. N concentration was determined according to the Kjeldahl method (AOAC, 1970). To determine the element levels of K, Ca, Mg, Cu, Zn, Fe, Mn, and Na, 0.200 g of ground sample was burned in an ashing furnace at 550 ^oC for 5.5 hours, and then 2 mL of 1/3 HCL

solution and 18 mL of distilled water were added to the ash obtained. This mixture was filtered by the blue band filter paper and taken into a vial. The plant nutrient concentrations were determined by the Atomic Absorption Spectrophotometer (AAS) (Bonomelli and Ruiz, 2010), in which chlorine was also determined. Finally, phosphorus was determined by spectrophotometer according to the Barton method (Barton, 1948).

Experimental Design and Statistical Analysis

The research was carried out according to a randomized factorial design with three replicates. Ten plantlets were used in each replicate. Variance analysis was performed on the data obtained by using JMP statistical package program (v8.00, SAS Institute Inc., USA), and the LSD test was used to determine different groups at a 5% significance level.

Results

In the study, the highest values in terms of shoot length, node number, shoot fresh, and dry weight was determined in Salt Creek rootstock (5.8 cm, 8 (n), 0.446 g, and 0.070 g, respectively) and then 140Ru (4.5 cm, 6.9 (n), 0.205 g and 0.040 g, respectively) rootstock. The lowest values were detected in 41B rootstock (3.4 cm, 5.1 (n), 0.136 g, and 0.029 g, respectively) (Table 1). It was found that the values of shoot properties decreased with increasing salt concentrations. It has been observed that there were significant differences among the rootstocks in terms of plant damage degree. The most severe damage was seen on 41B (3.3 scale degree) followed by SO4 (2.4 scale degree), while there was no damage on Salt Creek (0.0 scale degree) rootstock (Table 1).

The responses of root properties and chlorophyll values (SPAD readings) to different salt applications were parallel to the reactions of shoot properties (Table 2). In terms of these characteristics, Salt Creek had the highest values, followed by 140Ru. Together, 41B and 5BB made up the group with the lowest values. Average root length and number, root fresh and dry weights, and SPAD values were prominent in Salt Creek (7.4 cm, 5.4 (n), 0.429 g, 0.037 g, and 29.6, respectively) compared to other rootstocks. Root growth and SPAD values were determined to be inversely proportional to NaCl concentrations (Table 2).

Regarding plant viability and rooting rate, it was observed that all the plants of Salt Creek rootstock and the control group of applications developed without any problem (Table 3). While viability and rooting rates were over 70% in 140Ru rootstock, these rates remained at the level of 33.3% in 41B rootstock (Table 3).

According to the shoot and root tolerance ratios given in Table 4, the highest values were obtained from Salt Creek, and the lowest data were recorded from SO4 and 41B rootstocks. Shoot and root tolerance ratios of Salt Creek rootstock were found to be 1.118 and 1.097, respectively. Among the rootstock varieties, the order from the best to the lowest root tolerance was determined as Salt Creek, 140Ru, 5BB, SO4, and 41B (Table 4).

Table 1. Effects of different NaCl concentrations on shoot characteristics of different rootstocks.

Sources of Variation	Shoot length (cm)	Node number (n)	Shoot fresh weight (g)	Shoot dry weight (g)	Plant damage degree (0-5 scale)
Rootstock					
5BB	3.9 c [x]	6.5 b	0.163 c	0.024 d	1.6 c
41B	3.4 d	5.1 d	0.136 d	0.029 cd	3.3 a
Salt Creek	5.8 a	8.0 a	0.446 a	0.070 a	0.0 e
140Ru	4.5 b	6.9 b	0.205 b	0.040 b	1.2 d
SO4	3.5 d	6.0 c	0.172 c	0.032 c	2.4 b
LSD 5%	0.1	0.4	0.017	0.004	0.1
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NaCl Concentration g L^{-1}					
0	5.9 a	8.6 a	0.330 a	0.051 a	0.0 c
0.75	4.2 b	6.5 b	0.214 b	0.039 b	1.5 b
1.5	2.5 c	4.4 c	0.128 c	0.027 c	3.6 a
LSD 5%	0.1	0.3	0.013	0.003	0.1
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction					
LSD 5%	0.2	0.8	0.030	0.007	0.2
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 2. Effects of different NaCl concentrations on root properties and chlorophyll content of different rootstocks.

Sources of Variation	Root length (cm)	Root number (n)	Root fresh weight (g)	Root dry weight (g)	SPAD Readings
Rootstock					
5BB	4.4 b [x]	1.5 d	0.063 cd	0.006 d	22.5 c
41B	1.8 d	1.4 d	0.054 d	0.007 d	20.7 d
Salt Creek	7.4 a	5.4 a	0.429 a	0.037 a	29.6 a
140Ru	4.9 b	3.3 b	0.189 b	0.020 b	26.8 b
SO4	3.1 c	2.1 c	0.087 c	0.010 c	22.4 c
LSD 5%	0.7	0.4	0.030	0.003	0.8
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NaCl Concentration g L^{-1}					
0	6.9 a	4.3 a	0.238 a	0.024 a	29.6 a
0.75	4.5 b	2.6 b	0.180 b	0.017 b	26.0 b
1.5	1.5 c	1.2 c	0.075 c	0.007 c	17.5 c
LSD 5%	0.5	0.3	0.023	0.002	0.6
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction					
LSD 5%	1.2	0.6	0.051	0.005	1.4
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 3. Effects of different NaCl concentrations on plant viability and rooting rates of different rootstocks.

Sources of Variation	Viability rate (%)	Rooting rate (%)
Rootstock		
5BB	66.7 c [x]	58.0 c
41B	33.3 e	33.3 e
Salt Creek	100.0 a	100.0 a
140Ru	72.2 b	73.6 b
SO4	48.9 d	48.9 d
LSD 5%	3.92	6.25
p	<0.0001	<0.0001
NaCl Concentration g L^{-1}		
0	100.0 a	100.0 a
0.75	69.3 b	64.2 b
1.5	23.3 c	24.2 c
LSD 5%	3.04	4.83
p	<0.0001	<0.0001
Interaction		
LSD 5%	6.80	10.79
p	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 4. Effects of different NaCl concentrations on the shoot and root tolerance ratios of different rootstocks.

Sources of Variation	Shoot tolerance ratio	Root tolerance ratio
Rootstock		
5BB	0.539 c [x]	0.268 c
41B	0.352 d	0.000 e
Salt Creek	1.118 a	1.097 a
140Ru	0.630 b	0.551 b
SO4	0.413 d	0.125 d
LSD 5%	0.079	0.108
p	<0.0001	<0.0001
NaCl Concentration g L^{-1}		
0.75	0.720 a	0.605 a
1.5	0.500 b	0.211 b
LSD %5	0.050	0.069
p	<0.0001	<0.0001
Interaction		
LSD 5%	0.112	0.153
p	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

The tolerance rates of shoots and roots were more pronounced at a concentration of 0.75 g NaCl L^{-1}. When the shoot and root tolerance index table was examined (Fig 1), Salt Creek rootstock came first. According to the tolerance index, 140Ru and 5BB were in the same statistical group. Rooting of 5BB rootstock was evaluated to be slightly weaker compared to 140Ru rootstock, and the lowest data were obtained from SO4 and 41B rootstocks (Fig 1).

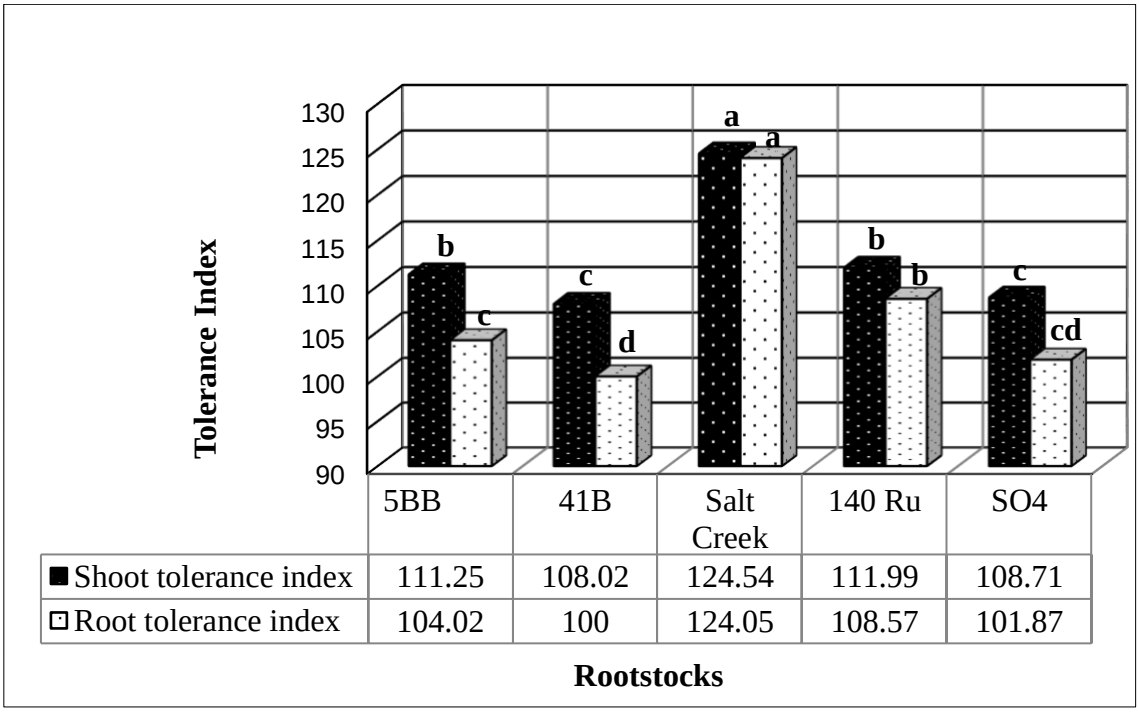

Fig 1. The effect of different NaCl concentrations applied to varying rootstocks on the shoot and root tolerance index.

A significant difference (P≤ 0.05) was found among the means indicated by different letters in the same indicator column.

According to the nutrient analysis performed on rootstock explants under *in vitro* salinity stress (Table 5), it was determined that the rootstock taking up the most N (3.46%), K (2.28%), Ca (0.66%), and Mg (0.34%) elements from the nutrient medium were found to be Salt Creek. The rootstock with the highest P uptake (0.34%) was SO4. It was determined that the rootstock with the lowest N (1.20%), P (0.12%), K (0.83%), Ca (0.23%), and Mg (0.11%) uptake was 41B. The effect of NaCl on macro element uptake of rootstock plantlets was negative. Adding 1.5 mg L^{-1} salt to the nutrient medium reduced the uptake by 17.9% in N, 12.5% in P, 17.7% in K, 18.5% in Ca, and 20.6% in Mg, compared to the control (Table 5).

The effect of different salt applications on the microelement uptake from the nutrient medium of the rootstock plantlets was different at the rootstock level (Table 6). Salt Creek was the rootstock that took up the highest amount of Cu (3.94 ppm), Mn (211.7 ppm), Zn (70.09 ppm), Na (3958.0 ppm), and Cl (30724.7 ppm) elements, except Fe element. In terms of Na and Cl element concentrations, Salt Creek was followed by 5BB. It was determined that the iron element was at the highest level in 140Ru rootstock (189.0 ppm). The rootstock that received the least microelements from the nutrient medium was 41B. Salt Creek rootstock can absorb macro and microelements in the best way is explained as that this rootstock continues to develop without being affected by salt stress. Whereas, it was evaluated that the low element concentrations in the 41B rootstock were caused by the fact that plants did not grow and died under salt stress conditions. In addition, it has been determined that increasing salt dose decreased the microelement uptake of plantlets.

As it can be seen from Table 7, which shows the nutritional correlations of rootstocks from nutrient media containing different salt concentrations, all nutrients are in a positive relationship with each other. The highest values of coefficients were found between Ca-Mg (1.00), N-K (0.99), K-Ca (0.98), K-Mg (0.97), and P-K (0.97).

Table 5. Effects of different NaCl concentrations on the macro element amounts (%) of different rootstocks.

Sources of Variation	N	P	K	Ca	Mg
Rootstock					
5BB	2.79 b [x]	0.24 c	1.77 b	0.41 c	0.22 c
41B	1.20 d	0.12 d	0.83 d	0.23 d	0.11 d
Salt Creek	3.46 a	0.32 b	2.28 a	0.66 a	0.34 a
140Ru	2.08 c	0.24 c	1.47 c	0.42 b	0.24 b
SO4	2.84 b	0.34 a	1.83 b	0.42 b	0.22 c
LSD 5%	0.26	0.01	0.10	0.01	0.01
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NaCl Concentration g L^{-1}					
0.0	3.91 a	0.40 a	2.54 a	0.65 a	0.34 a
0.75	2.81 b	0.30 b	1.92 b	0.51 b	0.27 b
1.5	0.70 c	0.05 c	0.45 c	0.12 c	0.07 c
LSD 5%	0.20	0.01	0.08	0.01	0.004
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction					
LSD 5%	0.45	0.03	0.18	0.02	0.01
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 6. Effects of different NaCl concentrations on the microelement amounts (ppm) of different rootstocks.

Sources of Variation	Cu	Mn	Fe	Zn	Na	Cl
Rootstock						
5BB	2.37 b [x]	148.5 c	143.9 b	61.58 b	3049.3 b	31949.6 a
41B	0.69 d	86.2 d	56.6 c	18.58 e	581.3 e	13119.8 b
Salt Creek	3.94 a	211.7 a	153.5 b	70.09 a	3958.0 a	30724.7 a
140Ru	1.90 c	214.5 a	189.0 a	42.53 d	1509.3 d	15330.0 b
SO4	2.42 b	188.0 b	142.9 b	53.72 c	1748.8 c	9170.3 c
LSD 5%	0.47	7.7	10.6	2.90	215.9	2494.9
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NaCl Concentration g L^{-1}						
0.0	3.41 a	276.7 a	223.6 a	78.05 a	1406.6 b	29175.7 a
0.75	2.69 b	197.2 b	160.5 b	58.28 b	3767.7 a	23200.9 b
1.5	0.70 c	35.4 c	27.5 c	11.56 c	1333.7 b	7800.0 c
LSD 5%	0.36	5.9	8.2	2.24	167.2	1932.6
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interaction						
LSD 5%	0.81	13.3	18.4	5.02	373.9	4321.3
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[x]: Significant difference (P≤ 0.05) was found among the means indicated by different letters in the same column.

Table 7. Correlation coefficients of uptake characteristics of plant nutrients obtained from different rootstocks grown under different NaCl concentrations.

Element	P	K	Ca	Mg	Cu	Mn	Fe	Zn	Na	Cl
N	0.96*	0.99	0.96	0.95	0.91	0.90	0.87	0.97	0.59	0.78
P		0.97	0.94	0.94	0.88	0.95	0.80	0.93	0.49	0.63
K			0.98	0.97	0.92	0.93	0.90	0.95	0.61	0.79
Ca				1.0	0.92	0.94	0.89	0.93	0.59	0.78
Mg					0.91	0.96	0.92	0.93	0.61	0.77
Cu						0.84	0.79	0.91	0.60	0.71
Mn							0.97	0.87	0.42	0.63
Fe								0.87	0.43	0.65
Zn									0.584	0.77
Na										0.72
Cl										1.00

*: Significant coefficients at P≤ 0.05

Discussion

Salinity has been known as one of the significant problems in the soils of the world. Because the soil salinity is an important factor limiting the growth of vine plants, this study was carried out to determine mineral nutrition preferences and the morphological response against the salt stress of the rootstocks consisting of 41B, 5BB, 140Ru, Salt Creek, and SO4 that are widely used in Turkey viticulture. In the study, shoot and root characteristics and chlorophyll content levels of rootstock plantlets decreased, and the degree of plant damage increased compared to control plantlets (without salt) (Table 1 and 2). According to Troncoso et al. (1999), shoot elongation decreases with an increasing salt concentration *in vitro* conditions similar to the results of our study. They ordered the rootstocks from sensitive to tolerant as 41B, 140Ru, and Salt Creek. Popescu et al. (2015) stated that the first symptom against salt stress was decreased growth in both *in vitro* and *in vivo* conditions. With the increase in salt concentrations, necrotic spots on shoot tips and chlorosis on leaves were also observed. The researchers determined that the SO4 and 5BB rootstock were moderately sensitive, and 140Ru rootstock was resistant. Troncoso et al. (1999) and Popescu et al. (2015) also stated that root growth and rooting rate also changed inversely with the increase in salt concentration in the growth medium. These results were by our findings, except for the SO4 rootstock.

Hamrouni et al. (2008) applied *in vitro* salt stress to 1103P, SO4, and 41B rootstocks for six weeks. They stated that the survival rate, reproduction, and

growth characteristics of vine plantlets decreased with salt concentrations. In addition, necroses were observed depending on the rootstock and NaCl concentrations, and the salt dose of 80 mM (4.7 g L-1) was determined to be the critical threshold. The same research revealed that the formation and development of roots are significantly affected by NaCl concentrations. Edriss et al. (2016), in their *in vitro* salt stress studies on the vine, stated that shoot length, the number of leaves, shoot fresh and dry weight, and plant vitality decreased depending on salinity. It was determined that root formation and chlorophyll contents of plantlets of Dogridge and Richter varieties were severely affected by increasing NaCl applications. At the same time, Salt Creek and Freedom rootstocks were less affected. Similar to our results, the researchers determined that the most tolerant rootstock to salt stress was Salt Creek. Also, they reported that Salt Creek rootstock continued to grow and develop by preserving its vitality at a high rate, even at 75 mM (4.4 g L^{-1}) salt concentration. Alizadeh et al. (2010) determined that the root's fresh and dry weights, number of roots per plantlet, and chlorophyll contents were negatively affected by NaCl in their *in vitro* studies. Stevens et al. (1996) and Uyar (2016) also stated that salt stress reduced root growth *in vivo*, and consequently, plant growth decreased. In addition, they reported that the total chlorophyll content decreased due to increased salinity.

Considering the viability and rooting rate in Salt Creek among the rootstocks and control (without salt stress) and 0.75 g L^{-1} among the concentrations, there was no significant problem (Table 3). Their rates were 100%, and the rates decreased by increasing NaCl doses. Similarly, Edriss et al. (2016), who determined the Salt Creek rootstock as the most resistant rootstock in *vitro* conditions, found that with increasing salt dose, plant vitality and the rooting rate decreased, and roots were more affected. Turhan et al. (2005) also reported that with the increase of salt concentration in vine rootstocks irrigated with salt water, the vitality of the cuttings decreased, and the plant roots were damaged. In addition, Kök (2012) stated that the rooting ability of some rootstocks irrigated with saltwater decreased. In our study, while 140Ru and 5BB rootstocks gave similar results, SO4 was the most affected rootstock. In another study conducted on rootstocks under *in vivo* conditions, Dardeniz et al. (2006) determined that irrigation with salt water gradually reduced plant viability. In their research, unlike our study, 41B was the most resistant rootstock, followed by 140Ru, and the most damaged rootstock was 5BB. In salt stress studies on grapevine rootstocks and varieties, it was concluded that the plants lost their vitality as the stress intensity increased (Salem et al., 2011; Desouky et al., 2015). Salem et al. (2011) and Desouky et al. (2015) found that the most tolerant rootstock was Salt Creek. Hamrouni et al. (2008) studied vine rootstocks in *vitro*, and Uyar (2016) studied grape varieties *in vivo* and stated that root and shoot viability decreased with the increase in salt stress intensity.

According to the shoot and root tolerance ratios given in Table 4, the highest values were in Salt Creek, and the lowest data were in SO4 and 41B rootstocks. The tolerance rates of shoots and roots were pronounced more at 0.75 g NaCl L^{-1} concentration. Regarding the shoot and root tolerance index (Fig 1), Salt Creek rootstock came first. Turhan et al. (2005), studying with 1103P, 420A, and 5BB grapevine rootstocks *in vivo*, determined that shoot and root tolerance rates decreased with the increase of the salt-water dose. In their study, salt concentration on shoot tolerance index was not significant, but the root tolerance index was significant, and 5BB gave the best result. In another salt stress study on 1103P, 41B, 140Ru, and 5BB vine rootstocks, Dardeniz et al. (2006) determined that shoot and root tolerance rates tended to decrease with increasing salt dose. But, the difference between the varieties in terms of root and shoot tolerance rates and tolerance index was not statistically significant. However, the best shoot tolerance index was determined in 140Ru, while the best root tolerance index was obtained from 41B rootstock. Müftüoğlu et al. (2006) and Uyar (2016) observed that shoot and root tolerance rates decreased with the increase in salt concentrations in their salt stress studies on grapevine varieties. Despite the literature mentioned above, the shoot and root tolerance index parameters were found to be significant by Sivritepe and Eriş (1999) and Uyar (2016), while Müftüoğlu et al. (2006) stated that these parameters were insignificant.

The mineral analysis of rootstock explants grown under salinity conditions (Table 5 and 6) showed that the rootstock taking up the most N, K, Ca, and Mg elements from the nutrient medium were found to be Salt Creek. The rootstock receiving the lowest N, P, K, Ca, and Mg was 41B. The effect of NaCl on macro and microelement uptake of rootstock plantlets was negative. Troncoso et al. (1999) reported in their *in vitro* studies that the contents of Na and Cl elements started to increase with salt stress and that the element Cl was taken into the plant more than Na. Although there were slight differences between the rootstock genotypes in their study, it was noticed that all element amounts decreased as the salinity increased. Similar results were obtained in our study. Edriss et al. (2016) found in their *in vitro* studies that the accumulation of N, Mg, K, Ca, P, Fe, Zn, and Mn in rootstocks decreased with increasing salt concentration, and Cl and Na contents increased with stress. They concluded that Salt Creek was the best rootstock taking the highest K and the lowest Cl and Na. In an *in vivo* study on 41B, 5BB, 1103P rootstocks, and Alphonse Lavallée variety, Babalık (2012) stated that Na and Cl uptake increased with salt stress. Still, Na and Cl levels decreased with water application. It was concluded that the amounts of K, N, P, Fe, Mg, and Zn decreased in general, although there were differences between genotypes as the water level decreased and salt doses

increased. Dag et al. (2015) stated that Na and Cl ions increase in the plant as the salinity of irrigation water increases. It has been determined that 140Ru rootstock accumulated Cl ions in the lowest amount and that chlorine ions were taken into the plant body more than Na. Alizadeh et al. (2010) stated that Na, Cl, and K ions increased with increasing salinity *in vitro* conditions. They observed that SO4 rootstock was sensitive, although it was one of the rootstocks accumulating the least toxic ions. Mohammadkhani et al. (2013) observed in their pot culture studies on Iranian varieties that Na and Cl elements were correlated positively, while potassium and nitrate minerals were negatively correlated depending on the severity of salt stress. Also, they stated that the Na element was accumulated more than Cl (2-5 times), but it is more likely that the effect of Cl may cause damages. Except for the excessive accumulation of Na, the results were consistent with our findings. Mohammadkhani et al. (2013) also declared that the decrease in the amount of nitrate caused by Cl antagonism and Cl accumulation was two times higher in the shoots than in the roots. Although it was stated that K is relatively high intolerant varieties, they concluded that Na and Cl elements limit plant growth and development. Uyar (2016) found in his *in vivo* study on Muscat of Hamburg and Isabella grape varieties that increasing NaCl doses increased Na ions in the plant. He concluded that K and Ca contents decreased with increasing salinity, and Mg ions gave similar results against salt stress. Mohammadkhani and Abbaspour (2018) examined the effect of NaCl on two sensitives and two tolerant Iranian varieties *in vivo*. They observed that Na and Cl ions accumulated three times more in the shoots of sensitive genotypes than tolerant varieties. This accumulation was much more in the roots intolerant varieties. In other words, sensitive varieties could not prevent the uptake of toxic ions into their bodies. They also stated that Cl ions accumulated more than Na. Fisarakis et al. (2005) reported that the K concentration of the leaf blade, petiole, and shoots of the plants increased, and NO_3-N and K concentrations decreased with salinity. Salt stress did not affect the concentrations of Ca and Mg in the shoots, P and Mg in the stem, and P, Ca, and Mg concentrations in the roots. Salinity has a negative effect on nutrient uptake (Najafi et al., 2007). Although the way salinity affects plant growth is still not fully understood, it has been reported that the salinity tolerance of plants can be changed by mineral nutrition (Fisarakis et al., 2005).

The nutritional correlations of rootstocks from nutrient media containing different salt concentrations, all nutrients are in a positive relationship with each other (Table 7). Since the conditions in tissue culture were controlled and the pH was initially adjusted to 5.8, it was evaluated that the rootstocks easily took the nutrients. At the same time, the elements affect each other's uptake positively. On the other hand, in *in vivo* cultivation, elements such as Mg, Ca, and K may negatively affect each other's uptake due to various climate and soil characteristics (Fozouni et al., 2012a; Esfandiari and Pourmohammad, 2013).

Conclusions

Considering the rapid increase in world population and the decrease in quality water, it is necessary to obtain sufficient yield and quality in salty soils and salty water. This situation makes it important to determine the growth conditions and nutritional levels of plants under stress. This study determined that plant elongation, node number, and plant vitality decreased compared to control plantlets with increasing salt stress in all rootstocks used. The decline in plant growth determined decreases in shoot fresh and dry weights. 41B and SO4 rootstocks were affected by NaCl more than Salt Creek and 140Ru rootstocks. With the increase in salt concentrations, it was observed that the root length and the number of roots per plantlet and root fresh and dry weights decreased. Chlorophyll and nutrient contents also decreased in plantlets compared to the control.

Parameters such as plant vitality, shoot length, node number, root number, and Na and Cl ions are important parameters in evaluating the tolerance to salinity. As a result of considering all the parameters examined, the rootstocks could be listed as 41B <SO4 <5BB <140Ru <Salt Creek from sensitive to tolerant. Since 41B and SO4 rootstocks are sensitive to salt stress, application of NaCl concentrations below 0.75 g L^{-1} may be proper. In addition, it is recommended to test intermediate concentrations between two doses of NaCl applied in this study for rootstocks that die at a dose of 1.5 g NaCl L^{-1}.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest in the publication.

Author contribution

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Bioinformatics analyses on molecular pathways and pharmacological properties of glabridin

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Abstract

Glabridin, a bioactive compound that originally isolated from the roots of licorice (*Glycyrrhiza glabra* L., Fam. Fabaceae), has a wide range of pharmacological properties for instance anti-inflammatory, anti-cancer, anti-microbial, anti-viral, anti-osteoporosis, anti-diabetic, anti-atherogenic, neuroprotective, estrogenic, and skin-whitening. Even though, biological activities and pharmacological properties of glabridin have already been determined, molecular signaling pathways, gene targets, and pharmacological properties based on bioinformatics analyses have not been fully elucidated. Thus, in the presented research, network-based bioinformatics approaches were applied to demonstrate targets of glabridin in human genomes and proteomes. The glabridin was input into the ChEBI database, and the targets of its were predicted using DIGEP-Pred, and then, top interacting genes were identified by GeneCards database. Afterward, STRING and KEGG enrichment database were used to construct a protein-protein interaction (PPI) network and molecular targeting pathway network, respectively. A total of 14 genes coding proteins such as UGT1A1, MAPK1, CYP2B6, MMP9, CHKA, CYP3A4, EGFR, PON1, SLC6A4, SRC, EPHX2, TYR, PTK2, and PPIG effected by glabridin were determined by gene set enrichment analysis. Furthermore, multiple pathways including endocrine resistance, bladder cancer, ErbB signaling pathway, VEGF signaling pathway, chemical carcinogenesis, proteoglycans in cancer, relaxin signaling pathway, and estrogen signaling pathway were also identified to be regulated by glabridin. This research showed that glabridin exhibits highly active pharmacological activity as an anti-infective agent, chemopreventive agent, membrane permeability inhibitor, melanin inhibitor, and apoptosis agonist. Taken together, this study is network-based scientific research that will be very useful in elucidating the biological, molecular and pharmacological properties of glabridin for clinical applications in detail.

Keywords: Bioinformatics, Glabridin, KEGG pathway, Network pharmacology, Protein-protein interactions

Introduction

A type of isoflavonoid, glabridin is an isoflavone and has many pharmacological activities including improving metabolic abnormalities to improve obesity, diabetes, and cardiovascular disease, protecting nervous system function, as an estrogen substitute, preventing Staphylococcus, Candida and other microorganism-caused infection (Vaillancour et al., 2021), anti-cancer anti-inflammatory, anti-osteoporosis (Li et al., 2021), antiviral (Gezici and Sekeroglu, 2020; Sekeroglu and Gezici, 2020), anti-atherogenic, regulation of energy metabolism, estrogenic and skin-whitening (Simmler et al., 2013). The main source of glabridin is licorice root (*Glycyrrhiza glabra* L., Fam. Fabaceae), and this magic plant has been used has widely used traditional Chinese medicine and has also many other bioactive components, such as glycyrrhizic acid, glycyrrhetinic acid, liquiritin, isoliquiritigenin, licochalcones apart from glabridin (Hosseinzadeh and Nassiri-Asl, 2015). As a major flavonoid extracted from licorice root, glabridin is found a small quantity about 0.2% in the licorice root. Its chemical structure is a prenylated isoflavone and the systematic name is 4-[(3R)-8,8-dimethyl3,4-dihydro-2H-pyrano[2,3-f]chromen-3-yl]benzene-1,3-diol (Li et al., 2021).

Glabridin was first described in 1976 and many scientific research was released about it up to now. After exploring glabridin, it has been used in many formulations and now it is a valuable natural product in food, dietary supplements (DSs) and cosmetic industries. Recent scientific literature claim that

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biological activity of glabridin primarily comes from its activity for down-regulating intracellular reactive oxygen species (ROS), binding to antioxidant effectors, and acting on estrogen receptors, potentially as a plant-based Selective Estrogen Receptor Modulator (phytoSERM) (Simmler et al., 2013).

However, a numerous studies have been performed for uncover biological properties of glabridin, network-based molecular and pharmacological activities of glabridin have not been proposed yet. Therefore, we aimed to clarify the potential interactions of glabridin by gene-set enrichment and network pharmacology analyses to provide a novel approach to reveal the therapeutic mechanisms of glabridin that will ease its future clinical applications in the treatment of diseases.

Materials and Methods

Chemical Compositions and Predicted Targets

Chemical Entities of Biological Interest (ChEBI) database, a part of ELIXIR Core Data Resources, was used for dictionary of molecular entities and chemical properties of glabridin (Hastings et al., 2016). The targets of glabridin were identified using DIGEP-Pred (Prediction of drug-induced changes of gene expression profile) based on structural formula of glabridin (Lagunin et al., 2013).

Gene Set Enrichment Analysis

GeneCards, The Human Gene Database, was used to determine probable interacting genes of glabridin. Based on this database, top interacting genes were analyzed using unique GeneCards identifiers (GC ids) and GeneCards Inferred Functionality Scores (GIFtS), provided by the GeneLoc Algorithm (Harel et al., 2009; Fishilevich et al., 2016).

Protein-Protein Interaction (PPI) Analysis

STRING database was used to annotate the role of probable interacting genes and proteins associated with glabridin. PPI network mapping was conducted on glabridin and protein targets using the Retrieval of Interacting Genes database with the species limited to "homo sapiens" and a confidence score > 0.4 (Wu et al., 2009; Athanasios et al., 2017).

KEGG Enrichment Analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) is an integrated database of genes and genomes used for mapping pathways at molecular level. KEGG enrichment analysis was performed for construction the network regulated by glabridin (Aoki-Kinoshita and Kanehisa, 2007; Kanehisa et al., 2017).

Results

Results of Chemical Compositions and Predicted Targets

Glabridin ($C_{20}H_{20}O_4$) belonging to the class of isoflavonoids, the most valuable natural compound, is found in the roots of licorice (*Glycyrrhiza glabra* L.). The systematic name of glabridin with an average molecular weight of 324.376 g/mol is 4-{8,8-dimethyl-2H,3H,4H-pyrano [2,3-f]chromen-3-yl}benzene-1,3-diol. Hispaglabridin A ($C_{25}H_{28}0_4$), 4'-O-Methylglabridin ($C_{21}H_{22}O_4$), 2'-O-Methylglabridin ($C_{21}H_{22}O_4$), (R)-Hispaglabridin A ($C_{25}H_{28}O_4$), (R)-Hispaglabridin B ($C_{25}H_{26}O_4$), 3'-Hydroxy-4'-methoxyglabridin ($C_{21}H_{22}O_5$), and 4'-O-Methylpreglabridin ($C_{21}H_{24}O_4$) are derivatives of glabridin, of which 4'-O-Methylglabridin, 2'-O-Methylglabridin, (R)-Hispaglabridin A, (R)-Hispaglabridin B belong to the class of isoflavonoids, while Hispaglabridin A and 4'-O-Methylpreglabridin are from the class of hydroxyisoflavans and flavonoids, respectively. The chemical structure of glabridin and its derivatives were given in the in the Figure. 1.

The targets of glabridin were investigated according to prediction of drug-induced changes of gene expression profile for proteins at the pharmacological activity (Pa) > 0.5. The findings were presented in the Table 1. Pa (probability to be active) means the chance that glabridin is belonging to the subclass of active compounds, while Pi (probability to be inactive) means the chance that glabridin is belonging to the subclass of inactive compounds. Based on the data presented in the table, glabridin exhibits quite active biological activities and pharmacological properties. Actually, capable of acting against infection as an antiinfective agent, expression inhibitor on Hypoxia-inducible factor 1-alpha, substrate of cytochrome P450, chemopreventive agent, membrane permeability inhibitor, melanin inhibitor, and apoptosis agonist were determined as the most important properties of glabridin (Pa > 0.7).

Table 1. Prediction of drug-induced changes of gene expression profile for glabridin

Pa	Pi	Activity
0,948	0,003	Antiinfective
0,911	0,005	HIF1A expression inhibitor
0,839	0,027	CYP2C12 substrate
0,786	0,004	Chemopreventive
0,775	0,014	Membrane permeability inhibitor
0,715	0,001	Melanin inhibitor
0,700	0,002	Skin whitener
0,676	0,017	Apoptosis agonist
0,658	0,002	RELA expression inhibitor

0,650	0,011	Histidine kinase inhibitor
0,648	0,017	Spasmolytic, urinary
0,640	0,003	NOS2 expression inhibitor
0,640	0,038	TP53 expression enhancer
0,618	0,071	Membrane integrity agonist
0,616	0,035	Antidyskinetic
0,611	0,014	Spasmolytic
0,609	0,012	AR expression inhibitor
0,597	0,062	Chlordecone reductase inhibitor
0,596	0,009	Lipid peroxidase inhibitor
0,590	0,101	Aspulvinone dimethylallyltransferase inhibitor
0,589	0,048	Antineoplastic
0,562	0,005	Antioxidant
0,533	0,029	Kinase inhibitor
0,519	0,009	CYP2E1 inhibitor
0,517	0,023	Cytostatic
0,507	0,148	Ubiquinol-cytochrome-c reductase inhibitor
0,503	0,104	Antiischemic, cerebral
0,502	0,107	Phosphatase inhibitor

Table 2. The list of top genes interacts with glabridin

	Symbol	Description	Category	GIFtS	GC id	Score
1	UGT1A1	UDP Glucuronosyltransferase Family 1 Member A1	Protein Coding	46	GC02P233760	1.81
2	MAPK1	Mitogen-Activated Protein Kinase 1	Protein Coding	50	GC22M021759	1.77
3	CYP2B6	Cytochrome P450 Family 2 Subfamily B Member 6	Protein Coding	45	GC19P040991	1.64
4	MMP9	Matrix Metallopeptidase 9	Protein Coding	51	GC20P046008	1.60
5	MIR148A	MicroRNA 148a	RNA Gene	19	GC07M025993	1.59
6	CHKA	Choline Kinase Alpha	Protein Coding	40	GC11M068052	1.35
7	CYP3A4	Cytochrome P450 Family 3 Subfamily A Member 4	Protein Coding	49	GC07M099759	1.32
8	EGFR	Epidermal Growth Factor Receptor	Protein Coding	51	GC07P055019	1.19
9	PON1	Paraoxonase 1	Protein Coding	44	GC07M095297	1.10
10	SLC6A4	Solute Carrier Family 6 Member 4	Protein Coding	46	GC17M030194	0.32
11	SRC	Proto-Oncogene, Non-Receptor Tyrosine Kinase	Protein Coding	50	GC20P037344	0.22
12	EPHX2	Epoxide Hydrolase 2	Protein Coding	46	GC08P027490	0.22
13	TYR	Tyrosinase	Protein Coding	45	GC11P089177	0.22
14	PTK2	Protein Tyrosine Kinase 2	Protein Coding	45	GC08M140657	0.22
15	PPIG	Peptidylprolyl Isomerase G	Protein Coding	40	GC02P169584	0.22

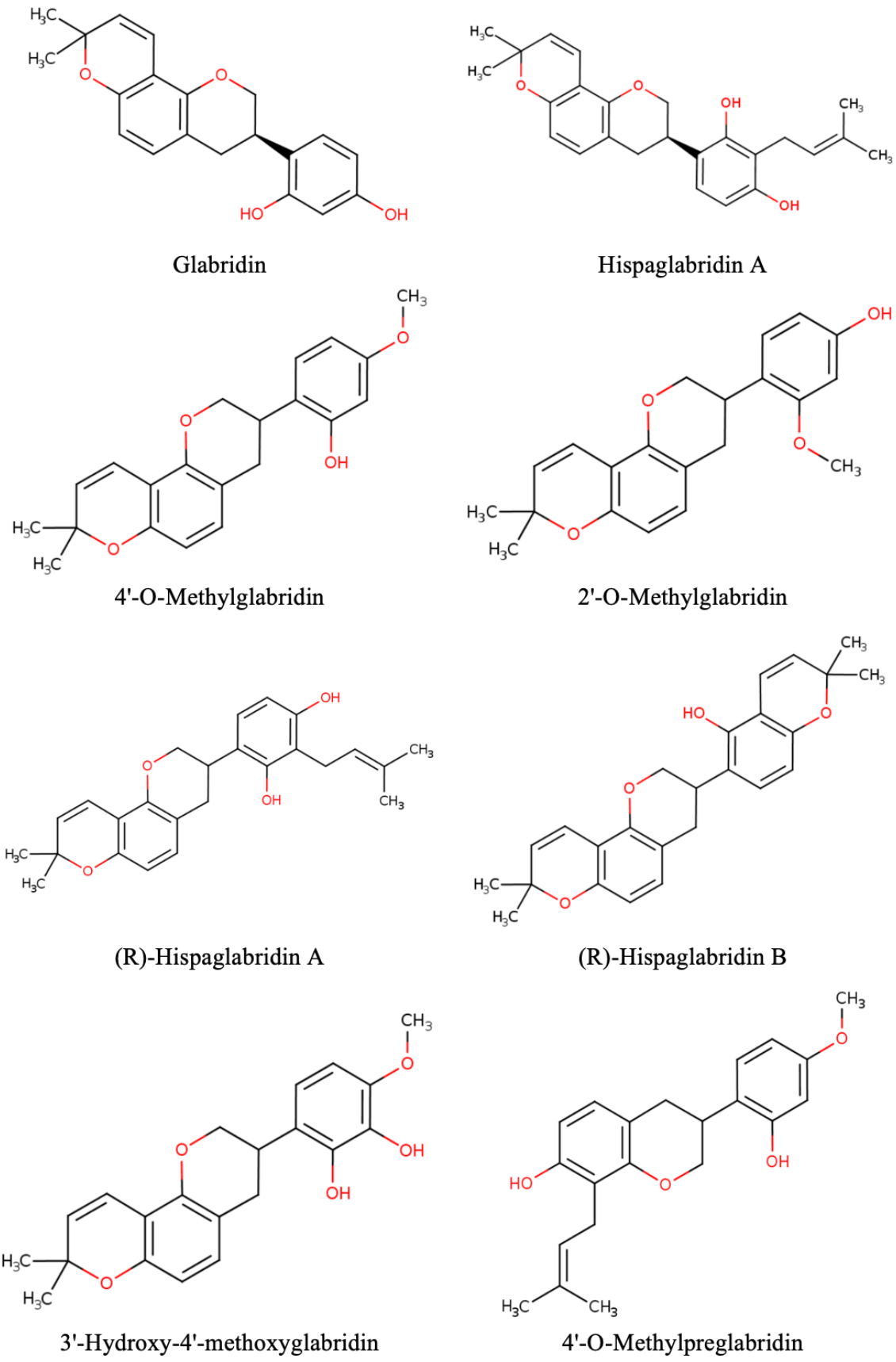

Figure 1. Chemical compositions of glabridin and glabridin derivatives

Results of Top Gene Enrichment Analysis

A total of fifteen genes regulated by glabridin were determined as the most interacting genes using gene enrichment analyses. All these genes, except MIR148A, are protein coding genes and MAPK1, MMP9, EGFR, SRC, UGT1A1, and CYP3A4 were found the most interacting genes, whereas PPIG, TYR, and PTK2 were identified as the least interacting genes with glabridin (Table 2).

