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SUBMISSION AND REVIEW PROCESS

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RESEARCH ARTICLES

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RESEARCH ARTICLE

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Investigation of Durum Wheat Genotypes (*Triticum durum* Desf.) in Terms of Quality and Some Agronomic Traits

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Abstract

Türkiye is one of the origin centers of durum wheat and among the important producer countries of durum wheat. The aim of this study is to examine some durum wheat genotypes with different characteristics in terms of some agronomic and quality characteristics and to determine the relationships between features. The study was carried out in four different environments with supplementary irrigated and based of rainfed in Diyarbakir province conditions. Trial design carry out according to Randomized Complete Blocks Split Plots Experiment Design and three replications. It was determined that there were significant differences at the $p<0.01$ level between genotypes in all the traits examined. According to the research results, change range of average values in durum wheat varieties were determined as; heading time (HT) 170.33-178.42 days, plant height (PH) 93.0-139.2 cm, the number of spikes per square meter (SN) 441.50-567.50 spikes/m^2, the number of grains per spike (GN) 40.7-65.5 grains/spike, thousand grain weight (TGW) 32.4-47.0 g, test weight (TW) 77.5-85.6 kg/hl, protein ratio (PR) 12.72-17.21%, sedimentation amount (SA) 9.58-25.08 ml, b yellowness value (YV) 18.27-27.90, vitreousness ratio (VR) 75.42%-85.42%. Plant height exhibited a positive correlation with protein content ($r = 0.728^{**}$), and sedimentation amount also demonstrated a positive correlation with the b yellowness value ($r = 0.649^{**}$). As a general trend, genotypes with spring attributes were positioned ahead of those with winter characteristics. It has been observed that winter genotypes have a heading time 5-6 days later than that of spring genotypes. Since Fırat-93 (TGW), Kunduru 1149 (PR), Urfa 2005 (TW and VR) and Candidate 1 (SA and YV) genotypes are at the forefront in terms of quality parameters. It would be beneficial to use these genotypes as parents and to protect them as genitor.

Keywords: Durum wheat, Agronomy, Quality, *Triticum durum* Desf.

INTRODUCTION

It has been reported that the origin center of wheat is the Mesopotamian region called the Fertile Crescent, it spread to Western Europe from here, and the Karacadağ region, which is located in the Diyarbakır, Mardin, and Şanlıurfa triangle, is one of the origine centers of wild wheat (Heun et al., 1997; Yıldırım and Atasoy, 2020).

Wheat production continues its potential to be a strategic product with 736 million tons according to 2018 statistical data. In addition, Russia, China, India, Ukraine, USA, Kazakhistan, Canada, Australia and Türkiye are the countries that draw attention with their durum wheat production amounts (FAO, 2019). Durum wheat can be consumed raw or processed into different products. As a matter

of fact, it is used extensively in the production of flour, semolina, bread, pasta, couscous, bulgur, and freekeh (Branković et al., 2018).

Some regions of Türkiye are ecologically very suitable for the cultivation of high quality durum wheat. In some years, unfavorable climatic conditions negatively affect durum wheat cultivation, but genetic structure, ecological conditions, agronomic practices are significantly effective on the quality durum wheat (Pehlivan and Ünver İkincikarakaya, 2017). It has been emphasized that wheat is one of the most important energy and protein sources of people in daily life, and 21% of the world population's protein needs and 19% of their calorie needs are met by wheat (Ali, 2017; Yıldırım and Atasoy, 2020).

Protein ratio is one of the important quality parameters in durum wheat and it has a positive and significant effect on grain vitreousness (Porceddu et al., 1973; Karaman, 2017). In durum wheat, test weight and thousand grain weight, which are the most important grain physical properties, affect the product and milling quality of the wheat and important for the flour and bulgur industry (Karababa and Ercan 1995; Karaman, 2017). In another study; it was emphasized that protein content, sedimentation amount, grain color and vitreousness are important features in categorizing wheat grain and flour (Turnbull and Rahman, 2002; Yıldırım and Atasoy, 2020). It was emphasized that plant height in durum wheat differs depending on the effect of climatic conditions, short wheat varieties are resistant to lodging and mostly early varieties, while plant height between 70-100 cm is reported to be optimum (Aykut et al., 2005; Özen and Akman, 2015).

The primary goal of this study is to assess different durum wheat genotypes, including spring, winter, and landrace varieties, for various agronomic and quality traits in the specific conditions of Diyarbakir province.

MATERIAL AND METHOD

The experiment was established in the Diyarbakir province and in total 4 environments (214-2015 based of rainfed and supplementary irrigated, 2015-2016 based of rainfed and supplementary irrigated) in based of rainfed and supplementary irrigated conditions in the 2014-2016 growing seasons. According to Randomized Complete Blocks Split Plots Experiment Design and three replications the main plots were designed as irrigation and the sub plots were designed as variety. Study material; 25 genotypes were created, including 20 modern, 1 landrace durum wheat cultivar and 4 candidate durum wheat lines. Durum wheat genotypes were planted in 7.2 m^2 plots with a six-row with trial seeder on 500 seeds per square meter. In the plots included in the irrigated application, irrigation was carried out once in the milk grain development stage in the 2014-2015 season, and twice in the 2015-2016 season, in the booting and milk grain development stage, in order to eliminate the drought stress. In the irrigated trials, water was given until the soil was saturated with water.

In rainfed and irrigated applications, 6 kg/da N + 6 kg/da P_2O_5 was given over the pure substance at the base with sowing. In addition, 8 kg/da N was applied as top fertilizer on the pure substance in the period between the end of tillering period and stem elongation. Harvesting was done with a parcel combine harvester on a net 6 m^2 area. In the table containing information on durum wheat material, the first 7 varieties have winter characteristics. Other genotypes are of spring character (Table 1).

In the first year of the study, the amount of precipitation was above the long-term average and in the second year it was below (Table 2). In addition, it was determined that the distribution of precipitation on a monthly basis was irregular in both seasons.

It has been determined that the soils of the trial area have a clay-loam texture, slightly alkaline and poor in terms of organic matter content (Table 3).

The heading time (day) was determined on the basis of the number of days until the period when 70% of the plants were spike at the rate of ½. Plant height (cm) was determined by measuring the part from the soil level to the top of the top spikelet of the 10 plant randomly selected from each plot in the dough formation period, in centimeters (Yürür et al., 1987). For the number of spike (piece) per square meter, the spike were counted before harvesting, taking into account 1 m length and 20 cm width on a row, and then the number of spike per 1 square meter was calculated by multiplying by 5. The number of grains (piece) per spike was determined by counting and averaging the grains obtained from 10 spike samples collected before harvest in each plot.

In order to determine the thousand grain weight (g), 4x100 kernels were counted and weighed separately and the average was multiplied by 10 (Williams et al., 1988). Test weight and protein ratio were determined by using NID In Model 9500 device and reading on the grain surface. For the sedimentation amount (ml), 3.2 g flour sample was weighed and placed in a 100 ml glass measuring cup, then 50 ml of bromine phenol solution was added and the homogeneous suspension obtained was shaken by hand several times. The prepared suspension was quickly placed in the device and shaken for 5 minutes. Then, 25 ml of the prepared lactic acid solution was added and it was shaken for another 5 minutes, the device was turned off and the tube was left on a flat surface for 5 minutes and the precipitation value was read in ml at eye level (ICC, 2008). Yellowness values (b value) of durum wheat genotypes were determined using semolina by Minolta Color Analyzer (CM-6220t). Vitreousness ratio (%) was determined by Grobecker sectioning tool. Vitreous grains were expressed as %.