Results of Protein – Protein Interaction (PPI) Network

The relationship of a total of 14 proteins between each other were constructed from STRING database with PPI enrichment p-value = 1.47e-06 (FDR < 0.05). PPPI network was presented in the Fig. 2. As can be seen in the Figure, SRC, MAPK1, PTK2, PPIG, and EGFR are the proteins that located in the center of the network (Figure. 2).

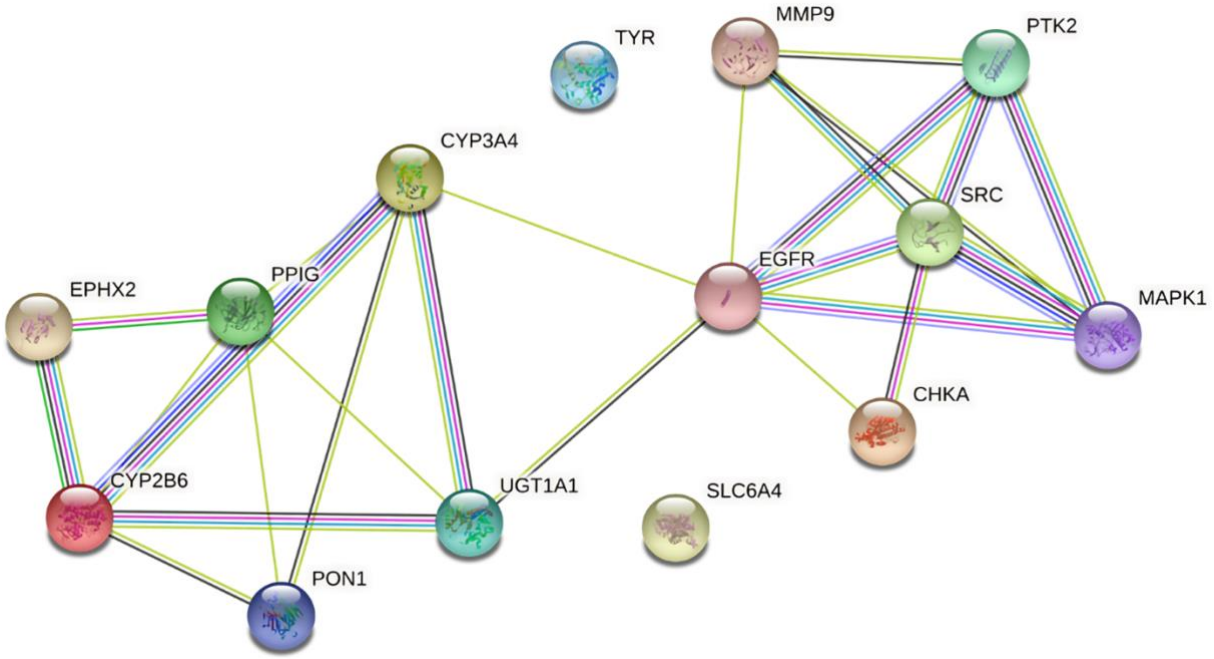

Figure 2. Protein-protein interaction proteins modulated by glabridin

Table 3. KEGG Enrichment analysis of proteins modulated by glabridin

#term ID	term description	observed gene count	background gene count	strength	false discovery rate	matching proteins in your network (labels)
hsa01522	Endocrine resistance	5	95	1.87	2.75e-06	MAPK1, EGFR, PTK2, MMP9, SRC
hsa05219	Bladder cancer	4	41	2.13	5.44e-06	MAPK1, EGFR, MMP9, SRC
hsa05205	Proteoglycans in cancer	5	196	1.55	3.05e-05	MAPK1, EGFR, PTK2, MMP9, SRC
hsa04012	ErbB signaling pathway	4	83	1.83	3.99e-05	MAPK1, EGFR, PTK2, SRC
hsa04915	Estrogen signaling pathway	4	133	1.62	0.00017	MAPK1, EGFR, MMP9, SRC
hsa04926	Relaxin signaling pathway	4	128	1.64	0.00017	MAPK1, EGFR, MMP9, SRC
hsa00830	Retinol metabolism	3	64	1.82	0.00063	CYP2B6, CYP3A4, UGT1A1

hsa00980	Metabolism of xenobiotics by cytochrome P450	3	69	1.78	0.00063	CYP2B6, CYP3A4, UGT1A1
hsa00982	Drug metabolism - cytochrome P450	3	64	1.82	0.00063	CYP2B6, CYP3A4, UGT1A1
hsa04370	VEGF signaling pathway	3	57	1.87	0.00063	MAPK1, PTK2, SRC
hsa04510	Focal adhesion	4	198	1.45	0.00063	MAPK1, EGFR, PTK2, SRC
hsa04520	Adherens junction	3	67	1.8	0.00063	MAPK1, EGFR, SRC
hsa04810	Regulation of actin cytoskeleton	4	209	1.43	0.00063	MAPK1, EGFR, PTK2, SRC
hsa05131	Shigellosis	4	218	1.41	0.00063	MAPK1, EGFR, PTK2, SRC
hsa05163	Human cytomegalovirus infection	4	218	1.41	0.00063	MAPK1, EGFR, PTK2, SRC
hsa01521	EGFR tyrosine kinase inhibitor resistance	3	78	1.73	0.00068	MAPK1, EGFR, SRC
hsa04540	Gap junction	3	87	1.68	0.00088	MAPK1, EGFR, SRC
hsa04912	GnRH signaling pathway	3	89	1.67	0.00089	MAPK1, EGFR, SRC
hsa05215	Prostate cancer	3	96	1.64	0.0010	MAPK1, EGFR, MMP9
hsa05231	Choline metabolism in cancer	3	96	1.64	0.0010	MAPK1, CHKA, EGFR
hsa05135	Yersinia infection	3	125	1.53	0.0020	MAPK1, PTK2, SRC
hsa05418	Fluid shear stress and atherosclerosis	3	130	1.51	0.0022	PTK2, MMP9, SRC
hsa04921	Oxytocin signaling pathway	3	149	1.45	0.0031	MAPK1, EGFR, SRC
hsa05161	Hepatitis B	3	159	1.42	0.0036	MAPK1, MMP9, SRC
hsa05206	MicroRNAs in cancer	3	160	1.42	0.0036	MAPK1, EGFR, MMP9
hsa04360	Axon guidance	3	177	1.37	0.0045	MAPK1, PTK2, SRC
hsa01100	Metabolic pathways	6	1447	0.76	0.0050	TYR, CHKA, CYP2B6, CYP3A4, UGT1A1, EPHX2
hsa04062	Chemokine signaling pathway	3	186	1.35	0.0050	MAPK1, PTK2, SRC
hsa04015	Rap1 signaling pathway	3	202	1.32	0.0059	MAPK1, EGFR, SRC

hsa05200	Pathways in cancer	4	517	1.03	0.0061	MAPK1, EGFR, PTK2, MMP9
hsa05213	Endometrial cancer	2	57	1.69	0.0116	MAPK1, EGFR
hsa00140	Steroid hormone biosynthesis	2	59	1.68	0.0120	CYP3A4, UGT1A1
hsa00590	Arachidonic acid metabolism	2	61	1.66	0.0124	CYP2B6, EPHX2
hsa04917	Prolactin signaling pathway	2	69	1.61	0.0145	MAPK1, SRC
hsa05100	Bacterial invasion of epithelial cells	2	70	1.6	0.0145	PTK2, SRC
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	2	67	1.62	0.0145	EGFR, SRC
hsa05212	Pancreatic cancer	2	73	1.58	0.0145	MAPK1, EGFR
hsa05214	Glioma	2	72	1.59	0.0145	MAPK1, EGFR
hsa05218	Melanoma	2	72	1.59	0.0145	MAPK1, EGFR
hsa05223	Non-small cell lung cancer	2	68	1.61	0.0145	MAPK1, EGFR
hsa05230	Central carbon metabolism in cancer	2	69	1.61	0.0145	MAPK1, EGFR
hsa00983	Drug metabolism - other enzymes	2	75	1.57	0.0146	CYP3A4, UGT1A1
hsa05204	Chemical carcinogenesis	2	75	1.57	0.0146	CYP3A4, UGT1A1
hsa05165	Human papillomavirus infection	3	325	1.11	0.0153	MAPK1, EGFR, PTK2
hsa05210	Colorectal cancer	2	82	1.53	0.0161	MAPK1, EGFR
hsa04151	PI3K-Akt signaling pathway	3	350	1.08	0.0180	MAPK1, EGFR, PTK2
hsa04976	Bile secretion	2	89	1.5	0.0180	CYP3A4, UGT1A1
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	2	88	1.5	0.0180	MAPK1, EGFR
hsa04657	IL-17 signaling pathway	2	92	1.48	0.0185	MAPK1, MMP9
hsa04916	Melanogenesis	2	95	1.47	0.0193	MAPK1, TYR
hsa04625	C-type lectin receptor signaling pathway	2	102	1.44	0.0217	MAPK1, SRC

hsa04928	Parathyroid hormone synthesis, secretion and action	2	103	1.43	0.0217	MAPK1, EGFR
hsa04066	HIF-1 signaling pathway	2	106	1.42	0.0225	MAPK1, EGFR
hsa04670	Leukocyte transendothelial migration	2	109	1.41	0.0229	PTK2, MMP9
hsa04726	Serotonergic synapse	2	108	1.41	0.0229	MAPK1, SLC6A4
hsa04668	TNF signaling pathway	2	112	1.4	0.0237	MAPK1, MMP9
hsa04919	Thyroid hormone signaling pathway	2	119	1.37	0.0257	MAPK1, SRC
hsa04935	Growth hormone synthesis, secretion and action	2	118	1.37	0.0257	MAPK1, PTK2
hsa04611	Platelet activation	2	122	1.36	0.0265	MAPK1, SRC
hsa04068	FoxO signaling pathway	2	127	1.34	0.0282	MAPK1, EGFR
hsa05224	Breast cancer	2	145	1.29	0.0353	MAPK1, EGFR
hsa05226	Gastric cancer	2	144	1.29	0.0353	MAPK1, EGFR
hsa04072	Phospholipase D signaling pathway	2	147	1.28	0.0355	MAPK1, EGFR
hsa04934	Cushing syndrome	2	153	1.26	0.0378	MAPK1, EGFR
hsa05160	Hepatitis C	2	156	1.25	0.0386	MAPK1, EGFR
hsa05225	Hepatocellular carcinoma	2	160	1.24	0.0399	MAPK1, EGFR
hsa05152	Tuberculosis	2	168	1.22	0.0431	MAPK1, SRC
hsa05202	Transcriptional misregulation in cancer	2	171	1.21	0.0440	PTK2, MMP9
hsa05203	Viral carcinogenesis	2	182	1.19	0.0488	MAPK1, SRC

Results of KEGG Enrichment Pathway

According to the KEGG enrichment pathway analyses, a total of 69 different pathways were defined as the probably modulated pathways by glabridin. The identified pathways were summarized in the Table 3, corresponding to 14 protein targets. As summarized in the Table 3, several target proteins are simultaneously involved in one pathway, while one target protein is also present in many pathways. A hierarchical clustering tree is schematized in Figure 3, summarizing the correlations between the major paths listed in the enrichment tab. Although pathways containing many common genes are clustered together; larger dots indicate more significant P values.

Accordingly, endocrine resistance, bladder cancer, proteoglycans in cancer, ErbB signaling pathway, estrogen signaling pathway, relaxin signaling pathway, retinol metabolism, metabolism of xenobiotics by cytochrome P450, drug metabolism - cytochrome P450, VEGF signaling pathway, focal adhesion, adherens junction, regulation of actin cytoskeleton, shigellosis, human cytomegalovirus infection, EGFR tyrosine kinase inhibitor resistance pathways and so forth were the most enrichment pathways-modulated by glabridin with the p-value cutoff (FDR<0.05). In addition, endocrine resistance,

bladder cancer, ErbB signaling pathway, VEGF signaling pathway, chemical carcinogenesis, proteoglycans in cancer, relaxin signaling pathway, and estrogen signaling pathway were determined as the top pathways associated with glabridin-regulated proteins with the lowest false discovery rate (FDR<0.05) (Figure. 3).

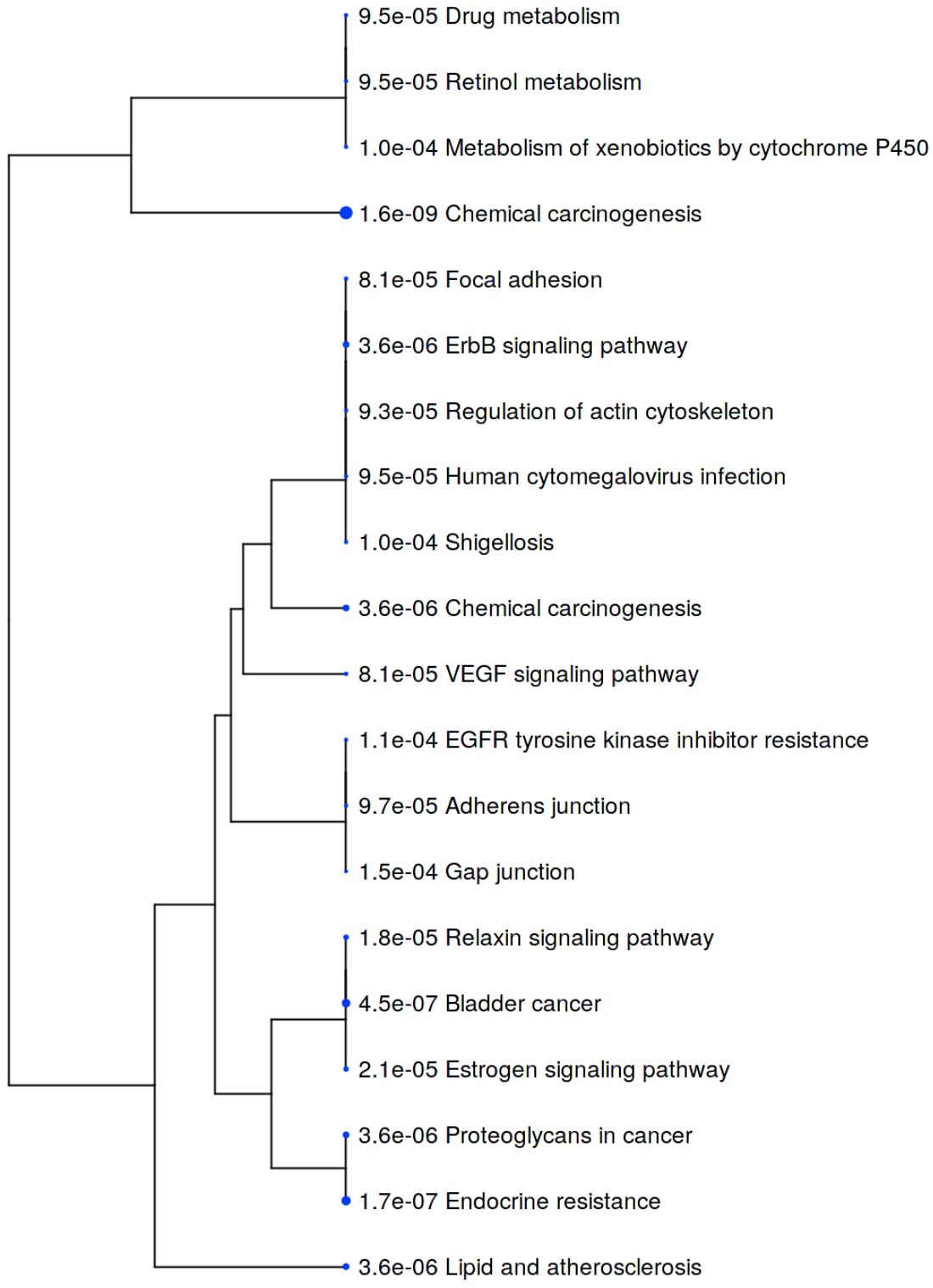

Figure 3. Chart of top related pathways construction with KEGG enrichment from STRING database

Discussion

Bioinformatics integrates systems-level network-based pharmacology and can provide insight into the molecular mechanisms of herbal formulas used for treatment of complex diseases. Currently, network-based analysis has been implemented to natural

compounds isolated form medicinal plants in order to search for multitargeted compounds that act in biological networks to explore multiple molecular mechanisms (Lee et al., 2018; Huang et al., 2020). Even though biological effects and pharmacological profiling of glabridin have been studied, network based genomic and proteomics prediction of glabridin and its target pathways has not been conducted yet (Simmler et al., 2013; Hosseinzadeh and Nassiri-Asl, 2015; Li et al., 2021). Thus, we investigated the potential interactions of glabridin by gene-set enrichment and network-based molecular pharmacology analyses to reveal the therapeutic mechanisms of glabridin in the current research. Glabridin regulates the activities of these genes in a way that causes an increase in the expression levels of some of the genes, when it causes a decrease in others. Glabridin both inactivates the active forms of the SRC (proto-oncogene, non-receptor tyrosine kinase) gene and enhances levels of phosphorylated SRC that functions as a proto-oncogene (Simmler et al., 2013; Su Wei Poh et al., 2015).

As for the PPI analyses of targeting proteins, SRC, MAPK1, PTK2, PPIG, and EGFR are identified as the core proteins that located in the center of the network. Among these proteins, SRC is a tyrosine kinase protein that involved in many biologicals signaling pathways such as gene transcription, immune response, cell adhesion, cell cycle progression, migration, and apoptosis. MAPK1, a serine/threonine kinase protein, is a major component of MAP kinase signal transduction pathway, and acts a significant role in the MAPK/ERK cascade, which mediates various biological functions including cell growth, adhesion, survival, and differentiation via the regulation of transcription, translation, cytoskeletal rearrangements. PTK2 is the other tyrosine kinase protein found in the center of network that it acts important roles in regulating cell migration, cell adhesion, metastasis, cell protrusions, cell cycle progression, cell proliferation and apoptosis, as well as reorganization of the actin cytoskeleton, and formation of focal adhesions. PPIG, peptidyl-prolyl cis-trans isomerase G, is a protein with catalytic activity that is involved in the folding, transport and assembly of proteins, besides in regulating pre-mRNA splicing. Lastly, EGFR, also called as proto-oncogene c-ErbB-1 and receptor tyrosine-protein kinase ErbB-1, is an essential protein participated in cell signaling pathways mainly associated with many types of cancer. Moreover, drugs that arrest epidermal growth factor receptor proteins are used in the treatment of some types of cancer (Hsu et al., 2011; Tsai et al., 2011; Huang et al., 2014; Lee et al., 2020; Li et al., 2021).

Additionally, target signaling pathways modulated by glabridin were determined in this research. It is well-known that multiple signaling pathways interact with each other in the metabolic processes normally occurred in living organisms. Based on the KEGG enrichment pathway analyses, most of the genes regulated by glabridin are closely associated with estrogen receptor modulator, oxidative stress, immune system, neurodegeneration, inflammation, and angiogenesis, as well as cancer. MAPK1, EGFR, PTK2, MMP9, and SRC genes are participated in endocrine resistance and estrogen signaling pathway, which are closely related to cancer development and progression. In agreement with the findings from this bioinformatics-based study, previous reports indicated that glabridin is used to treat of menopausal symptoms and thus has a

Herewith, glabridin prevent the cancer progression in many types of cancer through inhibition of migration, invasion, and angiogenesis. Glabridin also suppresses migration and invasion by transcriptionally inhibiting MMP9 (matrix metalloproteinase 9) via the modulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1) activity in human cancer cells. (Hsieh et al., 2016; Liu et al., 2019). Therefore, glabridin may function as a powerful immune stimulator and cancer preventive agent. Additionally, glabridin inhibits BRAF/MEK signaling pathway through arresting cell cycle and inhibiting proliferation in many types of cancers such as hepatocellular carcinoma, lung cancer, osteosarcoma. Meanwhile, it also reduces inflammation which caused by the inhibition of p38 mitogen activated protein kinase/extracellular regulated protein kinases (p38MAPK/ERK) signaling pathway that is a key pathway in the regulation of cellular processes including cell proliferation, survival, and differentiation. Besides, p38MAPK/ERK signaling pathway, glabridin induces cell death in the cancer cells by inducing apoptosis through the p38 MAPK and JNK1/2 pathways (Zhang and Li, 2016; Wang et al., 2016; Liu et al., 2019). Conversely, Glabridin induces the expression level of UDP-glucuronosyltransferase (UGT1A1) gene, which catalyzes the conjugation of bilirubin in the liver, supporting hepatoprotective properties of glabridin. Likewise, induction of the expression of UGT1A1 enzyme can improve the detoxification process and thereby releasing oxidative stress and contributing to reduce the burden of cancer development (Leung, 2001; Simmler et al., 2013). In contrast, the expression level of EGFR is suppressed by glabridin, resulting in decreased in cell proliferation, migration and angiogenesis as well as increased apoptotic process (Tsai et al., 2011; Zhu et al., 2019). It can be clearly stated that glabridin functions as a chemopreventive agents and apoptosis agonist. In addition to these activities, glabridin have been shown to induce the expressions of cytochrome P450 family 2 subfamily B member 6 (CYP2B6) and cytochrome P450 family 3 subfamily A member 4 (CYP3A4), which are involved in the metabolism of various endogenous substrates, including the metabolism of steroid, arachidonate, and retinol and also play a role in the oxidative metabolism of xenobiotics (Shahabi et al., 2014).

possible role in estrogen replacement therapy (ERT) (Tsai et al., 2011; Su Wei Poh et al., 2015). The genes of MAPK1, EGFR, MMP9, and SRC, whose expression levels are regulated by glabridin, are involved in relaxin signaling pathway signaling pathways. Relaxin, a polypeptide hormone with antifibrotic properties, inhibits fibrosis through numerous cellular targets and signaling pathways. Previous reports, supporting the findings from this study show that glabridin is an anti-inflammatory and anti-fibrotic agent (Ng et al., 2019). Furthermore, glabridin has also involved in ErbB signaling pathway and pathways of proteoglycans in cancer that revealed by previous studies. As revealed in previous studies, Insufficient ErbB signaling or expression of EGFR family are associated with the development of neurodegenerative diseases, whilst excessive ErbB signaling and increased EGFR expression are associated with the development of a wide variety cancer types (Zhang et al., 2016; Zhu et al., 2019; Karthikkeyan et al., 2020). In another research showed that glabridin regulates the MAPK1/3 and PI3K/AKT pathways (Karthikkeyan et al., 2020). Accordingly, glabridin has been shown to be a substantial pharmaceutical resource for drug targets, consistent with these findings from network-based molecular and pharmacological analyzes.

Conclusion

Glabridin is a natural isoflavonoid that found mainly in licorice roots and proven to possess remarkable biological and pharmacological activities in the human metabolism. In the current study, endocrine resistance, bladder cancer, ErbB signaling pathway, VEGF signaling pathway, chemical carcinogenesis, proteoglycans in cancer, relaxin signaling pathway, and estrogen signaling pathways involved in estrogen receptor modulator, oxidative stress, immune system, neurodegeneration, inflammation, and angiogenesis, as well as cancer were identified as the top pathways regulated by glabridin. Moreover, SRC, MAPK1, EGFR, PTK2, and MMP9, as well as CYP2B6, CYP3A4, and UGT1A1 were determined main core proteins involved in the top signaling pathways. Taken together, the targets of these core proteins may play a key role in the therapeutic potentials of glabridin. According to our network-based analysis, glabridin may exert various pharmacological effects through multiple targets, pathways, and biological processes, thus having potential use as a drug. Although it is rare to use glabridin alone as a medicine today, it is expected that glabridin will be most likely to be used in the future drug discovery. Further studies, especially clinical trials are necessary to confirm the metabolic efficacy of glabridin, as well as to reveal the possible side effects of glabridin.

Compliance with Ethical Standards

Conflicts of Interest: The authors declare no conflict of interest.

Author Contributions: The authors contributed the manuscript equally, both of the author read the manuscript and approved to submit the findings for publication.

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Aflatoxin analysis by LC-MS of local and imported black tea sold in Turkey

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Abstract

Tea is a popular drink throughout the world with known health benefits. Although it has been accepted as safe and healthy for centuries, recent research has reported that herbal tea could be contaminated by fungi and mycotoxins. The aim of this study was to investigate the presence of total aflatoxin and aflatoxin B1 in local and imported tea sold in the southeastern and eastern provinces of Turkey. A total of 79 samples were taken from tea originating from Turkey (Mardin; 7, Şırnak; 3, Van; 15, Diyarbakır; 13, Siirt; 9, Batman; 4, Gaziantep; 14, Kilis; 4, and Şanlıurfa; 10), Iran, Sri Lanka, and India. Analysis of the content of the samples was made in respect of total aflatoxin and aflatoxin B_1 using the Rapid Common Mass Spectrometry method (2006; 20: 2649-2659) with an LC-MS/MS device. The analyses were performed in an advanced, private, EU-accredited laboratory. According to the results obtained from the LC-MS/MS device, no total aflatoxin or aflatoxin B_1 was determined. That no aflatoxins were detected in the tea samples demonstrates that the harvesting, processing, drying and packaging stages of the local and imported teas sold in the southeast Anadolu and South Anadolu regions of Turkey are applied appropriately. These types of analyses should be applied in other regions to determine the presence of aflatoxin in tea in general in Turkey.

Keywords: Aflatoxin, Aflatoxin B_1, Black tea, Imported tea.

Introduction

Tea is an aromatic drink made by pouring hot or boiling water on the dried leaves obtained from the plant known as *Camellia sinensis*. After water, tea is one of the most important drinks in the world, with a history of 5000 years, and reported to be drunk by approximately two-thirds of the global population (McKay and Blumberg, 2002; Schwarz et al., 1994; Diby et al., 2017). In a 2017 statistical study from the UK, the USA, and Germany, it was shown that 30%-40% of the participants drank 2-3 cups of tea per day (Sedova et al., 2018). Tea plants are grown in the northern hemisphere at a latitude of approximately 42°, and in the southern hemisphere at 27°, in regions where the climate is hot with abundant rainfall. The countries where tea plants are generally grown are China, India, Sri Lanka, Kenya, Vietnam, Indonesia, Russia, Japan, Myanmar, Turkey, Bangladesh, Iran, Argentina, UgandaTanzania, Malawi, Thailand, Nepal, Rwanda, Burundi and Ethiopia (Kurt and Hacıoğlu, 2013; Takım and Aydemir, 2018). Tea plantations are widespread and tea production is intense in India, China, Sri Lanka, Indonesia, Kenya, Turkey and Japan, with 80% of the world tea produced in these countries (Harman, 2014; Amirahmadi et al., 2013).

The polyphenols found in the tea plant have antioxidant, antimicrobial, anticancer, anti-inflammatory and anti-viral effects on human health. Benefits have also been reported of lowering cholesterol, blood presssure, and the risk of cardiovascular disease, and reducing the risk of osteoporotic fractures in the elderly (Zhang et al., 2013; Khan and Mukhtar, 2007; Shen et al., 2013). However, although tea has many benefits, the presence of harmful polluting substances such as heavy metals, mycotoxins and pesticide remnants, can have a negative effect on human health (Abd El-Atya et al., 2014). Mycotoxins in tea can lead to serious health problems such as immunosuppression, and carcinogenic, genotoxic, hepatotoxic and nephrotoxic effects (Milićević et al., 2010; Santoz et al., 2009).

Aflatoxins are mycotoxins produced by several fungi, the most common of which are *A. flavus* and *A. parasiticus*. At least different aflatoxin types are produced naturally (B_1, B_2, G_1, G_2, M_1, M_2, Q_1)

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(Murphy et al., 2006). Aflatoxin B_1 produced by *A. flavus* and *A. parasiticus* is the strongest liver carcinogen (Lee et al., 1971). Aflatoxins have mutagenic, teratogenic and immunosuppressive activities, and long-term consumption of foods containing aflatoxins increases the risk of liver, stomach and colon cancer (Reiter et al., 2009).

During the harvesting, processing and storage of tea plants, there may be contamination with mycotoxins. Moulds and fungi which can spread to tea during processing and production include *Aspergillus, Penicillium, Pacelomyces, Cladosporium, Alternaria, Mucor, Fusarium, Rhizopus, Absidia* and *Trichoderma* (Abd et al., 2014). Poor farming practices, incorrect processing, drying, packaging and storage, and transportation under inappropriate conditions increase the risk of mycotoxin contamination by promoting fungal growth. Moreover, as the regions where tea is grown are warm, wet and humid, this provides the ideal environment for the development of toxicogenic mould (Sedova et al., 2018).

Mycotoxins are resistant to the traditional heat range of food processing (80°-121°C), so following normal cooking such as boiling or frying, or pasteurisation, there may be very little or no reduction in general toxin levels (Kabak, 2009). Therefore, in the preparation of tea contaminated with aflatoxin, it is not possible for the temperature of the boiling water to impair or decompose the toxins.

Different methods are used to determine the presence of aflatoxins in food and animal feed, such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbance analysis (ELISA). Of these methods, HPLC has been reported to be ideal as it has high sensitivity and specificity (0-320 µg/kg) (Braga et al., 2005).

The aim of this study was to evaluate the presence of total aflatoxin and aflatoxin B_1 in local and imported tea sold in the southeastern and eastern provinces of Turkey, using LC-MS/MS analysis.

Materials and Methods

Sample Collection

A total of 79 samples were taken from tea originating from Turkey, Iran, Sri Lanka, and India. The numbers of samples taken from Turkish provinces were: Mardin; 7, Şırnak; 3, Van; 15, Diyarbakır; 13, Siirt; 9, Batman; 4, Gaziantep; 14, Kilis; 4, and Şanlıurfa; 10 (Figüre 1). The samples were collected from city markets and cafes, placed in sterile sample bags and sent to the laboratory, where they were stored in a cool, dry environment until analysis. The analyses were performed in an advanced, private, EU-accredited laboratory. The analyses were made using the Rapid Common Mass Spectrometry method (2006; 20: 2649-2659) with an LC-MS/MS device.

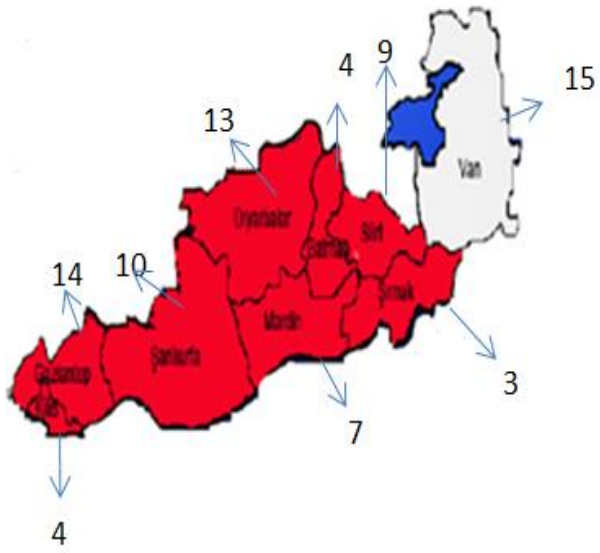

Figure 1. Cities and number of samples

Sample Preparation.

The ground tea samples (10g) were homogenised with a 40mL organic extraction solvent mixture (acetonitrile: water:acetic acid, 79:20:1) (Merck, Darmstadt, Almanya) for 60 mins in an orbital shaker (model 711 VDRI, Asal, Milan, Italy). The supernatant part was centrifuged for 10 mins at 3000 rpm (Allegra X-22R centrifuge, Beckman Coulter, Palo Alto, CA, USA). Then with single-stage extraction, 0.5 mL extract with the same amount of acetonitrile: water: acetic acid (20:79:1) was passed through a 0.22 µm filter and was injected into the LC-MS/MS (Sulyok et al., 2006).

LC – MS / MS device and conditions.

Determination and the amount determination were performed with a TurboIon Spray ESI source and a QTrap 4000 LC-MS/MS system (Applied Biosystems, Foster City, CA, USA) equipped with a 1100 Series HPLC system (Agilent, Waldbronn, Germany). LC analysis was performed using the Finnegan TSQ quantum ultra mass system (Thermo Scientific, CA, USA) formed from a paired pump, a degasser, a column oven and an automatic sampler. The analysis was made on a column over C18 columns of 150 mm, 4.6 and 3 µm fragment size. The column temperature was kept at 30°C. Capillary voltage was 3kV and nitrogen (Merck, Darmstadt, Almanya) was used as the spray gas. Source temperature and desolvation temperature were adjusted to 120°C and 400°C, respectively. Mycotoxins were analysed in selected reaction monitoring (SRM) channels (Sulyok et al., 2006).

Table 1. The results of the total aflatoxin and aflatoxin B_1 analyses in the tea samples

The province from which the sample was collected	Number of samples	Total Aflatoxin	Aflatoxin B1	Measurement Limit	Unit	Recycling	Analysis method
Mardin	7	ND	ND	0.15	µg/kg	93	Rapid Common Mass Spectrometry. 2006; 20: 2649-2659
Şırnak	3	ND	ND	0.15	µg/kg	93	Rapid Common Mass Spectrometry. 2006; 20: 2649-2659
Van	15	ND	ND	0.15	µg/kg	93	Rapid Common Mass Spectrometry. 2006; 20: 2649-2659
Diyarbakır	13	ND	ND	0.15	µg/kg	93	Rapid Common Mass Spectrometry. 2006; 20: 2649-2659
Siirt	9	ND	ND	0.15	µg/kg	93	Rapid Common Mass Spectrometry. 2006; 20: 2649-2659
Batman	4	ND	ND	0.15	µg/kg	93	Rapid Common Mass Spectrometry. 2006; 20: 2649-2659
Gaziantep	14	ND	ND	0.15	µg/kg	93	Rapid Common Mass Spectrometry. 2006; 20: 2649-2659
Kilis	4	ND	ND	0.15	µg/kg	93	Rapid Common Mass Spectrometry. 2006; 20: 2649-2659
Şanlıurfa	10	ND	ND	0.15	µg/kg	93	Rapid Common Mass Spectrometry. 2006; 20: 2649-2659

Results and Discussion

As seen in the analysis results in Table 1, total aflatoxin and aflatoxin B_1 were not determined in any of the 79 samples of tea.

Recently, studies have been carried out on the presence of aflatoxin in products such as spices, nuts and coffee. However, studies on the presence of aflatoxin in black teas have been limited. To the best of our knowledge, there has been no previous study related to aflatoxin analysis in imported tea in Turkey, and there is a limited number of studies of local tea. In a study by Tosun et al. (2016) 48 samples of herbal tea were collected from four local herb shops in the province of Manisa. Of the 48 samples analysed, 43 were determined as aflatoxin positive. The highest aflatoxin concentration (34.18µg/kg) was determined in a chamomile tea sample. In a study by Özden (2018) which was conducted to determine the levels of heavy metals and ochratoxin A in medicinal herbal teas, ochratoxin A was determined in only 1 chamomile tea sample of 21 samples. Omurtag and Yazicioglu (2004) investigated the presence of Fumonisin B_1 and B_2 in herbal tea and medicinal plants sold in Turkey. Fumonisin B_1 was not determined in 54 herbal teas and 61 medicinal plants but Fumonisin B_2 was detected in 2 samples. In another study in Istanbul by Hacibekiroglu and Kolak (2013) aflatoxin B_1 was determined in 2 of 15 samples of herbal tea.

There are studies in literature reporting aflatoxin determined in tea in other countries. In a 2010 study in Iran, Pouretedal et al. (2013) collected random samples from Tehran markets and showed that 30 of the 40 samples were contaminated with aflatoxins. The mean aflatoxin B_1 content of 11 contaminated samples was 10.0 ng/g and the total aflatoxin was mean 12.07 ng/g. In a study of Pu-Erh tea by Haas et al. (2013) 36 samples were examined, no aflatoxin or fumonisin was determined, and ochratoxin A was determined in 4 (11.1%) samples. Carraturo et al. (2018) examined 32 tea samples and reported the most widespread moulds to be *A. niger* strains, followed by *A. tubigensisoli*. Of the 32 samples examined, ochratoxin A was determined in 22 samples. A total of 91 different teas and herbal infusions were analysed by Monbaliu et al. (2010) and mycotoxin was determined in only one sample.

In the potable products, no mycotoxins were determined. In another recent study by Mannani et al. (2020) 76 (58.9%) of 129 herbal tea samples were found to be contaminated with aflatoxins, and 50 of the contaminated samples exceeded maximum levels. Pallarés et al. (2017) reported that they detected AFG1 in only two samples in 12 black tea bag samples they collected in Spain in a study they conducted. Contrary to the results reported in these studies related to the presence of aflatoxin in tea samples, no aflatoxin was detected in any tea sample in our study through Whatman filter paper and then concentrated at 40 °C in a rotary evaporator until dryness. The final obtained extract (4.8 g) was stored at 4 °C until use (Erkan et al., 2008).

Conclusion

Imported tea consumption is increasing day by day in Eastern and South Eastern provinces. It is said that imported tea consumption has many negative effects on health. However, these claims have not been scientifically proven. Therefore, the claims on this subject do not go beyond speculation. This study we have conducted has the quality to change the prejudices and prejudices about imported tea. In this study we conducted, a total of aflatoxin and aflatoxin B1 were not detected in imported and domestic tea samples that are popularly consumed by the people in Southeastern Anatolia and Eastern Anatolia in our country. The absence of aflatoxins in tea samples indicates that the harvesting, processing, drying and packaging stages of the local and imported teas sold in the Southeastern Anatolia and Eastern Anatolia regions are carried out properly. In order to determine the presence of aflatoxin in teas throughout Turkey, such analyzes should be made in teas in other regions.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest. Author contribution The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Resource use efficiency and economies of scale for ginger production in Ilam district, Nepal

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Abstract

This study was conducted in the Ilam district of Nepal to analyse resource use efficiency and economies of scale of ginger farming. A total of 160 farmers and 20 traders (collector, wholesaler and retailers) from the study area were randomly selected as the sample. The pre-tested systematic questionnaire, fitted in KoBo Toolbox, was used to obtain the primary data through face to face household interviews. The production function of ginger farming was in increasing return to scale, with a score of 1.1356. The result showed that expenditure on seed and labour was over utilised. Except for these two, other inputs of ginger farming were underutilised. Economies of scale for ginger production was 44 quintal in production quantity and 5.68 ropani (1 hectare=19.66 ropani) in area. To obtain economies of scale, ginger production and size must be increased by around double the present context. The study concluded that to increase the profitability of ginger farming, resources must be utilised in optimum conditions, and production scale must be increased.

Keywords: Economies of scale, Ginger farming, Resource use efficiency, Return to scale

Introduction

Ginger (*Zingiber officinale*) is an important commercial crop of Nepal, involving seventy thousand farming families among 3.8 million farming families of the nation (Pandey, 2012). Ginger is mainly being cultivated in the mid-hill region and on a small scale in the terai areas of Nepal. Ginger is an important, highly valued spice crop in the international market for its aroma, pungency, oil and oleoresin (ANSAB, 2011; Paudel and Timsina, 2017). Even though Nepal has an agriculture-based economy, it imports lots of agricultural goods. The import-export ratio of Nepal stands at 12.2, and to reduce that nation should look toward high-value crops such as ginger for the betterment of the economy (DOC, 2020; Karki, 2015). Nepal is the world's fourth largest ginger exporter in the world, after China, Netherlands and India, with the 24 MT of annual export. Main industrial buyers of gingers are the food, pharmaceutical and cosmetics industries. Nepal mostly exports ginger in fresh form and traditionally dried form as Sutho**.** Besides those, different forms of ginger, such as powder, candy, oil, pickle, etc., are also potential products for domestic and foreign markets (Adhikari, 2016; GIZ, 2017).

Nepal has a comparative advantage in ginger production due to the lower labour cost, well adapted local varieties, climatic suitability, low cost of production inputs etc. (TEPC, 2017). Even though ginger has such potential, most farmers are reluctant toward ginger farming on a commercial scale. To attract farmers toward the ginger farming first thing that needs to be ensured is its profitability. To ensure higher profitability and contribution of ginger in improving the livelihood of farmers, efficient cost structure and appropriate level of input utilisation must be understood and recommended (Pandey, Adhikari, Dhakal, and Timsina, 2015; Sonwani, Koshta, and Tigga, 2018). So, this study was conducted in the Ilam district of Nepal to analyse resource use efficiency and economies of scale of ginger farming.

Materials and methods

Study area

The study was conducted in the Ilam district of Nepal, which lies in a mid-hill region of eastern Nepal. The district covers a 1073 Km^2 area and

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consists population of 290,254 (CBS, 2011). Most of the area is covered by sub-tropical (40.1%) and upper tropical (33.5%) climatic zone (Lilleso et al., 2005). Four villages of Ilam district, namely Ekatappa, Bhanjyang, Barbote and Mage, were selected as the study area, as they were the district's main ginger-producing sites (DADO, 2016).

Selection of respondents

All the Ginger growing farmers, ginger traders, and other personals linked with ginger production and trading in Ilam were the study's target population. From those four villages, a total of 160 farmers and 20 traders (collector, wholesaler and retailers) were randomly selected as the sample.

Data collection and analysis

The pre-tested systematic questionnaire, fitted in KoBo Toolbox, was used to obtain the primary data through face to face household interviews in February 2018. Additional data were obtained through five focus group discussions (FGD) and key informant interviews (KII). Information obtained from interviews was cross-checked during FGD and KII. Stata and Microsoft Excel were used for data import, management and analysis processes. Secondary data were also used for cross-checking and interpretation.

Cost of production

Production is the process where inputs are transformed into outputs, which can produce utilities. Cost of production is the sum of expenditure on inputs for any kind of production (Raju and Rao, 2019). The cost of all the variable inputs of ginger farming was included in the estimation of production cost.

Total Cost = Cost of (Land rent + Seed + Nutrients + Protection measures + Labor)

Benefit-cost analysis (B:C ratio)

Benefit-cost analysis is the process of analysing the economic performance of an enterprise. B:C ratio represents the proportion of total return to the total cost of production. It represents the total return per unit of input expense (Shrestha, 2017; Baral, Marahatta and Shrestha, 2021), which was estimated using the following formula

B:C ratio = Total return/Total cost

Profit analysis

Profit is the financial gain obtained from expenditure on inputs. It is the surplus return after detecting the total cost of production (Debertin, 2013; Baral, Marahatta and Shrestha, 2021). Thus, net profit for any farm business can be written as;

$\Pi = TR - TC$

$\Pi = \sum P_y \times Y - \sum P_{xi} \times X$

Where,

Π = net profit TR = total return

TC = total cost Y = quantity of output

Xi = quantity of i^{th} input Py = price of output

Pxi = price of i^{th} input

Factors affecting ginger production

To estimate the factors affecting ginger production, the Cobb-Douglas production function was used. Such a production model was implemented by Islam et al. (2012) and Baral, Marahattha and Shrestha (2021). The production value was the dependent variable, and the cost of production inputs (seed, nutrients, protection measures, labour, and land rent) were the independent variables.

The estimating model for the coefficients of ginger production is the following:

$Yg = a\, L^{b1} S^{b2}\, N^{b3} P^{b4}\, Lr^{b5} eu$

By taking log on both sides, that can be presented in linear form as follows;

$\ln Yg = \ln a + b1\ln L + b2\ln S + b3\ln N + b4\ln P + b5\ln Lr$

Where, Yg= Ginger production (NRs),

L = Land rent (NRs)

S = Cost of seed (NRs)

N = Cost of nutrients (FYM + chemical fertilizer)

P = Cost of protection measures

Lr = Cost of labor (included animal cost)

a = Intercept

bi = Slope coeeficient

The sum of elasticity of production bi represent returns to scale. Returns to scale can be defined as the variation in output caused by given proportionate variation in all the factors of production simultaneously (Barkley and Barkley, 2013; Debertin, 2013).

Analysis of resource use efficiency

The resource use efficiency of ginger farming was estimated by dividing Marginal Value Product (MVP) to Marginal Factor Cost (MFC) of variable inputs (Rahman and Lawal, 2003). The resource use efficiency of ginger farming was estimated by using the following formulae and concepts (Abid et al., 2011; Karthick, Alagumani, and Amarnath, 2013; Mohammed, Abubakar, and Haruna, 2014; Ibitoye, Shaibu, and Omole, 2015; Umar and Abdulkadir, 2015);

R = MVP/MFC

Where,

R = Efficiency Ratio

MVP = Marginal Value Product

MFC = Marginal Factor Cost

The values of efficiency ratio were interpreted as follow,

a) If R < 1, it means that resources are over utilised. Therefore, if the quantity of such input is increased, profit will decrease, and for higher efficiency quantity of such input must be decreased.

b) If R > 1, it means that resources are underutilised. Therefore, if such input is increased, profit will increase, and form will be more efficient.

c) If R = 1, it means that the resource was being efficiently utilised, and no change in the quantity of such inputs is desired.

The value of MVP was estimated using the regression coefficient of each production input and the price of output,

MVP = MPPxi × Py

And, MPPxi = dy/dxi = biY/Xi

Where,

bi = Estimated regression coefficient of input xi Py = Unit price of output

Y = Geometric mean value of output

Xi = Geometric mean value of input being used

The current market price of input at the time of the study was used as MFC.

MFC = Pxi

Where, Pxi = Unit price of input xi

Again, the relative percentage change in MVP of each resource required to obtain optimal resource allocation or efficient production system, i.e. R = 1 or MVP = MFC, was estimated using the following equation;

D = (1 –MVP/MFC) × 100%

Or, D = (1 –1/R) × 100%

Where,

D = Absolute value of percentage change in MVP of each resource (Mijindadi, 1980)

Short-run cost function

Short-run average cost functions were estimated and plotted against the output of ginger farming. The average cost and quantity of output produced were assessed utilising the quadratic cost function. Short-run average cost functions were fitted and interpreted to determine the least cost of production or economies of scale of ginger production. Economies of scale are the level of production, where the cost of production per unit becomes lowest. The function of the following form was used in the study (Greer, 2011; Raju and Rao, 2019);

Average Cost = f (Quantity produced)

Which can be written as;

$Y_{ac} = a_1 + b_1 Q + b_2 Q^2$

Where, Y_{ac} = Average cost (NRs./quintal)

Q = Output quantity (quintal)

Q^2 = Square of output quantity

a = Intercept

bi = Slope coefficient

Result and discussion

Cost of ginger production

The average cost of ginger cultivation per ropani and Kg were estimated to be NRs. 12,400 and NRs. 16, respectively. ANSAB (2011) and Timsina (2010) suggested a similar cost of production in their findings, and contrary to that, Pandey et al. (2015) reported production cost to be 1.5 times more than the finding of this research. The seed cost was the highest contributor to the total cost, with a proportion of 42.31%. Similar to the findings of this research, ANSAB (2011), Timsina (2010), and Sonwani et al. (2018) all reported the cost of seed as the main contributor of ginger farming cost. Although Pandey et al. (2015) and Paudel et al. (2016) suggested a similar scenario, they suggested the share of the seed's cost to be almost 1.5 times more than a finding of this research.

Table 1. Cost of Ginger production in study area per ropani.

Particulars	Total (NRs.)	Share (%)
Seed	5250	42.34
Labor	3950	31.85
Land rent	2000	16.13
Nutrients	1050	8.47
Pesticides	150	1.21
Grand total cost	12400	100
The average cost of production (per Kg)	16	

Cost, return, profit and B:C ratio of ginger cultivation

The average cost, return and profit from ginger cultivation per ropani was NRs. 12,400, NRs. 17,050 and NRs. 4,650, respectively. Luitel (2009), Timsina (2010) and DADO (2016) suggested a similar cost of production but reported higher return and profit compared to this finding. That might result from a partial ban on import from the Indian Government and increased cases of root rot infestation (Adhikari, 2016). Average B:C ratio of ginger farming was estimated to be 1.38, with the range of 1.10 to 1.78. Similar results were also stated by Nmadu and Marcus (2012), Pandey et al. (2015) and DADO (2016). But Timsina (2010), Bhat et al. (2012) and Paudel et al. (2016) reported the B:C ratio of ginger farming at least twice of our findings.

Factors affecting ginger production

The result showed that labour cost and seed cost were significant at 1% level, while cost of nutrients and land rent were significant at 5% and positively affected ginger production. The cost of plant protection chemicals had a positive but insignificant effect on ginger production. Similarly, Paudel et al. (2016) and Islam et al. (2012) suggested that the cost of seed, labour, land rent and nutrient cost have a positive and significant effect on ginger farming. But, Paudel et al. (2017) suggested that the cost of plant protection measures also positively and significantly impacts ginger production.

The F value of the model was significant at a 1% level with a score of 107.70 that indicates independent variables of the model largely contributes to the total variation of the model. The sum of the coefficients was estimated to be 1.1356, which implies production function is an increasing return to scale. The result implies that if all the inputs specified in the function are increased by 100% return will increase by 113.56%. Similar to our finding, Ayodele and Sambo (2014) and Sakamma, Umesh, and Rangegowda (2018) suggested increasing return to scale, but Paudel et al. (2016) reported decreasing return to scale for ginger production function. The coefficient of multiple determinations (R^2) was estimated at 0.78 that indicates 78% of total variations of production function caused by independent variables of the model. Other details of resource use efficiency or

factors contributing to ginger production or return are shown in Table 3.

Resource use efficiency and required adjustment in marginal value product (MVP) of each resource

The result showed that expenditure on seed and labour was over utilised. Except for these two, other inputs of ginger farming were underutilised. The findings of the study showed that utilisation of production inputs was not at the optimum level. The level of resource utilisation must be adjusted to ensure efficient resource utilisation and optimum economic gain.

Similar to the finding of this research, Sakamma et al. (2018) and Anamayi and Anamayi (2018) reported that nutrients and plant protection are being underutilised and, seed and labour are over utilised. Contrary to our finding, Anamayi and Anamayi (2018) reported that nutrients and land are overutilised, and Mathew et al. (2017) reported underutilised seed and labour. Such findings show that resource use efficiency heavily relies on farmers' farming system, management, and cultivation practice.

Table 2. Cost, return and profit of ginger cultivation per ropani (NRs.) in the study area

Description	Minimum	Maximum	Mean
Cost	8310	16624	12400 (1408)
Return	12474	23760	17050 (1793)
Profit	1357	8802	4650 (1631)
B:C ratio	1.1	1.78	1.38

Note: Figures in parentheses indicates standard deviation

Table 3. Estimated values of coefficients and related statistics of Cobb-Douglas production function of ginger

Factors	Coefficient (bi)	Std. Error	t-value	P> | t |
Labor cost	0.1264***	0.0479	5.64	0.003
Seed or rhizome cost	0.3046***	0.0539	3.47	0.001
Land rent	0.3941**	0.0724	5.44	0.011
Cost of nutrients	0.2927**	0.0608	4.82	0.032
Cost of plant protection	0.0178	0.0131	1.35	0.178
Constant	2.4032	0.4504	5.34	0.000
F-value	107.70***			0.001
R square	0.78			
Adjusted R-square	0.77			
Return to scale	1.1356			

Note: *** and ** indicate 1% and 5% levels of significance, respectively.

Table 4. Estimated resource use efficiency and required adjustment in MVP of each resource for ginger production

Inputs	Bi	MVP	MFC	R	D-value	Decision
Labor cost	0.1264	0.55	1	0.55	-81.81	Over utilised
Seed or rhizome cost	0.3046	0.99	1	0.99	-1.09	Over utilised
Land rent	0.3941	3.36	1	3.36	70.24	Underutilised
Cost of nutrients	0.2927	4.75	1	4.75	78.95	Underutilised
Cost of plant protection	0.0178	2.02	1	2.02	50.50	Underutilised

Economies of scale for ginger production

It was determined that ginger production economies in the study area were 44 quintals in quantity term and 5.68 ropani in area term. Comparing these economies of scale with the current context of farming (2.74 ropani and 21.24 quintal), ginger farming is operating way below its optimum condition. To obtain the least cost condition or economies of scale, ginger production and area must be increased by around double the present context. Further details are shown in Table 5.

Table 5. Cost function used to derive economies of scale for ginger production

Explanatory variables	Coefficient	Std. error	T	P>t
Production (Quintal)	-21.068	3.609	-5.84	0.001
Square of production (Quintal)	0.241	0.042	5.68	0.001
Constant	1899.508	50.961	37.27	0.001
Economies of scale = 44 quintal = 5.68 ropani area under ginger production per farm				

N = 160, F (2,157) = 17.24, Prob > F = 0.001, R-squared = 0.18 and adjusted R-squared = 0.17

Conclusion

A study of resource use efficiency and economies of scale for ginger production showed that ginger farming performs with very low profit on expenditure. As average B:C ratio of ginger farming was estimated to be just 1.38. This showed that the production level of ginger farming is not at its optimum level. It is supported by the findings of this research, as analysis of production function showed that production of ginger farming was in increasing return to scale (RTS=1.13). That clearly indicates increased production level can increase the profitability of ginger farming. Besides that, analysis of cost function also showed that to obtain the least cost condition or economies of scale, ginger production and area must be increased by double the present context in the case of an individual farming family. Expenditure on seed and labour was over utilised, but other inputs of ginger farming were underutilised. The utilisation of production inputs was not at the optimum level. The level of resource utilisation must be adjusted to ensure efficient resource utilisation and optimum economic gain. To sum up the research findings, it can be stated that to increase the profitability of ginger farming, resources must be utilised to optimum condition, and production scale must be increased.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

Corresponding author conducted the research and prepared manuscript. Other authors contributed in research design. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before

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A first insight into genetic diversity of Jerusalem artichoke accessions collected from different regions of Turkey assessed by ISSR markers

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Abstract

The study aimed to assess genetic diversity among Jerusalem artichoke (*Helianthus tuberosus* L.) accessions using ISSR (Inter Simple Sequence Repeat) markers. Twenty-five Jerusalem artichoke accessions collected from the different regions of Turkey have been used for molecular analysis. Nine primers used in the study produced a total of 62 bands. A total 57 of these are polymorphic. 57 of them resulted in polymorphic. Allele's average number was 6.33. Polymorphic information content (PIC) varied between 0.219 and 0.340. Examined accessions were classified into two main groups in a dendrogram. Genetic similarities varied from 0.001 to 0.815, with an average of 0.292. Also, Principal Coordinate Analysis (PCoA) of molecular data was conducted. These findings present valuable diversity data for recognizing the Jerusalem artichoke germplasm, preserving genetic richness, and determining parents for genetic enhancement.

Keywords: Genetic diversity, Gene-Pool, *Helianthus tuberosus* L., ISSR

Introduction

Jerusalem artichoke is classified in the Helianthus genus of the *Asteraceae* family. Helianthus genus is of American origin and has around 50 species currently grown in the World.The worldwide distribution of these species is limited. Sunflower (*Helianthus annuus* L.) and Jerusalem artichoke (*H. tuberosus* L.) are the most prominent species of this genus (Heiser, 1978). The sunflower is grown as an oilseed crop in general, while Jerusalem artichoke is grown as a vegetable, fodder crop, and as a reserve of inulin for food and industrial purposes (Heiser, 1978). This plant is grown for edible tubers. The root length varies between 1 and 3 meters and is an important part of the plant's biomass. Jerusalem artichoke can tolerate yearly rain varying from 31 to 282 cm with an average temperature scale of 6.3-26.6 °C and a pHof 4.5-8.2. Even if temperatures sub-zero kill the leaves and stems of the Jerusalem artichoke, the tubers can survive for a long time without being damaged by frost. Tolerance of tubers to low temperatures allows them to be stored under the ground during the cold winter until harvested. Jerusalem artichoke should ideally be planted in well-drained soil. Besides, good aeration of the soil before planting is advantageous throughout the cultivation because of its aggressive growth. Another advantage of rapid growth is the low pesticide need and the increase in the amount of biomass per area. The high biomass rate per area leads to good resistance to pests. The tubers of Jerusalem artichoke includes different kind of vitamins, minerals. Also it contains the complex carbohydrate inulin which can all promote good health in humans. Because of these properties, Jerusalem artichoke is considered not only as a human or animal food but also as a promising plant for ethanol production (Kays et al., 2007).

Turkey has a rich genetic diversity for many plant species. Preservation of this richness will enable both the improvement of new varieties andthe transfer of genetic resources to future generations (Balkaya et. al., 2001). The rich genetic diversity of Turkey's geography has great potential for crop breeding programs. Local accessions have been considered as the initial population for the development of many new commercial varieties. In addition to these local varieties, wild relatives of plant species are also used in breeding programs. However, for different reasons, this wealth is at risk of extinction. To reduce this risk, studies have been carried out within the framework of the National Program for Conservation of Plant Genetic Resources / Diversity since the 1960s (Tan, 2010). The low genetic diversity in breeding programs limits the rate of genetic progress expected fromsome crops. The use of germplasm stored in gene

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banks as a source of genetic diversity strongly influences the development of high yield varieties (Broughton et al., 2003). Despite all these efforts, a comprehensive characterization study has not been carried out in Turkish Jerusalem Artichokes' genetic resources. In a study conducted by the same author between the years 2018-2020, Jerusalem artichokes samples were collected from different cities of Turkey. During these trips, a predominance of traditional family agriculture was observed. Also, all farmers emphasized that they cultivate Jerusalem artichoke in very small areas such as 100 or 200-meter square. The main advantage emphasized by the local farmers was that Jerusalem artichoke isvery easy to cultivate (Hanci and Tuncer, 2019).

To use any gene source in breeding studies, the distribution of the genetic similarity of the cultivated species and their wild relatives must be known in detail. This can be possibly more effective by various methods, such as the use of technologies based on DNA polymorphism. DNA molecular markers are widely used to detect differences and similarities between accessions or to identify similarities or differences between varieties and their parents (Karaca et al., 2002). Today, molecular markers can be used in many plant species for several purposes such as genotypic identification, systematic and characterization, QTL (Quantitative Trait Loci) mapping, marker-assisted selection, and conservation of genetic resources (Vardar-Karlıtepe et al., 2010). Despite the important economic potential, the genetic diversity of the Turkish Jerusalem artichoke remained undiscovered yet, concerning traits such as DNA-based variation. This investigation aimed to assess the genetic diversity of Turkish Jerusalem artichoke accessions using ISSR markers for the first time. These genetic similarity data will guide the selection of parents in crossbreeding programs to be implemented in the future.

Materials and Methods

Twenty-five locally grown Jerusalem artichoke tubers, which were collected from eleven cities of Turkey (Table 1), were used as plant material. These accessions are preserved in the Hanci's cool-season vegetable collection under the Erciyes University. None of these materials are commercial varieties. Tuber characteristics of 15 accessions have been described and published previously during the establishment of this collection. High variabilitywas detected for tuber color, with five classes: red (7 accessions); light red (3 accessions); fawn (1 accession); light yellow (3 accessions); yellow (11 accessions). Also, high variation was observed in tubers weight ranging between 6,03 g (40/03) to 118,55 g (50/02). Accessions 50/02, 19/03, and 50/01 were distinct from all other ones due to high tuber weight (Hanci and Tuncer, 2019). Some of the collected tubers were planted in 10-liter pots in November 2018. The other part was stored in the refrigerator and planted in the same size pots in March 2019.

Table 1. Jerusalem artichoke samples assessed in the study and their geographic locations

Code Number	Geographic location	Coordinate	Code Number	Geographic location	Coordinate
06*1	Ankara / Beypazarı	40°09'45.7"N 31°55'27.5"E	50*1	Nevşehir / Gülşehir	38°44'25.7"N 34°37'57.5"E
06*2	Ankara / Bala	39°32'41.8"N 33°07'26.0"E	50*2	Nevşehir / Avanos	38°42'52.2"N 34°50'41.1"E
07*1	Antalya / Alanya	36°34'26.8"N 31°59'33.6"E	50*3	Nevşehir / Avanos	38°43'04.8"N 34°51'49.9"E
19*1	Çorum / Sungurlu	40°10'05.9"N 34°23'02.8"E	50*4	Nevşehir / Ürgüp	38°37'37.2"N 34°54'52.0"E
19*2	Çorum / Sungurlu	40°10'32.7"N 34°26'37.4"E	55*1	Samsun / Vezirköprü	41°07'56.9"N 35°27'09.1"E
19*3	Çorum / Feruz	40°42'57.0"N 34°52'35.5"E	55*2	Samsun / Havza	40°59'14.8"N 35°38'10.2"E
19*4	Çorum /Center	40°34'25.5"N 34°57'34.4"E	58*1	Sivas / Gemerek	39°11'42.2"N 36°04'54.1"
19*5	Çorum / Bayat	40°38'41.0"N 34°15'33.6"E	61*2	Trabzon/ Ortahisar	41°00'41.3"N 39°36'21.0"E
38*1	Kayseri / İncesu	38°37'38.3"N 35°11'56.4"E	66*1	Yozgat/ Center	39°49'15.7"N 34°48'19.0"E
38*2	Kayseri / Pınarbaşı	38°44'34.8"N 36°25'49.9"E	66*2	Yozgat / Boğazlıyan	39°48'05.4"N 34°47'25.3"E
40*1	Kırşehir /Center	39°09'10.1"N 34°10'00.3"E	66*3	Yozgat / Boğazlıyan	39°47'53.9"N 34°45'43.2"E
40*2	Kırşehir / Center	39°11'43.3"N 34°08'56.6"E	77*1	Yalova / Center	40°39'31.0"N 29°16'30.8"E
40*3	Kırşehir / Mucur	39°02'51.5"N 34°26'28.3"E			

Table 2. Various parameters of ISSR primers observed in the study

Marker	Tm (°C)	Band Size (bp)	Total number of alleles	Number of polymorphic alleles	% Polymorphism	Observed Heterozygosity	PIC*
DBDA$(CA)_7$	45	370-990	3	3	100	0.435	0.340
$(CT)_8$TG	48	210-1590	10	9	90	0.336	0.279
$(CA)_8$R	45	420-1090	8	7	70	0.292	0.249
VHV$(GT)_7$G	45	250-890	4	4	100	0.332	0.279
HVH$(CA)_7$T	45	90-320	3	3	100	0.250	0.219
$(AG)_7$YC	45	130-790	7	6	86	0.269	0.233
HVH$(TCC)_7$	60	1200-1790	7	6	86	0.304	0.258
BDB$(CA)_7$C	45	120-990	15	14	88	0.329	0.273
$(CA)_8$YG	45	110-800	5	5	71	0.417	0.330

*PIC: polymorphism information content

Bulked samples of four tubers of the same accessions were used for DNA isolation using an EasyPure® Quick Gel Extraction Kit (TransGen Biotech Co., Ltd, China). Tissue samples were taken from twenty days old fresh shoots and frozen in liquid nitrogen. Then these frozen tissues have disintegrated until they turned into a powder-like appearance using a tissuelyser (TissueLyser II Retsch, Qiagen Retsch GmbH., Hannover, Germany). DNA was obtained following the procedures specified by the producers of the kit. The DNA quality was examined on 1.3 % agarose gel.

A set of nine ISSR (Inter Simple Sequence Repeat) markers were preferred for their powerful polymorphism, according to the conclusions of previous studies (Wangsomnuk et al., 2011). Polymerase chain reaction (PCR) was performed using a thermal cycler model SC 300 (Australia). Each reaction tube (30 µL) contained 15 µL of 2X AmpMasterTMTaq PCR Mix (components: Taq DNA polymerase 1.25U; dNTP mixture 100 × 4 = 400 µL; reaction buffer with 1.25 mM MgCl2 1X; and loading dye&stabilizer 1X) and 25 ng/µL template DNA. The reaction conditions were of 95˚C for 2 min; then 45 cycles at 94 ˚C for 30 s, annealing temperature 45, 49 or 51˚C for 30 s, and 72˚C for 90 s; and a final extension at 72˚C for 5 min (Tan, 2010). After the amplification, PCR products were electrophoretically analyzed in 4 % agarose gel in 1X TBE buffer.

For data analysis, each scorable band was defined as a single locus/allele. The loci were scored as present (1) or absent (0), and the bi-variate 1-0 matrix was generated genetic distances were calculated using PAST3 software (Wangsomnuk et al., 2011). Cluster analysis was performed on molecular data using the Unweighted Pair Group Method with Arithmetic Means (UPGMA). The dendrogram was formed using Dice's value. The polymorphic information content (PIC) and observed heterozygosity (H) were calculated to define the informativeness and the discriminatory capacity of each ISSR marker using the PICcalc software (Mornkham et al., 2010). PIC was defined using the formula (Hildebrand et al., 1992):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2\, p_j^2$$

where pi and pj are the population frequency of the ith and jth allele, respectively.

Genetic similarities between accessions were also determined with a coefficient based on the proportion of shared alleles and a principal coordinate analysis (PCoA) using the PAST3 software.

Results and Discussion

A total of 62 different alleles were identified using nine ISSR primers in 25 Jerusalem artichoke accessions (Table 2). Fifty-seven of these alleles were polymorphic (92 %). The size of the alleles varied from 90 to 1790 bp. The number of polymorphic alleles varied between three and 14. The most distinguished polymorphic alleles were obtained from marker BDB(CA)7C. The markers DBDA(CA)7 and HVH(CA)7T produced just three polymorphic alleles. The mean value of polymorphic alleles per locus was 6.33. The PIC values ranged from 0.219 to 0.3405. Observed heterozygosity varied from 0.250 to 0.435. The abundance of allelic variation is of importance concerning both evolutionary and breeders' aspects (Wouw et al., 2010). Genetic similarities between Jerusalem artichoke accessions varied from 0.001 to 0.815, with an average of 0.292. The highest genetic similarity value (0.815) was found between accessions 40*3 and 66*1 (Figure 1), which were collected from the Kırşehir and Yozgat provinces, respectively. The other genetically close accessions are 61*2 and 50*3 (collected from Trabzon and Nevşehir province, respectively). But, all these accessions are not phenotypically similar. UPGMA analysis showed that the accessions were divided into two main groups. (Figure 1). No correlation was observed between the distributions in these groups and the geographical origins. The Group-I was consisted of five accessions. Allmembers of this group were collected from different provinces. There is no ecological similarity between these collection sites. For example, although accession 61*2 is collected from a very humid environment, accessions 66*3, 40*3, 50*3, and 19*1 were collected from central Anatolian cities with an arid climate. The genetic similarity value of these accessions were 0.408.

Figure 1. Dendrogram of all 25 Jerusalem artichoke accessions, generated for polymorphic loci through nine ISSR markers using UPGMA.

Group-II consisted of 20 accessions. The genetic similarity value of these accessions was found to be 0.370. In this group, as in the previous one, the geographic origin was not determinant. The only interesting point in this group is that all red tuber colors' accessions (19*1, 06*1, 19*4, 50*2, 58*1, 66*1, 66*2) are placed in this cluster (Hanci and Tuncer, 2019). Within this group, 40*2 (collected from Kırşehir province) is located in a different branch compared to the other members. However, no phenotypic features could be observed to explain the reason for this. The ISSR data explained 38.19 % of thevariation in the principal coordinate analysis by coordinates 1 and 2. The first coordinate explained 20.73 %, the second coordinate explained 17.46 % by PCoA analysis (Figure. 3).

At the end of thesame analysis, the first six principal coordinates showed 68.45 % of the total variation. The PCoA graph indicated that 25 Jerusalem artichokeaccessions did not separate as main groups and there was partially similarity between the patterns constructed by the UPGMA cluster. The most extensive investigations concerning the DNA-based genetic relationships among Jerusalem artichoke accessions were performed by Wangsomnuk et al., 2011. In this study, 147 Jerusalem artichoke accessions collected from nine countries were examined using 30 RAPD (Random Amplified Polymorphic DNA) markers. Thirteen markers produced 357 scorable bands. It was observed that 94.3% of these bands were polymorphic (Wangsomnuk et al., 2011). Also, low genetic richness was detected among wild and cultivated accessions. These results are consistent with the findings of this study. In this study, 57 polymorphic bands with nine ISSR markers were obtained. The ratio of these bands to total bands is 92 %.

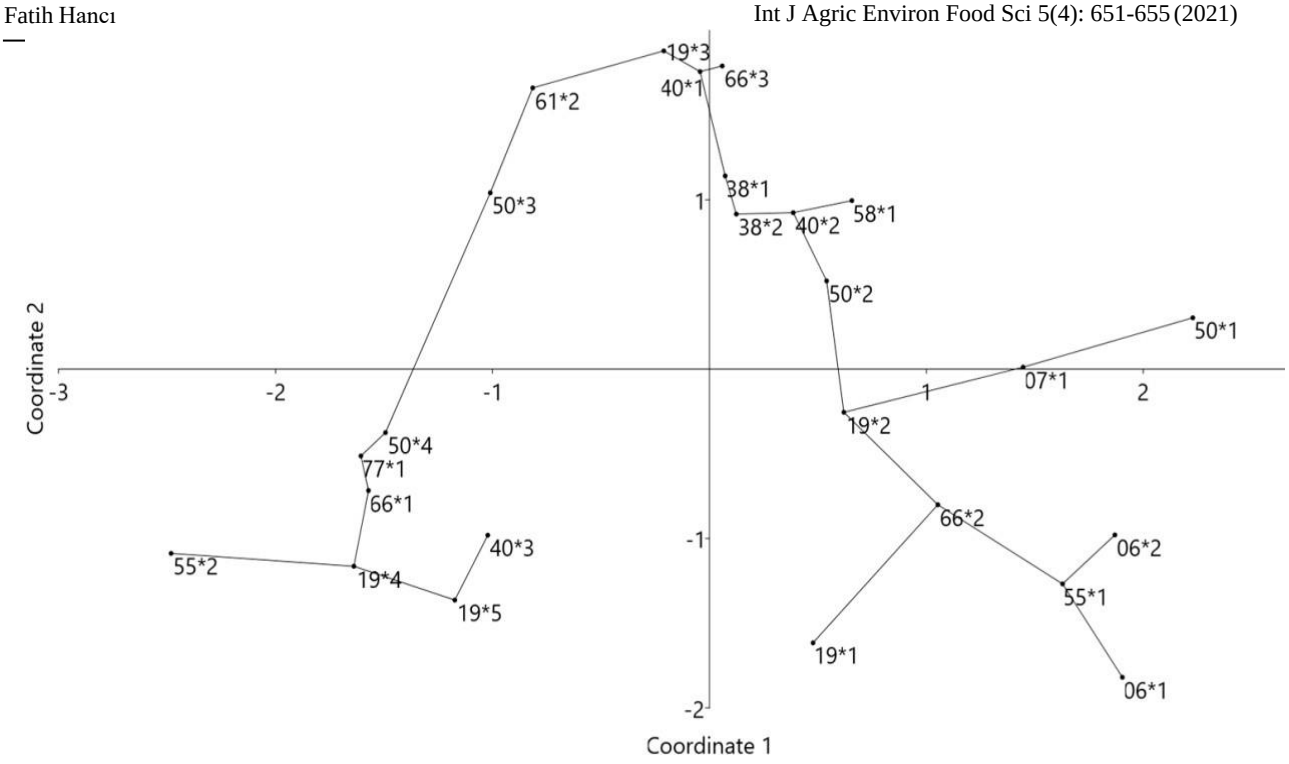

Figure 3. Distribution of Jerusalem artichoke accessions based on principal coordinate analysis

In another study, (Wangsomnuk et al., 2011) the efficacy of RAPD, ISSR, and SRAP (Sequence Related Amplified Polymorphism) marker in characterizing the Jerusalem artichoke accessions was compared. Forty-seven different Jerusalem artichoke accessions were used in that study. A total of 92 (80%) polymorphic bands from six ISSR primers; 296 (87.1 %) polymorphic bands from 13 RAPD primers; and 194 (88.6 %) were detected from nine combinations of SRAP primers. The polymorphism rate obtained from this study (92 %) was found to be high. (Wangsomnuk et al., 2011).

Mornkham et al. (2012) developed 43 EST-SSR markers using 40,362 Jerusalem artichoke ESTs. Then they tested these EST-SSR markers in six Jerusalem artichoke populations. At the end of the study, these SSR loci showed a large genetic richness between accessions. In this study, allele numbers range from 2 to 7, with an average of 3.95 alleles per loci. However, the number of polymorphic alleles per loci was higher in our study (6.33). The reasons for this difference are assumedto be different marker types (dominant ISSR and co-dominate EST-SSR) and the size of the gene pool examined (Jung et al., 2014).