In the study, variance analysis, LSD and correlation

Table 1. Information on the durum wheat genotypes used in the study

Variety/Candidate	Spring or Winter	Breeder Organization or Origin
Gökgöl-79	Winter	DTARI
Tunca 79	Winter	DTARI
Kunduru 1149	Winter	TZARI
Yelken 2000	Winter	TZARI
Meram-2002	Winter	BDIARI
Selçuklu-97	Winter	BDIARI
Dumlupınar	Winter	TZARI
Güneyyıldızı	Spring	GAP IARTCD
Artuklu	Spring	GAP IARTCD
Fırat-93	Spring	GAP IARTCD
Aydın-93	Spring	GAP IARTCD
Altıntoprak-98	Spring	GAP IARTCD
Ceylan-95	Spring	GAP IARTCD
Diyarbakır-81	Spring	GAP IARTCD
Fuatbey 2000	Spring	EMARI
Sham-1	Spring	EMARI
Sarıbaşak	Spring	EMARI
Pitagora	Spring	MAI
Urfa 2005	Spring	HUFA
Cesare	Spring	PSI
Sorgül	Spring	Landrace variety
Candidate 1	Spring	CIMMYT
Candidate 2	Spring	CIMMYT
Candidate 3	Spring	CIMMYT
Candidate 4	Spring	CIMMYT

GAP IARTCD: GAP International Agricultural Research and Training Center Directorate, PSI: Progen Seed Inc., TZARI: Transitional Zone Agricultural Research Institute, DTARI: Directorate of Trakya Agricultural Research Institute, BDIARI: Bahri Dagdas International Agricultural Research Institute, EMARI: Eastern Mediterranean Agricultural Research Institute, HUFA: Harran University Faculty of Agriculture, MAI: Maro Agriculture Inc. CIMMYT: The International Maize and Wheat Improvement Center

Table 2. Climate data of Diyarbakir province

	Maximum and minimum temperature (°C)		Average temperature long years (°C)	Precipitation (mm)		
Months	**2014-2015**	**2015-2016**		**2014-2015**	**2015-2016**	**Long years (mm)**
September	39.8-10.5	39.1-14.0	24.8	27.4	0.0	4.1
October	30.0-4.7	32.1-7.5	17.2	34.2	84.2	34.7
November	19.7-(-3.6)	21.0-(-1.8)	9.2	97.6	10.4	51.8
December	16.0-(-4.2)	17.0-(-5.9)	4.0	73.6	31.6	71.4
January	13.0-(-10.1)	11.2-(-19.0)	1.8	64.6	77.4	68.0
February	15.3-(-3.1)	21.8-(-5.6)	3.5	55.2	69.2	68.8
March	20.0-(-4.4)	21.1-(-5.1)	8.5	127.0	55.6	67.3
April	27.5-1.2	28.8-(-0.3)	13.8	48.6	29.0	68.7
May	34.2-4.7	32.9-5.2	19.3	48.2	41.4	41.3
June	39.3-9.2	40.5-11.6	26.3	7.4	18.4	7.9
Total				**583.8**	**417.2**	**484.0**

Table 3. The soils analysis results of 2014-2016 experiment areas

Soil Structure	Total Salt (%)	Ph	Lime CaCO3 (%)	Phosphorus P_2O_5 (kg/da)	Organic Matter (%)	Saturation with water (%)
Clayey- loamy	0.25-0.06	7.8-7.9	6.3-13.1	1.28-2.36	0.676-1.33	77-64

analyzes were performed in the J.M.P (5.0.1) package program and the differences between the groups were evaluated at the level of $p<0.01$ or $p<0.05$ according to the LSD test (Kalaycı, 2005). Also, since the variances of the years were homogeneous, the combined analysis was performed.

RESULTS AND DISCUSSION

In the study, it was determined that there was a significant difference at the level of 1% between genotypes in all the traits examined (Table 4, 5 ve 6).

In the study, the mean of heading time varied between 170.33 and 178.42 days. It was observed that the mean of heading time (177.83 days) for the winter genotypes was 5-6 days late more than the spring genotypes (172.39 days). Yelken 2000 durum wheat variety was the latest and Artuklu was the earliest durum wheat variety. Regarding the time to heading time, Sakin et al. (2004) 191.7-205.0 days, Şahinter (2015) 154.4 days, Tanrikulu (2018) 103.50-107.75 days, Enes et al. (2021) 128.00-141.00 days reported that. Differences between wheat genotypes in terms of heading times are highly related to heredity, but the effect of ecological conditions is also important (Yıldırım et al., 2005).

In the experiment, the average plant height differed between 93.0 and 139.2 cm. It was determined that the mean plant height of winter genotypes (110.7 cm) was 8.6 cm longer than spring genotypes (102.1 cm). Kunduru 1149 durum wheat variety gave the longest and Candidate 3 gave the shortest plant height. Regarding plant height; Ertekin (2011) 84.5-98.3 cm, Enes et al. (2021) stated that it is 71.75-117.00 cm reported that. It has been reported that the effect of heredity on plant height is high, but it is shaped under environmental conditions. In addition, it was emphasized that plant height had an indirect effect on yield and yield components (Sakin et al., 2004).

The average number of spikes per square meter varied between 441.50 and 567.50 spikes. It has been determined that the average number of spikes per square meter is 20 spikes less in winter genotypes (489.5 spikes/m^2) compared to spring genotypes (509.5 spikes/m^2). Candidate 1 had the highest number of spike and Dumlupınar durum wheat variety had the lowest number of spike. Regarding the number of spikes per square meter; Özen and Akman (2015) 423-492 spikes/m^2, Naneli et al. (2015) 428.3-565.0 spikes/m^2, Doruk Kahraman and Gökmen (2022) 217.7-462.7 spikes/m^2 reported that.

The average values for the number of grains per spike differed between 40.7 and 65.5 grains. It was determined that the average number of grains per spike of winter genotypes (51.7 grains/spike) was 1.9 grain less than the spring genotypes (53.6 grains/spike). It was determined that Candidate 2 had the highest number of grains per spike, while the Sorgül (40.7 grains/spike) landrace durum wheat variety was the least (Table 5). The number of grains in the spike; Özen and Akman (2015) 21.9-45.9 grains, Doruk Kahraman and Gökmen (2022) 9-23 grains reported that. Higher values were observed in our study. It is thought that this situation is caused by variety, ecological differences and agronomic practices.

Thousand grain weight changed between 32.4 and 47.0 g. It was observed that the average thousand grain weight of the winter genotypes (36.5 g) was 2.7 g lower than the spring genotypes (39.2 g). Fırat-93 variety gave the highest thousand grain weight and Tunca 79 variety gave the lowest thousand grain weight. Thousand grain weight; Güngör and Akgül (2015); 30.5-42.7 g, Yıldırım and Atasoy (2020); 47.18-53.82 g, Enes et al. (2021); it has determined that it differs between 26.52-37.96 g reported that. In the study, the average test weight was between 77.5 and 85.6 kg/hl. Average test weight of the winter genotypes was 3.3 kg less than the spring genotypes. Urfa 2005 durum wheat variety gave the highest test weight and Selcuklu-97 variety gave the lowest weight. Regarding the test weight; Yıldırım and Atasoy (2020) 81.75-84.71 kg/hl, Enes et al. (2021) 67.40-72.20 kg/hl, Bayhan (2022) 82.52-89.74 kg/hl reported that. High test weight in durum wheat indicates a low and healthy grain structure of disease and pest damage (Atlı et al., 2010).

In the study, the average protein content varied between 12.72% and 17.21%. The average protein content in winter genotypes was 1.31% higher than in spring genotypes. The highest protein content was observed in Kunduru 1149 durum wheat variety and the lowest protein content in Candidate 2. Regarding the protein ratio; Altay et al. (2021) 14.85-17.00%, Enes et al. (2021) reported values ranging between 15.85-19.40%, and Bayhan (2022) varying between 12.45-19.74%.

In the study, the average sedimentation amount varied between 9.58 and 25.08 ml. It was observed that the sedimentation amount of winter durum wheat was 0.92 ml lower than the spring genotypes (Table 6). The sedimentation amount of Candidate 1 was the highest and the Ceylan-95 durum wheat variety was the lowest. It was emphasized that the samples with a sedimentation amount of <15 ml were very weak, between 16-24 ml weak, between 25-36 ml good, and those with >36 ml very good gluten quality (Elgün et al., 2002). Regarding the amount of sedimentation; Doğan and Cetiz (2015) 13.3-27.6 ml, Yıldırım and Atasoy (2020) 13.00-29.00 ml, Enes et al. (2021) 18.50-25.00 ml and Bayhan (2022) 8.70-29.70 ml reported that. In the study, b yellowness value was found to differ between 18.27 and 27.90. It was observed that the b yellowness value of winter durum wheat varieties was 0.5 units less than spring varieties. While Candidate 1 had the highest b yellowness value, Diyarbakır-81 durum wheat variety had the lowest value. For the yellowness value (b); Bayhan (2022) 18.41-29.42%, Altay et al. (2021) reported that it was 19.63-21.63%.