Conclusion

This study contains the first investigation carried out to exhibit the DNA-based genetic diversity between 25 Jerusalem artichoke accessions collected from different geographical localities of Turkey. For this purpose, nine ISSR markers were used, and these markers were found to be quite successful according to the obtained polymorphic band ratio and polymorphism information content (PIC) values. The genetic richness was expected to be narrow because the Jerusalem artichokes are generally vegetatively propagated, and that their homeland is continental America. The richness of genetic diversity observed in this study may be explained as follows: (a) These plants have been grown in very different ecological regions of Turkey for many years, and variations may have arisen through natural selection; (b) the emergence of new genotypic variations as a result of generative reproduction, even in low probability; (c) and spontaneous mutations may have caused genetic variation. The findings of this study can be used by breeders in the selection of various parents for future breeding programs because the particular core subsets can promote the association mapping of genes controlling ecologically relevant features such as inulin, oil characters, and disease resistance [Brown, 1989; Wangsomnuk et al., 2011). However, further characterization studies in this gene pool must continue to establish an effective selection scheme. For this purpose, the concentration of different compounds, such as inulin, will be determined under the same project. Also, studies on the use of high biomass for different purposes are ongoing.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Performance of some *Prunus* rootstocks to transmit micronutrients to leaves

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Abstract

This study was conducted to investigate the intake of micro plant nutrients of promising genotypes in the selection study of some wild plums that can be rootstock for apricots in Malatya and Elazığ region. The study was carried out in 2020 on the land of Malatya Apricot Research Institute and in the Soil, Plant and Water Analysis Laboratory of the Kahramanmaraş Eastern Mediterranean Transitional Zone Agricultural Research Institute. Soil samples were conducted from 0-30 cm and 30-60 cm depths in order to determine the micronutrients in the soil from the area where the trial was established. According to the analysis results, it was determined that the micronutrient elements examined in the top soil (0-30 cm), except boron, were at sufficient levels. As a result of the analysis of leaf samples taken from 69 rootstocks selected in June, scoring was made by applying weighted grading to the amounts obtained. This method has been applied for the first time in the world with this study. At the end of the study, in the leaf contents, iron 33.65-101.00 mg kg^{-1}, manganese 19.01-106.27 mg kg^{-1}, copper 4.15-13.03 mg kg^{-1}, zinc 9.25-35.55 mg kg^{-1} and boron 19.54-35.55 mg kg^{-1} varied between. It has been determined that obtained these values are highly similar to the reference values, and when compared with other literature data, manganese is high, iron is relatively low, and other micronutrients elements are sufficient.

Keywords: Plant nutrition, Scion, Selected rootstocks, Soil and leaf analysis, Weighted grading

Introduction

Fruit trees, consist of two different plants as rootstock and scion produced by grafting. Although these two different plant parts have different genetic structures, they are in mutual symbiotic relationship (Shahkoomahally et al., 2020). With the variety that forms the scion part, the breeding of the plant in the rootstock part contains different criteria (Hernández et al., 2010). In fruit trees, which have a long generation period, since the breeding of the variety takes a long time also, it is the most practical method to reproduce these varieties by grafting. With the grafting becoming necessary, the importance of using rootstock has increased one more time (Taaren et al., 2016). The foremost criterion for a rootstock is the intake of plant nutrients from the soil at desired rates (Yahmed et al., 2020). Nawaz et al. (2016) also reported that the intake of plant nutrients at desired rates is closely related to the yield and quality of the variety grafted on the rootstock. However, considering the demands from producers and consumers, and the rapid changes in biotic and abiotic climate and soil conditions, the importance of rootstock breeding studies is better understood (Tombesi et al., 2011; Gündeşli, 2018). Rootstocks affect resistance to soil biotic factors such as growth force (Beckman et al., 1992; Layne, 1994), yield, quality, nematode as well as also the uptake and use of plant nutrients (Boyhan et al., 1995) with the phenological properties of the fruit varieties grafted on them. The factor that plays an important role in the emergence of all these features is the healthy transmission of plant nutrients from the rootstock to the scions.

Plum rootstocks provide dwarfing in the growth strength in apricot varieties grafted on them. Such situations in which vegetative growth is suppressed causes an increase in leaf nutrient content and the nutrient competition between vegetative growth and fruits in favor of fruit (Faust, 1989). Failure of the developed rootstocks to adapt well to different soil conditions causes difficulties in the transmission of plant nutrients, as well as problems in the graft compatibility rate and post-grafting development.

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Fe, Mn, Cu, Zn and B are known as micronutrients in plant nutrition. Although these elements are uptaken very little by plants, they have very important roles in plant metabolism. They are essential elements as catalysts in chlorophyll formation, oxidation and reduction mechanisms in plants. It has been reported that micronutrients are an important source in the mobility of nutrients in the vegetative tissues of plants, but there is not enough information about these mobility mechanisms (Pearson and Rengel, 1994).

This research study was conducted on the land of Malatya Apricot Research Institute and in Soil, Plant and Water Analysis Laboratory belonging to Kahramanmaraş Eastern Mediterranean Transitional Zone Agricultural Research Institute in 2020, in order to determine the transmission of micronutrients from soil to leaves in different plum species obtained by selection breeding in Malatya and Elazığ provinces.

Materials and Methods

Four different species of Plum genotypes (Prunus cerasifera, Prunus divaricata, Prunus domestica and Prunus spinosa) determined by selection breeding from Malatya and Elazığ regions were used as material in this study. Myrobolan 29C (Prunus cerasifera) was used as a control plant. From these rooted genotypes, a garden was established on the land of Malatya Apricot Research Institute in October 2019, with a distance of 1.5 m x 1 m above and between rows. Three samplings of each genotype were planted. Leaf samples were taken from single-year seedlings. Full-grown leaf samples were taken from each of these seedlings that had completed one year of age.

Soil samples

A total of 40 soil samples were collected from depths of 0-30 cm and 30-60 cm by zigzagging walking (Z-shaped) among the rootstocks used in the study in order to represent the study area. 20 soil samples taken from a depth of 0-30 cm were thoroughly mixed in a clean bucket and made into a single sample of 2 kg. The same procedure was done by taking it from 30-60 cm depth also. A total of 2 samples were obtained. Soil samples brought to the soil preparation room were laid in drying containers and the large stones and pieces of branches inside were cleaned and left to dry. The dried soil samples were beaten with wooden mallets and passed through a 2 mm sieve and made ready for analysis. Soil texture in soil samples made ready for analysis was determined according to the modified Bouyoucus hydrometer method (Klute, 1986). The soil reaction (pH) was measured by pH meter with glass electrodes in soil (sature the soil reaction (pH) was measured by pH meter with glass electrodes in soil (saturated sludge) saturated with water prepared as reported by Richards (1954). Total salt contents (%), electrical conductivity values (EC) of soils were calculated by measuring with electrical conductivity device from saturated sludge (Richards, 1954). Lime ($CaCO_3$) (%) was determined volumetrically in Scheibler calcimeter (Klute, 1986). SOM (%) was determined by the Walkley-Black method modified by Richards (1954). The amounts of available iron, manganese, copper and zinc (mg kg^{-1}), as Lindsay and Norvell (1978) reported, were determined with the Agilent 5100 brand ICP-OES device measuring of the filtered solutions obtained from soils extracted with DTPA solution (Klute, 1986). Boron contents that can be taken by the plants were determined in the ICP-OES device according to the method reported by Klute (1986).

Leaf samples

Collecting leaf samples:

In June, leaves were selected, which completed the development from the middle part of their sprouts of the seedlings were selected. 150 leaves were collected from each iteration. The samples taken were numbered and placed on the paper bags. The collected leaf samples were brought to the laboratory without waiting. Here, plants were laid out on papers with their own numbers written. Unhealthy and worn leaves were cleaned and discarded. Then, the dust on it was cleaned by pre-washing. Next, it was passed through the 0.1 N HCl solution and washed with pure water. The washed leaves were laid loosely and left to dry in the drying cabinet at 65 °C until their weight did not change (about 48 hours). The dried samples; it was stored in the refrigerator until it was analyzed in plastic bags in a labeled way (Lilleland and McCollam, 1961; Steyn, 1961; Sannoveld and Dijk, 1982; Kacar, 2008).

Determination of nutrients uptaken by plants:

The dried leaf samples were ground in a tungsten coated hand mill. 0.30 g was taken from the milled plant parts and analyzed according to wet digestion method in a pressurized microwave oven with 0.5 ml nitric acid (HNO_3, d= 1.42) and 2 ml hydrogen peroxide (H_2O_2, 30 %) as reported by Miller (1998). After wet digestion, samples were filtered and Fe, Mn, Cu, Zn and B amounts were determined in Agilent 5100 brand ICP-OES device. The accuracy of the results was also checked with the certified values of the relevant minerals in reference plant materials obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Evaluation of the results

After the leaf samples were analyzed in triplicate, the measured grading method modified by Uğur and Kargı (2018) was applied to the obtained results (Table 1). This method was used for the first time in the world with this study. With this method, each plant nutrient was given a score according to its minimum and maximum values. The scoring was based on the coefficient obtained from the minimum and maximum difference. After collecting their scores took from each plant nutrient of the rootstock candidates, the total points that micronutrients

received were obtained. After applying the re-modified weighed grading to these scores, the general status of the rootstocks in the transmission of nutrients was determined.

The adequacy levels of the micronutrient contents determined by leaf analysis were evaluated according to Table 2.

Table 1. Basis value ranges for the scores used in the weighted grading.

Iron			Copper			Manganese			Zinc			Boron		
Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
33,65	101	6,74	4,15	13,03	0,89	19,01	106,27	8,73	9,25	35,55	2,63	19,54	75,55	5,60
Scores			Scores			Scores			Scores			Scores		
1	33.65	40,39	1	4.15	5,04	1	19.01	27,74	1	9.25	11,88	1	2.63	25,14
2	40,4	47,14	2	5,05	5,94	2	27,75	36,48	2	11,89	14,52	2	25,15	30,75
3	47,15	53,89	3	5,95	6,84	3	36,49	45,22	3	14,53	17,16	3	30,76	36,36
4	53,90	60,64	4	6,85	7,74	4	45,23	53,96	4	17,17	19,80	4	36,37	41,97
5	60,65	67,39	5	7,75	8,64	5	53,97	62,70	5	19,81	22,44	5	41,98	47,58
6	67,40	74,14	6	8,65	9,54	6	62,71	71,44	6	22,45	25,08	6	47,59	53,19
7	74,15	80,89	7	9,55	10,44	7	71,45	80,18	7	25,09	27,72	7	53,2	58,80
8	80,90	87,64	8	10,45	11,34	8	80,19	88,92	8	27,73	30,36	8	58,81	64,41
9	87,65	94,39	9	11,35	12,24	9	88,93	97,66	9	30,37	33	9	64,42	70,02
10	94,40	<	10	12,25	<	10	97,67	<	10	33,01	<	10	70,03	<

Table 2. Micro plant nutrients required for the growth of most plants and some characteristics related to them (Çepel, 1996; Jones and Jacobsen, 2001; Epstein and Bloom, 2005).

Name of the element	Chemical icon	Content in dry matter (mg kg^{-1})	Available shape for plant
Iron	Fe	100 (50-250)	Fe^{+2}, Fe^{+3}
Manganese	Mn	50 (20-200)	Mn^{2+}
Copper	Cu	6	Cu^{+}, Cu^{+2}
Zinc	Zn	20	Zn^{2+}
Boron	B	20 (6-60)	BO_3^{-3}, $B_4O_7^{-2}$

Results and Discussion

Soil properties according to analysis results

According to soil analysis results; the soils of the research area were determined as loamy, slightly alkaline and non-saline. The study area soils were found extremely calcareous also at both depths (0-30 cm and 30-60 cm). The fact that the soils are very calcareous can be due to the parent material. Topsoil (0-30 cm) contains well, subsoil (30-60 cm) contains moderate organic matter. In a depth of 0-30 cm, available iron, manganese, copper and zinc were determined to be sufficient for plants. But, at a depth of 30-60 cm, zinc may have been binded to clay minerals, organic matter or lime, converting into an unavailable form for plants. It has been found that the boron that can be taken by the plants is not to be sufficient for the plant also at both depths (Table 3). Yılmaz et al. (2020), in a their study conducted in Malatya soils, reported that 25.42% of Malatya soils had very little and little boron deficiency and the reasons for this were due to the fact that the soils were the slightly alkaline and calcareous. It is thought that the fact that the soils of the study area are loam texture, that is, in a permeable structure, may also cause the boron to be washed.

Evaluations in Table 3; texture was made according to Bouyoucos (1951), and pH was evaluated according to USDA (1998), and total saline was evaluated according to USDA (2018), and lime was evaluated according to FAO (2006), and organic matter was according to Ülgen and Yurtseven (1995), and available iron, manganese, copper and zinc were according to Lindsay and Norvell (1978), and also available boron was according to Wolf (1971).

Results related to the transmission of nutrients from the soil to the leaves

Leaf iron contents in all rootstocks were distribution between 33.65 mg kg^{-1} and 101.00 mg kg^{-1} (Table 4 and 5). The highest leaf iron contents were found in 23 KV 03 (*P. spinosa*), 23 KK 12 (*P. cerasifera*) and 23 MR 03 (*P. divaricata*) rootstocks, and determined as 101.00 mg kg^{-1}, 95.66 mg kg^{-1} and 95.63 mg kg^{-1}, respectively (Table 4). The lowest iron contents were found in 23 KK 13 (*P. divaricata*, 33.65 mg kg^{-1}, Table 5), 23 AK 12 (*P. domestica*, 40.23 mg kg^{-1}, Table 4) and 23 KK 11 (*P. domestica*, 41.04 mg kg^{-1}, Table 4) rootstocks. It was determined that the average leaf iron content of all rootstocks was 58.48 mg kg^{-1} (Table 5). When leaf iron contents of the rootstocks are examined in

general, it is seen that leaf iron contents of 55 of seventy rootstocks are compatible with the reference values. Mestre et al. (2015), in a their conducted on peaches, it is understood that the results they received around 59.8-86.3 mg kg^{-1} on average were similar to our study. The leaf iron contents of rootstocks were found to be high according to the results of Jimenez et al. (2008). The other fifteen rootstocks used in the study showed no leaf degradation that would cause a high degree of chlorosis. Iron content of rootstocks is closely related with chlorosis. This also directly affects the content of leaf chlorophyll. In rootstocks, iron deficiency in microelements and, accordingly, chlorosis is an important criterion. Rootstocks are requested to transfer sufficient amount of iron to the scion grafted onto itself, it at high pH. This situation, which is increased the quality of the leaf and the amount of chlorophyll, also increases the efficiency of photosynthesis. In general, it has been reported that iron uptake mechanism of the root system in rootstocks is in two different ways (Tagliavani & Rombola, 2001). Gündeşli et al. (2020) reported in a study they conducted that these mechanisms may differ in terms of the operating speed in rootstocks, therefore the selection of appropriate rootstocks is important. We can say that this situation is foreseen as an important criterion in rootstock selection. Plants uptake iron with their roots from the soil. If they do not get enough, the deficiency is eliminated by foliar fertilization. In this sense, rootstock becomes more important (Mayer et al., 2015). Because rootstock means the root of the plant to be grafted on. It is understood that most of the rootstocks used in the study are promising.

Table 3. Some physical and chemical properties of the research area soils.

Soil Properties	Value (0-30 cm)	Evaluation	Value (30-60 cm)	Evaluation
Sand (%)	47.4		47.4	
Silt (%)	34.0		34.0	
Clay (%)	18.6		18.6	
Texture		Loam		Loam
pH	7.72	Slightly alkaline	7.76	Slightly alkaline
Total saline (%)	0.042	Non-saline	0.041	Non-saline
Lime (%)	37.72	Extremely calcareous	38.38	Extremely calcareous
Organic matter (%)	3.25	Good	2.67	Good
Available iron (mg kg^{-1})	6.49	Good	8.02	Medium
Available manganese (mg kg^{-1})	6.65	Sufficient	6.26	Sufficient
Available copper (mg kg^{-1})	4.14	Sufficient	1.80	Sufficient
Available zinc (mg kg^{-1})	0.95	Sufficient	0.40	Low
Available boron (mg kg^{-1})	0.87	Low	0.87	Low

Leaf manganese contents of rootstocks varied between 19.01 mg kg^{-1} and 106.27 mg kg^{-1} (Table 4). In the distribution where the average manganese content was 50.24 mg kg^{-1} (Table 5), the manganese contents of 32 rootstocks were determined above the average value. When compared with the reference values, it is understood that the leaf manganese content is at the desired level. Looking at the results obtained from similar studies, Karlıdağ et al. (2019) determined the average leaf manganese contents as 32.09 mg kg^{-1} in apricot, and Milosevic and Milosevic (2011) found the average leaf manganese content between 20.71 mg kg^{-1} and 68.82 mg kg^{-1} in their study. These results appear to be similar to our results. Jimenesa et al. (2018) found the manganese contents of leaves between 36.74-74.32 mg kg^{-1} in a study they conducted on peaches. Similar values have been also reported by Mestre et al. (2017). Although these results are somewhat high, it is seen that they are generally compatible with the results obtained from our study.

It is understood from the tables that leaf copper contents in selected rootstocks range between 4.15 mg kg^{-1} and 13.03 mg kg^{-1} (Table 4 and 5). In the distribution where the average copper content is around 8.64 mg kg^{-1} (Table 5), it is seen that 32 rootstocks are above the average value, and when compared with the reference values, almost leaves of all rootstocks have high copper content. When compared with the sufficiency levels, it was also determined that there was no copper deficiency in the leaves of all rootstocks (Table 2). This situation can be explained by the fact that there is no problem in transmitting the copper nutrient, which is taken from the soil enough, to the leaves and is accumulated in the leaves. In other words, in all the rootstocks, there appears to be no problem in uptaking copper from the soil and transmitting it to the leaves.

It is seen in Tables that the leaf zinc content of all selected rootstocks varied between 9.25 mg kg^{-1} and 35.55 mg kg^{-1} (Table 4). Zinc contents of the rootstocks an average were found as 18.80 mg kg^{-1} (Table 4), and 25 of rootstocks were determined above the average sufficiency amounts. In the rest of the rootstocks, it was determined that the obtained leaf zinc contents were at sufficient levels and there was no any deficiency. In fact, it is seen that there is

a widespread lack of microelements in the territory of Turkey where agriculture is carried out. Zinc and iron deficiency are the leading them (Eyüpoğlu et al., 1993; Aliyazıcıoğlu et al., 2013). Research has reported that the most accurate and practical way of uptaking zinc in plants and transferring it to products would be the selection of genotypes with good zinc intake (Çakmak et al., 1998; Ullah et al., 2017). It is welcomed that the zinc values of the rootstocks used in our study are realized at the expected levels.

In the rootstocks used in our study, leaf boron contents showed a distribution between 19.54 mg kg^{-1} and 75.55 mg kg^{-1} (Table 4). The average boron value was found to be 23.49 mg kg^{-1} (Table 5). Considering this average value, it is understood that the leaf boron contents of the rootstocks used in the study are at optimum values and there will be no boron deficiency or toxicity. Especially due to its active role in many physiological events such as fertilization and fruit formation, and due to the losses of yield and quality in its deficiency, this nutrient element is also requested to be found between 6-60 mg kg^{-1} in plant leaves (Jones et al., 1991). In a previous study, it was reported that the average leaf boron content varied according to varieties and showed distribution between 60-80 mg kg^{-1} (Çakmak, 2002). The values (23.62-92.54 mg kg^{-1}) related to the leaf boron contents obtained from the study conducted by Milosevic and Milosevic (2011) on apricots can be given this as an example. Kacar and Fox (1967) reported that boron concentrations in 20 soils, they collected from different parts of Turkey ranged from 0.70-4.55 mg kg^{-1} and that 25% of soils had boron deficiency. Although there is an available boron deficiency in both depths in soils of the working garden, no deficiency was detected in the leaves of the rootstocks. It is thought that the reason for this is that the plants completed their deficiency by uptaking the available boron from irrigation water. Or it may have uptaken it from the deeper soil where their roots reached.

As a result of the weighted grading applied to the data obtained from this research, it is seen that high differences occur in the microelement transmission in all rootstocks and each genotype transmits different microelement at a level that can be considered good. It is understood that the scores in the total scoring range between 9.00 and 37.00 and the average score is 23.49 (Table 4 and 5). Considering the general distribution, it is seen that 40 of the rootstocks have an average value and above, and 10 of them get scores close to the average. It is understood that the remaining 20 rootstocks are around 10 points (Tables 4 and 5). Forcada et al. (2020) reported that the difference in nutrient transmission between rootstocks is accepted as normal and this is due to genetic variation, therefore it is important to select the appropriate rootstock. While the rootstocks that got the highest scores according to the micronutrients they absorbed, they were 23 KK 18 (*P. cerasifera*), 23 MR 04 (*P. domestica*) and 23 AR 18 (*P. cerasifera*), the scores of these rootstocks were also determined as 37.00, 34.00 and 33.00 (Table 4), respectively. The rootstocks with the lowest scores were also determined as 44 YY 06 (P. domestica) (14.00), 44 AK 13 (P. domestica) (13.00) and 44 YY 18 (P. domestica) (9.00) (Table 5).

Conclusion

In the majority of Turkey's soil, the soil reaction (pH) is known to be slightly alkaline. This situation causes major problems in the uptake of many microelements, especially iron, and malfunctions in plant growth and consequently yield losses. In modern fruit growing, this deficiency is tried to be overcome by appropriate fertilization programs. However, not using the appropriate rootstock greatly reduces the effectiveness of these programs. Therefore, in the studies of rootstock breeding, it is very important that the efficiency of the rootstock to uptake plant nutrients from the soil is high. In this research which we have done, the study data on the selected rootstocks uptaking the plant nutrients from the soil and transmitting them to the leaves were found promising. In the study, 70 rootstocks were used (Table 4). These rootstocks were compared with the control rootstock and it was examined to what extent they took nutrients from the soil. At the end of the study, it was determined that most of the rootstock candidates (46 of them) had higher leaf nutrient content than the control rootstock. These performances of rootstock candidates mean that they are promising considering the values of the control rootstock. As a result of the study, no selection was made, and these results will be taken into account in the future selection.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

Table 4. Transmission status and scoring list of micronutrients uptaken by all selected rootstocks.

Line number	Code	*Species*	Fe (mg kg^{-1})	Fe scores	Mn (mg kg^{-1})	Mn scores	Cu (mg kg^{-1})	Cu scores	Zn (mg kg^{-1})	Zn scores	B (mg kg^{-1})	B scores	Micro total
1	23 KK 18	*P.cerasifera*	90.50	9	54.24	5	11.36	9	35.55	10	37.58	4	37.00
2	23 MR 04	*P.domestica*	88.32	9	35.24	2	10.20	8	23.41	6	66.91	9	34.00
3	23 AR 18	*P.cerasifera*	64.11	5	64.10	6	10.50	8	26.37	7	55.90	7	33.00
4	23 KK 05	*P.cerasifera*	65.56	5	63.51	6	9.75	6	22.56	5	72.47	10	32.00
5	23 KK 12	*P.cerasifera*	95.66	10	40.28	3	9.25	7	17.74	4	63.52	8	32.00
6	23 KV 03	*P.spinosa*	101.00	10	61.83	5	8.02	5	17.58	4	60.14	8	32.00
7	44 AK 06	*P.cerasifera*	62.38	5	101.46	10	12.34	10	21.12	5	29.24	2	32.00
8	23 MR 03	*P.divaricata*	95.63	10	53.81	4	8.80	7	12.12	2	64.24	8	31.00
9	44 AK 02	*P.divaricata*	58.55	4	60.12	5	10.80	9	17.04	3	72.42	10	31.00
10	44 YY 11	*P.cerasifera*	75.90	7	106.27	10	9.42	7	19.23	4	30.55	2	30.00
11	44 YY 16	*P.cerasifera*	57.41	4	66.86	6	13.03	10	27.59	7	36.24	3	30.00
12	23 KK 15	*P.cerasifera*	49.99	3	81.54	8	8.56	5	20.59	5	62.19	8	29.00
13	23 KK 16	*P.spinosa*	68.20	6	51.94	3	9.13	7	24.60	6	49.12	7	29.00
14	23 KK 04	*P.cerasifera*	52.74	3	43.48	3	10.66	8	18.34	4	75.55	10	28.00
15	23 KV 02	*P.domestica*	55.68	4	40.38	4	11.48	9	19.25	4	56.01	7	28.00
16	44 AK 03	*P.divaricata*	55.43	4	36.83	3	9.02	7	18.25	4	70.29	10	28.00
17	23 KK 09	*P.cerasifera*	50.78	3	71.08	6	11.13	8	17.04	3	55.68	7	27.00
18	23 KL 01	*P.cerasifera*	61.63	5	38.95	3	10.36	7	17.76	4	61.52	8	27.00
19	23 KV 01	*P.cerasifera*	60.15	4	44.57	3	9.16	7	23.60	6	55.67	7	27.00
20	23 AK 12	*P.domestica*	40.23	1	64.22	6	7.93	5	27.40	7	53.95	7	26.00

Table 4. Transmission status and scoring list of micronutrients uptaken by all selected rootstocks (continuation)

Line number	Code	*Species*	Fe (mg kg^{-1})	Fe scores	Mn (mg kg^{-1})	Mn scores	Cu (mg kg^{-1})	Cu scores	Zn (mg kg^{-1})	Zn scores	B (mg kg^{-1})	B scores	Micro total
21	23 KK 03	*P.cerasifera*	60.83	5	66.43	6	8.69	6	24.08	6	34.39	3	26.00
22	23 PA 05	*P.domestica*	48.51	3	39.65	3	7.93	5	21.39	5	74.86	10	26.00
23	44 YY 02	*P.cerasifera*	62.08	6	39.29	3	10.86	8	17.83	4	37.88	5	26.00
24	23 AR 09	*P.spinosa*	58.93	4	42.00	3	10.20	8	17.09	3	57.17	7	25.00
25	23 KK 02	*P.cerasifera*	52.55	3	30.20	2	10.22	7	28.41	8	43.32	5	25.00
26	23 KK 14	*P.cerasifera*	60.91	5	46.21	4	7.02	5	16.59	3	60.25	8	25.00
27	44 AK 17	*P.divaricata*	52.18	3	69.91	6	8.69	6	13.05	2	63.63	8	25.00
28	44 YY 24	*P.cerasifera*	74.78	7	68.45	6	7.95	5	17.70	4	34.69	3	25.00
29	23 AR 15	*P.spinosa*	50.83	3	86.45	8	6.53	3	19.55	4	51.45	6	24.00
30	44 AK 01	*P.cerasifera*	52.66	3	24.85	1	8.67	7	21.69	5	66.03	8	24.00
31	44 YY 05	*P.domestica*	78.97	7	33.52	2	10.41	7	25.76	7	22.24	1	24.00
32	44 YY 10	*P.domestica*	63.01	5	44.34	3	9.55	7	19.90	5	38.57	4	24.00

33	44 YY 17	*P.domestica*	58.49	4	28.27	2	10.02	7	27.24	7	39.66	4	24.00
34	44 YY 19	*P.cerasifera*	55.89	4	45.82	4	9.34	7	20.78	5	30.86	4	24.00
35	44 AK 05	*P.divaricata*	55.10	4	41.12	3	10.23	8	15.27	3	45.68	5	23.00
36	44 AK 10	*P.cerasifera*	58.62	4	50.71	4	8.44	5	20.40	5	43.16	5	23.00
37	44 AK 15	*P.divaricata*	59.72	4	37.65	3	7.97	5	14.79	3	62.37	8	23.00
38	44 YY 04	*P.cerasifera*	64.38	5	40.73	3	9.81	7	21.66	5	33.21	3	23.00
39	44 YY 08	*P.cerasifera*	53.19	3	82.41	8	7.48	4	22.08	5	32.62	3	23.00

Table 4. Transmission status and scoring list of micronutrients uptaken by all selected rootstocks (continuation)

Line number	Code	*Species*	Fe (mg kg^{-1})	Fe scores	Mn (mg kg^{-1})	Mn scores	Cu (mg kg^{-1})	Cu scores	Zn (mg kg^{-1})	Zn scores	B (mg kg^{-1})	B scores	Micro total
40	44 YY 15	*P.domestica*	90.83	9	48.92	4	6.65	3	20.85	5	29.63	2	23.00
41	23 KK 11	*P.domestica*	41.04	2	34.72	2	8.18	6	29.03	8	37.52	4	22.00
42	23 KK 17	*P.cerasifera*	49.31	3	72.52	7	7.78	5	14.64	3	38.04	4	22.00
43	23 KV 04	*P.spinosa*	50.59	3	59.11	5	6.61	3	10.78	3	64.14	8	22.00
44	44 AK 16	*P.divaricata*	59.14	4	48.37	4	7.54	5	13.70	2	54.58	7	22.00
45	44 YY 22	*P.divaricata*	68.69	6	59.37	5	6.96	3	16.28	3	37.74	5	22.00
46	44 YY 23	*P.divaricata*	54.09	4	42.27	3	9.16	7	14.30	3	42.59	5	22.00
47	Kontrol	*P.cerasifera*	58.48	4	50.24	4	8.64	5	18.80	4	46.08	5	22.00
48	23 AR 13	*P.spinosa*	49.41	3	35.62	2	7.44	4	17.02	3	67.03	9	21.00
49	23 KK 06	*P.cerasifera*	53.96	4	61.01	5	10.15	7	19.46	4	19.54	1	21.00
50	44 AK 09	*P.cerasifera*	57.92	4	51.65	4	8.61	5	14.44	3	42.09	5	21.00
51	44 YY 12	*P.cerasifera*	60.65	5	42.86	3	9.24	7	20.36	5	24.63	1	21.00
52	23 AR 05	*P.spinosa*	43.65	2	28.66	2	7.87	5	12.62	3	62.92	8	20.00
53	23 MR 05	*P.divaricata*	44.48	2	44.33	4	7.48	4	14.91	3	58.27	7	20.00
54	44 AK 04	*P.cerasifera*	52.69	3	66.67	6	7.38	4	14.47	2	42.90	5	20.00
55	44 DR 04	*P.cerasifera*	44.97	2	57.15	5	6.12	3	13.49	2	54.58	7	19.00
56	44 YY 01	*P.domestica*	52.01	3	44.79	3	8.90	7	19.39	4	27.54	2	19.00
57	23 KK 07	*P.cerasifera*	54.74	4	19.01	1	8.33	6	22.46	5	26.48	2	18.00
58	44 AK 14	*P.divaricata*	48.61	3	33.59	2	8.73	7	18.16	4	28.56	2	18.00
59	23 AR 04	*P.spinosa*	48.31	3	57.76	5	5.81	2	9.25	1	52.32	6	17.00

Table 5. Transmission status, and minimum, and maximum, and standard deviation values, and scoring list of micronutrients taken by all selected rootstocks.

Line number	Code	*Species*	Fe (mg kg^{-1})	Fe scores	Mn (mg kg^{-1})	Mn scores	Cu (mg kg^{-1})	Cu scores	Zn (mg kg^{-1})	Zn scores	B (mg kg^{-1})	B scores	Micro Total
60	23 AR 10	*P.cerasifera*	58.65	4	38.35	3	5.78	2	11.75	1	58.64	7	17.00
61	23 KK 08	*P.cerasifera*	44.30	2	45.01	3	7.52	4	16.36	3	42.24	5	17.00
62	44 YY 03	*P.domestica*	47.52	3	26.44	1	8.24	5	23.35	6	24.36	2	17.00
63	44 YY 13	*P.domestica*	53.50	3	56.47	5	7.58	4	16.60	3	27.96	2	17.00
64	44 YY 20	*P.divaricata*	54.11	4	54.95	5	7.46	4	10.24	1	36.34	3	17.00
65	44 YY 07	*P.domestica*	54.27	4	35.75	2	7.10	4	18.91	4	29.81	2	16.00
66	44 YY 09	*P.cerasifera*	49.12	3	52.51	4	8.38	5	14.40	2	26.18	2	16.00
67	23 KK 13	*P.divaricata*	33.65	1	53.22	4	4.15	1	12.02	2	47.67	6	14.00
68	44 YY 06	*P.domestica*	55.57	4	33.73	2	7.27	4	14.65	3	22.29	1	14.00
69	44 AK 13	*P.domestica*	42.95	3	24.51	1	6.87	3	19.48	4	28.08	2	13.00
70	44 YY 18	*P.domestica*	45.19	2	38.30	3	5.69	2	11.72	1	23.91	1	9.00
Minimum			33.65		19.01		4.15		9.25		19.54		9.00
Maximum			101.00		106.27		13.03		35.55		75.55		37.00
Average			58.48		50.24		8.64		18.80		46.08		
Standard deviation			13.4806		17.12727		1.646738		5.014842		15.44921		

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Aroma profiles and mineral composition of Buffalo kaymak collected from markets in the Çukurova region of Turkey

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Abstract

The aim of this study was to analyze the chemical composition in terms of dry matter, protein, fat and ash contents, mineral content by laser ablation inductively coupled plasma mass spectrometry and atomic absorption spectroscopy methods and aroma profile by solid phase microextraction (SPME) method of buffalo kaymak (clotted cream) samples collected from the Çukurova region in Turkey. The results of our analyses showed that the kaymak samples contained an average of 85.31% dry matter, 78.00% fat, 4.01% protein, and 0.44% ash. Forty volatile compounds were identified comprising four aldehydes, three ketones, eight acids, five alcohols, six esters, six amines, and eight miscellaneous compounds. The average compositions of Ca, K, Na, Mg, Cu, Fe, Mn, and Zn were 408.96 mg L^{-1}, 542.42 mg L^{-1}, 238.84 mg L^{-1}, 289.39 mg L^{-1}, 0.12 ppm, 5.65 ppm, 0.08 ppm, and 14.70 ppm, respectively. When comparing these results with those in the literature using of kaymak samples from different locations, the samples from the Çukurova region had higher dry matter, fat, Ca, K, Na, Mg, and Fe contents and lower Mn content.

Keywords: Traditional dairy product, Flavor, Mineral content

Introduction

Kaymak is a traditional dairy product that is produced mainly in eastern Turkey and central Anatolia (especially in Afyonkarahisar province) and is generally from either buffalo or cow milk. Kaymak is also known as “kajmak”, “kaimak”, “gemagh”, or “geymar” in some countries in the Middle East, Central Asia, India, and the Balkans (Simsek et al., 2018). The origin of kaymak, which has an important place in Turkish culinary culture, is based on the Ottoman Cuisine and it was a food sent as a gift to the rulers of other countries in that period (Demirgul, 2018). In Turkish cuisine, this product can be served at breakfast as plain (Çekal and Doğan, 2021), or can accompany some desserts (Zengin and Gürkan, 2019), as well as used instead of butter or oil in the preparation of some dishes (Sandikci and Ozkan, 2017), and used in the production of traditional desserts such as “Ekmek kadayıfi”, “Baklava” and “Lokum” (Demirgul, 2018). Especially “Afyon kaymağı” which is produced by buffalo milk in Afyonkarahisar province, is a product that revives gastronomy tourism today (Zengin and Gürkan, 2019). Kaymak is one of the most popular dairy products made from buffalo milk (Yaman et al., 2017) and contains at least 60% milk fat by weight (Kara and Ince, 2018). To produce kaymak, buffalo milk is heated to 95°C for 30 min and cooled to room temperature. One day after preheating, the milk is placed into a perforated container used to separate the cream layer from the milk. During this step, the milk is slowly heated again to 95°C for 45 min and cooled to room temperature, after which it is refrigerated for 1 night and the kaymak layer, which forms at the top, is separated (Saglam and Seker, 2018).

The Anatolian water buffalo (*Bubalus bubalis*) reared in Turkey are from the Mediterranean buffalo and are a subgroup of river buffalo (Gecgel et al., 2019). According to regional statistics, the total number of buffalo in Turkey in 2020 was 192,489 (head), of which 3,557 were in the Mediterranean region, which has the least number of head in Turkey. There were 437 and 55, respectively, head of buffalo in Adana and Mersin Provinces, or the Çukurova

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region located in the eastern Mediterranean, in 2020. The total amount of buffalo milk in 2019 was 79341 T in Turkey, 1181 T in the Mediterranean region, 155 T in Adana, and 24 T in Mersin. Thus, the production of buffalo milk and its dairy products is limited; therefore, these products are valuable in the Çukurova region.

Geographical location can result in differences in compositions of buffalo milk and its dairy products. Location affects the steps in processing the milk and the overall quality of the final product (Akgun et al., 2016). There are only a few studies on buffalo milk and dairy products from specific regions in Turkey. Bulut et al. (2010, 2011) have investigated organochlorine pesticide residues buffalo milk, buffalo butter, and kaymak samples from Afyonkarahisar Province. Kara and Ince (2014, 2018) have determined aflatoxin M1 levels in buffalo milk and kaymak samples from the same province. Kara and Ince (2016) have also researched malathion and malaoxon contaminations in buffalo milk samples from Afyonkarahisar province. Çınar et al. (2019) have studied the compositions of general and fatty acids in buffalo milk samples from six different provinces.

Only a few studies have been conducted on buffalo kaymak, especially on their mineral contents and aroma profiles. Kan and Küçükkurt (2018) have investigated the amount of heavy metals and Şenel (2011) has studied the aroma characteristics of Afyon kaymak samples.

In the Çukurova region, buffalo milk and its products are produced from very few family businesses; therefore, the number of products on the market is quite low. Buffalo milk is not easily accessible, especially in cities such as Adana and Mersin; therefore, these products are quite valuable in this region. With the slow food movement, the gastronomic value of these products is increasing in the region (Çavuş et al., 2019; Guzeler et al., 2020). There is no information on the characteristics of buffalo dairy products in the Çukurova region and limited published data on the mineral composition and aroma profile of buffalo kaymak samples; therefore, the present study was conducted to determine the mineral compositions and aroma profiles of kaymak samples collected from Çukurova markets. In terms of ensuring the sustainability of traditional products, it is thought that it is important to determine the chemical and aromatic components of these products. This study also provides information on some general compositional properties of kaymak samples.

Materials and Methods

The present study used 20 samples of buffalo (*Bubalus bubalis*) kaymak collected from 20 different local producers in Mersin and Adana Provinces in the Çukurova region in spring season in 2019. Samples were transported to Çukurova University, Faculty of Agriculture, Department of Food Engineering, Milk Technology Laboratory, and some of the compositional properties, mineral contents, and aroma profiles were assessed.

Chemical composition

The amount of dry matter was determined gravimetrically by drying the samples at 100°C until the weight was constant (IDF, 2005). Fat ratios of the samples were determined using the Gerber method (TSE, 2002). The micro Kjeldahl method was used to determine total nitrogen content, and total nitrogen results were multiplied by 6.38 to determine protein content (IDF, 2014). The percentage of ash content was calculated using ~5 g samples placed into porcelain crucibles, drying in an oven, and cooling in a desiccator before burning the samples at 550°C, cooling again in the desiccator, and weighing (Kurt et al., 2007).

Aroma profile analyses

Solid phase microextraction (SPME) fibers were preconditioned by placing on an injection port of the Agilent 7890B gas chromatograph (Agilent Technologies, Agilent, Avondale, USA) at 250°C for 5 min and then placed in a gas-phase flask for extraction for 60 min. Desorption from cheese samples was conducted at 250° for 3 min and a programmed temperature route was used for chromatography. The temperature was kept at 35°C for 3 min and then increased to 140°C at 4°C min^{-1}. The temperature was maintained at 140°C for 1 min and then increased to 250°C over 3 min. The transfer line temperature was set at 250°C. Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. For mass spectroscopy, electron ionization was conducted at 70 eV, the ion source temperature was 230°C, the mass scanning range was m/z 33–450 AMU, and the emission current was 100 µA (Dan et al., 2019).

Mineral composition

The concentrations of Cu, Fe, Mn, and Zn in the samples were determined using laser ablation inductively coupled plasma mass spectrometry (LA-ICP/MS) (Perkin Elmer Nexion 2000 P) as applied by Khan et al. (2014). For this task, 2 mg sample was placed into Teflon tubes and 5 mL 65% HNO_3 and 1 mL 30% H_2O_2 were added to the tube. The samples were heated in a microwave oven (Berghof Speedwave MWS-2, Eningen, Germany) at 170°C for 10 min, 200°C for 15 min, and 100°C for 10 min, after which 1 mL distillate obtained by heating in the microwave was removed and increased to 10 mL using distilled water. The mineral content was determined using LA-ICP/MS.

The Perkin Elmer PinAAcle 900T atomic absorption spectroscopy device (AAS, Norwalk, CT, USA) was used to determine Ca, Mg, K, and Na concentrations. Three grams of the sample were placed into Teflon cups and 5 mL deionized water and 5 mL concentrated HNO_3 were added. The cups were shaken to mix the solution and then placed into a microwave mineralization device. After heating, the samples were filtered through filter paper and completed to 50 cm^3 using deionized water. The samples were measured using the AAS device at specific wavelengths (Capcarova et al., 2017).

Results and Discussion

Chemical Composition

The mean values for the chemical compositions of the kaymak samples collected from Çukurova markets are provided in the Figure 1.

The average dry matter content in the kaymak samples from the Çukurova region was 85.31%, which was higher than that in buffalo kaymak samples assessed by Şenel (2011) from Afyonkarahisar Province (65.92%) and Kocatürk et al. (2019) from Kütahya Province (57.48–64.41%). Siddique et al. (2011) and Parmar and Khamrui (2017) have determined similar percentages (83.00–89.00 and 87.00%, respectively) for the dry matter contents in buffalo kaymak samples produced in Rawalpindi and Islamabad in Pakistan and Karnal in India.

The fat content of the buffalo kaymak samples from Afyonkarahisar and Kütahya in Turkey that were assessed by other researchers was quite a bit lower (at ~5.00–40.00% lower) than those in samples from the Çukurova region (Siriken and Erol, 2009; Kocatürk et al., 2019).

The protein content of the kaymak samples was similar to the findings (0.83–5.90, 3.30, and 3.50%, respectively) of other researchers from Afyonkarahisar Province (İzmen and Eralp, 1967; Yılmaz Baytok, 1999; Anon., 2005).

The ash content of the kaymak samples was also similar to the findings of other researchers (0.50 and 0.40%, respectively) from Afyonkarahisar Province (Yılmaz Baytok, 1999; Anon., 2005).

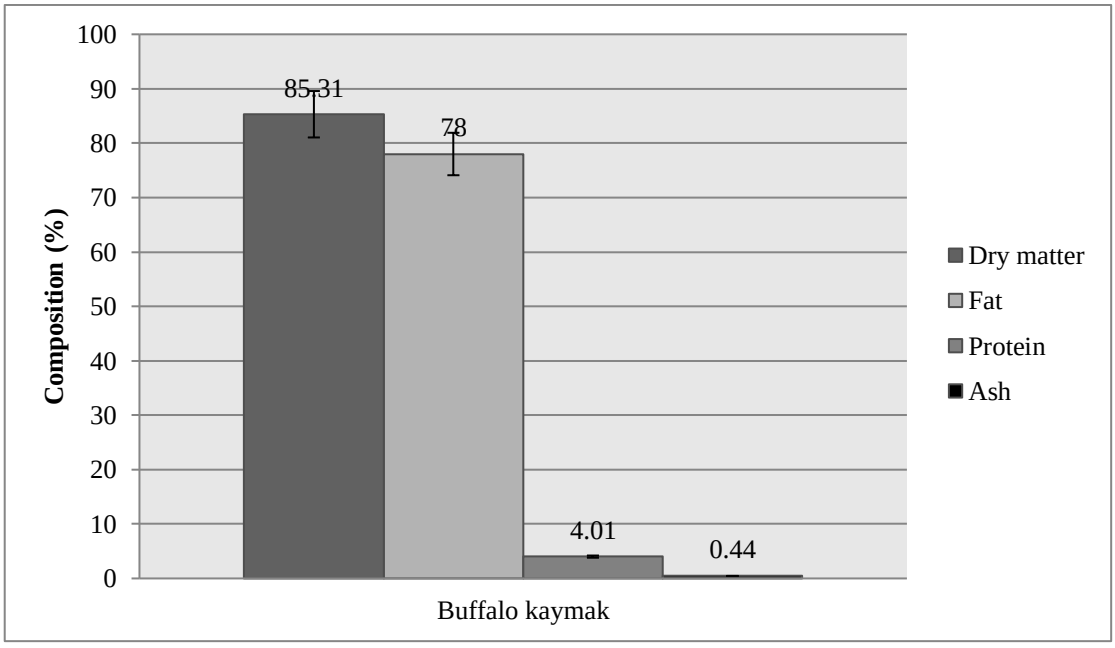

Figure 1. Chemical composition of buffalo kaymak samples collected from Çukurova markets.

Volatile aroma profile.

Forty volatiles comprising four aldehydes, three ketones, eight acids, five alcohols, six esters, six amines, and eight miscellaneous compounds were identified in the buffalo kaymak samples from the Çukurova region (Table 1).

Aldehydes

Aldehydes were not major compounds in the buffalo kaymak samples. Acetaldehyde had the highest level among the aldehydes; however, it was at lower levels than other volatiles in the samples. Acetaldehyde is produced by the activities of *Lactobacillus bulgaricus* in the mixed starter cultures (Chen, 2017), and these cultures are not used to produce kaymak; therefore, the level of acetaldehyde in buffalo kaymak is low. According to Tamime and Robinson (2007), acetaldehyde contributes a unique flavor in butter and cream products. Şenel (2011) has also identified acetaldehyde in Afyon kaymak samples made with buffalo milk and has stated that Afyon kaymak samples have low acetaldehyde concentrations that is close to that of butter because of the high fat content in kaymak. Peterson and Reineccius (2003), and Karaca et al. (2018) have reported acetaldehyde in butter samples. The presence of 3-methylbutanal depends on the activities of *Streptococcus lactis* var. *maltigenes* and causes a malty off-flavor in butter (Mallia et al., 2018). Lee et al. (1991), and Peterson and Reineccius (2003) have identified 3-methylbutanal in butter samples. Çakmakçı and Hayaloğlu (2011) have identified hexanal in Ispir kaymak samples made with cow milk.

Table 1. Flavor compounds of buffalo kaymak samples supplied from Çukurova markets.

Compounds		Relative peak area (%)	RT (min)	Molecular formula
Aldehydes	Acetaldehyde	1.210±0.254	4.466	C_2H_4O
	3-Methylbutanal	0.460±0.420	6.315	$C_5H_{10}O$
	Hexanal	0.163±0.022	14.751	$C_6H_{12}O$
	3-Hydroxybutanal	0.189±0.119	45.087	$C_4H_8O_2$
Ketones	2-Heptanone	5.102±3.024	16.330	$C_7H_{14}O$
	Acetoin	7.484±5.865	20.836	$C_4H_8O_2$
	2-Nonanone	0.778±0.255	25.466	$C_9H_{18}O$
Acids	3,4-Bis(methoxycarbonyl)benzoic acid	0.382±0.184	4.997	$C_{11}H_{10}O_6$
	Acetyl acetate;formic acid	0.214±0.034	27.904	$C_5H_8O_5$
	Butyric acid	11.855±7.785	34.176	$C_4H_8O_2$
	1,3,4-Trihydroxy-5-oxo-cyclohexanecarboxylic acid	0.213±0.118	36.251	$C_7H_{10}O_6$
	Hexanoic acid	9.825±6.095	41.994	$C_6H_{12}O_2$
	Heptanoic acid	0.176±0.020	44.981	$C_7H_{14}O_2$
	Caprylic acid	3.593±1.948	47.425	$C_8H_{16}O_2$
	Capric acid	0.743±0.376	51.813	$C_{10}H_{20}O_2$
Alcohols	Cyclobutanol	0.283±0.044	3.047	C_4H_8O
	3-Phenyl-2-butanol	0.128±0.035	14.379	$C_{10}H_{14}O$
	Isoamyl alcohol	4.073±3.120	17.597	$C_5H_{12}O$
	2-Ethyl-4-methylpentan-1-ol	0.242±0.067	29.181	$C_8H_{18}O$
	2,3-Butanediol	0.300±0.214	30.733	$C_4H_{10}O_2$
Esters	Ethyl acetate	19.578±5.225	5.655	$C_4H_8O_2$
	Vinyl formate	0.842±0.490	5.911	$C_3H_4O_2$
	Vinyl acetate	3.336±1.055	7.990	$C_4H_6O_2$
	Methyl butyrate	0.239±0.033	8.303	$C_5H_{10}O_2$
	Ethyl butyrate	4.046±1.775	10.143	$C_6H_{12}O_2$
	Ethyl hexanoate	1.804±0.741	18.812	$C_8H_{16}O_2$
Amines	sec-Butylamine	0.315±0.104	3.158	$C_4H_{11}N$
	1-Cyclohexylethanamine	0.385±0.109	3.277	$C_8H_{17}N$
	2-Amino-5-methylhexane	1.904±1.519	3.798	$C_7H_{17}N$
	1-Aziridineethanamine	0.182±0.026	4.349	$C_4H_{10}N_2$
	Octodrine	0.130±0.010	7.124	$C_8H_{19}N$
	2-Formylhistamine	0.289±0.117	24.037	$C_6H_9N_3O$
Miscellaneous	Carbon dioxide	10.531±2.701	2.901	CO_2
	Ethylene oxide	0.209±0.090	3.501	C_2H_4O
	Formamide	6.366±4.238	6.855	CH_3NO
	2-(Methylamino)ethanol	0.362±0.122	7.455	C_3H_9NO
	4,7,7-Trimethylbicyclo[4.1.0]hept-2-ene	0.139±0.049	9.614	$C_{10}H_{16}$
	D-Limonene	0.409±0.231	17.194	$C_{10}H_{16}$
	Oxime-, methoxy-phenyl-	0.527±0.111	39.196	$C_8H_9NO_2$
	1-Deoxy-d-mannitol	0.151±0.062	49.763	$C_6H_{14}O_5$

RT: Retention time

The values are expressed as the average ± standard deviation.

Ketones

Acetoin and 2-heptanone were the major ketones in the aroma compounds. Acetoin is a reduced form of diacetyl and produces a mild flavor and creamy consistency (Cheng, 2010). Diacetyl and acetoin are known as "butter aroma compounds" (Oberman et al., 1982). Ergöz (2017) has also identified acetoin, 2-heptanone (milk flavor), and 2-nonanone (cooked milk flavor) in buffalo butter and has suggested that acetoin contributes to form the characteristic aroma of butter from buffalo cream. Gokce et al. (2014) and Roosta et al. (2014) have reported the presence of acetoin in butter samples. Çakmakçı and Hayaloğlu (2011) have stated that 2-pentanone, 2-heptanone, and 2-nonanone are the most abundant ketones in Ispir kaymak samples. The presence of 2-pentanone was not observed in buffalo kaymak collected from the Çukurova region, but 2-heptanone and 2-nonanone were detected in all samples. Şenel (2011) has detected acetone, 2-butanone, and diacetyl in Afyon kaymak made from buffalo milk and none of these compounds were identified in buffalo kaymak samples collected from the Çukurova region; consequently, it is possible that the aroma profile and volatile compounds of these products can vary by location.

Acids

Acids were the largest group of volatile compounds in the buffalo kaymak, with butyric acid, hexanoic acid, and caprylic acid being the most abundant. Butyric acid contributes to the flavor of butter and cream and has many health benefits, such as anticancer properties (Kwak et al., 2013). Şenel (2011) has identified butyric, stearic, oleic, linoleic,

and linolenic acids as the characteristic free fatty acids in Afyon kaymak and has identified hexanoic, caprylic, and capric acids in the samples. Hashemi et al. (2017) and Kocatürk et al. (2019) have detected butyric, hexanoic, caprylic, and capric acids in kaymak samples made from cow milk and buffalo kaymak samples produced in Kütahya, Turkey. Çakmakçı and Hayaloğlu (2011) have identified butyric and hexanoic acids in Ispir kaymak. Erfani et al. (2020) have reported that butyric acid and heptanoic acid produce the buttery, sweet, cheesy, and rancid flavors, while hexanoic acid and caprylic acid produce the pungent, musty, cheesy, and rancid flavors in ghee. Ergöz (2017) has also identified butyric, hexanoic, and caprylic acids in buffalo butter samples.

Alcohols

Alcohols were not major compounds in the buffalo kaymak samples, the most abundant of which was isoamyl alcohol. This alcohol produces the fruity flavor in dairy products (Costa et al., 2019); however, no study was found on the presence of isoamyl alcohol in kaymak or butter samples. Çakmakçı and Hayaloğlu (2011) have reported the presence of ethanol, 2-furanmethanol, 2-pentanol, 1-pentanol, 2-heptanol, 2-propanol, 1-hexanol, 2-nonanol, and terpinen 4-ol in Ispir kaymak. Ergöz (2017) has identified butanol, n-hexanol, 2-pentadecanol, n-octanol, n-hexadecanol, and benzyl alcohol in buffalo butter samples. None of those alcohols were detected in the buffalo kaymak samples from the Çukurova region.

Esters

Esters were a large group of compounds in the buffalo kaymak, with ethyl acetate being at the highest level among all volatile compounds. Erkaya and Şengül (2011) have reported that ethyl acetate contributes a pineapple flavor. Ethyl butyrate and vinyl acetate were also important esters in buffalo kaymak. Çakmakçı and Hayaloğlu (2011) have reported that ethyl acetate, methyl butyrate, ethyl butyrate, and ethyl hexanoate were detected in Ispir kaymak samples. We could not find any study on the presence of vinyl acetate in the buffalo kaymak, cream, or butter.

Amines

Amines were not major compounds in the buffalo kaymak samples. It is known that in dairy products, lactic acid bacteria are the primary producers of amines (Perin and Nero, 2017). No studies were identified on the presence of amine groups in kaymak, cream, butter, or ghee samples, which could indicate their low concentrations and the absence of a starter culture in these products.

Miscellaneous

Carbon dioxide and formamide were the major miscellaneous compounds. It has been suggested that formamide contributes an ammonia-like flavor (Hohn, 1999). Li et al. (2020) have identified D-limonene in samples of butter made from cow milk. Demirkol et al. (2016) have detected oxime-, methoxy-phenyl- in butter samples collected from Çanakkale, Turkey. No information was found in the literature on the presence of other miscellaneous compounds in buffalo kaymak.

Mineral composition

The mineral contents in milk and dairy products vary depending on the animal species, breed, nutrition, lactation stage, and health (Paszczyk et al., 2019). The data on mineral composition in terms of major and minor elements in buffalo kaymak is provided in Table 2.

Ateteallah and Hassan (2017) have assessed the Na, Ca, and K levels of some dairy products made with buffalo milk, such as fermented milk beverage, cream, and cheeses, collected from Egypt. They have found that the average Na, Ca, and K levels in buffalo creams are 165, 176, and 242 ppm, respectively. The kaymak samples from Çukurova had higher levels of these minerals than cream samples from Egypt.

Kan and Küçükkurt (2018) have determined the levels of heavy metals in Afyon kaymak samples made from buffalo milk. According to their study, Afyon kaymak samples contained 0.09 mg kg^{-1} Cu, 2.72 mg kg^{-1} Fe, 0.56 mg kg^{-1} Mn, and 8.27 mg kg^{-1} Zn. The kaymak samples from the Çukurova region had higher Cu, Fe, and Zn levels and lower Mn levels than the Afyon kaymak samples.

Enb et al. (2009) have also investigated the heavy metal compositions in buffalo milk and milk products from Egypt. They found that Cu, Fe, Mn, and Zn levels in the buffalo cream samples were 0.922, 4.520, 0.360, and 19.570 mg kg^{-1}, respectively. They have also found that buffalo cream had higher Cu, Fe, Mn, and Zn levels than cream made from cow milk. The levels of Cu, Mn, and Zn in the kaymak samples from the Çukurova region were lower than those in creams from Egypt, while the Fe level was higher.

Table 2. Mineral contents of buffalo kaymak samples collected from Çukurova markets.

Minerals	Mean	SD	Min.	Max.
Ca (mg L^{-1})	408.96	47.36	361.14	455.85
K (mg L^{-1})	542.42	106.02	440.66	652.24
Mg (mg L^{-1})	289.39	33.58	255.40	322.55
Na (mg L^{-1})	238.84	73.82	153.61	281.92
Cu (ppm)	0.12	0.14	0.03	0.28
Fe (ppm)	5.65	3.31	2.22	8.84
Mn (ppm)	0.08	0.08	0.00	0.17
Zn (ppm)	14.70	7.01	8.55	22.34

Sayed et al. (2012) have also investigated the heavy metal composition in buffalo milk and some dairy products, such as cream, butter, ghee, and cheese, from Egypt. They have determined that the Mg levels in cream, butter, and ghee are 0.016, 0.010, and 0.0012 mg kg^{-1}; Cu levels are 0.986, 1.040, and 0.86 mg kg^{-1}; and Fe levels are 2.470, 2.54, and 3.91 mg kg^{-1}, respectively. They have indicated that Mn

levels are 0 mg kg^{-1} in buffalo cream, butter, and ghee samples. When the results were compared with these compositions in the kaymak samples, Mg and Fe levels in the buffalo kaymak were quite higher than that in the buffalo cream, butter, and ghee samples, while the Cu level was lower. Tokuşoğlu et al. (2004) have indicated that the Cu level in kaymak made from cow milk is 0.098 mg kg^{-1}, which is lower than that in buffalo kaymak from the Çukurova region.

Conclusion

Kaymak is a valuable product for Turkish cuisine in terms of gastronomy and culinary culture. It is very important to know the aromatic properties, chemical composition and mineral contents of this product in order to define the properties well and to ensure the sustainability of local variations of this product.

In this research, kaymak samples obtained from the milk of buffaloes grown in Çukurova region were examined, compared with other regions and countries and recorded. As a result of the research, it was determined that Çukurova buffalo kaymak has a higher dry matter and fat content than the kaymak of other regions and countries in the literature. Thus, the nutritional value of desserts, meals or commercial products obtained by using this kaymak will be higher. Kaymak is a product with a very short shelf life. For this reason, it is consumed by drying in some regions. The drying efficiency of Çukurova buffalo kaymak with high dry matter is much higher and the drying cost is less. Çukurova buffalo kaymak, which is close to the kaymak of other regions and countries in terms of protein content, can be put on the market as a functional product with increased nutritional value if it is enriched in protein.

It has been determined that the aromatic compounds of kaymak differ according to the regions. This is due to the milk composition that changes depending on the health status of buffaloes, lactation period, feeding, climatic and seasonal conditions. Unlike other regions, isoamyl alcohol, which gives the product a fruity aroma, has been detected in Çukurova buffalo kaymak. The presence of vinyl acetate in buffalo kaymak indicates an undesirable aroma originating from the packaging material. For this reason, we suggest that different packages than polypropylene packages can be used for these products on the market.

As a result of mineral analysis, it is thought that Çukurova buffalo kaymak can be enriched in terms of copper, manganese and zinc. Thus, an alternative functional product can be brought to the market.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

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Consent for publication

Not applicable.

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Exceedance probability assessment of bathing water quality standards in lake Van based on a geostatistical analysis

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Abstract

Monitoring bathing water quality (BWQ) is highly important in Turkey both for public health issues and tourism income. Lake Van is one of the largest lakes of Turkey and serves as one of the most important tourist attractions in the eastern part of Turkey. This study aims to assess critical bathing sites in Lake Van by using historical BWQ data that was collected twice a month during the swimming season from June 15 to August 31, between the years 2010 and 2020. To avoid public health hazards, it is very important to determine the spatial dimension of inland water pollution and provide visual tools for its presentation. Geostatistical data analysis and the determination of critical locations have been done by a spatial interpolation method, named Probability Kriging (PK) using Geographic Information System (GIS) based software ArcMap 10. Probability maps for exceeding the threshold values identified for the two microbiological water quality parameters of *Escherichia coli* and intestinal enterococci (IE) were generated, and used for the identification of four critical regions: İskele (4), west side of Gevaş (1), Edremit (3) and Muradiye/Erciş (7). Insufficient wastewater treatment plant capacity serving to high population in these regions may indicate the most pressing issues disturbing the BWQ.

Keywords: Geostatistics, Kriging, Bathing water quality, Lake Van

Introduction

Turkey is a semi-island Mediterranean country serving as the bridge between Europe and the Middle East, therefore, nationwide monitoring of the bathing water quality (BWQ) is highly important to protect public health and the aquatic environment and also to increase national tourism income. Along with the long coastline on the Aegean Sea, Mediterranean Sea, and the Black Sea, Turkey has several inland waters such as Lake Van, Lake Salda, Lake Sapanca, and Lake Hazar that are also used for recreational purposes. In Turkey, the Ministry of Environment and Urban Planning (MEUP, former Ministry of Environment and Forestry) is responsible to set the standards to be met by the bathing waters. Before 2006, BWQ standards were provided under the Water Pollution Control Regulation. In January 2006, Turkey released its first "Bathing Water Quality Regulation", in line with the former European Council Directive 76/160/EEC. Recently, in May 2019 Turkey revised its BWQ standards and released "the Regulation on the Management of Bathing Water Quality" that is in compliance with the current European Council Directive 2000/60/EC. The regulation aims to protect human health, environment and prevent pollution of bathing waters from all kinds of contamination especially microbiological (MoH, 2019). A striking example of such contamination is the occurrence of mucilage, which may host a variety of microbial species that potentially could spread marine diseases and economically harm local businesses (Danovaro et al., 2009, Keleş et al., 2021).

Pollution enters water bodies through different sources and is found in various forms. One of the most prevalent pollutions observed in water environments is fecal contamination coming from sewage and animals. Fecal contamination occurs because of poorly treated sewage, overflow of sewage due to the stormwater accumulation, faulty or leaky septic systems, runoff from the urban areas and the other sorts of waste that comes from the animals (EEA, 2018a). Using contaminated waters for swimming or recreational purposes may cause spread of illnesses due to the presence of pathogens. Indicator microorganisms are non-pathogenic

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microorganisms that are present strictly in pathogen-contaminated water. They do not multiply in water, but can be reliably detected even at low concentrations and are more numerous than pathogens and have similar survival times to pathogens (Thomas et al., 2007). Quantification of the indicator microorganism concentrations is preferred, because it is easier, faster, and more reliable than examining pathogens in water samples for assessing their potentially harmful impacts (Ortega et al., 2009). With the knowledge about the indicator organisms, it is aimed to forecast the probability of a pathogenic organism's existence. Although the use of bacterial indicators to measure water quality is common, there is no universal agreement on which indicator organisms are most useful, and there are no federal regulations that require a single standard for bacterial indicators. Total coliform, fecal coliform, and fecal streptococci have been used commonly as microbiological BWQ parameters. Yet, with the newest European regulation, *Escherichia coli* (*E. coli*) and Intestinal enterococci (IE*)* were chosen as fecal indicator bacteria to be monitored (EC, 2006).

Lakes among other aquatic environments are comparatively more stagnant and thus the impact of human activities on their water quality is greater in comparison to rivers. While investigating the water quality in the lakes, it is important to evaluate the biological, physical, and chemical parameters of the environment and their changes over time (Kaymak et al., 2021). Numerous research studies have been conducted so far to understand the causes of coastal pollution around the recreational waters, to find measures for its prevention, and to accurately monitor BWQ. For instance, an integrated approach of load estimation was used to determine pollution loads from nearby sources of domestic wastewater, runoff, and industrial wastewaters to estimate the total pollution load of Cartagena Bay in Columbia, and the study emphasizes the importance of calculating confidence intervals for each load value by combining different load estimation methods for land-based pollution loads in coastal areas (Tosic et al., 2018). In another study, remote sensing, and geographic information system (GIS) technologies were used together to assess pollution load on Burullus Lake via Landsat images. (El-Zeiny and El-Kafrawy, 2017). Coastal water pollution is a very complex phenomenon that is impacted by many different parameters, such as the configuration of the coastal area, hydrodynamic features of the coastal sediment, and local weather conditions (El Mrini et al., 2012; Mali et al., 2018). The accuracy of field monitoring is extremely important for studying such a complex natural phenomenon, and a lot of effort has been put to develop sensors, and remote sensing tools (Hafeez et al., 2019; Zielinski et al., 2009). An equally very important task is to develop data analysis tools that will enable decision-makers to perform accurate and reliable analyses leading to proper, and responsible decisions. Especially for countries such as Turkey, where tourism is quite important, the development of inexpensive, widely adaptable, and simple tools for BWQ data analysis is extremely important.

Geostatistics has been widely used for environmental data such as groundwater, soil, water quality related spatial data analysis (Jang, 2018). Geostatistics provides an accurate tool of BWQ estimation, especially where monitoring actions are limited and a high number of observations are impossible to obtain due to financial reasons ("Geostatistical Appl. Precis. Agric.," 2010). Kriging is one of the most commonly applied geostatistical interpolation methods (Bostan, 2017) and can be used for estimating BWQ in non-monitored areas (Malcangio et al., 2018; Jang, 2018). Probability kriging is a special form of kriging, which provides estimations of spatial data based on the available field data and a comparison to a threshold value. It provides the exceedance probability of the threshold limit value in a given location which then can be put into visual aids such as probability maps and provide visual and easy to follow tools for the general public or decision-makers. This study aims to use a geostatistical tool to estimate the general BWQ of the largest inland bathing site of Turkey, Lake Van, and determine the most critical bathing sites in the coastal zone of Lake Van. To this purpose, for the first time in the literature, historical microbiological water quality data collected during a ten-year period (2010 – 2020) from the coastal line of Lake Van has been processed via ArcGIS 10 software and the probability maps along with error estimations are presented.

Materials and Methods

Study Area

Lake Van is the largest body of water in Turkey with a total area of 3,750 km^2 and the depth of the lake reaches up to 450 meters (Doğan et al., 2016). The eastern part of the lake is in the territory of Van province, while the western part of the lake is in the territory of Bitlis province. Lake Van houses the most important inland bathing water sites of Turkey, and there are more than 30 beaches ("General Directorate of Public Health," 2021). Lake Van is located between the geographical coordinates of 38.5° N 43° E (Degens et al., 1984), which come up to the Eastern part of Anatolia in Turkey (Figure 1). Lake Van is the largest soda lake on earth (Tomonaga et al., 2017) and among the closed lakes around the world, it takes fourth place in terms of volume (607 km^3) (Kadioğlu et al., 1997). The water level is 1,648-meter-high according to sea level. The long axis of Van Lake which lies between the southwest of the Tatvan Bay and the Erciş Gulf in the northeast is 130 km and the axis between Ahlat Bay and Gevaş Bay is 80 km. The lake is surrounded by mountains (Özalp et al., 2016). The lowest place

on the edge of the lake is east of Reşadiye and it is 1800 meter-high. The shallowest parts of the lake are the Erciş Bay and Van Bay, where the depth of water is around 50 meters. A depth of 451 meters was measured between Ahlat and Adilcevaz. The water of Lake Van is bitter, salty, and soda rich. The main reason for this is the accumulation of salty water in the lake and the continuous condensation due to evaporation.

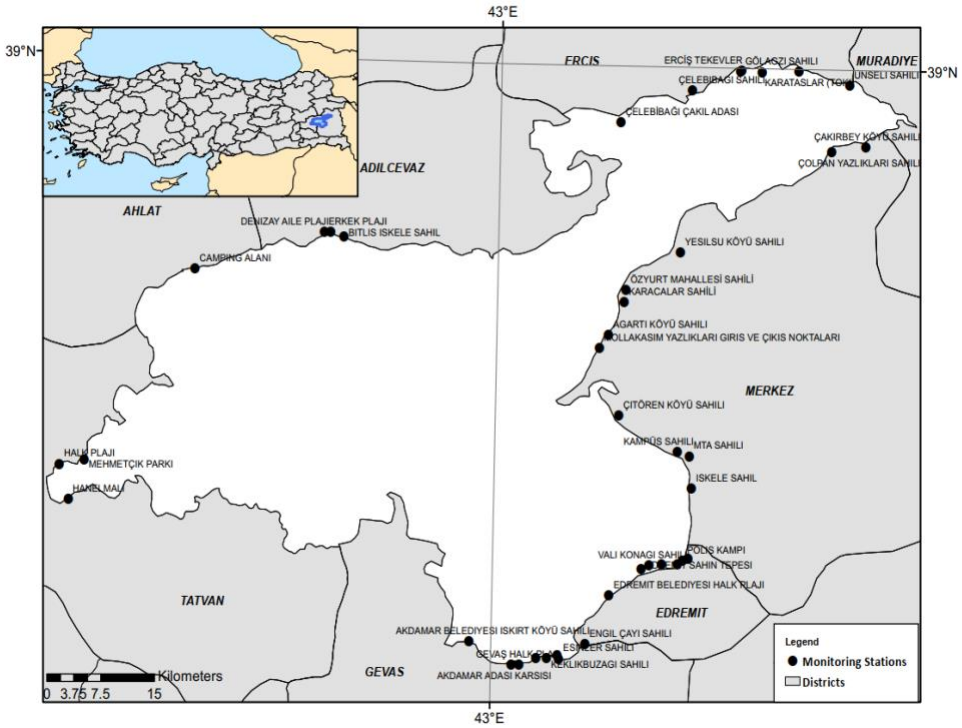

Figure 1. Map of monitoring stations and the study area, Lake Van

Sample Collection and Data Set

There are 40 monitoring stations in the Van Lake coastal line (Figure 1), and the data used in this study has been obtained from the Ministry of Health of the Republic of Turkey ("General Directorate of Public Health," 2021). Water samples were collected twice a month from each monitoring station during swimming season periods (June 15 - August 31) between 2010 and 2020. As stated in the Bathing Water Quality Regulation, samples were taken from each monitoring station, 15 days before the start of the swimming season (MoH, 2019). In order to represent the swimming conditions as much as possible, the sample must be taken at least 1 meter deep and 30 cm below the water surface. Within the scope of this study, 40 monitoring stations and a total number of 2,325 records were used.

Geostatistical Approach

The application of probabilistic methods to region-based variables has been known as geostatistics, which implies that any of these region-based variables have random and spatial properties (Varouchakis, 2019). The technique aims to develop a model for the spatial pattern, with the help of the variogram, since the variogram defines the spatial variability of the random variables between two points (Narany et al., 2014). The empirical variogram that is used for the analysis of the spatial variability between two points is defined as follows (Goovaerts et al., 2005);

$$\boldsymbol{\gamma}(\boldsymbol{h}) = \frac{\mathbf{1}}{\mathbf{2}\,\boldsymbol{N}(\boldsymbol{h})} \sum_{\boldsymbol{\alpha}=\mathbf{1}}^{\boldsymbol{N}(\boldsymbol{h})} [\boldsymbol{z}(\boldsymbol{x}_{\boldsymbol{\alpha}} + \boldsymbol{h}) - \boldsymbol{z}(\boldsymbol{x}_{\boldsymbol{\alpha}})]^2 \quad (1)$$

In Equation 1, *N (h)* represents the number of data pairs, h is the lag vector and z (x) is the value of the spatial variables at the data collection points. The experimental variogram needs to fit the theoretical variogram, which may be defined with eleven different functions.

Probability Kriging

The method used under this study for estimations of exceedance probability is called probability kriging (PK). PK is a modified version of co-kriging where only one variable is estimated using two spatial variables, indicator and uniform. The uniform value is defined as the z (x_α) in Equation 2 and the other variable is defined as I (x_α). The probability kriging equation is defined as (Adhikary et al., 2010);

$$\mathbf{I}(\mathbf{x_0}) = \sum_{\boldsymbol{\alpha}=\mathbf{1}}^{\mathbf{n}} \boldsymbol{\lambda}_{\boldsymbol{\alpha}}\, \mathbf{I}(\mathbf{x}_{\boldsymbol{\alpha}}) + \sum_{\boldsymbol{\alpha}=\mathbf{1}}^{\mathbf{n}} \boldsymbol{\xi}_{\boldsymbol{\alpha}}\, \mathbf{z}(\mathbf{x}_{\boldsymbol{\alpha}}) \quad (2)$$

where λ_α and ξ_α are the weights associated with the two spatial variables.

For decreasing the variance of the error and ensure unbiased conditions the weights are defined

as $\sum \lambda_\alpha = 1$ and $\sum \xi_\alpha = 0$. PK can be used to produce a probability map of the occurrence that exceeds the specified threshold value at a specified location. This method is appropriate to determine the critical bathing sites, which may exceed the threshold of BWQ set by the authorities, and also provides a standard error map. This information is useful to decision-makers especially when they need to make decisions on where and how many monitoring stations should be opened. Based on the provided maps, decision-makers can also decide whether they should close some bathing sites for remediation immediately, or to monitor slightly problematic areas with an increased frequency of sampling.

In spatial data analysis, a semivariogram is used to illustrate the spatial correlation of collected data points and yet in kriging, the experimental variogram may not be used directly since the kriging algorithm requires a model fit to describe the continuity of the data. To this purpose, several positive definite models (*i.e.,* mathematical functions) are used in the modeling step. Geographic Information System (GIS) based software ArcMap 10 was used to get semivariogram parameters for a total of eleven theoretical models including but not limited to exponential, stable, spherical, and k-Bessel. Among these eleven model options available in the software, best fitting models were chosen considering error functions for both *E. coli* and IE datasets. Specifically, the best-fitting model for each data set was founded by the comparison of the mean standardized error (MSE) and root mean square standardized error (RMSSE) values. For choosing the best model, MSE should be closed to zero (0) and the RMSSE should be close to 1 (McCoy and Johnston, 2002). The MSE and RMSSE were defined as (Audu, 2015; Zhang and Wang, 2010);

$$\mathbf{MSE} = \frac{\mathbf{1}}{\mathbf{n}} \sum_{\alpha=1}^{\mathbf{n}} \left\{ \frac{\mathbf{z}^*(\mathbf{x_\alpha}) - \mathbf{z}(\mathbf{x_\alpha})}{\mathbf{p_\alpha}} \right\} \quad (3)$$

$$\mathbf{RMSSE} = \sqrt{\frac{\mathbf{1}}{\mathbf{n}} \sum_{\alpha=1}^{\mathbf{n}} \left\{ \frac{\mathbf{z}^*(\mathbf{x_\alpha}) - \mathbf{z}(\mathbf{x_\alpha})}{\mathbf{p_\alpha}} \right\}^2} \quad (4)$$

where z (x_α) are actual values, z* (x_α) are estimated values, n states the number of observation points and p_α is the standard error prediction at the corresponding location

Determination of the Critical Bathing Sites

Coastal and inland bathing waters in Turkey are monitored and regulated through the provisions of Regulation on the Management of Bathing Water Quality (MoH, 2019). In the previous regulation, the parameters of BWQ were chosen as Total coliform, fecal coliform, and fecal streptococci. However, based on the current regulation, Turkey switched to the *Intestinal Enterococci* (IE) and *Escherichia coli* as BWQ parameters. According to the most recent regulation, FC and FS are considered equivalent to *E. coli* and IE, respectively. As a result of this, in all data up to 2019, the BWQ was taken in terms of FC and FS, as of 2020, the data sets were switched to IE and *E. coli*. According to the current regulation, waters used for recreational and swimming purposes must meet BWQ criteria given in Table 1. The 95-percentile evaluation process used in this study is defined as per the regulation (MoH, 2019) (i) taking the log10 of the dataset and if the obtained logarithmic value is equal to zero, take the minimum log10 value which is equal to 1, (ii) calculation of arithmetic mean of the log10 values (μ), (iii) calculation of the standard deviation of log10 values (σ). After these three steps, 95-percentile is calculated with antilog (μ + 1.65 σ).

Table 1. BWQ standards to be satisfied for "excellent" quality inland bathing waters in Turkey (MoH, 2019)

Parameter (CFU/100 mL)	**Guide Value**
Escherichia coli	500*
Intestinal enterococci	200*

* 95-percentile should be taken into consideration.

With a conservative approach, the threshold values were selected as the "excellent" quality parameters defined by the most recent Bathing Water Quality regulation, which is 500 CFU/100 mL for *E. coli* and 200 CFU/100 mL for IE (Table 1). The probability maps for the exceedance of identified thresholds are plotted and lead to the identification of the critical bathing sites of the study area.

Results and Discussion

Data Set Analysis

Data were taken from the Ministry of Health between the years 2010 and 2020. Prior to data input to GIS, the collected data were listed according to their location, then the geographical location *i.e.* XY coordinates of each monitoring site was assigned. Each BWQ parameter was expressed in the terms of Colony Forming Units (CFU) per 100 mL *i.e.*

CFU/100 mL. Table 2 represents important properties such as maximum, minimum, and mean values of the *E. coli* and IE data sets used in this study. The distribution of the data shows that the null values and exceedance numbers of threshold limits for the study area were widespread (Figure 2 and Figure 3).

Table 2. Characteristics of raw datasets

Parameter	*E. coli*	IE
N	2,325	2,325
Maximum	10,000	10,000
Minimum	0	0
Mean	49	181
Stdev	385	751

N: total number of measurements
Stdev: standard deviation
All concentration units are in CFU/100 mL.