Table 4. Mean values and groups of investigated characteristics

Genotypes	HT (day) Irrigation * Genotype				PH (cm) Irrigation * Genotype				SN (spikes /m²) Irrigation * Genotype			
	Rainfed	İrrigated	Mean		Rainfed	İrrigated	Mean		Rainfed	İrrigated	Mean	
Gökgöl 79	176.20	178.20	177.17	b	97.5	105.8	101.7	gh	470.8	618.3	544.58	ab
Tunca 79	175.50	177.30	176.42	b	93.3	103.3	98.4	h-j	431.7	560.8	496.25	b-h
Kunduru 1149	177.30	179.20	178.25	a	133.3	145.1	139.2	a	394.2	568.3	481.25	d-ı
Yelken 2000	178.00	178.80	178.42	a	102.5	110.8	106.8	ef	435.0	508.3	471.67	f-ı
Meram-2002	177.30	179.00	178.17	a	97.5	106.7	102.1	gh	495.0	528.3	511.67	b-g
Selçuklu-97	177.50	179.20	178.33	a	89.2	103.3	96.3	ı-k	405.0	554.2	479.58	d-ı
Dumlupınar	177.00	179.20	178.08	a	128.3	134.2	131.2	b	399.2	483.8	441.50	ı
Güney Yıldızı	169.80	171.50	170.67	j	98.3	107.5	102.4	gh	405.0	515.0	460.00	h-ı
Artuklu	169.30	171.30	170.33	j	90.8	101.7	104.4	e	460.8	567.5	514.17	b-g
Fırat-93	169.70	172.50	171.08	ıj	95.8	105.3	96.2	jk	450.8	508.3	479.58	d-ı
Aydın-93	171.80	173.30	172.58	fg	96.7	107.5	110.6	d	535.0	503.3	519.17	a-f
Altıntoprak 98	169.30	171.70	170.50	j	101.7	112.5	102.1	gh	427.2	524.2	475.67	e-ı
Ceylan-95	172.70	175.20	173.92	de	88.3	100.8	109.0	d	388.0	545.0	466.50	g-ı
Diyarbakır-81	172.50	176.20	174.33	cd	111.7	115.8	112.2	d	415.0	668.3	541.67	ab
Fuatbey 2000	171.50	175.00	173.25	ef	94.2	106.7	102.3	fg	459.2	583.8	521.50	a-f
Sham-1	170.70	172.30	171.50	hı	106.7	120.8	100.7	ı-k	485.8	519.2	502.50	b-h
Sarı Başak	171.00	173.50	172.25	gh	106.7	116.7	103.4	gh	460.0	565.0	512.50	b-g
Pitagora	168.80	172.20	170.50	j	90.8	98.3	94.6	jk	462.5	515.8	489.17	c-ı
Urfa 2005	171.70	173.80	172.75	fg	106.7	113.3	110.1	de	490.5	508.0	499.25	b-h
Cesare	173.80	176.20	175.00	c	94.2	103.3	98.8	hı	440.0	538.3	489.17	c-ı
Sorgül	172.70	175.00	173.83	de	118.3	127.5	123.0	c	463.3	594.7	529.00	a-d
Candidate 1	170.70	172.80	171.75	hı	85.6	92.5	88.9	l	446.7	688.3	567.50	a
Candidate 2	172.50	173.80	173.17	ef	89.2	97.5	93.5	k	485.0	565.0	525.00	a-e
Candidate 3	171.80	173.50	172.67	fg	86.7	99.2	93.0	k	468.0	603.3	535.67	a-c
Candidate 4	171.80	174.00	172.92	fg	89.2	99.2	94.2	k	475.0	612.2	543.58	ab
Av. of winter gen. (1-7)	178.70	176.97	177.83	a	105.9	115.6	110.7		433.0	546.0	489.5	
Av. of spring gen. (8-25)	173.54	171.23	172.39	b	97.3	107	102.1		456.5	562.5	509.5	
Year		**				**				*		
Irrigation		**				**				**		
Year* Irrigation		**				**				ns		
Genotype		**				**				**		
Year * Genotype		**				**				**		
Irrigation * Genotype		ns				ns				**		
Year* Irrigation * Genotype		*				**				**		
CV (%)	0.6				4.9				12.6			

*, 5%, and **, significant at 1%, ns: not significant, Av. of winter gen.: Average of winter genotype, Av. of spring gen.: Average of spring genotype

HT: Heading time, PH: Plant height, SN: Number of fertile spike per square meter

Table 5. Mean values and groups of investigated characteristics

Genotypes	GN (grains/spike) Irrigation * Genotype				TGW (g) Irrigation * Genotype				TW (kg/hl) Irrigation * Genotype				PR (%) Irrigation * Genotype			
	Rain.	Irrig.	Mean		Rain.	Irrig.	Mean		Rain.	Irrig.	Mean		Rain.	Irrig.	Mean	
Gökgöl 79	62.0	49.4	55.7	c-e	32.6	36.1	34.4 lm		79.3	81.2	80.3	j	16.13	13.63	14.88	c-f
Tunca 79	54.0	48.0	51.0	e-h	30.6	34.2	32.4 n		79.8	81.8	80.8	j	16.02	13.67	14.84	c-g
Kunduru 1149	52.7	45.7	49.2	f-h	40.9	44.5	42.7 c		81.9	82.7	82.3	h	18.35	16.07	17.21	a
Yelken 2000	55.9	48.2	52.0	d-h	35.4	41.5	38.5 fg		80.6	83.8	82.2	h	17.03	13.27	15.15	cd
Meram-2002	55.0	50.1	52.6	c-g	33.3	38.4	35.8 jk		77.9	80.4	79.2	k	17.43	12.52	14.98	c-e
Selçuklu-97	53.6	52.8	53.2	c-g	27.7	31.6	29.6 o		75.7	79.2	77.5	l	17.85	12.52	15.18	c
Dumlupınar	52.4	43.9	48.2	g-ı	39.7	44.5	42.1 cd		80.0	81.4	80.7	j	18.70	15.65	17.18	a
Güney Yıldızı	52.1	53.5	52.8	c-g	34.3	39.8	37.1 h-j		82.2	84.9	83.6	ef	15.68	13.35	14.52	d-j
Artuklu	52.6	52.8	52.7	c-g	37.8	43.5	40.6 e		84.1	86.3	85.2	a-c	15.05	12.77	13.91	j-l
Fırat-93	40.5	45.9	43.2	ıj	44.5	49.5	47.0 a		83.5	85.4	84.4	d	15.88	14.57	15.23	c
Aydın-93	49.0	57.7	53.4	c-g	36.0	40.8	38.4 f-h		84.7	85.9	85.3	ab	15.27	13.72	14.49	e-k
Altıntoprak 98	49.0	43.9	46.5	h-j	39.0	46.6	42.8 c		82.2	84.4	83.3	ef	15.57	13.95	14.76	c-h
Ceylan-95	55.1	55.0	55.0	c-f	37.5	44.6	41.0 de		82.3	85.1	83.7	ef	15.20	12.42	13.81	l
Diyarbakır-81	56.1	49.3	52.7	c-g	37.8	44.9	41.4 de		80.8	84.5	82.7	gh	15.27	12.43	13.85	kl
Fuatbey 2000	56.7	54.9	55.8	c-e	40.3	43.8	42.1 cd		84.1	84.9	84.5	d	15.22	13.23	14.23	g-l
Sham-1	55.2	52.6	53.9	c-g	31.6	39.0	35.3 kl		81.7	84.7	83.2	fg	16.20	13.35	14.78	c-h
Sarı Başak	64.1	63.7	63.9	ab	32.8	39.2	36.0 ı-k		83.2	86.2	84.7	cd	15.33	12.60	13.97	jl
Pitagora	57.2	50.1	53.7	c-g	35.2	40.8	38.0 f-h		82.8	84.9	83.8	ef	15.47	13.15	14.31	f-l
Urfa 2005	57.0	59.2	58.1	bc	35.0	39.6	37.3 g-ı		84.8	86.3	85.6	a	15.45	13.38	14.42	e-l
Cesare	57.2	57.8	57.5	cd	36.9	44.1	40.5 e		83.9	86.0	85.0	b-d	15.10	12.73	13.92	j-l
Sorgül	41.6	39.9	40.7	j	36.5	41.1	38.8 f		79.7	81.0	80.3	j	17.13	14.87	16.00	b
Candidate 1	55.3	53.6	54.5	c-f	29.5	36.9	33.2 mn		79.9	84.4	82.2	h	15.97	12.02	13.99	ı-l
Candidate 2	66.3	64.7	65.5	a	29.1	37.2	33.2 mn		79.6	83.6	81.6	ı	14.45	10.98	12.72	m
Candidate 3	55.2	54.8	55.0	c-f	33.5	42.3	37.9 f-h		81.3	85.6	83.5	ef	16.13	12.18	14.16	h-l
Candidate 4	49.2	51.0	50.1	e-h	41.5	47.6	44.5 b		82.5	84.8	83.6	ef	16.12	13.12	14.62	c-ı
Av. of winter gen. (1-7)	55.1	48.3	51.7		34.3	38.7	36.5		79.3	81.5	80.4		17.36	13.90	15.63	
Av. of spring gen.(8-25)	53.8	53.4	53.6		36.0	42.3	39.2		82.4	84.9	83.7		15.58	13.1	14.32	
Year		*				**				**				**		
Irrigation		ns				**				**				**		
Year* Irrigation		ns				**				ns				**		
Genotype		**				**				**				**		
Year * Genotype		ns				**				**				**		
Irrigation * Genotype		ns				**				**				**		
Year* Irrigation * Genotype		ns				**				**				ns		
CV (%)	13.7				4.2				4.2				5.4			

*, 5%, and **, significant at 1%, ns: not significant, Av. of winter gen.: Average of winter genotype, Av. of spring gen.: Average of spring genotype, Rain.: Rainfed, Irrig.: Irrigated, Av.: Average, GN: Number of grains per spike, TW: Test weight, TGW: Thousand grain weight, PR: Protein ratio

Table 6. Mean values and groups of investigated characteristics

Genotypes	SA (ml) Irrigation * Genotype				YV(b value) Irrigation * Genotype				VR (%) Irrigation * Genotype			
	Rainfed	İrrigated	Mean		Rainfed	İrrigated	Mean		Rainfed	İrrigated	Mean	
Gökgöl 79	15.17	16.50	15.83	fg	23.54	22.19	22.86	ef	81.50	81.17	81.33	a-e
Tunca 79	17.50	16.00	16.75	ef	24.33	23.59	23.96	c	82.83	81.50	82.17	a-e
Kunduru 1149	10.50	11.17	10.83	ıj	22.32	21.87	22.10	fg	82.17	80.17	81.17	a-e
Yelken 2000	14.33	13.67	14.00	h	24.36	23.17	23.77	cd	84.00	82.67	83.33	a-d
Meram-2002	20.00	17.67	18.83	cd	19.26	19.26	19.26	kl	82.00	76.50	79.25	c-f
Selçuklu-97	19.00	15.67	17.33	d-f	22.57	21.73	22.15	fg	85.33	82.67	84.00	a-c
Dumlupınar	18.83	17.33	18.08	de	20.86	20.08	20.47	j	79.33	80.50	79.92	b-f
Güney Yıldızı	19.67	17.17	18.42	cd	24.97	24.08	24.52	c	86.17	83.50	84.83	a
Artuklu	16.17	13.83	15.00	gh	22.30	20.75	21.52	gh	81.83	85.17	83.50	a-c
Fırat-93	15.67	16.17	15.92	fg	20.14	19.91	20.02	jk	75.00	75.83	75.42	f
Aydın-93	16.50	13.33	14.92	gh	22.90	21.58	22.24	fg	89.67	80.83	85.25	a
Altıntoprak 98	22.00	19.67	20.83	b	22.93	21.70	22.31	fg	83.83	80.83	82.33	a-e
Ceylan-95	9.83	9.33	9.58	j	19.42	17.95	18.69	lm	81.50	82.83	82.17	a-e
Diyarbakır-81	9.50	10.00	9.75	ıj	19.17	17.37	18.27	m	80.00	76.33	78.17	ef
Fuatbey 2000	10.83	10.33	10.58	ıj	21.02	21.53	21.27	hı	86.50	82.00	84.25	ab
Sham-1	12.33	10.33	11.33	ı	23.16	22.02	22.59	ef	83.17	83.00	83.08	a-d
Sarı Başak	20.83	15.67	18.25	c-e	20.92	20.22	20.57	ıj	81.33	87.50	84.42	ab
Pitagora	22.17	17.33	19.75	bc	26.33	26.40	26.36	b	85.17	84.00	84.58	ab
Urfa 2005	15.33	13.33	14.33	gh	22.82	22.00	22.41	ef	89.00	81.83	85.42	a
Cesare	25.83	21.50	23.67	a	26.21	25.11	25.66	b	79.67	79.17	79.42	c-f
Sorgül	13.33	14.00	13.67	h	21.19	20.06	20.63	ıj	80.17	77.17	78.67	d-f
Candidate 1	28.00	22.17	25.08	a	28.59	27.21	27.90	a	83.33	80.00	81.67	a-e
Candidate 2	24.50	18.00	21.25	b	23.34	23.00	23.17	de	82.50	82.83	82.67	a-e
Candidate 3	20.83	14.83	17.83	de	24.43	23.33	23.88	cd	85.17	84.17	84.67	ab
Candidate 4	26.67	20.50	23.58	a	24.64	24.23	24.44	c	78.67	85.17	81.92	a-e
Av. of winter gen. (1-7)	16.48	15.43	15.95		22.46	21.70	22.08		82.45	80.74	81.60	
Av. of spring gen. (8-25)	18.33	15.42	16.87		23.03	22.14	22.58		82.93	81.79	82.36	
Year		*				ns				**		
Irrigation		**				**				ns		
Year* Irrigation		ns				ns				**		
Genotype		**				**				**		
Year * Genotype		**				ns				ns		
Irrigation * Genotype		**				ns				ns		
Year* Irrigation * Genotype		ns				ns				ns		
CV (%)	12.3				4.4					7.2		

*, 5%, and **, significant at 1%, ns: not significant , Av. of winter gen.: Average of winter genotype, Av. of spring gen.: Average of spring genotype, SA. Sedimentation amount, YV(b): b yellowness value, VR: Vitreousness ratio

Table 7. Correlation results for the investigated traits

Features	HT	PH	SN	GN	TGW	TW	PR	SA	YV (b)
PH	0.421*								
SN	-0.2012	-0.3662							
GN	-0.0652	-0.3668	0.2322						
TGW	-0.2226	0.3493	-0.218	-0.477*					
TW	-0.688**	-0.0945	0.0175	0.2182	0.512**				
PR	0.527**	0.728**	-0.433*	-0.673**	0.2529	-0.3952			
SA	-0.1706	-0.530**	0.1624	0.1566	-0.1916	-0.0643	-0.2224		
YV(b)	-0.2124	-0.471*	0.1833	0.1882	-0.3165	0.1113	-0.191	0.649**	
VR	-0.2383	-0.2075	-0.0097	0.526**	-0.409*	0.27	-0.313	0.0074	0.37

*, 5%, and **, significant at 1%, HT: Heading time, PH: Plant height, SN: Number of fertile spike per square meter, GN: Number of grains per spike, TGW: Thousand grain weight, TW: Test weight, PR: Protein ratio, SA. Sedimentation amount, YV(b): b yellowness value, VR: Vitreousness ratio

In the durum wheat, b yellowness value was reported to be associated with heredity at the rate of 86.6%, and it was shaped under the influence of ecological conditions at the rate of 8.5% (Manthey, 2001).

In the study, the average vitreousness ratio varied between 75.42% and 85.42%. Average vitreousness ratio of spring durum wheat varieties was 0.76% higher than winter genotypes. Urfa 2005 durum wheat variety had the highest, Fırat-93 variety had the lowest vitreousness. Regarding the vitreousness ratio; Altay et al. (2021) determined that it was 90.25-97.25% and Bayhan (2022) 85.08-99.68%. Grain hardness in durum wheat; it was emphasized that associated with protein, starch ratio and grain vitreousness (Stenvert and Kingswood, 1977; El-Khayat et al., 2006).

According to the results of the correlation analysis, it was determined that the heading time (r=-0.688**) was negatively correlated with the test weight and positively correlated with the protein ratio (r=0.527**). This situation can be explained by the fact that the genotypes are exposed to more heat stress during the grain filling period and cause the grain to become wrinkled as the heading period is prolonged in the region. It was observed that plant height was positively correlated with protein ratio (r=0.728**) and negatively correlated with sedimentation (r=-0.530**) and yellowness value (b) (r=-0.471*). The number of spike per square meter (r=-0.433*) and the number of grains per spike (r=-0.673**) were negatively correlated with the protein ratio (Table 7). In addition, the sedimentation amount (r=0.649**) was positively related to the b yellowness value, and the vitreousness ratio (r=-0.409*) was negatively related to the thousand grain weight (Bayhan, 2022).

CONCLUSION

As a general trend, genotypes with spring attributes were positioned ahead of those with winter characteristics. It has been observed that there are 5-6 day difference between winter and spring genotypes in terms of the heading time. It is noteworthy that Fırat-93 has high thousand-grain weight, Kunduru 1149 protein content, Urfa 2005's vitreousness, Candidate 1's sedimentation, b yellowness values and spike number per squar meter are well above the trial average. In the study; it was found that plant height was positively correlated with protein ratio (r= 0.728**), and b yellowness value (r= -0471**) was negatively correlated. In addition, it was determined that the vitreousness ratio was negatively (r= -0.409) related to the thousand grain weight. In quality-oriented breeding programs; it was concluded that it would be beneficial to use Fırat-93, Kunduru 1149, Urfa 2005 and Candidate 1 genotypes as parents and to protect as genitor. Especially the fact that Candidate 1 is in the first place in terms of sedimentation amount, yellowness value (b value) and number of spikes per square meter strengthens its being a variety candidate.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, 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.