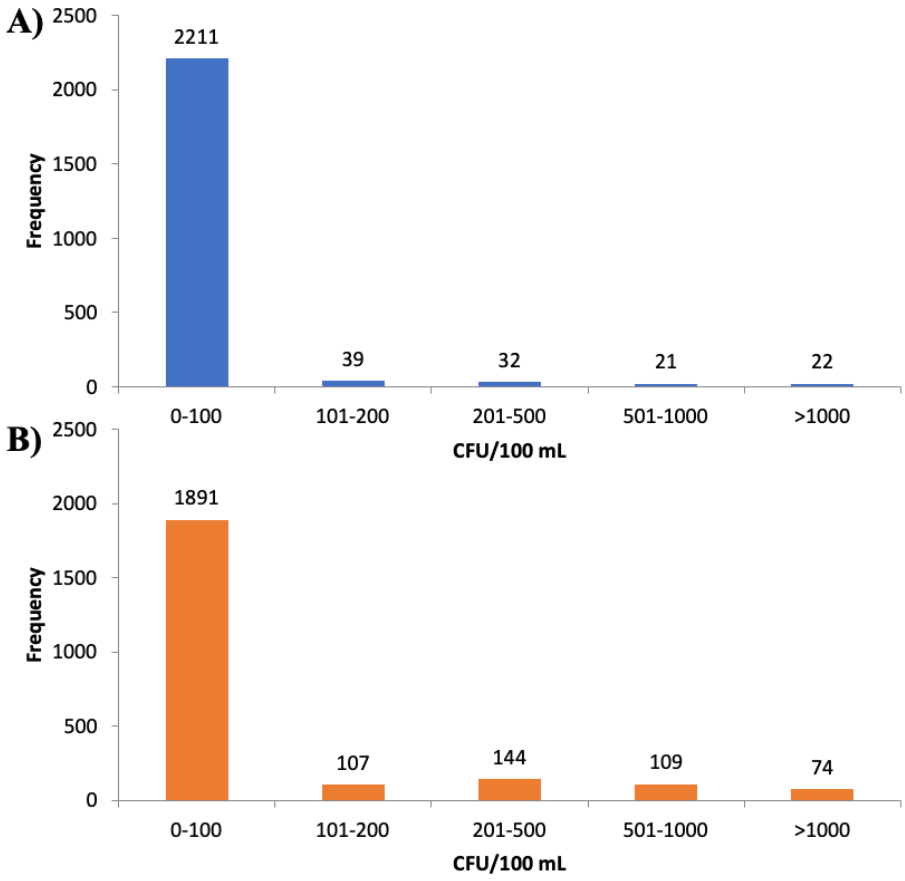

Figure 2. The histogram of the monitored parameters A) *E. coli* and B) IE

The complete dataset was used in the histogram (Figure 2) and the exceedance plot (Figure 3). As shown in Figure 2A, 98% of the data collected fall below the guide value of 500 CFU/100 mL for *E. coli*, indicating Lake Van water quality mostly was not a concern regarding this parameter. In the IE dataset, 86% of the data collected fall below the guide value of 200 CFU/ 100 mL specified for this parameter. The temporal variation of the exceedances provided in Figure 3B shows that most of the exceedances occurred in 2015 and 2016 for the IE parameter. For the *E. coli* data set (Figure 3A), a relatively higher number of exceedances were recorded between 2015 to 2018 over the 10-year period.

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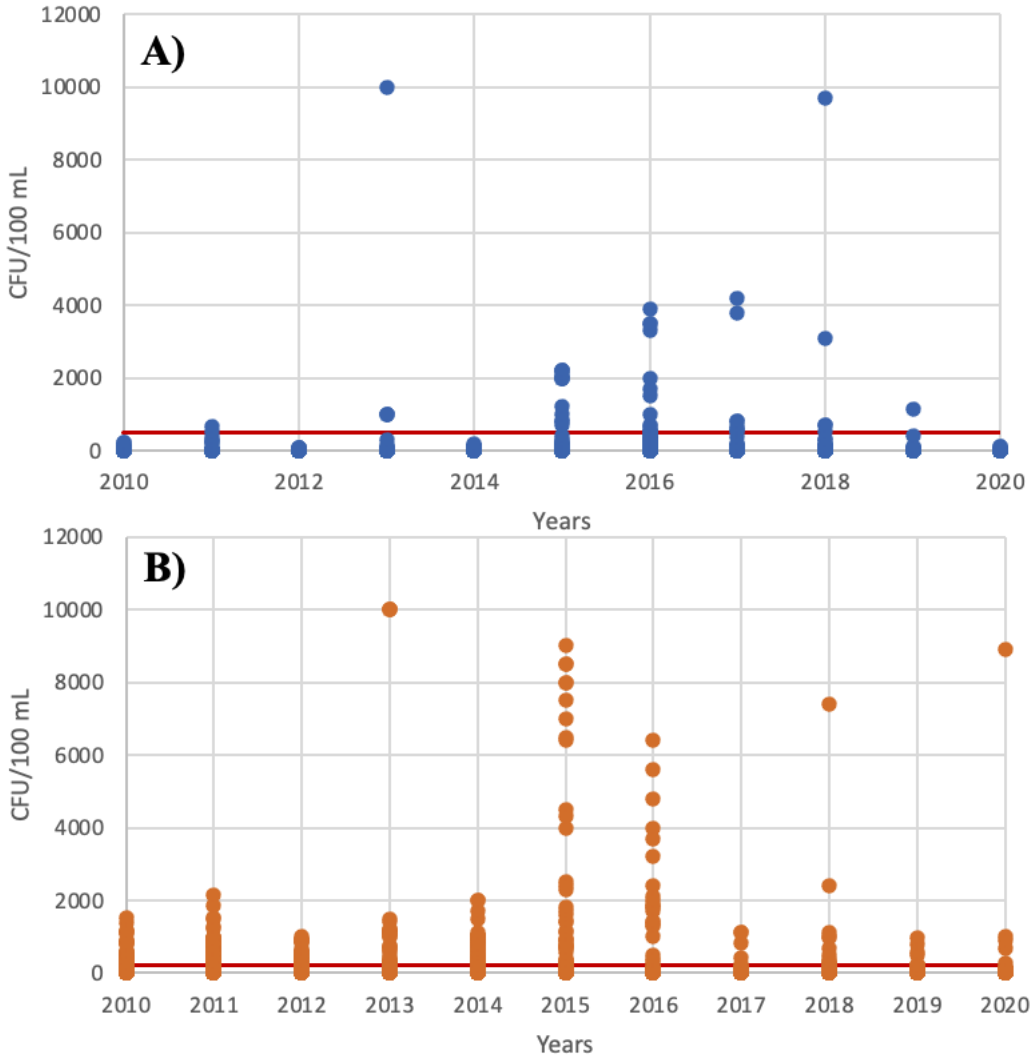

Figure 3. Temporal changes of exceedance number of threshold limits for A) *E. coli* and B) IE. The threshold limit value for each parameter is represented by the red line (Table 1).

Geographical Information System (GIS) Analysis

PK provides a value of probability that the regulatory limit of BWQ standards (the threshold limit for each BWQ parameter) is overcome at interpolation points. Probability maps that represent the prediction to exceed the threshold values were generated according to the semivariograms shown in Figure 4. As described in the Materials and Methods part, best-fitting model is chosen according to MSE and RMSSE values (Table 3).

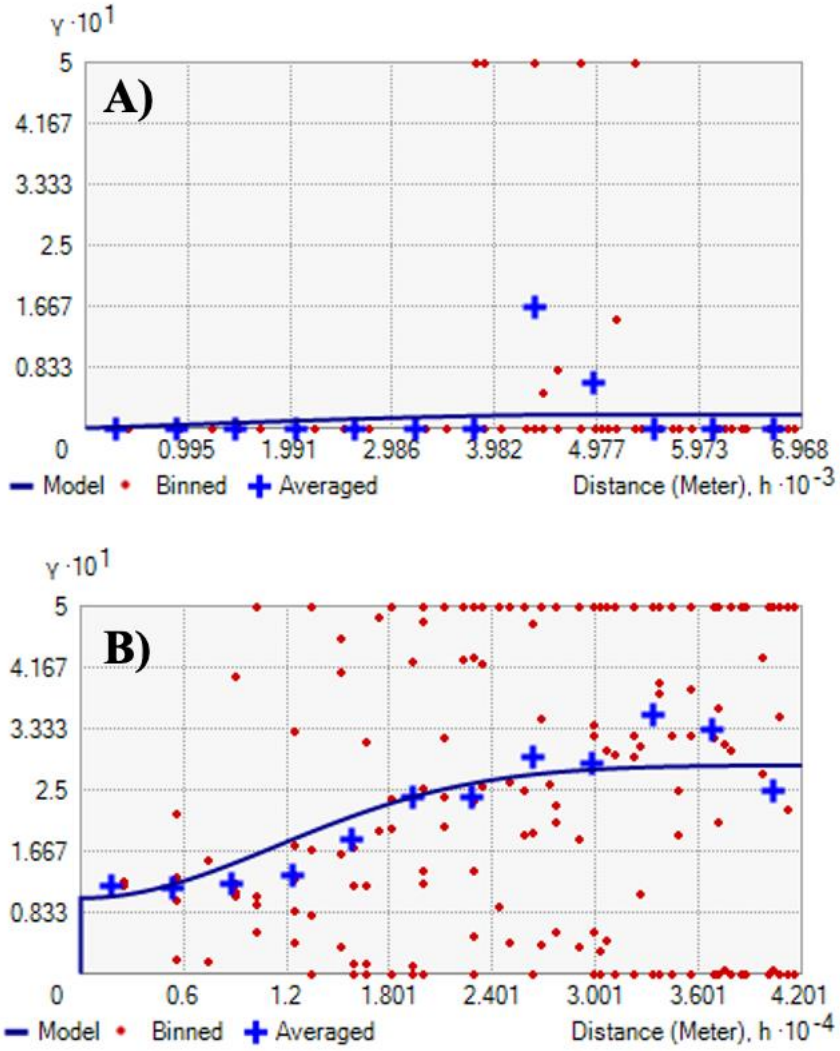

Figure 4. Variogram of datasets A) *E. coli* and B) IE

Table 2. Chosen semivariogram parameters and error values

BWQ parameter	Fitted Model	MSE[a]	RMSSE[b]	Range (m)	Nugget	Partial sill
E. coli	Circular	-0.100	1.008	4,645	0	0.0184
IE	Gaussian	0.044	1.010	5,000	0.1029	0.0180

a Mean standardized error
b Root mean square standardized error

Using PK, the probability maps for exceedance of thresholds are presented in Figure 5. For a better interpretation of results, the study area was divided into nine designated areas listed in Table 4, and also raw data properties that were presented for complete dataset in Table 2 was examined for each designated area in order to provide information on the raw datasets in addition to the 95-percentile evaluation. According to Table 4, mean values of *E. coli* were below the threshold limit for all of the designated areas, however, for the mean concentration of IE in four regions namely, west side of Gevaş (1), Edremit (3), İskele (4), and Muradiye/Erciş (7) were above the threshold limit. Based on this, there is an apparent high expectation to observe a higher probability of exceedance of IE threshold limit yet to confirm spatial analysis have been conducted. For the *E. coli* dataset, Figure 5A shows the most critical location as Region 4, İskele where the mean concentration is the highest with 132 CFU/100 ml (Table 4).

Table 3. Data set analysis of designated areas in study area

Designated Area		West side of Gevaş	East side of Gevaş	Edremit	İskele	Agartı/ Çıtören/ Mollakasım	Northern side of Merkez	Muradiye/ Erciş	Adilcevaz/ Ahlat	Tatvan
Region Number		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
E. coli	N	186	196	546	268	282	246	370	138	93
	Max	10,000	2,100	4,200	3,900	300	300	3,500	700	400
	Min	0	0	0	0	0	0	0	0	0
	Mean	98	18	46	132	8	6	78	8	9
	Stdev	1,018	88	290	504	34	27	357	60	43
IE	N	186	196	546	268	282	246	370	138	93
	Max	10,000	810	7,000	10,000	2,500	1,000	9,000	500	550
	Min	0	0	0	0	0	0	0	0	0
	Mean	175	78	194	603	53	39	199	26	38
	Stdev	918	227	546	1592	198	112	898	52	70

N: total number of measurements

Stdev: standard deviation

All concentration units are in CFU/100 mL.

A maximum probability for exceedance of threshold value of 80% to 100% percent was found for the İskele region (4). In the other regions, the exceedance probability of the BWQ of Lake Van does not exceed 10%. North and south sections of the İskele region (4) are likely to exceed the threshold value by 30 to 60 percent due to their proximity to this identified critical area. For IE, critical site numbers and the exceedance probabilities were higher when compared to the *E. coli* dataset as expected based on the histogram (Figure 2B) frequency of the exceedances plot (Figure 3B) for this dataset. As shown on the probability map (Figure 5B) a significant portion of the coastal area of Lake Van including the west side of Gevaş (1), Edremit (3), İskele (4), Muradiye/Erciş (7) depict a probability of exceeding the threshold limit close to 100%. Among these critical sites, Iskele region (4) was also identified as a critical site according to the *E. coli* dataset. Therefore, definitely, more attention must be paid to this particular area. The authorities such as European Commission and World Health Organization (WHO) discuss whether monitoring of both *E. coli* and IE is necessary for monitoring of water quality in bathing sites is necessary or if one of these parameters is sufficient (WHO, 2018; Tiwari et al., 2021). Even though early work suggested monitoring of only *E. coli*, the results presented herein clearly indicated the importance of monitoring both *E. coli* and IE simultaneously for the case of Lake Van. There are also areas that show qood water quality in the Lake Van coastal zone, for instance, the probability of exceeding the threshold value in the east side of Gevaş (2) was relatively low when compared to the neighbors of this site. Although Mollakasım region (5) and northern side of Merkez (6) are located in between the critical areas, the probability of exceeding the threshold value does not exceed 10% in these regions. The other regions Agartı (5), northern side of Merkez (6), Adilcevaz (8), and Tatvan (9) regions depict a low probability of exceeding the threshold limits.

In summary, based on the information presented on the exceedance probability maps (Figure 5), the territory of İskele region (4) is identified as the most critical area, since it shows a high threshold overcoming probability for both parameters. This could be due to the presence of a chronic problem in this area. Regions of the west side of Gevaş (1), Edremit (3), and Muradiye/Erciş (7) also need attention since they show high overcoming probabilities based on the IE dataset. In another recently published work focusing on Lake Van water quality, similar results were attained for the year 2015 (Aydin et al., 2021). In their study, researchers collected samples between June and September 2015, and analyzed over 200 water samples for their total coliform, fecal coliform, and enterococcus content, which indicated lowered water quality in the same 4 regions and additionally in Region 5 namely, Agartı/Mollakasım/Çıtören (Aydin et al., 2021). Data used in our study covers years between 2010 – 2020 and the analysis conducted herein clearly indicates the problems with İskele (4), Gevaş (1), Edremit (3) and Muradiye/Erciş (7) were chronic; yet, over the ten-year period Region 5 conditions were improved since 2015.

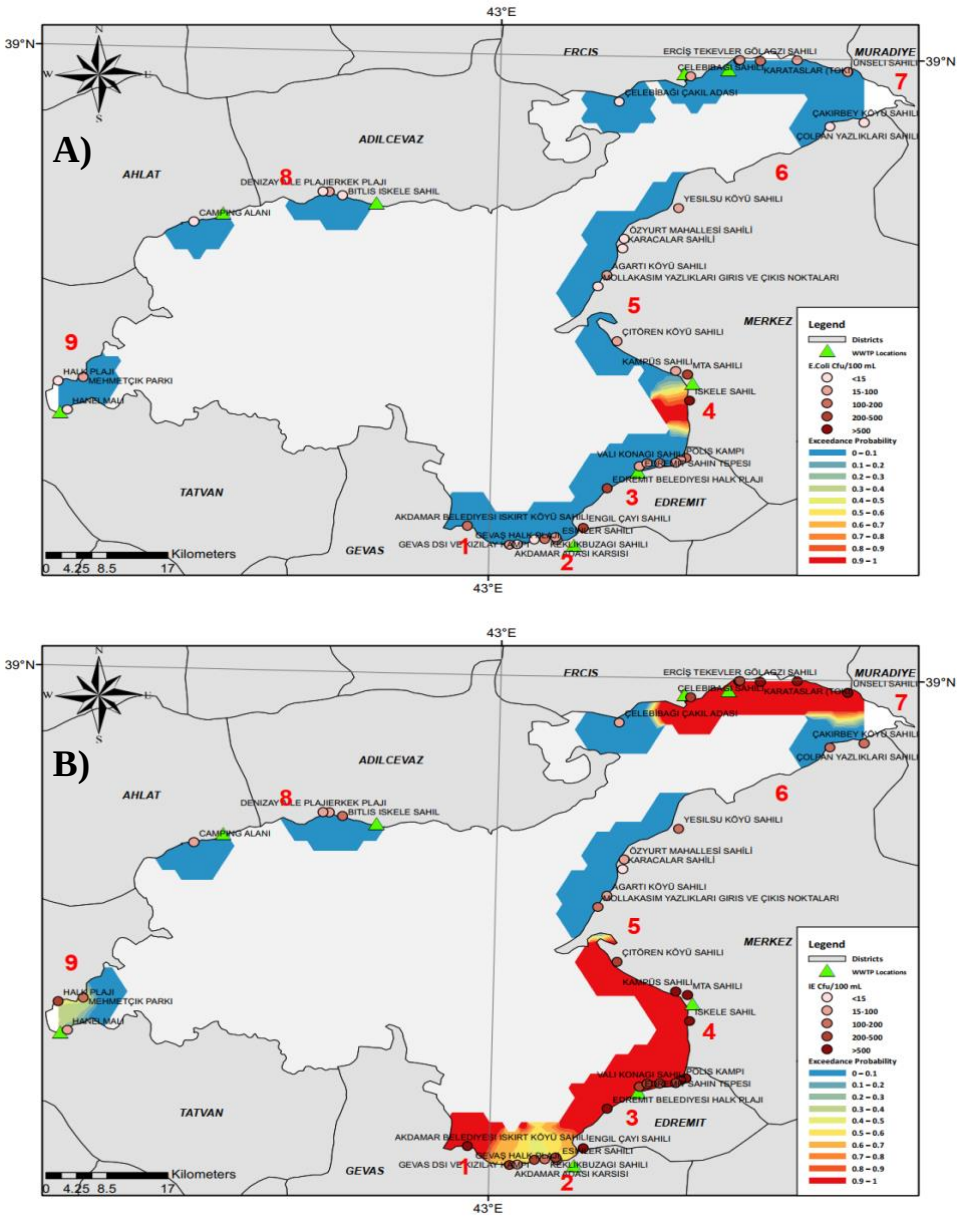

Figure 5. Probability map of threshold exceedance for datasets of A) *E. coli* and B) IE

GIS also provides a standard error map in the form of error variance. PK tool of GIS uses the methodology of an indicator variable, which is a binary, and computes maps of probability by classifying the dataset to 0 or 1 based on the threshold value. If the values are less than the threshold they are specified as 0, and if the values are higher than the threshold then they are set a value of 1. After the interpolation of the variable, the expected value of the variable is calculated by the prediction map. This expected value may be considered to exceed the threshold value of the expected variable. So, the error map represents the error of the probability that the threshold value is exceeded. Figure 6 shows the error maps for each BWQ parameter. As expected, the error percentage for two BWQ parameters is increasing while moving away from the coastal zones (Figure 6). Also, the percentage error is increasing with increasing distance between the two monitoring stations. Since our range was calculated as 4,645 meters for the *E. coli* dataset and 5,000 meters for the IE dataset in Table 3, if the number of monitoring stations within this range is high, the margin of error is minimized. This means that increasing the frequency of the monitoring stations will result in more reliable outputs. To give an example, sites like Camping Alanı in Region 8 and Çelebibağı Çakıl Adası (in Erciş, West of Region 7) has not enough monitoring stations within their range that is the reason why they have the highest error in the probability maps. Also, between the west side of Gevaş (1) and Tatvan (9)

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the frequency of monitoring station number is significantly less than the frequency of stations between the Edremit (3) and İskele (4), which has the lowest error percentage for all parameters. Since the frequency of the monitoring stations has higher in the İskele region, error for IE stands within a range of 32-36%. On the other hand, the error range is between 45 to 50% between Region 1 and Region 9 since there are not enough monitoring stations along this path. In the *E. coli* dataset, the standard error of the predictions is lower, because lower number of exceedances were recorded in comparison to the IE dataset. Nevertheless, İskele Region (4) is proven to be a problematic area since the error of prediction for this region is around 10 % and 30 % for *E. coli* and IE datasets, respectively.

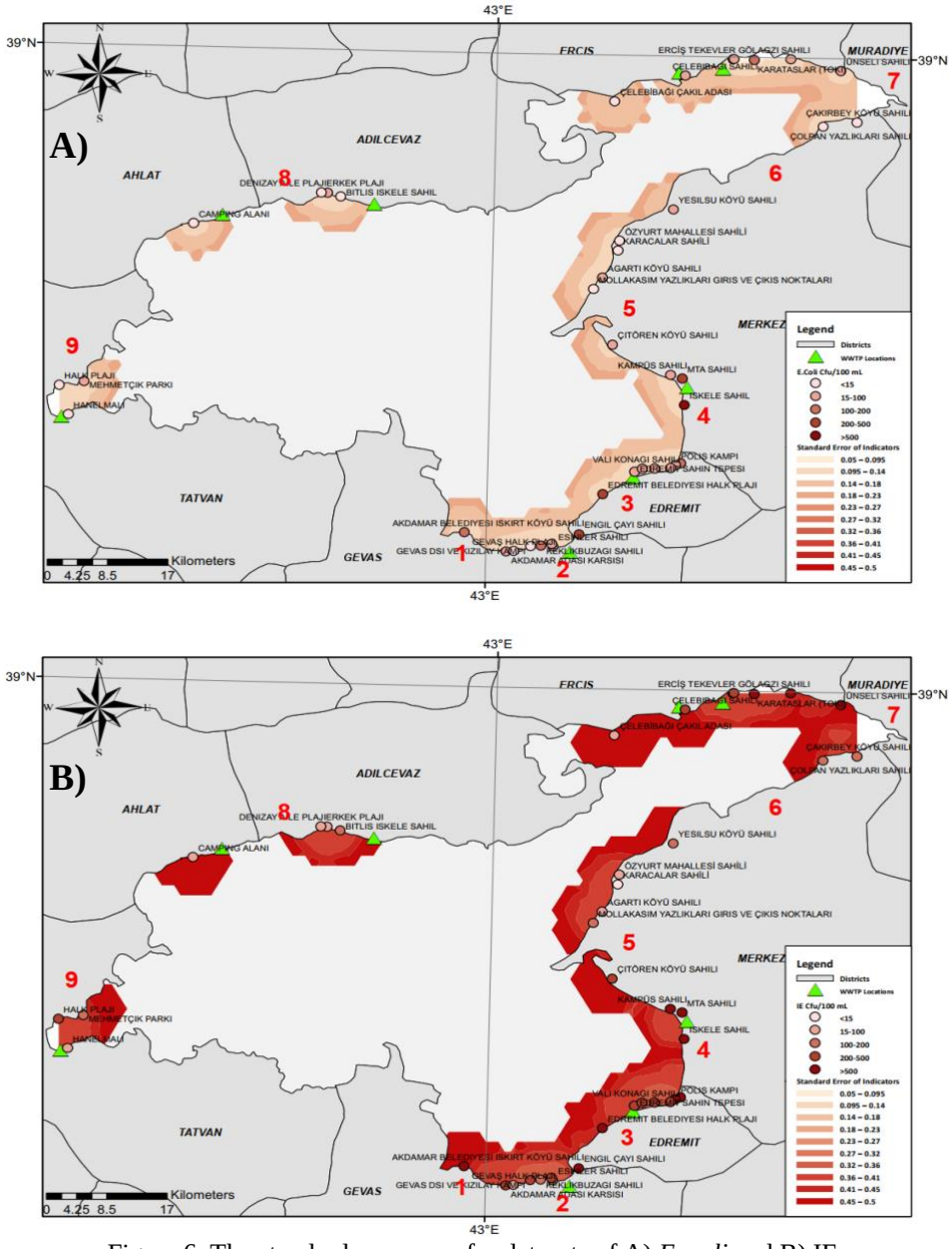

Figure 6. The standard error map for datasets of A) *E. coli* and B) IE

Even though the use of microbiological indicators for prediction of water quality in Lake Van provides information about the presence and the degree of fecal contamination; unfortunately, source tracking by just monitoring these parameters is impossible. For source tracking, culture-independent measurement methods such as real-time quantitative polymerase chain reaction (q-PCR) based assays are necessary, however, they are not preferred due to the economic burden on monitoring agencies (Tiwari et al., 2021). Lake Van is a closed water body, therefore, discharges and water draining from farms to the lake could make a significant contribution to its pollution. To evaluate the impact of the wastewater treatment plant (WWTP) discharges on the lake the discharge locations of WWTPs were also shown in probability maps generated (Figure 5). The treatment capacity of a WWTP is designed based on the population served, thus the capacity of each WWTP is different. Therefore, the load of the

discharge to the lake from each WWTP may differ. The WWTP that is located in the İskele (4) district has the highest loads of discharge, and is serving to the highest population in the Lake Van area. Mostly, there is a strong correlation between the discharge, and the quality of nearby surface waters, thus, there is a need to assess the treatment performance of the WWTPs in the identified critical areas (Sanders er al., 2013). In fact, recently a monitoring study has been conducted in Edremit coastline to determine any potential negative impacts of WWTPs on Lake Van through impact analysis, and the results indicated that the discharge from Edremit WWTP significantly impacts lake water quality especially in terms of the provided organic pollution load (as measured by chemical oxygen demand and biological oxygen demand) (Ozguven and Yetis, 2020). Clearly, in the case of the Lake Van area, especially for the Edremit (3), İskele (4) and Erciş (7) regions, there might be a need for an additional WWTP or a capacity increase of the current WWTP. In another recent study, where heavy metal pollution over the Edremit coast (Region 3) of Lake Van is investigated, it was stated that the chromium and copper concentrations measured in the effluent water of Edremit WWTP are at a level that exerts pressure on Lake Van (Yetiş and Özgüven, 2020). Additionally, in a recent field study, the presence of animal-related pollution was reported for Karasu river (freshwater) at the specific location, where it is flowing into Lake Van, *i.e.* between Çıtören Köyü beach and Kampüs beach on Figure 5 (PEMAT, 2018). During the fieldwork, the measurements of water quality parameters confirmed the presence of pollution, and the lake water was classified under Class IV (the most polluted) according to the Turkish Water Pollution Control Regulation (MoEF, 2004).

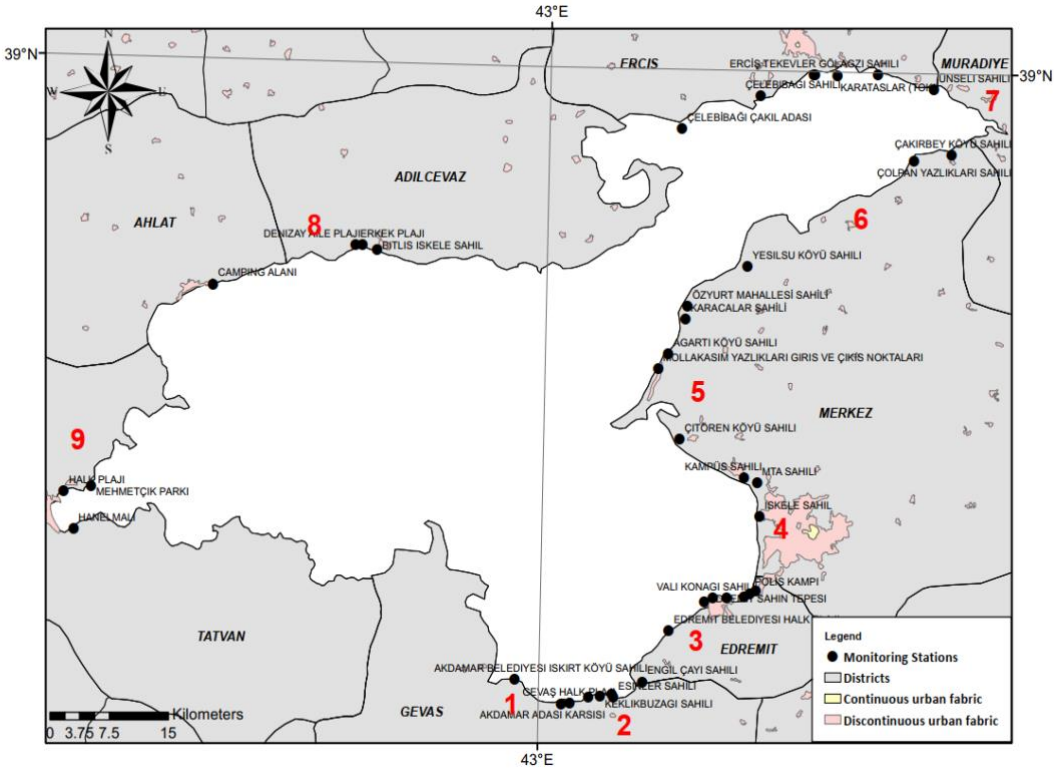

Figure 7. Urbanization Map around the Lake Van

Another supporting information is gathered by plotting the data about the urban and rural population densities that were obtained from the Coordination of Information on the Environment (CORINE) available as a free source. The data which was taken from CORINE (EEA, 2018b) illustrates the Continuous Urban Fabric and Discontinuous Urban Fabric (Figure 7). Continuous Urban Fabric is the class assigned to residential buildings, public service and commercial buildings and intercities of non-vegetated or bare surfaces. In addition to that, Discontinuous Urban Fabric illustrates individual houses, small and large blocks of flats, parking areas and such (EEA, 2020). Based on this information, it can be said that the urban fabric is a good tool for the assessment of the population around a given area. As it can be seen from Figure 7, there were 3 critical locations around Lake Van based on population. These 3 locations are İskele (4), Erciş (7) and Edremit (3) regions of the lake coastal zone. Apparently, there is a link between the disturbed BWQ parameters and population (Figure 7), which may indicate that there needs to be additional measures taken in order to prevent contamination of nearby water bodies in highly populated urban areas.

Conclusion

In this study, the critical bathing sites in the Lake Van coastal zone, that may pose risk to swimmers were identified via geostatistical evaluation of microbiological water quality data collected for ten years from 2010 – to 2020 by the Ministry of Health. Data collected from 40 monitoring stations were critically analyzed in terms of BWQ parameters, namely *E. coli* and IE. The results obtained in this study prove that PK is a good tool for evaluation of the critical sites of BWQ and can be effectively used by the decision-makers to determine the critical locations, where a higher number of monitoring stations are needed. In these critical sites, an increased frequencies of the sample collection may be necessary to ensure the safety of the swimmers and the locals. The most critical site of Lake Van is identified as İskele (4) region, with the highest percentage of exceedance probability of the regulatory standards observed in all BWQ parameters. The other three critical sites are regions of the west side of Gevaş (1), Edremit (3), and Muradiye/Erciş (7), which need more attention. The overlap between the exceedance probability maps, and increased urbanization underlines that highly populated areas may represent the high-risk areas especially related to the capacity of the WWTPs. Therefore, especially in highly populated areas the capacity of the WWTPs and their performance is quite important to lower the amount of contamination.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential, or perceived conflict of interest.

Author contribution

Mert Sanli carried out data analysis and writing the original draft. Yasemin Dilsad Yilmazel designed the study, supervised, wrote, reviewed and edited the original draft. Both authors approve the final version of this manuscript. Both authors verify that the tables, figures and the main text are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Effects of exogenous glycine betaine application on some physiological and biochemical properties of cotton (*G. hirsutum* L.) plants grown in different drought levels

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Abstract

Drought stress significantly reduces the growth and yield of cotton plants; therefore, reducing the damage caused by drought stress and improving the plant growth are highly important. The aims of this study were to investigate some of physiological and biochemical properties of cotton plants exposed to different drought levels and to determine the extent of improvement obtained by exogenous glycine betaine treatment for the damages caused by the adverse effects of drought. Three drought levels were created using PEG (Polyethylene glycol 6000) solution (0%, 10% and 20%). The drought groups were also divided into 2 subgroups by using 0 (control: glycine betaine solution was not applied) and 2 mM glycine betaine solution. The pot experiment was established in a growth chamber with 3 replications and the experiment repeated twice. The changes in the contents of contents of chlorophyll a and b, carotenoid, total dissolved protein, total dissolved carbohydrate and some nutrients were investigated. Chlorophyll a and b, calcium, magnesium, sodium and phosphorus concentrations were decreased, while carotenoid, total dissolved protein and carbohydrate, potassium contents increased with the increasing drought level. The results revealed that adverse effects of drought stress on cotton plants were alleviated by exogenous application of glycine betaine.

Keywords: Chlorophyll-a, Chlorophyll-b, Protein Content, Nutrient Accumulation

Introduction

Cotton plant (*G. hirsutum* L.) is originated from the genus Gossypium of the Malvaceae family, and is a product of great economic importance for humanity with its widespread and compulsory use, and for the producer countries with the added value and employment opportunities created. The population growth and rising living standards increase the demand for cotton. Cotton is an important raw material of the gin industry for the processing, the textile industry with its fiber, the oil and feed industry with its seed, and the paper industry with its lint. Oil obtained from the cotton seeds is increasingly used as a raw material in the production of biodiesel as an alternative to petroleum (GTB, 2020). In 2019/2020 growing season, 24.4% of cotton production in the world was carried out in India, 22.2% in China, 17.42% in United States of America, 6.1% in Pakistan, 9.3% in Brazil and 3.3% in Turkey (USDA, 2019). The amount of cotton produced in Turkey during 2019/2020 period was 814 thousand tons of fibers while 2.2 million tons of the cotton production was seed cotton. Cotton fiber yield in 2019/2020 season was 1870 kg ha^{-1}. Eighty eight percent of cotton production in Turkey is carried out in Sanliurfa, Aydin, Hatay, Diyarbakir, Adana and Izmir provinces.

The share of Şanlıurfa province in cotton production is 42% in the country (UPK, 2019; Özüdoğru, 2019).

The drought stress is one of the most serious abiotic stress factors that restrict the growth and

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development of the plant and decrease the yield (Pandey et al., 2012). A series of reactions occur in plant metabolism due to drought stress. Reactive oxygen species (ROS) are produced in the plant cell with the exposure to drought stress. These reactive substances cause stress in the photosynthesis mechanism and cause oxidation in the cell membrane and deformation in its structure (Foyer and Noctor, 2009; Dietz and Pfannschmidt, 2011). In addition, nucleic acids negatively affect plant metabolism due to the oxidation in carbohydrate and protein metabolism (Clemens, 2001; Ali et al., 2012). Drought affects carbon and nitrogen metabolisms of the plant, causing decreases in photosynthesis production; thus, negatively affect the plant growth and development (Lawlor and Tezera, 2009). The decline in the photosynthesis mechanism causes stomata closure (Chaves et al., 2009; Aranjuelo et al., 2011). Stomatal closure mechanism directly affects the carbon metabolism of plants; therefore, the severity of drought significantly affects the stomatal closure mechanism (Bota et al., 2004). Plants generally increase their antioxidant contents in response to drought stress and alter total soluble sugar, total amino nitrogen and polyphenol levels (Başal and Aydın, 2006; Chaves et al., 2009). Loka and Oosterhius (2014) stated that the total dissolved carbohydrate content and glutathione reductase level of the pistils increased, while photosynthesis and respiration of the cotton plants significantly decreased during the flowering stage at different drought levels. Plants develop aforementioned mechanisms to survive and to have the least damage from the stress. Synthesis of osmolite is one of the mechanisms developed to prevent from drought stress damages. The osmotic pressure changes when plants are under drought stress, and plants produce non-toxic, low molecular weight and highly soluble organic materials called osmolites to compensate for the changing osmotic pressure (Serraj and Sinclair, 2002). The most common compatible osmotic preservatives are betaines, polyols and sugars (mannitol and trehalose) and amino acids (proline). The osmolites improve the resistance of plants to drought, heavy metals and stress factors (McNeil et al., 1999).

Glycine betaine, one of the osmolite types, occurs naturally in many plant species and living organisms. The glycine betaine is a substance that can be synthesized in high quantity in the chloroplast, regulates the photosynthesis and thylakoid membrane structure of the cell and helps maintain the integrity of the cell membrane structure (Allakhverdieva, 2001). The glycine betaine is synthesized in some plants and accumulated against stress factors (Yancey, 1994; Subbarao et al., 2001), while they may accumulate at a very small amount in some plants or may not accumulate. The researchers indicated that even very small amount of accumulation in plants may help alleviate the stress damage (Agboma et al., 1997a, 1997b; Yang and Lu, 2005; Zhang et al., 2013).

Materials and Methods

Plant material

Drought tolerant CANDIA cotton variety of Bayer Company was used in the study. This variety is widely used in the Southeast regions of Turkey due to better yield and fiber quality criteria.

Sterilization of Seeds and Planting

Cotton seeds were pre-treated with 70% ethanol for 30 seconds to ensure sterilization. Then, the seeds were soaked in 10% NaOCl solution for 10 minutes. The seeds were washed 3 times with double distilled water (ddI -H_2O) to prevent the possible adverse effect of NaOCl (Wu et al., 2011; Can, 2013).

The pots were washed with distilled water and sterilized, super coarse perlite (0-5 mm) was filled into the pots. Cotton seeds were germinated in magenta boxes. Five cotton seedlings were transplanted in each pot.

Setting The Growing Conditions in The Growth Chamber

The ambient temperature of the plant growth chamber was adjusted to a daily average of 27± ℃ (30 °C/26 °C) (Reddy et al., 2004; Salvucci and Crafts-Brandner, 2004). The lighting of the growth chamber was set to 14 hours light and 10 hours dark. Fluorescent lamps designed for plants were used in the light phase. Light intensity was measured as 350-400 µmol m^{-2} sec^{-1}. The humidity level of the growth chamber was set to 65-70% (Rahman et al., 2004).

For 30 days when the plants reached the 5-6 leaf stage, ½ Hoagland nutrient solution was applied (Hoagland, 1920). Drought stress treatments were started on the 31st day. The drought treatments (0 and 10, 20%) were carried out by adding PEG 6000 solution to the Hoagland nutrient solution. Stress treatments contained 0 mM (glycine betaine not given: control group) and 2 mM glycine betaine solution, which was added to the nutrient solution and applied with the drought stress treatment.