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

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RESEARCH ARTICLE

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Some Quality and Microbiological Traits of Tokat Tarhana Obtained from Different Wheat Cultivars Under Various Drying Conditions

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Abstract
This study was carried out to evaluate the variations in some quality traits of Tokat red pepper tarhana samples according to the cultivars and drying techniques. For this aim, five white grained bread wheat cultivars such as Altınöz, Candaş, Gökkan, Şahika, and Yakamoz were used as material. And, tarhana samples were dried under open air, airflow oven and vacuum. The study was conducted with three replications according to the split plot design in random plots. All quality traits showed statistically significant variations for the bread wheat cultivars (C), drying techniques (DT), and DT×C interactions. Thus, airflow oven showed the highest values among the drying techniques for the contents of ash (8.5892%), total titration acidity (27.433%) and salt (6.842%). On the other hand, open air drying technique presented the highest values for moisture (14.556%), pH (4.349) and the density of lactic acid bacteria (LAB) (1.4×10^4 CFU g^{-1} for M17 agar, 2.4×10^4 CFU g^{-1} for MRS agar. Also, Gökkan *cv* had the highest percents for all quality traits except LAB density. These findings showed that the most suitable bread wheat cultivar for Tokat red pepper tarhana was Gökkan *cv*, and the most favorable drying technique was open air drying. In addition, it is understood from the results that wheat cultivar and drying technique which used in tarhana production were essential factors. So, tarhana sector should choose the best wheat cultivar and drying technique to be used in tarhana production for consumer taste and healthy diet. From this study, it is understood that the open air drying is the most sustainable technique in terms of both production and consumption.
Keywords: Bread wheat, Flour, Quality traits, Tarhana

INTRODUCTION

When Tokat tarhana is mentioned, red pepper tarhana, named after Turkey's Tokat province, comes to mind. Of course, tarhana, which is enjoyed by almost everyone all over Turkey, is not limited to this region. When a detailed literature review is made, the origin of tarhana is from Central Asia to Anatolia; It will be understood that it spread widely from the Balkans to the Middle East. So and so, some researchers say that tarhana was passed to the Turks from Central Asia due to the relations of the Turks with the Chinese, and from there to Anatolia; they reported that it spread from Anatolia to the Balkans, Europe and the Middle East. While "Tarhana" finds a usage area with the same name in Türkiye; "Kishk" in Syria, Palestine, Jordan, Lebanon and Egypt; it is called "Kushuk" in Iraq and Iran; "Thanu" or "Tahonya"n Hungary; Known as "Talkuna" in Finland (Siyamoğlu, 1961; Merdol, 1968). It is known as "Trahanas" in Greece (Lazos et al., 1993). About 60 years ago, when two types of tarhana named "Göce" and "Aegean" were mentioned in Turkey (Siyamoğlu, 1961); today, it is reported that this number is

around 50 and this diversity is due to the changes in the raw material used locally and the differences in the way of presentation (Aksu et al., 2012).

Although its name and content are similar according to regions and countries; "Tarhana" is defined as follows according to the standard numbered 2282 of TSE (2004): "It is a nutrient obtained by drying, grinding and sifting wheat flour, wheat flour, semolina or their mixture with yoghurt, pepper, salt, onion, tomato and flavor-scented, harmless herbal substances after mixing and kneading and fermenting". In the same standard, four types of tarhana are defined as "göce tarhana", "flour tarhana", "mixed tarhana" and "semolina tarhana". These definitions have been determined depending on the use of semolina and wheat flour/crumbs in tarhana production. Mixed tarhana is obtained by using wheat flour/crushed or two different products from semolina, and semolina tarhana is obtained by using semolina instead of flour in its production (TSE, 2004). In Turkey, other tarhanas named according to their content and the region they are located in can be listed as follows: ball tarhana, Thrace, white tarhana, Gediz, cranberry, minced meat, Beyşehir, Göçmen, Kastamonu fresh tarhana, Sivas, Kahramanmaraş, Turnip tarhana, beet tarhana, milk tarhana, dough tarhana, meat tarhana, grape tarhana and sweet tarhana (Coşkun, 2014). In addition, the amount of yoghurt and salt added in tarhana production is also effective on the fermentation efficiency. It has been reported that the fermentation efficiency of tarhana increases with increasing the amount of substitute yogurt, but the time decreases with the addition of salt, and the use of lactic acid bacteria as probiotics in food production has increased in recent years (İbanoğlu and İbanoğlu, 1999).

The main foods used in tarhana production are yogurt and cereals. The type and amount of cereals and yoghurt used vary according to the region to which the tarhana belongs (Akbaş and Coşkun, 2006). Cereals are sources that we consume in our daily diet and meet our needs of carbohydrates, minerals, proteins, dietary fiber and vitamins. Nutritional values and sensory properties of milk and its products are higher when compared to cereal products. However, as a result of the fermentation of cereal grain and dairy products, a significant increase is observed in both sensory properties and nutritional values (Blandino et al., 2003). Flour Tarhana; it is a type of tarhana obtained by mixing yoghurt, onion, tomato pepper, salt and flavoring herbal products that are harmless to health, kneading with wheat flour and leaving to fermentation, then drying, grinding and finally sifting (Esimek, 2010). Dried or fresh ingredients such as tomatoes, onions, peppers are added to the mixture. These products are prepared by washing, cutting and sifting the main material of flour. Cooking is done for fresh vegetables. The thinning process is applied to the cooked and mixed materials (Atasoy, 2018). Although the materials used and their amounts vary according to the region, homemade or industrial tarhana production is generally done in four basic stages. These are: i) process of preparation and mixing, ii) fermentation, iii) drying and iv) grinding (Özdemir et al., 2007). Yogurt bacteria (*Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*) and sourdough (*Saccharomyces cerevisiae*) perform the fermentation process in tarhana. With this process, the acidic and sour taste peculiar to tarhana is obtained. Ventilation should be provided in the room where the fermentation will take place and the temperature should be 30-35 °C. Thanks to the lactic acid bacteria formed during the fermentation, the pH of the environment is lowered and a bacteriostatic effect is created on pathogenic microorganisms. As the nutritional value of tarhana increases with fermentation, it also becomes an easy-to-digest product (Çopur et al., 2001).

In this research, which concerns tarhana in general, but specifically studied with Tokat red pepper tarhana, answers to the following questions were sought: First, what is the most suitable cultivar and the most suitable drying technique in terms of the quality characteristics examined? Second, has the drying technique×bread wheat cultivar interaction been found to be statistically significant on this quality criterion? After that, what is the best drying technique for the preservation of LAB in tarhana? Finally, which drying technique is most suitable for food safety?

MATERIALS AND METHODS

Material

Wheat grain materials were obtained from Republic of Türkiye, Ministry of Agriculture and Forestry, Eastern Mediterranean Agricultural Research Institute. All of the bread wheat cultivars used in the study are white grained, and their names are: Altınöz, Candaş, Gökkan, Şahika, and Yakamoz (Anonymous, 2023). The other materials used in the tarhana samples and their amounts were given in Table 1.

Table 1. The formulation of Tokat red pepper tarhana

Materials	Quantity (g)
Yogurt	130
Wheat flour	350
Tomatoe	200
Onion	100
Red pepper	400
Fresh dill	15
Salt	30
Fresh mint	15
Chickpea	15

Method

Tarhana production

Traditional tarhana production method based on spontaneous gradual fermentation method was applied to five bread wheat cultivars mentioned in the material. Firstly, vegetables such as onions, red peppers, and tomatoes were cut, followed by the cooking process by adding fresh mint and dill. Then, thinning was performed by shredding. Boiled chickpeas were then subjected to this process and included in the mixture. After this mixture was cooled, yoghurt, flour and salt were added and kneaded. This mixture was left to ferment for 5-7 days in a controlled manner by being ventilated at certain intervals, and then drying and grinding processes were started. For the drying process, three methods such as open air, airflow oven, and under vacuum were used.

Evaluated traits and the methodology

Of the investigated properties, moisture content was determined according to AACC (1990). TSE (2004) was used to determine the ash content. Protein content of tarhana samples was determined by Kjeldahl method (AACC, 1990) according to TS 2282 (TSE, 2004). The amount of nitrogen obtained was multiplied by the factor of 6.25 and the total protein was calculated as percent. Fat content of tarhana samples were calculated as percent by extraction with petroleum ether using automatic soxhlet device (AACC, 1990). The pH of the tarhana samples was determined according to TSE (2004). Total titration acidity was determined by the titration of tarhana samples (1 g) with 0.1 M NaOH concentration and expressed as percent lactic acid (Nes et al., 1996). Salt percentage of the samples were indicated by TSE (2004). For the density of lactic acid bacteria; 10 g of tarhana sample was homogenized in 90 mL of sterile physiological saline (SFS). Other dilutions were made by transferring 1 mL of the 10^{-1} dilution prepared in this way to 9 mL SFSs. It was sterilized by autoclaving before taking 0.1 mL from the appropriate diffusions. It was cooled down to 45-50 °C and poured into sterile petri dishes and inoculated on MRS Agar (for *Lactobacillus spp.*) and M17 Agar (for *Streptococcus* and *Lactococcus spp.*) media under aseptic conditions with 0.1 mL spread method. Petri dishes were incubated for 2 days at 30 °C for mesophiles and 40 °C for thermophiles under anaerobic conditions for MRS Agar, aerobic conditions for M17 Agar. At the end of the incubation, the colonies in the petri dishes were counted and expressed as CFU g^{-1}. Data except of lactic acid bacteria were evaluted by the JMP statistical program.

RESULTS AND DISCUSSION

The mean squares of cultivar, drying technique and the interaction of cultivar×drying technique obtained from variance analyzes of the investigated traits such as the contents of moisture, ash, protein, fat, pH, total titration acidity, and salt in Tokat tarhana are given in Table 2. According to Table 2; all sources of variation (cultivar, drying technique and the interaction of cultivar×drying technique) showed significant differences at $p<0.01$.

Moisture content

Tarhana is usually dried after fermentation to reduce its moisture content to less than 10% and to prevent clumping and microbial spoilage. Drying not only reduces moisture in tarhana, but also ensures that it has a bacteriostatic effect. The low humidity and relatively low pH inhibit the growth of pathogenic and spoilage microorganisms, resulting in a shelf life of more than one year. Tarhana is not hygroscopic and can be stored for 1-2 years without spoiling due to its low pH (3.50-5.00) and moisture content. It was stated by Erkan et al. (2006) that the difference in moisture content of tarhana samples was due to the properties of the components used in the formulation and the drying methods.

Mean values for moisture content in Tokat tarhana dried under various techniques and from some bread wheat cultivars were presented in Table 3. While the highest moisture content in the tarhana samples was obtained from the samples of open air drying (14.56%), the lowest humidity was determined in the samples of airflow oven drying (4.31%). When evaluated on the basis of the cultivars, the highest moisture content was determined in Gökkan *cv* with 9.22%, the lowest humidity was determined in Altınöz *cv* with 7.77%. Tarhana samples obtained from bread wheat varieties showed higher moisture in open air drying technique compared to

Table 2. The mean squares of cultivar, drying technique and the interaction of cultivar×drying technique obtained from variance analyzes of the investigated traits in Tokat tarhana.

Variation source	d_f	Mean of squares						
		Moisture	Ash	Protein	Fat	pH	TTA	Salt
Drying technique (DT)	2	437.997**	11.760**	6.211**	3.977**	0.010**	323.539**	6.719**
Error1	6	0.131	0.020	0.062	0.068	0.000	0.308	0.001
Cultivar (C)	4	2.584**	4.187**	5.890**	4.173**	0.028**	26.589**	3.076**
DT×C	8	4.203**	0.389**	1.082**	3.743**	0.004**	4.258**	0.232**
Error2	24	0.105	0.026	0.043	0.109	0.000	0.593	0.005
CV (%)		3.8	2.0	1.6	6.3	0.2	3.2	1.2

d_f: degree of freedom; CV: coefficient of variation; TTA: total titration acidity; **: significant at $p<0.01$

Table 3. The Mean values for moisture content in Tokat tarhana dried under various techniques and from some bread wheat cultivars (%).

Cultivars (C)	Drying Techniques (DT)			Mean
	Open air	Airflow oven	Under vacuum	
Altınöz	14.980^{b*}	2.215^{gh}	6.165^{k}	7.787^{d}
Candaş	14.350^{c}	4.949^{h}	5.726^{i}	8.342^{bc}
Gökkan	15.971^{a}	4.246^{e}	7.451^{j}	9.222^{a}
Şahika	13.645^{d}	6.333^{gh}	5.905^{fg}	8.628^{b}
Yakamoz	13.833^{cd}	3.912^{f}	6.810^{j}	8.185^{c}
Mean	14.556^{a}	4.331^{c}	6.411^{b}	8.433
LSD_{DT}			0.324	
LSD_{C}			0.315	
$LSD_{DT\times C}$			0.545	

*: There is no difference at the 0.05 probability level between the mean values with the same letter group. LSD_{DT}, LSD_{C}, and $LSD_{DT\times C}$ show the least significant differences between the mean values in terms of drying techniques, bread wheat cultivars and drying technique×cultivar interaction, respectively.

other drying techniques; it caused the KT×Ç interaction, in other words, the cultivars showed different moisture xontent in different drying techniques (Table 3).

ASH content

ASH refers to the inorganic residue remaining after the combustion or complete oxidation of organic matter in a food sample. Determining the ash content of a food is part of close analysis for nutritional assessment and is an important quality attribute for some food ingredients. In a study, the results of ash analysis in tarhana samples sold on the market varied between 1.91% and 3.97% (Göçmen et al. 2003). In the study of 27 homemade tarhanas produced in Isparta region, the ash analysis results were determined between 1.63% and 3.19% (Soyyigit, 2004). As the salt content increases, the ash values also increase. In addition, since the moisture content is different according to the drying techniques, the amount of ash in the products was different according to the drying techniques.

Mean values for ash content in Tokat tarhana dried under various techniques and from some bread wheat cultivars were presented in Table 4. While the highest ash content in tarhana samples was obtained from the airflow oven drying (8.592%), the lowest value was from the samples of open air drying (6.937%). Whean the mean values were evaluated on the basis of bread wheat cultivars, the highest ash content was determined in Gökkan *cv* with 9.136%, and the lowest ash was from Altınöz *cv* with 7.462%. Tarhana samples obtained from bread wheat cultivars showed higher ash content values in airflow oven drying technique compared to other drying techniques; so, DT×C interaction was occured, in other words, the cultivars showed different ash contents in different drying techniques (Table 4).

Protein content

It has been reported in some studies that the main reason for the change in the protein content of tarhana may be the type and amount of yogurt used in making tarhana (Temiz and Pirkul, 1991). It has been reported that the average protein values obtained from 13 different tarhana samples taken from industrially produced areas are 14.49% (Şimşekli and Doğan, 2015). In our study, it was observed that the amount of protein changed in different bread wheat cultivars and drying techniques.

Mean values for protein content in Tokat tarhana dried under various techniques and from some bread wheat cultivars were presented in Table 5. In the tarhana samples, the highest protein was obtained from the samples that were dried in the airflow oven (13.543%) while the lowest protein was from the samples of open air drying (12.404%). When evaluated on the basis of cultivars, the highest protein was determined in Gökkan *cv* with 14.167%, and the lowest protein was indicated in Şahika *cv* with 12.339%. Tarhana samples obtained from bread wheat varieties showed higher protein in airflow oven drying technique than the other drying techniques; so, the inraction of DT×C was occured (Table 5).

Fat content

Fats, which have an important place in human nutrition; it provides heat and energy to the body. In a study conducted; they examined five different commercial tarhana samples. It has been reported that the fat ratios in the examined tarhana samples are between 2.70% and 5.40% (O'Callaghan et al., 2019). When we look at another study, five different tarhana samples were examined and it was reported that the fat content of these examined tarhana samples was between 1.00% and 9.00% (Özdemir et al., 2007).

Mean values for fat content in Tokat tarhana dried under various techniques and from some bread wheat cultivars were showed in Table 6. When Table 6 was evaluated; the highest fat content in tarhana samples was obtained from the samples dried under vacuum (5.867%), while

Table 4. The Mean values for ash content in Tokat tarhana dried under various techniques and from some bread wheat cultivars (%).

Cultivars (C)	Drying Techniques (DT)			Mean
	Open air	Airflow oven	Under vacuum	
Altınöz	6.823^{gh*}	8.105^{de}	7.459^{f}	7.462^{c}
Candaş	6.731^{gh}	8.469^{c}	8.327^{cd}	7.842^{b}
Gökkan	7.501^{f}	10.147^{a}	9.761^{b}	9.136^{a}
Şahika	6.993^{g}	8.138^{de}	8.090^{de}	7.740^{b}
Yakamoz	6.636^{h}	8.099^{de}	7.914^{e}	7.550^{c}
Mean	6.937^{c}	8.592^{a}	8.310^{b}	7.946
LSD_{DT}			0.127	
LSD_{C}			0.157	
LSD_{DTxC}			0.273	

*: There is no difference at the 0.05 probability level between the mean values with the same letter group. LSD_{DT}, LSD_{C}, and LSD_{DTxC} show the least significant differences between the mean values in terms of drying techniques, bread wheat cultivars and drying techniquexcultivar interaction, respectively.