Elemental Analysis of Plant Samples

Plant samples were separated into the parts and dried in an oven at 70 °C until the weight becomes a constant value. After reaching a constant weight, the plants were weighed and ground in a plant grinding mill (Can, 2013). Then, 0.3 g of plant samples were weighed, and 5 ml HNO_3 was added over the plant samples. The samples were filtered into polystyrene tubes using a filter paper and allowed to cool. Total volume of filtrates were completed to 15 ml with deionized water. The concentration of sodium (Na), magnesium (Mg), potassium (K), phosphorus (P) and calcium (Ca) in solution was determined using an ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometer) (Varian-Vista, axial) instrument in the Elemental Analysis Laboratory.

Photosynthetic Pigments in Leaf Tissues

Chlorophyll a, chlorophyll b and carotenoid contents of leaves were determined using the

methods described by Lichtenthaler (1987). Absorbance values of the supernatants at 663, 645 and 470 nm wavelengths were measured with a spectrophotometer. Calculations were performed using the following equations;

Chlorophyll-a = $\Delta A_{663} \cdot 12.7 - \Delta A_{645} \cdot 2.69$

Chlorophyll-b = $\Delta A_{645} \cdot 22.9 - \Delta A_{663} \cdot 4.68$

Carotenoid = $\Delta A_{480} + 0.114 \cdot \Delta A_{663} - 0.638 \cdot \Delta A_{645} / 112.5$

Total Soluble Carbohydrate Content

Total soluble carbohydrate content of leaves was determined using the phenol-sulfuric method (Dubois et al., 1956). 0.05 g of dry samples were weighed and placed into new tubes, and 70% ethyl alcohol was added to the tubes. The solution was kept in a hot water bath at 80 ℃ for 60 minutes. After that, the tubes were centrifuged at 3500 rpm for 20 minutes. After the centrifugation, 1000 µl of supernatant was transferred to new test tubes, 300 µl 5% phenol and 2000 µl concentrated sulfuric acid (H_2SO_4) were added and vortexed. The absorbance values of the solutions were determined by a spectrophotometer at 490 nm wavelength. Glucose solution at different concentrations was used as the standards. Total soluble carbohydrate content was calculated by creating a standard curve. Soluble total carbohydrate was determined as dry weight (mg mL^{-1}).

Total Protein Content

Total protein content was determined by the method of Bradford (1976) using BSA (Bovine Serum Albumin) standards. The absorbance of the solutions were measured at 595 nm using Shimadzu UV spectrophotometer instrument against blank. The curve was created using different concentrations as the standards. Soluble total protein content was determined as wet weight (mg mL^{-1}).

Statistical Analysis

The study was repeated twice, and only the leaf samples were analyzed in the study. Growth parameters were determined 2 times (n=10). Other analyzes were replicated 3 times. The third analysis was carried out by combining the leaves of the 1st and 2nd experiments. Statistical evaluation of the data was performed using the SPSS software. The effects of exogenous glycine betaine treatment on physiological and biochemical properties of cotton plants under drought stress levels were assessed by variance analysis (ANOVA). When ANOVA indicated significant difference, a post hoc test was used to group the treatments.

Results and Discussion

Results

Chlorophyll a, b and Carotenoids Content

The chlorophyll a content in the glycine betaine not applied group, decreased as drought severity increased. However, chlorophyll a content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest chlorophyll a content was obtained in the control (7.58 µg g^{-1}) when glycine betaine was not exogenously applied. The highest chlorophyll content in this group was 7.12 µg g^{-1}

The chlorophyll b content in the glycine betaine not applied group, decreased as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, chlorophyll b content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest chlorophyll a content in drought stress group was obtained in the control (8.05 µg g^{-1}) when glycine betaine was not exogenously applied. The highest chlorophyll content in this group was 7.99 µg g-1 ($p < 0.05$) when exogenous glycine betaine was applied.

The carotenoid content in the glycine betaine not applied group, increased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, carotenoid content decreased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest carotenoid content in drought stress group was obtained in the 20% PEG 6000 (1.53 µg g^{-1}) treatment when glycine betaine was not exogenously applied. The highest carotenoid content in this group was 1.53 µg g^{-1} ($p < 0.05$) when exogenous glycine betaine was applied.

Total Dissolved Carbohydrate and Protein Content:

Total dissolved carbohydrate content in the glycine betaine not applied group, increased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total dissolved carbohydrate content decreased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest total dissolved carbohydrate content in drought stress group was obtained in the 20% PEG 6000 (19.26 µg g^{-1}) treatment when glycine betaine was not exogenously applied. The highest total dissolved carbohydrate content in this group was 17.6 µg g^{-1} ($p<0.05$) when exogenous glycine betaine was applied.

Total dissolved protein content in the glycine betaine not applied group, increased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total dissolved protein content decreased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest total dissolved protein content in drought stress group was obtained in the 20% PEG 6000 (4.37 µg g^{-1}) treatment when glycine betaine was not exogenously applied. The highest total dissolved protein content in this group was 3.21 µg g^{-1} ($p<0.05$) when exogenous glycine betaine was applied.

Some of Nutrient Contents:

Total Ca content in the glycine betaine not applied group, decreased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total Ca content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest Ca content in drought stress group was obtained in the control (427.53 µg g-1) treatment when glycine betaine was not exogenously applied.

The highest Ca content in this group was 425.53 µg g-1 ($p<0.05$) when exogenous glycine betaine was applied.

Total K content in the glycine betaine not applied group, increased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total K content decreased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest K content in drought stress group was obtained in the control (633.45 µg g^{-1}) treatment when glycine betaine was not exogenously applied. The highest K content in this group was 629.13 µg g^{-1} ($p<0.05$) when exogenous glycine betaine was applied.

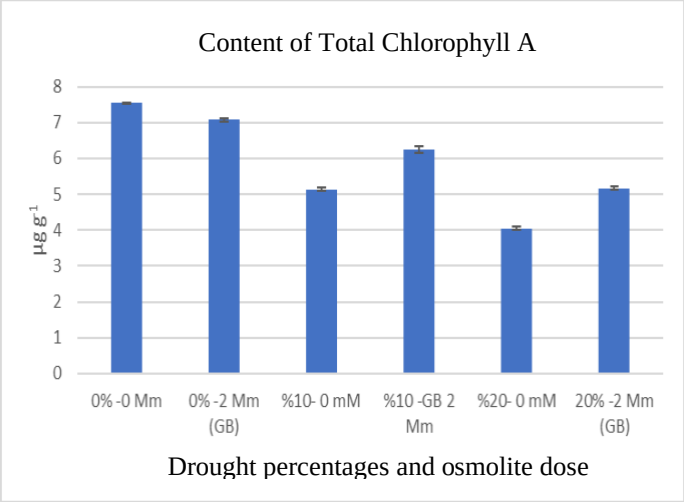

Figure 1. Effect of exogenous glycine betaine application (2 mM) to cotton plants grown in different drought severity on chlorophyll a content (error bars show standard deviation) (N=9)

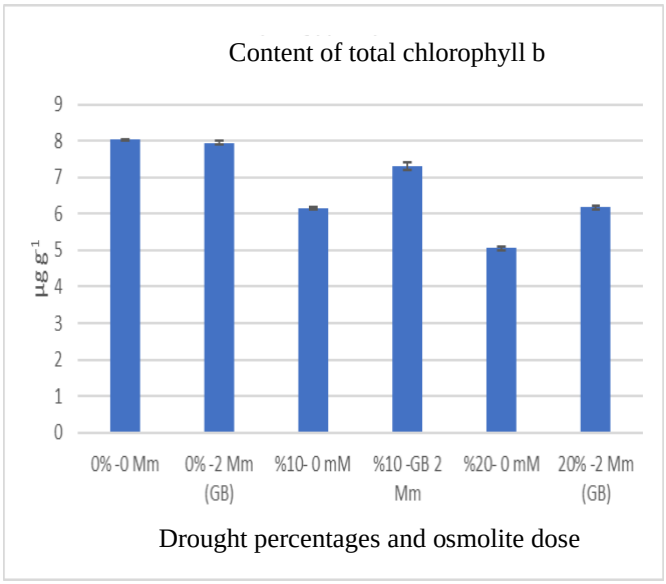

Figure 2. Effect of exogenous glycine betaine application (2 mM) on cotton plants grown at different drought severity on chlorophyll b content (error bars show standard deviation) (N=9)

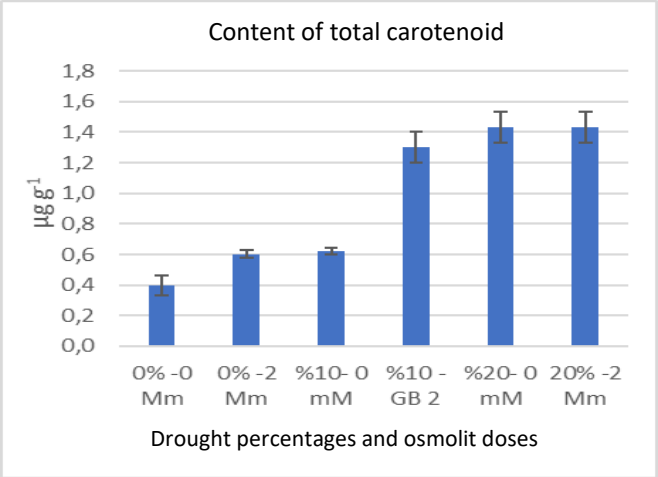

Figure 3. Effect of exogenous glycine betaine application (2 mM) on total carotenoid content of cotton plants grown at different drought severity (error bars show standard deviation) (N=9)

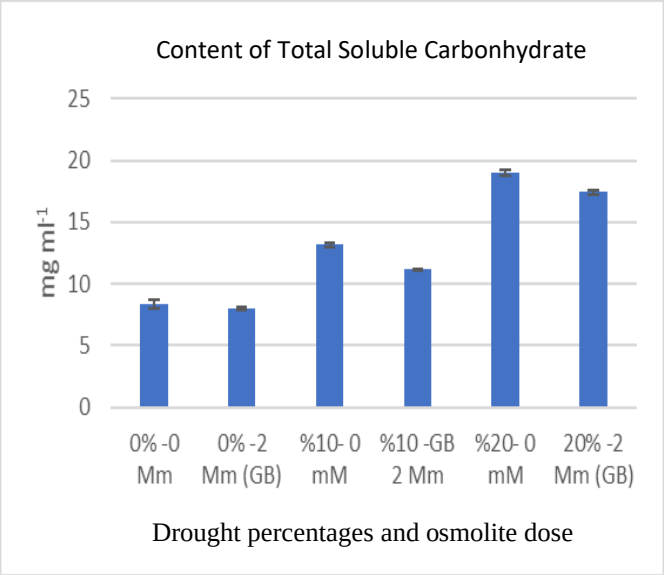

Figure 4. Effect of exogenous glycine betaine application (2 mM) on total dissolved carbohydrate content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

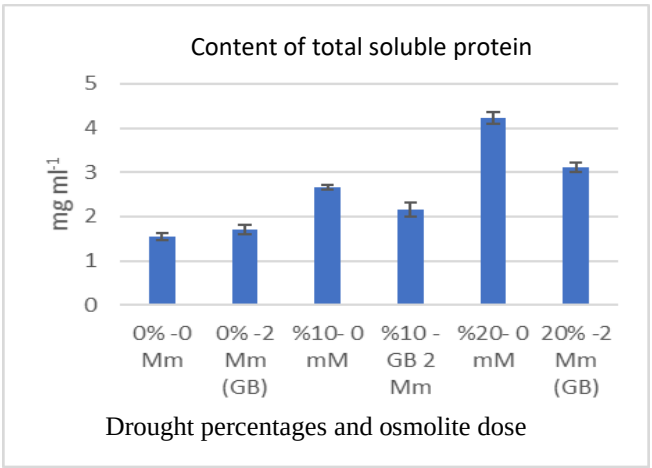

Figure 5. Effect of exogenous glycine betaine application (2 mM) on total dissolved protein content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

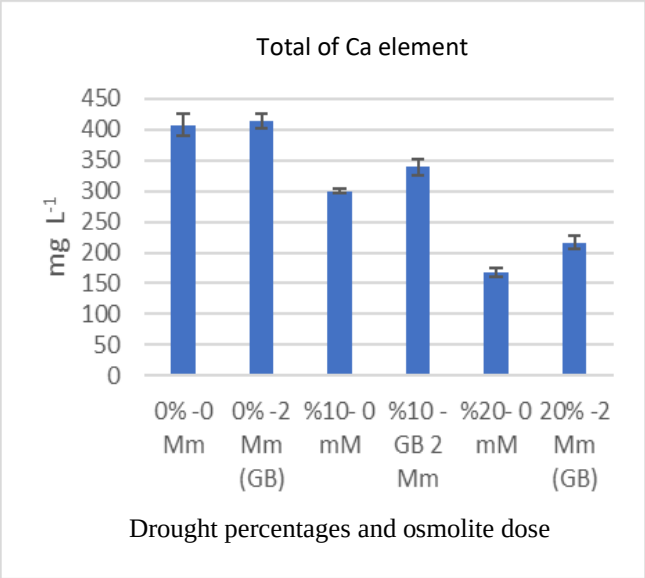

Figure 6. Effect of exogenous glycine betaine application (2 mM) on total Ca content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

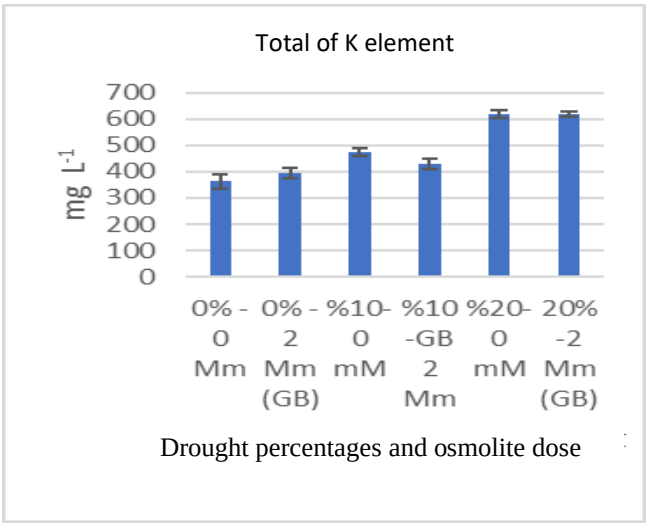

Figure 7. Effect of exogenous glycine betaine application (2 mM) on total K content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

Magnesium Content

Total Mg content in the glycine betaine not applied group, decreased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total Mg content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest Mg content in drought stress group was obtained in the control (195.5 µg g^{-1}) treatment when glycine betaine was not exogenously applied. The highest Mg content in this group was 196.73 µg g^{-1} ($p<0.05$) when exogenous glycine betaine was applied.

Sodium Content

Total Na content in the glycine betaine not applied group, decreased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total Na content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest Na content in drought stress group was obtained in the control (149.8 µg g-1) treatment when glycine betaine was not exogenously applied. The highest Na content in this group was 171.17 µg g-1 ($p<0.05$) when exogenous glycine betaine was applied.

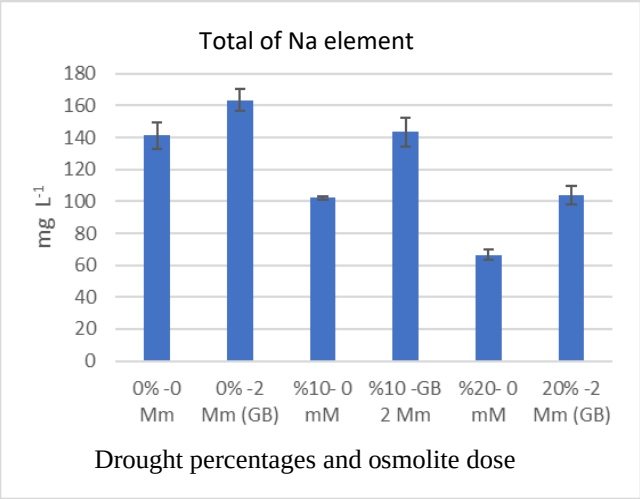

Figure 9. Effect of exogenous glycine betaine application (2 mM) on total Na content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

Total P content in the glycine betaine not applied group, decreased compared to the control as drought severity that was created by application of 0, 10 and 20% PEG 6000 increased. However, total P content increased with glycine betaine (2 mM) application along with the exogenous drought treatment. The highest P content in drought stress group wasobtained in the control (238.4 µg g^{-1}) treatment when glycine betaine was not exogenously applied. The highest P content in this group was 272.12 µg g^{-1} ($p<0.05$) when exogenous glycine betaine was applied.

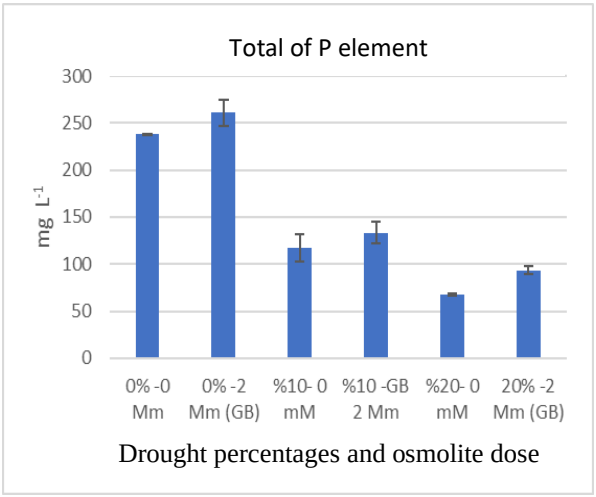

Figure 10. Effect of exogenous glycine betaine application (2 mM) on total phosphorus content of cotton plants grown at different drought severity (error bars show standard deviation) (N = 9)

Discussion

Photosynthesis mechanism is slowed down and chlorophyll structure is decreased under stress conditions (Pettigrew et al., 2005). In addition, the drought stress causes a decrease in water content and stomata closure. This situation results in a decrease in CO_2 concentration, and led to photooxidation and photoinhibition (Flexas and Medrano, 2002). The results are in agreement with the Thimmanaik et al. (2002), Parida et al. (2007), Guerfel et al. (2009) and Nikolaeva et al. (2010) who reported that chlorophyll was degraded and photosynthetic pigment content decreased due to the indirect chain reaction.

Increasing the severity of drought may reduce the chlorophyll content; thus, the plant become more sensitive to drought stress (Anju et al., 1994). The presence of glycine betaine in chloroplasts protects the thylakoid membranes, and maintains photosynthetic efficiency by providing membrane integrity (Yokoi et al., 2002; Yang and Lu, 2005).

The findings indicated reductions in chlorophyll a and b contents with the drought severity, however, exogenous glycine betaine application improved the damage caused by the drought stress. The results revealed that cotton plants maintain the photosynthesis mechanism resilient against the drought stress.

Carotenoid is an antioxidant that is bound with weak bonds to proteins in the cell, and prevents the pigments from being oxidized and degraded (Çınar, 2003; Kalefetoğlu and Ekmekçi, 2005). The antioxidant content generally increases as the drought severity increases. Increasing carotenoid content during the drought stress activates the antioxidant mechanism of the plant and reduces the negative impact of drought.

The photosynthesis mechanism of plants exposed to drought stress decelerates, which affects the carbohydrate mechanism and causes deterioration (Pelleschi et al., 1997; Kim et al., 2000). Kim et al. (2000) reported that metabolic processes such as photosynthesis and respiration are important to provide intracellular carbohydrate content. The findings of Kerepesi and Galiba (2000) who reported that increasing drought severity increases the dissolved carbohydrate content in plant cells coincides with the findings of Kim et al. (2000), Sanchez et al. (2004) and Zali and Ehsanzadeh (2018). The findings indicated that cotton plant has a deteriorated carbohydrate mechanism due to drought, and this metabolism can be improved by application of exogenous glycine betaine.

Photosynthesis electron transport mechanism and photophosphorylation mechanism of chloroplasts, which were isolated from plants grown under drought conditions decreased (Smirnoff, 1993). The PS II in the isolated chloroplasts was affected by the drought (He et al., 1995). The D1 and D2 regions in the PS II reaction mechanism are responsible for photoinhibition, and these regions go through transformations under stress (Baker, 1991). The increase in total protein content may be related to the proteins in the degraded region due to the impact of increased reactive oxygen species under stress conditions. In addition, drought stress not only affects photosynthesis reactions, but also affects nucleic acid and protein synthesis. Inhibition in nucleic acid synthesis also decreases the protein synthesis mechanism (Çırak and Esendal, 2006). Protein synthesis rate decreases, while protein structures change and their structures are fragmented (Chartzoulakisa, 2002; Parida et al., 2007).

Drought stress increases the soluble protein content of some plants, and proteins, stimulated by the effect of drought stress, develop an adaptation mechanism against drought (Bray, 1993; Han and Kermode, 1996; Riccardi et al., 1998; Can, 2013). The synthesis of some proteins from the dehydrin family increases with drought stress and plays an important role in protecting other proteins and their structural integrity. The results obtained are in accordance with the findings of Bray (1993) and Close et al (1996) who attributed to the increase in the dissolved protein content with the degradation and fragmentation of their structures.

Calcium uptake of plants decreases due to the drought stress. The Ca element is used in osmotic regulation of plants and plays an important role in the signal formation mechanism against stress factors (Bartels and Sunkar, 2005). Jenne et al. (1958) attributed the decrease in Ca content to competing with P and K ions under stress conditions and stated that the intracellular P, K and Ca contents reached 40, 71 and 91% under stress conditions. The plants may have used Ca element in their metabolisms to develop drought signal.

Potassium increases the internal balance (homeostasis) by regulating the stomata to reduce the effect of drought stress in plants, regulating the intracellular osmatic pressure, protein and energy mechanism (Beringer and Trolldenier, 1978; Marscher, 1995). In addition, the K element is also used to control the transpiration mechanism and reduces stress damage by balancing the osmatic pressure (Andersen et al., 1992). Potassium production of plants in arid regions is more important than organic matter synthesis to protect against the stress. Plants reallocate the energy to increase stress tolerance and the energy released in osmotic balance increases with K uptake in stress-inducing conditions. Previous studies indicated that the K content of plants increased under arid conditions (Morgan, 1992; Yaşar et al., 2006).

Havlin et al. (1999) reported that the uptake mechanism of Mg, Fe and P ions are disrupted due to drought stress, their uptake into the cell is prevented and some toxicities start to occur in plants. These ratios under stress conditions vary between the species. Our results are similar to the findings of Can (2013) who reported that the K content increased and Ca, Mg, P and Na contents of cotton genotypes grown under different drought stress conditions decreased due to the increasing drought severity.

Conclusion

Cotton is considered the white gold of our country and is one of the vital sources of the economy. Drought is one of the major abiotic stress factors that limit the growth of many plants such as cotton and reduce crop yield. Reactive oxygen species (ROS) are produced under drought stress conditions. The ROS causes damage to the plants by changing the physiology and biochemistry of the plants. Exogenous osmolite application is an alternative mean to reduce the damage caused by ROS. Glycine betaine is the most common osmolite used against drought stress. Exogenous application of glycine betaine was reported to increase plant tolerance to heavy metals (Cao et al., 2013; Ali et al., 2015) and other stresses factors such as drought, salinity, high and low temperature (Yang et al., 2008; Iqbal et al., 2009; Islam et al., 2009; Chen and Murata, 2011).

This improvement demonstrates that glycine betaine is an alternative product that can be used to reduce crop loss during drought conditions. However, further studies are needed to investigate the effect of application before the drought in preventing the drought damage. In addition, the physiological and biochemical changes occur in exogenous glycine

betaine application at different development stages of the cotton plant can be studied. Determining the activity of enzymatic components of the antioxidant system and the isoenzymes and even investigating the gene expression will be effective in elucidating the effect of osmotic preservatives on the drought tolerance mechanism.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Ethics committee approval is not required.

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Evaluation of the use of prepaid water meter on some irrigation management performance indicators: A case study

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Abstract

Volume based pricing in irrigation is an important application method in solving excessive and illegal water use problems. It is essential to use a properly selected water meter to accurately measure the amount of water. In this study, the irrigation system performance of Kayack Water User Association (WUA) was comparatively analyzed for the years 2012 to 2017. The prepaid water meter has been used for water measurement in Kayacık irrigation scheme since 2015. While it could be irrigated on 11.754 ha in 2012, the irrigated area reached 19.528 ha in 2017. It was determined that there was a significant water saving and economic recovery after the installation of prepaid water meters in the irrigation scheme. The quantity of water, which was 7,414 m3ha-1 in 2012, decreased to 3,617 m3ha-1 in 2017. The cost recovery ratios were 76% and 107% in 2013 and 2017, respectively. Consequently, prepaid water meter usage provides so many advantages in service delivery, economics and productivity. In addition, the quantity of water discharged into the drainage canal has significantly decreased. It is recommended that prepaid water meter usage should be prevalence in irrigation operation.

Keywords: Measuring irrigation water, Prepaid water meter, Service delivery performance, Financial performance, Production performance

Introduction

The state must effectively protect, develop and control distribute water resources to all who will use it. The demand for more efficient irrigation water use is growing. Countries have to find new solutions due to new developments and occasions in water resources management for more efficient water resources management.

Considering the amount of water per capita of 1380 m3 in Turkey, unfortunately, it is among the countries that no longer have enough water. By the end of 2018, approximately 74% of Turkey's water resources were appropriated for agricultural purposes (DSI, 2018). In this situation, the effective use of water and irrigation systems to save water are the most important matters to be taken into consideration.

Furthermore, irrigation water use efficiency (IE) and water productivity (WP) are closely related to other basic concepts of ongoing environmental resource management. The water is used by a variety of agricultural, environmental, urban, industrial, and recreational users (Bos et al., 2009).

In many countries, there are many different problems related to irrigation water management, such as excess and illegal water use, lack of technical experience for irrigation systems used, drainage, salinity, waterlogging, and irrigation water pricing. Improving IE and WP can provide less stress on water resources, reduce losses of water and nutrients to water resources, increase production and provide overall benefits. Thus, this increase potentially allows a greater area to be irrigated with a given volume of water (Koç, 2013). In some cases, water losses can be reused elsewhere; in other cases, they cannot be recovered due to salt water sinks. Onfarm water management practices, improved water distribution, and infrastructure can reduce these avoidable water losses (De Fraiture et al., 2014).

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If the extension services have provided the necessary knowledge, helping farmers to adapt and apply new practices, more benefits can be provided from improved irrigation technology. In many cases, the application of advanced irrigation technology has led to increased water prices without reaping all the potential benefits through water efficiency. Farmers usually have insufficient instruments and stimulations to know their crops' water consumption, actual irrigation practices' feedback to water management applications, and thus actual irrigation-efficiency levels (Levidov et al., 2014).

According to the experts, appropriate irrigation scheduling should provide improvements to higher water management performance. Appropriately managed irrigation not only increases crop yields and productivity but also declines pest infestations and precisely delivers and manages nutrients (Sedara and Sedara, 2020). The farmer should not be able to control the timing and the amount or volume of irrigation water in the practical application of the irrigation techniques and methods due to the non-economical pricing of water, the high cost of irrigation scheduling, insufficient education and demonstration, etc. In some cases, irrigation water price covers less than 30% of the total cost in many countries (Chartzoulakis and Bertaki, 2015). If irrigation is driven by non-economic objectives, farmers should not repay the full cost. Irrigation becomes increasingly uneconomical if the farmers are unable to pay for marginal (future) costs (Moll and Berkof, 2018).

In this context, water pricing based on volumetric of water is the most profitable method because of sustainability interaction between the amount of water used in irrigation and refund for it. So many studies on volume based pricing and efficient collection of irrigation water charges are a driving factor. (Ünver and Gupta, 2003). It is advised that the infrastructure for shifting to volumetric pricing should be established (Çakmak et al., 2010).

On the other hand, there are significant advances in the adoption of more efficient irrigation technologies with promoted government support. Adoption of efficient irrigation technologies by farmers can be promoted by introducing volume based pricing applications (Çakmak, 2010). Selection of a water measurement method for volumetric pricing should also consider past practice. When improved water measurement methods are needed, suggesting changes that build on established practice is often easier to institute than extreme changes (Anon., 2001). In order for this method to be applied effectively, a measurement device that records the quantity of water used for each field/land unit is needed. The reliability of the measurement device for volume based water fees is an important requirement (Burt, 2007). Suitably preferred and well-kept water meters can provide the most precise and easy way to measure the quantity of water in flowing into the farm and/or field (Baum et al. 2012; Golin et al. 2015).

However, individual consumption measurement is difficult in some situations. There is a risk of tampering with water meters by irrigators if there are drastic limitations on their use or increases in their water charges (Molle, 2009). Prepaid water meters perform accurate water measurements and guarantee the collection of water charges as well. The total water usage of the water users is calculated and provided payment in advance in the prepayment system. With a subscriber card, which belongs to only one water meter device, the water user can take as much credit as he wants. As soon as the smart card is scanned by the water meter device, present credits are transported to the device. The water user uses water on a schedule, and controlled water use is provided this way.

In this study, Kayacık irrigation system was taken as the material for benchmarking irrigation performance for the years between 2012 and 2017. Kayacık Irrigation Facility's irrigation, maintenance, and management responsibilities have been undertaken by Kayacık WUA. Approximately 60% of the people in the region, where the study area is located, earn their living from agriculture. Since 2015, the prepaid water meter has been used for water measuring in Kayacık Irrigation Facility. The main purpose of this study is to compare the irrigation system performance indicators before and after using the prepaid water meters. While evaluating the performance indicators of the irrigation system, performance indicators were used as suggested by the International Programme for Technology and Research in Irrigation and Drainage (IPTRID) and benchmark values for performance categorized (Malano and Burton 2001). Water delivery, financial performance, and productive performance were examined within the scope of evaluations.

Materials and Methods

Kayacık irrigation area is located in the Euphrates-Tigris basin, in the Oğuzeli district of Gaziantep province. The monthly mean precipitation is 438 mm and the avarage monthly temperature is 16.4 ^{o}C.

State Hydraulic Works built Kayacık irrigation scheme and put it into operation. While irrigation area was 600 ha in first year, it reached 10 800 ha by increasing each year in 2012. It was fully operational in 2012. It was started to be operated completely in 2012. The water resource of irrigation project area is Kayacık Dam fed by Aynifar, Tuzel, and Sacir creeks. The irrigation conveyance systems include an open canal system of 5% and a pipeline irrigation network of %95. Operation, maintenance, and management duties of the irrigation facility were undertaken by Kayacık WUA in 2006. Some data regarding the irrigated area and irrigation ration according to the years are given in Table 1.

Table 1. Spatial change based on command and a irrigated area

Years	Irrigation command area (ha)	Irrigated area (ha)	Irrigation ratio (%)
2012	10.800	11.754	109
2013	10.800	11.116	103
2014	10.800	8.245	76
2015	10.800	9.866	91
2016	10.800	14.543	135
2017	10.800	19.528	181

The domain crop pattern in the irrigation district consists of mainly cereals, cotton, and maize, and some fruits and vegetables are cultivated as well. Crop patterns based on years are given in Table 2.

Table 2. The crop pattern of Kayacık irrigation

ars	Cereals (ha)	Cotton (ha)	Maize (ha)	Vegetable (ha)	Fruit (ha)	Off season Irrigation (ha)
2012	7.368	-	4.215	-	171	-
2013	4.660	339	6.041	39	37	-
2014	5.393	459	2.229	112	51	-
2015	4.305	172	4.699	605	85	-
2016	7.004	269	1.872	1.229	818	3.351
2017	10.140	886	1.007	5.924	1.072	499

According to the Evaluation Report of Irrigation Facilities Operated and Transferred by DSI for 2012, farmers irrigated the entire irrigated area by surface method, including furrow and border (Anon., 2013). Over time, farmers started to irrigate by sprinkler and drip irrigation methods instead of surface irrigation methods (Anon., 2018).

Volume-based irrigation started to apply by installing the prepaid water meter in irrigation scheme in 2014. Some water distribution problems related to the use of prepaid water meters were observed in the first years, but they started to be used successfully in the following years.

In this study, some performance indicators in Kayacık irrigation scheme are compared before and after the use of the prepaid water meter. The water delivery, financial, and productive efficiency performances of the irrigation system were evaluated within the scope of this study. These performance indicators have been suggested by the IPTRID/World Bank study on benchmarking in the irrigation sector. Benchmark values for performance have been determined in this manner (Malano and Burton, 2001). Some performance indicators used in this study are shown in Table 3.

Table 3. The performance indicators considered in the benchmark study (Malano and Burton, 2001).

Domain	Indicators
Service delivery performance	Seasonal relative irrigation supply
	Delivered irrigation water per unit command area during the season (m^3 ha^{-1})
	Delivered irrigation water per unit irrigated area during the season (m^3 ha^{-1})
Financial performance	Cost recovery ratio
	Maintenance cost to revenue ratio
	Total MOM cost per unit area (US$ ha^{-1})
	Revenue collection performance
	Employed staff numbers per unit area (persons ha^{-1})
Productive efficiency	Output(net revenue) per unit command area (US$ ha^{-1})
	Output(net revenue) per unit irrigated area (US$ ha^{-1})
	Output per(net revenue) unit irrigation supply (US$ m^{-3})

All of the data in this study came from a technical report on irrigation scheme monitoring and evaluation prepared by DSI and Kayak WUA and used to determine the performance indicators. Also, field observations were made for 6 years.

All of the data in this study came from a technical report on irrigation scheme monitoring and

Service delivery performance

Results and Discussion

Within the scope of service delivery performance,

the seasonal relative water supply ratio (RWS) (Table 4), total seasonal water delivery per command area used to determine the performance indicators. Also, evaluation prepared by DSI and Kayak WUA and field observations were made for 6 years. (WDC) and total seasonal water delivery per irrigated area (WDI) (Table 5) were computed. Required data are daily measured water entering the irrigation system and periodic volume of crop water requirement during the irrigation season.

Table 4. The annual relative water supply ratio

Years	Total seasonal volume of irrigation water inflow (m^3)	Total volume of water required by crop (m^3)	RWS
2012	87.150.000	69.889.284	1.25
2013	81.872.010	85.037.400	0.96
2014	57.480.000	51.086.020	1.13
2015	66.400.000	74.646.156	0.89
2016	68.412.500	75.507.256	0.91
2017	70.640.000	98.382.064	0.72

For calculating RWS, two data points are required: daily measured water inflow to the irrigation system and periodic volume of crop water requirement during the irrigation season. RWS was the highest (1.25) in the irrigation season of 2012. After the prepaid water meter was installed, this ratio started to decline. If the water supply ratio is 1.0, the water diverted to the irrigation scheme is enough. If it is less than 1.0, it is not enough. And if it is greater than 1.0, it means that much more was used (Beyribey, 1997). While it was possible to irrigate only 11.754 ha in 2012 with the water entering the irrigation scheme, the real irrigated area reached 19.528 ha in 2017. Therefore, an increasing of 7.774 ha was obtained.

Table 5. Water delivery per command and irrigated area by years

Years	Seasonal volume of irrigation water inflow (m^3)	Irrigation command area (ha)	Irrigated area (ha)	WDC (m^3 ha^{-1})	WDI (m^3 ha^{-1})
2012	87.150.000	10.800	11.754	8.069	7.414
2013	81.872.010	10.800	11.116	7.580	7.365
2014	57.480.000	10.800	8.245	5.322	6.971
2015	66.400.000	10.800	9.866	6.148	6.730
2016	68.412.500	10.800	14.543	6.334	4.704
2017	70.640.000	10.800	19.528	6.541	3.617

For assessing WDC, daily measured water inflow to the irrigation system and total command area were used during the irrigation. WDC was the lowest value in the irrigation season of 2014 with 5.322 m^3 ha^{-1}, and the highest WDC (8.069 m^3 ha^{-1}) in the irrigation season of 2012. WDI was calculated by using the data points of total daily measured water inflow to the irrigation system and total seasonal irrigated crop area. WDI was the lowest value in the irrigation season of 2017 with 3.617 m^3 ha^{-1} and the highest value in the irrigation season of 2012 with 7.4140 m^3 ha^{-1}. As can be seen the data from Table 5, both WDC and WDI were higher compared to the data before installing the prepaid water meter. The quantity of water used in irrigation has started to decrease after installing the prepaid water meter.

Furthermore, the prepaid water meter use has reduced adverse environmental effects such as soil salinization and the height of the water table caused by excess watering because of the less irrigation water used.

Financial Performance

The cost recovery ratio (CR) (Table 6), maintenance cost to revenue ratio (MCR) (Table 7), operating cost per unit area (OC) (Table 8), revenue collection performance (RCP) (Table 9) and employed staff numbers per unit area (SNC and SNI) (Table 10) were considered to revealed financial performance. These performance indicators were calculated to equalities given in Table 3.

For calculating CR, revenues collected from water users during season and annual total management, operation and maintenance (MOM) cost values were used. CR was analyzed, considering revenue collected from the irrigated farmers. According to Table 6, CR started to increase with the installation of the prepaid water meter, dependently the improvement in revenue collected from irrigators.

Table 6. Cost recovery ratio

Years	Revenue collected from water users (US$)	MOM cost (US$)	CR (%)
2012	608.985	717.242	85
2013	779.417	1.029.042	76
2014	480.267	585.857	82
2015	786.879	753.404	104
2016	631.251	598.747	105
2017	725.006	678.664	107

The maintenance expenditure and revenue collected from water users during irrigation season are required data points for determining MCR. As shown in Table 7, MCR was the lowest with 2% in 2012 and the highest with 70% in 2013. It is understood that collected revenue was enough to make amends for the maintenance costs for all but 2012.

Table 7. Maintenance cost to revenue ratio

Years	Maintenance expenditure (US$)	Revenue collected from water users (US$)	MER (%)
2012	12.006	608.985	2
2013	544.297	779.417	70
2014	183.941	480.267	38
2015	249.431	786.879	32
2016	185.503	631.251	29
2017	231.458	725.006	32

The OC is calculated by considering total income and total service income collected from water users data points. OC was highest in 2013, at 93 U $/ha^{-1}, but it fell in subsequent years (Table 8).

Table 8. Operating cost per unit area

Years	Operation expenditure (US$)	Irrigated area (ha)	OC (US$ ha^{-1})
2012	717.242	11.754	61
2013	1.029.042	11.116	93
2014	585.857	8.245	71
2015	753.404	9.866	76
2016	598.747	14.543	41
2017	67.664	19.528	35

For analyzing RCP, required data points are the number of MOM personnel employed and the command area serviced by irrigation facility. As can be seen from Table 9, RCP was 100% after the installation of the prepaid water meter.