Table 5. The Mean values for protein content in Tokat tarhana dried under various techniques and from some bread wheat cultivars (%).

Cultivars (C)	Drying Techniques (DT)			Mean
	Open air	Airflow oven	Under vacuum	
Altınöz	11.373^{f*}	13.317^{c}	12.833^{de}	12.508^{d}
Candaş	12.490^{e}	12.743^{de}	13.460^{c}	12.898^{c}
Gökkan	12.883^{d}	14.780^{a}	14.837^{a}	14.167^{a}
Şahika	11.250^{f}	12.873^{d}	12.893^{d}	12.339^{d}
Yakamoz	14.023^{b}	14.003^{b}	13.437^{c}	13.821^{b}
Mean	12.404^{b}	13.543^{a}	13.492^{a}	13.146
LSD_{DT}			0.223	
LSD_{C}			0.201	
LSD_{DTxC}			0.348	

*: There is no difference at the 0.05 probability level between the mean values with the same letter group. LSD_{DT}, LSD_{C}, and LSD_{DTxC} show the least significant differences between the mean values in terms of drying techniques, bread wheat cultivars and drying techniquexcultivar interaction, respectively.

Table 6. The Mean values for fat content in Tokat tarhana dried under various techniques and from some bread wheat cultivars (%).

Cultivars (C)	Drying Techniques (DT)			Mean
	Open air	Airflow oven	Under vacuum	
Altınöz	5.507^{def*}	3.123^{i}	6.667^{ab}	5.099^{c}
Candaş	4.553^{gh}	5.440^{def}	6.487^{ab}	5.493^{b}
Gökkan	6.213^{bc}	5..680^{cde}	5.593^{cd}	5.939^{a}
Şahika	3.213^{i}	4.133^{h}	5.217^{ef}	4.188^{d}
Yakamoz	5.113^{f}	6.843^{a}	5.043^{fd}	5.667^{ab}
Mean	4.920^{b}	5.044^{b}	5.867^{a}	5.277
LSD_{DT}			0.234	
LSD_{C}			0.321	
LSD_{DTxC}			0.556	

*: There is no difference at the 0.05 probability level between the mean values with the same letter group. LSD_{DT}, LSD_{C}, and LSD_{DTxC} show the least significant differences between the mean values in terms of drying techniques, bread wheat cultivars and drying techniquexcultivar interaction, respectively.

the lowest fat was found in the samples dried in open air (4.920%). From the analysed of cultivars for fat content, the highest value was determined in Gökkan *cv* with 5.939%, the lowest value in Şahika *cv* with 4.188%. Tarhana samples obtained from bread wheat cultivars showed higher oil in vacuum drying technique than the other drying techniques, resulting in DT×C interaction (Table 6).

pH value

Color, flavor, and texture are important quality attributes and major factors influencing food sensory perception and consumer acceptance. Most food products have a pH between 3.50 and 7.00. pH has a significant effect on pigments (eg chlorophyll, carotenoids, anthocyanins etc.) and is responsible for the color of fruit, vegetables and meat. Therefore, knowledge of pH is very important to produce safe, quality and value-added products. pH values of 13 tarhana samples produced and offered for sale in the Kahramanmaraş region varied between 3.00 and 4.22 (Yörükoğlu and Dayısoylu, 2016). In the tarhana production study using legume flour instead of wheat flour, pH values changed between 3.80-4.20 (Atasoy, 2018).

In tarhana samples, the highest pH was obtained from the samples dried in the open air (4.349), while the lowest pH was determined in the samples dried in the oven (4.298). When evaluated on the basis of cultivars, the highest pH was found in Gökkan *cv* with 4.370 and the lowest pH in Altınöz *cv* with 4.240. Tarhana samples obtained from bread wheat cultivars showed higher pH in open air drying technique compared to other drying techniques, causing DT×C interaction, in other words, cultivars show different pH values in different drying techniques (Table 7).

Table 7. The Mean values for pH value in Tokat tarhana dried under various techniques and from some bread wheat cultivars.

Cultivars (C)	Drying Techniques (DT)			Mean
	Open air	Airflow oven	Under vacuum	
Altınöz	4.233^{h*}	4.237^{gh}	4.250^{g}	4.240^{d}
Candaş	4.360^{c}	4.270^{f}	4.300^{e}	4.310^{c}
Gökkan	4.397^{b}	4.353^{c}	4.360^{c}	4.370^{a}
Şahika	4.320^{d}	4.280^{f}	4.400^{b}	4.333^{b}
Yakamoz	4.433^{a}	4.350^{c}	4.350^{c}	4.378^{a}
Mean	4.349^{a}	4.298^{b}	4.332^{a}	4.326
LSD_{DT}			0.018	
LSD_{C}			0.009	
$LSD_{DT×C}$			0.015	

*: There is no difference at the 0.05 probability level between the mean values with the same letter group. LSD_{DT}, LSD_{C}, and $LSD_{DT×C}$ show the least significant differences between the mean values in terms of drying techniques, bread wheat cultivars and drying techniquexcultivar interaction, respectively.

Table 8. The Mean values for TTA in Tokat tarhana dried under various techniques and from some bread wheat cultivars (%).

Cultivars (C)	Drying Techniques (DT)			Mean
	Open air	Airflow oven	Under vacuum	
Altınöz	20.000^{g*}	29.583^{ab}	27.250^{c}	25.611^{b}
Candaş	16.583^{i}	25.583^{de}	24.250^{f}	22.139^{d}
Gökkan	20.417^{g}	30.417^{a}	28.750^{b}	26.528^{a}
Şahika	20.250^{g}	24.750^{ef}	26.250^{cd}	23.750^{c}
Yakamoz	17.917^{h}	26.833^{cd}	26.833^{cd}	23.861^{c}
Mean	19.033^{c}	27.433^{a}	26.667^{b}	24.378
LSD_{DT}			0.496	
LSD_{C}			0.749	
$LSD_{DT×C}$			1.298	

*: There is no difference at the 0.05 probability level between the mean values with the same letter group. LSD_{DT}, LSD_{C}, and $LSD_{DT×C}$ show the least significant differences between the mean values in terms of drying techniques, bread wheat cultivars and drying techniquexcultivar interaction, respectively.

Table 9. The Mean values for salt content in Tokat tarhana dried under various techniques and from some bread wheat cultivars (%).

Cultivars (C)	Drying Techniques (DT)			Mean
	Open air	Airflow oven	Under vacuum	
Altınöz	5.483^{hi*}	6.310^{de}	5.813^{g}	5.869^{d}
Candaş	5.200^{j}	6.760^{c}	6.430^{d}	6.130^{b}
Gökkan	6.147^{f}	8.080^{a}	7.740^{b}	7.322^{a}
Şahika	5.530^{h}	6.420^{d}	6.190^{ef}	6.047^{c}
Yakamoz	5.373^{i}	6.640^{c}	6.260^{ef}	6.091^{bc}
Mean	5.547^{c}	6.842^{a}	6.487^{b}	6.292
LSD_{DT}			0.039	
LSD_{C}			0.075	
$LSD_{DT\times C}$			0.129	

*: There is no difference at the 0.05 probability level between the mean values with the same letter group. LSD_{DT}, LSD_{C}, and $LSD_{DT\times C}$ show the least significant differences between the mean values in terms of drying techniques, bread wheat cultivars and drying technique×cultivar interaction, respectively.

Table 10. The Mean values for lactic acid bacteria density in Tokat tarhana dried under various techniques and from some bread wheat cultivars (CFU g^{-1}).

Cultivars	Drying Techniques					
	Open air		Airflow oven		Under vacuum	
	M17	MRS	M17	MRS	M17	MRS
Altınöz	1.7×10^4	2.4×10^4	<10	<10	<10	<10
Candaş	2.1×10^4	3.1×10^4	<10	<10	<10	<10
Gökkan	0.8×10^4	0.3×10^4	<10	<10	<10	<10
Şahika	1.4×10^4	2.3×10^4	<10	<10	<10	<10
Yakamoz	1.0×10^4	4.0×10^4	<10	<10	<10	<10
Mean	1.4×10^4	2.4×10^4	<10	<10	<10	<10

CFU: Coloni-Forming Units; M17: aerobic agar medium; MRS: anaerobic agar medium

Total titration acidity (TTA)

The increase in acidity in foods is due to organic acids produced as a result of fermentation. This acidity not only adds flavor to tarhana, but also extends its shelf life. In a study, as a result of the examination of 27 home tarhana procured from Isparta region, the acidity values were determined between 4.91% and 36.62%. On average, it reached a value of 15.13% (Soyyigit, 2004).