Table 9. Revenue collection performance

Years	Revenue collected from water users (US$)	Revenue due (US$)	RCP
2012	608.985	663.943	0.92
2013	779.417	820.458	0.95
2014	480.267	496.266	0.97
2015	786.879	786.879	1.00
2016	631.251	635.645	0.99
2017	725.006	725.006	1.00

SNC and SNI were calculated by dividing the number of MOM personnel employed by commend area and irrigated area services by system, respectively. SNC and irrigated SNI area are presented in Table 10. SNCs were almost the same between 2012 and 2017. However, SNI declined

because of the increase in irrigated area because of the measuring and distributing of water through the prepaid water meter.

Table 10. Staff numbers per unit area

Years	Total number of MOM staff	Irrigation command area (ha)	Irrigated area (ha)	SNC (person ha^{-1})	SNI (person ha^{-1})
2012	26	10.800	11.754	0.0024	0,0022
2013	26	10.800	11.116	0.0024	0.0023
2014	28	10.800	8.245	0.0026	0.0034
2015	28	10.800	9.866	0.0026	0,0028
2016	26	10.800	14.543	0.0024	0.0018
2017	26	10.800	19.528	0.0024	0.0013

Production performance

Indicators of OUC and OUI (Table 11) and (OUIS) (Table 12) were used to analyze production performance.

OUC and OUI were calculated by dividing the total seasonal value of agricultural production by the command area and total command area, respectively. OUC was calculated as the highest in 2017 at 3.793 U.S.$ ha^{-1} and the lowest in 2014 at 743 U.S.$ ha^{-1}. Correspondingly, OUI was calculated as the highest in 2017 at 2.098 U.S.$ ha^{-1} and the lowest in 2014 at 973 U.S.$ ha^{-1}.

Table 11. Output per unit command and irrigated area

Years	Seasonal agricultural output (US$)	Command area (ha)	Irrigated area (ha)	OUC (US$ ha^{-1})	OUI (US$ ha^{-1})
2012	16.041.096	10.800	11.754	1.485	1.365
2013	13.883.560	10.800	11.116	1.286	1.249
2014	8.024.879	10.800	8.245	743	973
2015	16.977.217	10.800	9.866	1.572	1.721
2016	20.398.898	10.800	14.543	1.889	1.403
2017	40.967.985	10.800	19.528	3.793	2.098

Required data points are the total seasonal agricultural production value and daily measured water entering the irrigation system. As it can be seen from Table 12, OUIS was the highest in 2014 with 1.98 U.S.$ m^{-3} and the lowest in 2013 with 0.17 U.S.$ m^{-3}. This performance indicator has changed by the quantity of water used and the annual market value of each crop.

Table 12. Output per unit irrigation delivery

Years	Seasonal agricultural production (U.S.$)	Seasonal volume of irrigation water entering (m^3)	OUIS (U.S.$ m^{-3})
2012	16.041.096	87.50.000	0.18
2013	13.883.560	81.872.010	0.17
2014	8.024.879	57.480.000	0,14
2015	16.977.217	66.400.000	0.26
2016	20.398.898	68.412.500	0.30
2017	40.967.985	70.640.000	0.58

Conclusion

The performance indicator values obtained from this study revealed that remarkable advancements were achieved after installing prepaid water meters in the irrigation scheme. Irrigation efficiency has increased in cases where irrigation water is charged on volume basis. With the use of prepaid water meters, the cost recovery rate and the ratio of maintenance fee to water usage service fee have increased significantly. Punctual collection of irrigation water charges and on-time and complete operation, maintenance, and repair services have ensured that it has high-performance values. The production per both the unit command area and the irrigated area tends to decrease before the prepaid water meter is used, it starts to increase after use. According to field observations, adverse environmental effects caused by excessive irrigation have decreased and performance indicators have improved. According to field observations, adverse environmental effects caused by excessive irrigation have decreased and performance indicators have

improved. Significant progress has been made in terms of soil salinization and water table height. Consequently, it has been determined that performance indicators have improved over time thorough the prepayment meter was installed in the irrigation facility. The reason for this time-dependent improvement is that farmers adopt the use of prepaid water meters over time and gain habit. It is possible to recover irrigation costs and adjust pricing as an instrument to manage water demand in agriculture by using the prepaid water meter where appropriate and applicable technically, socially, and economically. The prepaid water meter used as a management tool that can result in water savings and thus effects energy used from pumping water. According to the results of this study, the use of prepaid water meters in Kayacık irrigation is very effective in terms of water saving, increasing efficiency and economic benefit, and reducing adverse environmental effects. Especially, under conditions of water shortage, all farmers are able to benefit from water more equitably and efficiently. In addition, the energy cost of pumping irrigation per hectare reduces thanks to water saving provided by using the prepaid water meter. In addition, it is possible to cultivate second crop in the same area.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Breaking seed dormancy and regeneration in *Cannabis sativa* L.

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Abstract

Cannabis sativa L. is an important medicinal plant species that grow under natural conditions and has been legalized in 20 out of 81 Turkish provinces. The female inflorescence is a highly branched compound raceme with indeterminate habit of growth. This results in different maturing of seeds on the inflorescence and induce physiological dormancy on seeds. The study aimed to improve seed germination percentage using various concentrations of GA_3, GA_3 + BAP, germination on water and water solidified with agar, MS or Gamborg B5 medium. The results showed that the best seed germination was noted on Gamborg B5 medium. Different explants were used to regenerated plantlets on Gamborg B5 medium. All explants were suitable for callus regeneration variably. Only the stem nodes of Samsun Vezirköprü were suitable to induce shoots and plantlets. These plantlets were acclimatized on clay loam soils and transferred to field condition during October 2020, where they acclimatized successfully. These studies provide an effective insight into the mechanism seed dormancy in *C. sativa.* Further studies using other plant growth regulator concentrations will improve shoot regeneration and aid in utilizing the methods for breeding purpose.

Keywords: Hemp, *In vitro*, *Mass* propagation, Seed germination, TDZ

Introduction

Cannabis sativa L. (family *Cannabaceae*) is an importnt plant species that has been cultivated in many Asian and European countries as annual herbaceous, multi-purpose plant species used in medicinal or palliative care systems since 2700's years before the Common Era (BCE) (Schäfer, 2005, Schumacher, et al., 2020). Cannabidiol and Cannabidiolic acid are the important and abundant phytocannabinoids in *C. sativa* cultivars in general, but some of them biosynthesize cannabigerol as the major constituent compounds (Hanuš, et al., 2016) that are evaluated as non-psychotropic compounds with potential therapeutic uses. They are considered neuroprotective, anti-rheumatoid anxiolytic, anti-nausea, anti-spasmodic and used for the treatment of arthritis (Bonini, et al., 2018, Hanuš, et al., 2016,) and cancer (Sánchez et al., 2001, Blázquez et al., 2004, Śledziński, et al., 2018), appetite loss and prevent vomiting (Abrams, 2016).

The stem is herbaceous in the first development period, with high sap, and takes a corrugated arthritis body appearance in the later stages of growth. The plants contain 70% cellulose (with ~22% hemicellulose and ~45% carbon). Its fiber is used to make a durable and cost-effective thread (Seher et al., 2020). It is also a rich source of raw material in paper, medicinal or pharmacological products (e.g., phytocannabinoids, terpenes, and phenolic compounds) (Bonini, et al., 2018, Hanuš, et al., 2016).

It is allelopathic and could be used in soil phytoremediation, with the ability to prevent or suppress both weeds and soil pathogens (Adesina, et al., 2020).

Its cultivation in many countries of the world including Turkey remained banned for many years in accordance with global trends because of psychoactive contents. However, its cultivation has been allowed in 20 out of 81 provinces according to the "Regulation on *Cannabis* Growing and Control." (Official Gazette, 2016, 2021). *C. sativa* plants are produced industrially in 36 countries in the world (Aydogan, 2020). The global market US $ 4 billion in 2017, US $ 4.7 billion in 2018, for *C. sativa* is expected to reach US $ 11 billion in 2025 (FAO, 2019).

When the data of TUİK for 1998-2018 is reviewed, there is a decline in *C. sativa* production,

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which reached to total production of 160 tons in 2001(TSI, 2020).

C. sativa is a dichotomous plant with male and female floral organs on different plants with an indeterminate type of growth habit. The most important step in production of *Cannabis* is understanding mechanism of seed germination, plant survival and its harvest of seeds, alkaloids and fibre from the plants at economic level. The Inflorescence is highly branched with a compound raceme and pistillate flowers (Spitzer-Rimon, et al. 2019, Bernstein et al. 2019). The development of the inflorescence is acropetal and lateral racemes are produced prior to terminal flower differentiation (Spitzer-Rimon, et al. 2019, Hall et al., 2012).

The most important step in production of *Cannabis* is understanding mechanism of floral and pollination biology along with seed germination (Spitzer-Rimon, et al. 2019, Strzelczyk et al. 2021). The *Cannabis* or hemp seed germination testing protocols are mentioned in ISTA rules (ISTA 2021), Quality plants and seed production is very difficult in *Cannbis* due to unavailability of registered cultivars and varieities world over in general.

Therefore, seed germination percentage and germination performance depends on the environment and varies from plant to plant. This ends up in several types of dormancies that could be released using various phytohormones or chemical treatments (Ewel, et al., 2019, Jovičić, et al. 2019, Green, et al., 2016, Geneve, 2016) that could end up in variable results. Therefore, there is need to establish simplest methods for successful germination and cultivation (Sera, et al.2017).

Chemical pre germination treatments help to stimulate and increase seed germination and quality (Walck, et al., 2005). Therefore, it is necessary to evaluate chemical treatments to improve its seed germination to enhance production and yields (Chahtane, et al., 2017). Hence, there is a need to design experiments for increased seed germination considering the ever-increasing need for plant biomass and pharmacological products, the World over.

The current study was conducted to break seed dormancy and evaluate the effect of different osmo and hydro priming treatments to successful germination of Samsun Vezirköprü and Uşak populations under *in vitro* conditions.

Materıals and Methods

Seed Material

Seeds belonging to the Uşak and Samsun Vezirköprü populations used in the study were obtained from the Department of Field Crops, Faculty of Agriculture, Uşak University, Turkey.

Methods

Sterilization of Equipment

All laboratory equipment made of glass used in the study were sterilized by keeping them in the oven at 160 ° C for 2 hours. The rest of the material including culture boxes and culture media used in the study were sterilized using autoclave under 4.5 kPa atmospheric pressure and 121 °C for 20 minutes. The forceps and scalpels were cleaned with 70% (v / h) alcohol and then sterilized at 250 °C with a steril 250 sterilizer device in a laminar airflow cabinet.

Surface Sterilization of Seeds

The seeds were shaken in a laminar flow cabinet under sterile conditions on a magnetic stirrer for 15 minutes by dipping and shaking them in 50, 70, and 90% commercial bleach (ACE - Turkey containing 5% sodium hypochlorite-NaOCl) at room temperature. Thereafter, the seeds were rinsed for 3 × 3 min with sterile distilled water at room temperature.

Contamination in the culture medium or over explants was monitored for one week after planting the seeds in the culture medium. The serilized mature seeds were rinsed 3 × 3 min and used as control treatment. The experiments were repeated 3 times.

Germination of Seeds and Regeneration of *C. sativa* Seeds

Sterilized *C. sativa* seeds were cultured in 3 replications with 5 seeds in each Petri dish, and they were kept in the growth cabinet for 10 days to form seedlings. These experiments were also repeated 3 times.

The optimal concentration of bleach was determined after the sterilization and was used in the rest of the studies.

Following treatments were given to break seed dormancy and seed germination

Seed Dormancy Break

i. Treatment with MS medium containing 0.2, 0.4, 0.6, 0.8, 1, 1.2 mg/l gibberellic acid (GA_3) (6 treatments)

ii. Treatment with MS medium containing 0.4 mg/l GA_3 + 0.5, 0.8, 1 mg/l BAP (4 treatments)

iii. Treatment with water and solidified with agar.

iv. Treatment with MS or Gamborg B5 medium.

Regeneration

Upper portion of the leaf, central portion of the leaf, the lower portion of the leaf, petiole, Stem node, and internode explants of 10 days-old *C. sativa* plantlets were treated with ½ ×Gamborg B5 containing 0.1, 0.2, 0.3, 0.4, 0.5 mg/l TDZ solidified with 3.5, 5 g/l agar supplemented with 10 g/l sucrose.

The pH of the nutrient medium was adjusted to 5.7 ± 0.1 using 1 N NaOH or 1 N HCl. Subsequently, sterilization was provided by keeping the respective culture medium under 4.5 kPa pressure and at 121° C for 20 minutes.

The seeds were cultured in a dark in a growth chamber for 12 days at 25 ± 1 °C. Thereafter, the explants were taken from these plantlets as described above.

The cultures were transferred to a chamber with 16 hours light and 8 hours dark photoperiod at 24°C temperature.

Acclimatisation

The growing plants were acclimatised in seedling trays covered with two vented covers. The plantlets continued to grow in these vented trays until they showed the sighns of growth (15 days). Thereafter, the plants were transferred to one litre plastic pots filled with peat moss and transferred to the greenhouse.

Evaluation of Data for Statistical Analysis

The experimental pattern consisted of 3 replications for each treatment using 100 × 10 mm Petri dishes. IBM SPSS 26 computer program was used for the analysis of variance. The results of each experiment were compared with One Way ANOVA. LSD or Duncan test was performed to separate statistically different means in each experiment unless otherwise mentioned. The arcsin transformation was applied to the percent values before statistical analysis (Snedecor, & Cochran, 1967).

Results and Discussion

Effect of Sterilization Treatments on *C. Sativa* Seeds

Effect of Sterilization treatments on *C. Sativa* seeds

Sodium hypochlorite often purchased as bleach, is the most commonly used chemical for the surface sterilization of seeds. Commercial bleach is 5-5.25% sodium hypochlorite. Seed material is often immersed/mixed in this solution singly or with a magnetic stirrer for 10 - 20 minutes or more.

Optimization studies are carried out to experimentally determine a balance between concentration and time due to phytotoxicity for each explant type. The results showed that all concentrations of commercial bleach were appropriate for seed sterilization. However, the percentage of seed germination varied using 50, 70, and 90% concentration of commercial bleach showing a range of 33.333-42.222% and 35.556-53.33 for population Uşak and Samsun Vezirköprü respectively (Figure 1). The maximum germination percentage was determined as 53.33%. Similarly, the use of bleach has been found appropriate in many previous sterilization studies of other plants (Kai, et al., 2007, Daud et al., 2012, Hesami, et al., 2019, Ines, et al., 2013, Bello, et al., 2018).

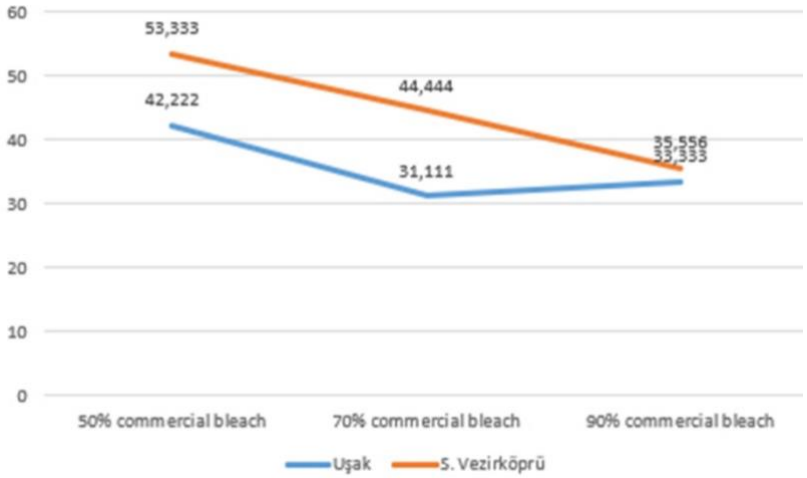

Figure 1. Effect of different concentrations of commercial bleach on *C. sativa* seeds germination percentage

Seed Germination

Effect of GA_3 Doses on Germination

Apart from these possible causes of dormancy, which directly or indirectly affect seed metabolism of carbohydrates, proteins, and other reserves in the germination process, dormancy can also be attributed to a balance between growth-regulating hormones that play a fundamental role in the seed germination process. The results indicated a germination percentage of 13.333-40.000% in population Uşak and 20.000-53.333% in population Samsun Vezirköprü. The highest germination in both populations was noted after treatment with 0.4 mg/l GA_3 (Figure 2).

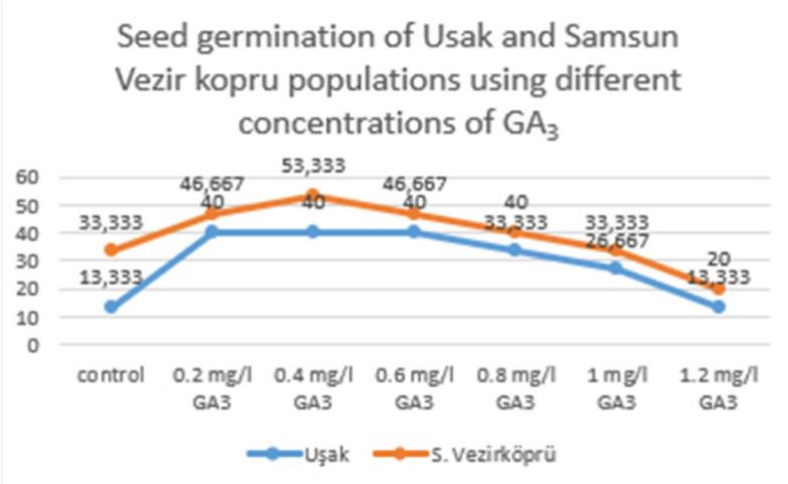

Figure 2. *C. sativa* seeds germinating using different concentrations of GA_3

Gibberellic acid plays a role in overcoming dormancy as well as in controlling the hydrolysis of reserves. The presence of sufficient levels of this acid in seeds stimulates the synthesis, activation, and secretion of hydrolytic enzymes, especially α-amylase, releasing reducing sugars and amino acids necessary for embryo growth (Khan, 1971). External application of Gibberellic acid (GA_3) is one of the hormones suggested to control and break seed dormancy and induce germination (Ritchie, & Gilroy, 1998, Greipsson, 2001).

Effect of GA_3 and different BAP Concentrations on Seed Germination;

The previous experiment showed the highest germination percentage of *C. sativa* seeds of two populations using 0.40 mg/l GA_3. Using this concentration as control, this study compared the effects of 0.40 mg/l GA_3 Control treatment, 0.50 mg/l BAP+0.40 mg/l BAP, 0.40 mg/l GA_3 + 0.80, 1mg / L BAP (Figure 3).

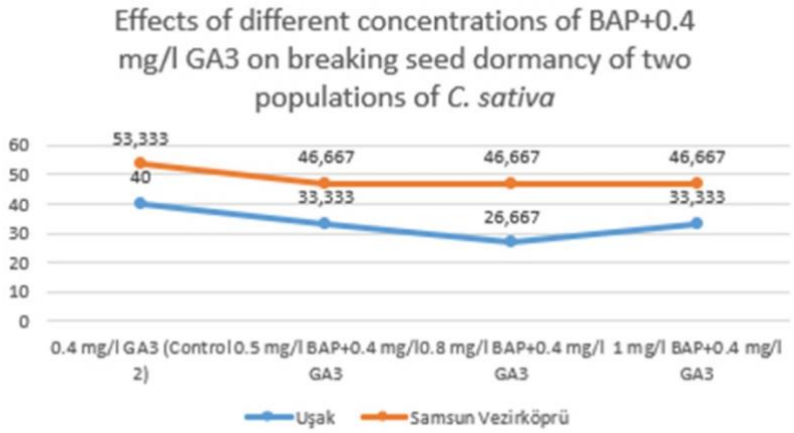

Figure 3. Determination of the effect of different concentrations of BAP+GA_3 on seed germination of *C. Sativa*

Plant hormones have been found to play an important role in the germination process (Nadjafia, et al., 2006). The experimental results showed that BAP+GA_3 combinations had significant inhibitory effects on seed germination percentage and were not as effective, as when GA_3 was used singly. The germination rate varied between 20-33.333% on the Uşak population. The seed germination percentage of the Samsun Vezirköprü population ranged between 26.667-46.667%. The highest seed germination percentage did not show an improvement over control treatment using 0.40 mg/l GA_3 singly.

Effect of Pure Sterile Water on Germination;

Hydro-priming and osmopriming seed pretreatment techniques have been applied to enhance the germination of *C. sativa* populations (Ashraf, et al., 2005, Paparella, et al., 2015).

This experiment compared the effects of water and water solidified with agar on seed germination. It was observed that seed germination percentage using water singly was superior compared to the using water solidified with agar. It showed germination of 50% in Uşak and 63.33% in S. Vezirköprü population (Figure 4). Agar was inhibitory ending up with a germination percentage of 24.67% in the Uşak and 26.67% in the

VezirköprüSamsun Vezirköprü population. The results of this study show that hydro-priming application is effective in overcoming dormancy in *C. sativa* seeds compared to osmopriming using water solidified with agar. It was found that *C. sativa* seeds have physical dormancy due to hard seed coats.

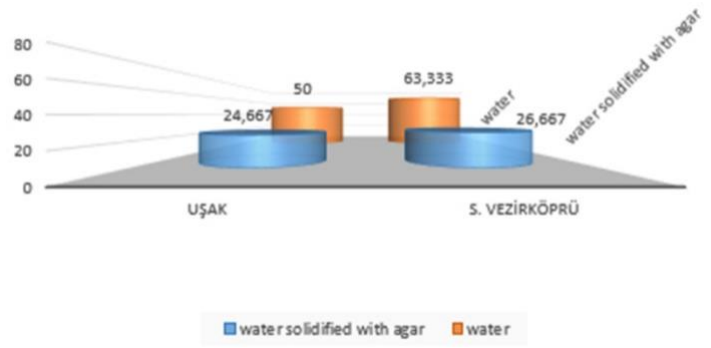

Figure 4. Determination of the effect of sterile pure water on germination in *C. Sativa* plants

Effects MS and Gamborg B5 Medium on Seed Germination;

MS medium is the most widely used medium for plant tissue cultures. It was developed for tobacco tissue culture by Murashige and Skoog (MS) (Sattar, et al., 2010, Owen, et al., 1991, Sarwar, et al., 2009, Khan, et al., 1999). The key feature of MS medium is the very high concentration of nitrate, potassium, and ammonia. Glycine, one of the vitamins, is present in MS medium, which is not present in Gamborg B5 medium (Gamborg, et al., 1976). Inorganic nutrient levels in Gamborg B5 medium are lower compared to the MS medium. Nicotinic Acid, Pyridoxine HCl, and Thiamine HCl are present at a higher percentage compared to MS medium. Inhibition was detected in MS medium containing high nitrogen and K and small amounts of vitamins compared to Gamborg B5 medium.

C. sativa showed seed germination of 26,666% and 33,333% using Uşak and Samsun Vezirköprü population on MS medium (Figure 5). However, an improved seed germination percentage of 66.666% in Uşak and 73.33% in S. Vezirköprü population was noted using Gamborg B5 medium. The results of this study showed that Gamborg B5 medium was more effective in seed germination compared to the MS medium. Thereafter, all seeds were germinated using this methodology.

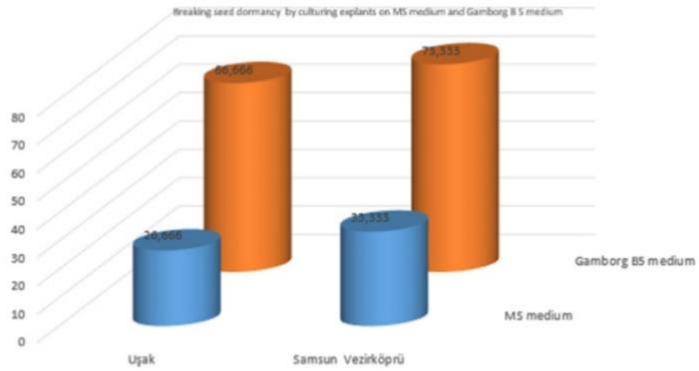

Figure 5. A comparison between Germination percentage on MS and Gamborg B5 medium

Micropropagation Studies

TDZ concentrations have been found to be beneficial in shoot proliferation in a number of explants taken from several plant species. TDZ can inhibit shoot elongation and regeneration (Mok, & Mok, 1985, Gribaudo, & Fronda, 1991, Mok, et al., 1982, Khawar, 2004). Callus formation was noted on all explants but they did not induce any

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adventitious shoots except on calli induced on stem nodes. Shoot induction was noted on stem node explants only (Figure 6). Either callus formation or shoot induction was noted on the Uşak population. Callus induction was noted on the upper portion of the leaf, central portion of the leaf, lower portion of leaf, petiole, internode, and stem nodes explants of Samsun Vezirköprü population showing the percentage of 33.333-66.67%, 33.333-50%, 25-66.667%, 0.667-21.667%, 66.67-83.333%, 25.00-66.667% respectively (Table 1).

Minimum and maximum callus formation was noted on 0.1 and 0.5 mg/l TDZ with the exception of petiole and stem node explant; where maximum callus formation was noted on 0.4 mg/l TDZ. Each increase in the concentration of TDZ from 0.1 mg/l to 0.3 mg/l showed promotory effect on callus induction from stem node explants with shoot regeneration of 75.335% and 4.593 shoots per explant (Figure 6 a,b,c,d,e, f, g, h) Samsun Vezirköprü population.

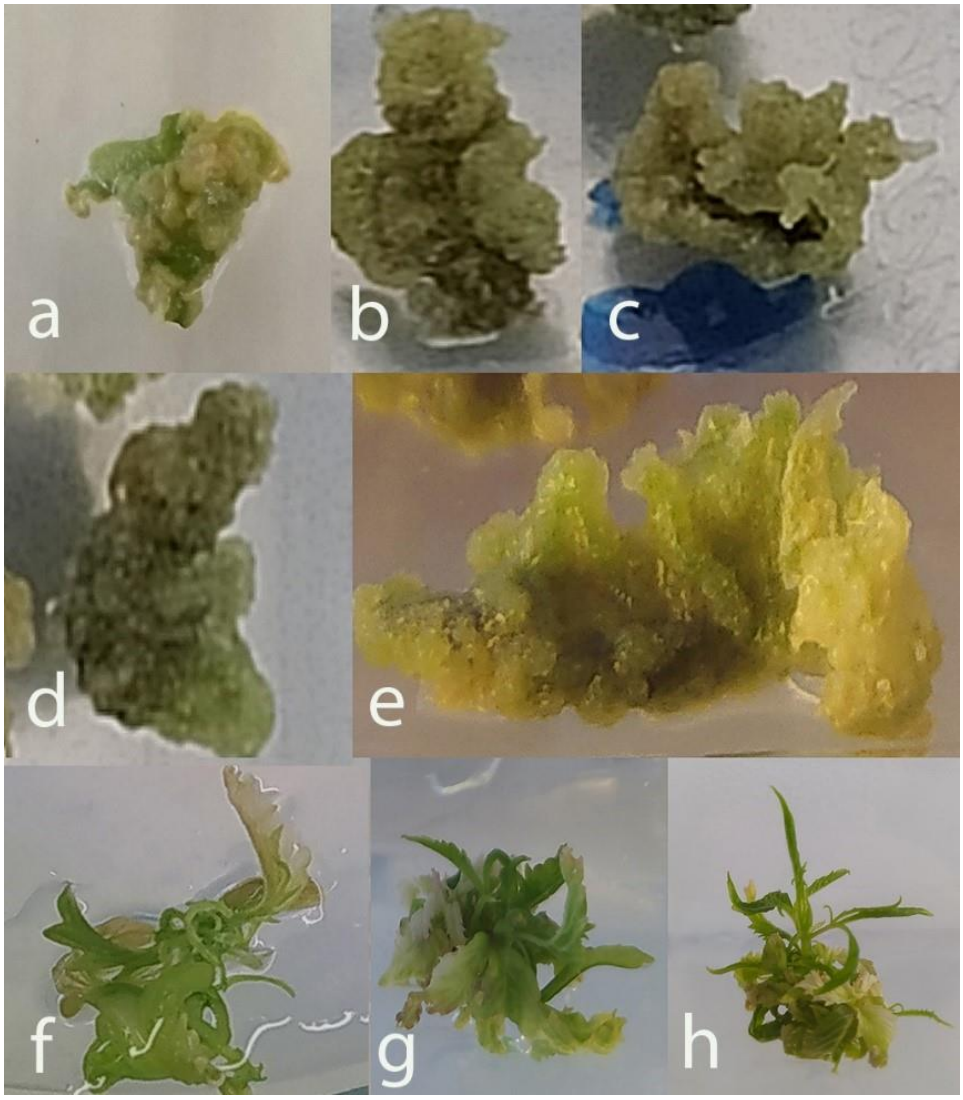

Figure 6. Observing the effect of TDZ doses on different explants (a), upper portion of leaf, (b), central portion of leaf, (c), lower portion of leaf, (d), petiol, (e), stem node (f) ınternode explants Fig a, b, c, d, f, g, h

Table 1. Duncan test results to determine the effects of varying TDZ doses on different explants of *C. Sativa* plants

Explant type	0.1 mg/l TDZ			0.2 mg/l TDZ		
	Swelling percentage (%)	Shoot regeneration percentage (%)	Number of shoots per explant	Swelling/precallusing percentage (%)	Shoot regeneration percentage (%)	Number of shoots per explant
Upper portion of leaf	33,333b*	0.000	0.000	41,667ab	0.000	0.000
Central portion of leaf	33,333b	0.000	0.000	41,667ab	0.000	0.000
Lower portion of leaf,	25,000b	0.000	0.000	50,000ab	0.000	0.000
Petiol	0,667b	0.000	0.000	8,333b	0.000	0.000
Internode explants	25,000b	0.000	0.000	41,667b	0.000	0.000
Stem node	66,667b	0.000	0.000	58,333b	0.000	0.000
	0.3 mg/l TDZ			**0.4 mg/l TDZ**		
Explant type	**Swelling/precallusing percentage (%)**	**Shoot regeneration percentage (%)**	**Number of shoots per explant**	**Swelling/precallusing percentage (%)**	**Shoot regeneration percentage (%)**	**Number of shoots per explant**
Upper portion of leaf	66,667a	0.000	0.000	58,333ab	0.000	0.000
Central portion of leaf	66,667a	0.000	0.000	50,000ab	0.000	0.000
Lower portion of leaf,	50,000ab	0.000	0.000	41,667ab	0.000	0.000
Petiol	66,667a	0.000	0.000	21,667a	0.000	0.000
Internode explants	66,667b	0.000	0.000	25,000b	0.000	0.000
Stem node	16,667a	0.000	0.000	83,333a	75.334	0.000
Explant type	**0.5 mg/l TDZ**					
	Swelling percentage (%)	**Shoot regeneration percentage (%)**	**Number of shoots per explant**			
Upper portion of leaf	33,333b	0.000	0.000			
Central portion of leaf	33,333b	0.000	0.000			
Lower portion of leaf,	33,333b	0.000	0.000			
Petiol	8,333b	0.000	0.000			
Internode explants	25,000b	0.000	0.000			
Stem node	58,333b	75.335	4.593			

*****All nean values given in a column are significantly different using Duncan's Multiple Range Test at 0.05 level of signifcance

Rooting and Acclimatisation

Adaptation of plants obtained from laboratory studies to external conditions is important for ensuring sustainability. All developing shoots on stem node explants rooted in the regeneration medium. Therefore no separate medium containing auxins were used for rooting. These plants were transferred to transparent plastic pots for acclimatization in the growth room (Figure 7).

The *C. sativa* has high adaptability and has spread from the subtropical zone to the temperate zone climate line. *C. sativa* grows naturally or is grown on a limited scale. The amount of moisture in the soil is important before planting. The plant has a high water requirement and the rainfall requirement is high. Since the *C. sativa* plant is very sensitive to temperatures below 0°C, it is badly damaged at temperatures lower than -5 ° C, it needs at least 150 days of maturity not lower than 0°C degrees for seed production and 120 days for quality fiber production (Merve and Orhan, 2020).

The most suitable soils for *C. sativa* plants are medium-heavy, well-drained, airy, deep, fertile in nutrients, soil pH between 6-7.5, loose, loamy and rich in organic matter, calcareous, alluvial soils. Sandy soils, slightly acidic, slightly arid, loamy, and heavy soils, and soils with low permeability and poor drainage are not suitable for *C. sativa* cultivation (Merve and Orhan, 2020). However, when the climatic conditions are evaluated (Figure 8), the *C. sativa* plant, which has a very low resistance to temperatures below 0°C, is completely

damaged at temperatures lower than -5 ° C (Merve, & Orhan, 2020). Therefore it was determined that the plants that acclimatized well during October could not withstand the colds of December when the temperature drops below 5°C and it was damaged.

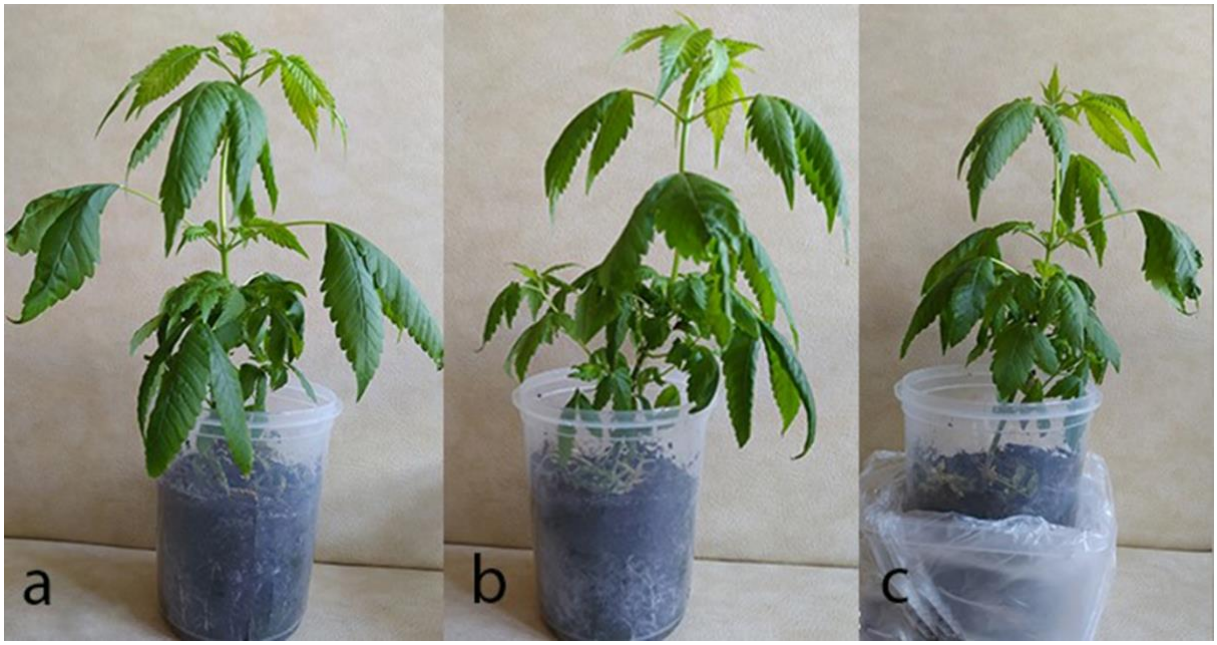

Figure 7. The acclimatization of the plants of *In vitro* cultured plantlets of (a, b, c) Samsun Vezirköprü population to external conditions in the growth cabinet before transfer to external conditions

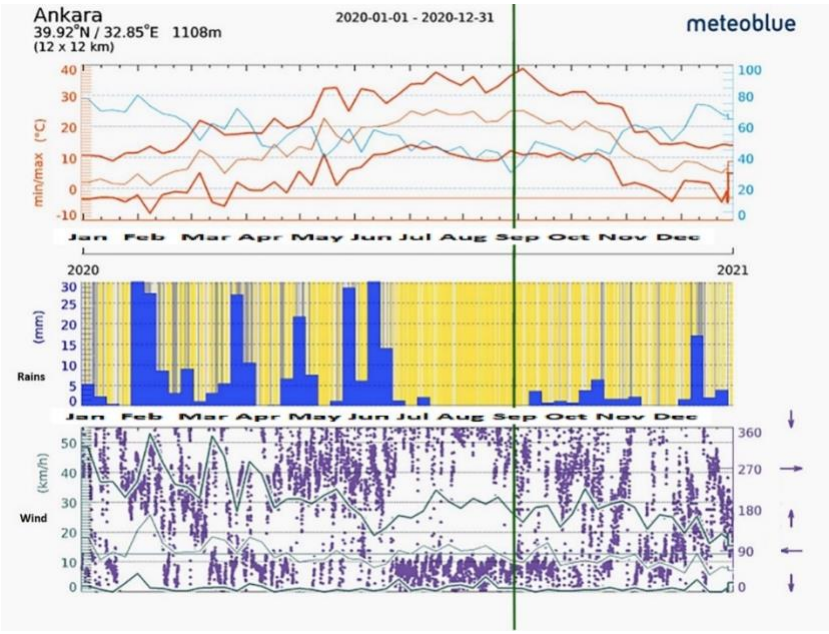

Figure 8. Temperature, precipitation and wind data of the trial site for 2020 (https://www.meteoblue.com/tr/hava/historyclimate/weatherarchive/ankara_t%c3%bcrkiye_323786?fcstlength=1y&year=2020&month=2)

Conclusion

The Uşak and Samsun Vezirköprü populations of *C. sativa* were used in the study. The results of this study optimized conditions for seed germination of two *C. sativa* populations. Samsun Vezirköprü population was found vigorous compared to the Uşak population. The results indicated that the *C. sativa* is suitable for spring sowing under hot humid continental climate of Ankara. It was indicated that further studies should be addressed to the phytochemical behavior of these populations. These studies will be of significant importance for further studies related to the breeding of new cultivars from these populations.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

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Data availability

Not applicable.

Consent for publication

Not applicable.

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