The highest TTA in the tarhana samples was obtained from the samples of dried in the airflow oven (27.433%), while the lowest from the samples of dried in the open air (19.033%). When evaluated for the cultivars, the highest TTA was from Gökkan *cv* with 26.528%, and the lowest TTA was from Candaş *cv* with 22.139). Tarhana samples obtained from bread wheat cultivars showed higher TTA in airflow oven drying technique compared to other drying techniques; this stuation caused the DT×C interaction (Table 8).

Salt content

Salt; by inhibiting many enzymatic reactions in foods, it contributes to activating reactions that facilitate the characterization of color, texture and additionally flavor properties (Roy et al., 2021). In addition, salt added to food; it triggers the growth and development of yeast and fermented bacteria. Thus, it supports proteins and other binders in foods to achieve the desired texture. In a study, 13 tarhana samples in Kahramanmaraş region were examined and their physical and chemical analyzes were examined. It has been reported that the salt content of 13 tarhana samples is between 4.37-6.47% (Yörükoğlu, 2016).

From the evaluation of Table 9, the highest salt content in the tarhana samples was found in the oven-dried samples (6.842%); it is understood that the lowest salt content was obtained from the samples (5.547%) of open air dried. When the salt contents of tarhana samples were evaluated on the basis of cultivars, the highest salt was found in Gökkan *cv* with 7.322%, the lowest salt was determined in Altınöz *cv* with 5.699%. Tarhana samples obtained from bread wheat varieties showed higher salt content in airflow oven technique than other drying techniques; thus, DT×C interaction was formed (Table9).

Lactic acid bacteria density (LABD)

No lactic acid development was observed in the samples of tarhana produced in airflow oven drying and vacuum drying (Table 10). It is thought that the lactic acid bacteria under these conditions are killed because they are treated for a long time at 80 °C in an oven and at

60 °C in vacuum. LABD also changed according to the agar medium in which the bacteria grew and the wheat varieties used in tarhana. The highest LABD (2.4×10^4 cfu g^{-1}) was determined in MRS agar while the lowest density (1.4×10^4 cfu g^{-1}) was from M17 agar which they were as mean of wheat cultivars in open air drying (Table 10). Also, according to the wheat cultivars; the highest LABD values were 2.1×10^4 cfu g^{-1} (Candaş *cv*) in M17 agar; 4.0×10^4 cfu g^{-1} (Yakamoz *cv*) in MRS agar.

CONCLUSION

The findings of this study are generalizable qualification not only for Tokat red pepper tarhana, but also for all fermented products that require drying in which cereals such as tarhana are included. However, the effects of bread wheat cultivars and applied drying techniques on tarhana quality will be mentioned here. The results of the study can be summarized as follows: i) The bread wheat cultivars used in tarhana were found to be statistically effective on the investigated quality parameters. ii) The drying techniques applied showed significant differences for all quality parameters. iii) The wheat cultivars used showed significant differences in terms of quality traits in different drying techniques. In other words, the interaction of drying technique×wheat cultivar was found to be statistically significant. iv) While lactic acid bacteria density (LABD) is adversely affected by drying in the airflow oven and under vacuum; LAB could only survive in the drying environment in the open air. v) When LABD is evaluated in terms of drying in the open air; LAB production also varied on different agar media and different wheat cultivars. vi) For Tokat red pepper tarhana, it was concluded that Gökkan *cv* was the most suitable among the wheat varieties used. vii) If a generalization is to be made, the open air drying technique is a technique that should be considered in terms of food safety, as it is a drying method based on a renewable (solar) energy source, since it is based on sustainable production. viii) In tarhana, drying in the open air seems to be a most sustainable method for also consumption because of continuity of lactic acid bacteria production after drying, too.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

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. However, SGÖ had MSc thesis and made the laboratory studies together with CB (her 2nd supervisor). BB undertook the article writing and the 1st supervisory role of SGÖ's thesis. 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.

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

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Data availability

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RESEARCH ARTICLE

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Agricultural Environmental Kuznets Curve: A Panel Data Approach

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Abstract

This study employs a panel regression model to empirically examine the association between environmental degradation and agricultural performance across a sample of 150 nations over the period of 2000-2020. Agricultural methane emissions serve as a metric for quantifying environmental damage. The measurement of agricultural performance is represented by two variables, namely, the net value added for agriculture and the livestock production index. While agricultural production is a significant source of methane emissions, it is noteworthy that the majority of existing literature mostly focuses on carbon dioxide (CO_2) emissions. The primary contribution of this study lies in the utilization of methane emissions as a surrogate measure for assessing the extent of environmental degradation. The findings substantiate the credibility of the agricultural Environmental Kuznets Curve (EKC), indicating a curvilinear association between agricultural net value added and methane emissions, characterised by an inverted U shape. In addition, it is worth noting that animal production exerts a substantial adverse influence on methane emissions. Hence, the development in net value-added in the agricultural sector might lead to a reduction in environmental degradation. Therefore, the results indicate that the use of agricultural production techniques and agricultural technology approaches is recommended in order to promote a more environmentally sustainable global context.

Keywords: Environmental Kuznets Curves, Panel Data, Agriculture, Net Value Added, Methane

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INTRODUCTION

Climate change poses a significant risk to the agricultural sector and the overall stability of food production and availability. The productivity of farming systems in numerous locations is at risk due to the escalating temperatures and occurrences of extreme weather events. According to projections, the global population is anticipated to reach over 9-10 billion individuals by the year 2050. In light of this demographic trend, it is crucial to acknowledge the pivotal position that agriculture plays in providing sustenance for this expanding populace. The importance of agricultural economic growth has been increasingly evident in the current era of worldwide trade and self-sufficiency, particularly in light of the recent COVID-19 pandemic. Given the projected increase in global population, the agricultural industry is faced with the dual challenge of meeting the growing demand for food while also mitigating its environmental impact, particularly in terms of carbon emissions. Emissions of greenhouse gases (GHGs) are widely recognised as the primary catalyst for anthropogenic climate change. Agricultural operations are responsible for around 10-14% of

global anthropogenic greenhouse gas (GHG) emissions. These emissions mostly consist of enteric fermentation (methane, CH_4), synthetic fertiliser application (nitrous oxide, N_2O), and tillage (carbon dioxide, CO_2) (IPCC, 2012).

The practices of livestock husbandry and field crop cultivation exert significant strain on the natural environment and contribute to the amplification of greenhouse gas emissions. In the realm of field crop production, the utilisation of fuel and the implementation of tillage operations contribute to the emission of carbon dioxide (CO_2), while the oxidation of soil organic carbon (SOC) also takes place. The utilisation of nitrogen fertilisers results in the release of nitrous oxide (N_2O). One particular source of greenhouse gas emissions within the cattle business is methane (CH_4) produced by ruminant animals. Specifically, in the year 2018, the emissions of methane (CH_4) resulting from the process of enteric fermentation occurring within the digestive systems of ruminant cattle remained the most significant contributor to greenhouse gas emissions at the farm level. This emission accounted for a total of 2.1 gigatonnes of carbon dioxide equivalent (Gt CO_2eq) (Faostat, 2020). The emissions resulting from agricultural activities, specifically those associated with crops and livestock, showed a notable increase between the years 2000 and 2018. In fact, at the end of this period, the emissions had expanded by around 14% in comparison to the levels observed in 2000.

Climate change is the result of prolonged alterations in temperature patterns. Since the 19th century, anthropogenic activities, specifically the combustion of fossil fuels such as coal, oil, and gas, have emerged as the primary catalyst for climate change. The combustion of fossil fuels leads to the release of greenhouse gases, which subsequently results in the retention of solar radiation and the subsequent elevation of temperatures. Alongside the phenomenon of global warming, the proliferation of agricultural activities emerges as a significant catalyst for worldwide environmental transformations. While agricultural activities play a significant role in fostering economic development, they also give rise to greenhouse gas emissions and contribute to environmental degradation through processes such as deforestation, land utilisation, and the use of fossil fuels, fertilisers, machinery, and the burning of crop residues. Agricultural operations have been found to be linked to detrimental environmental consequences. The possibility for soil damage can arise from alterations in land-use practices, as evidenced by activities such as the cultivation of previously uncultivated regions, the lack of implementation of soil conservation techniques, and the occurrence of excessive grazing. In addition, the agricultural sector contributes to the degradation of water quality through the contamination of surface and groundwater caused by the extensive use of chemical fertilizers. Furthermore, the increase in agricultural production requires a higher utilization of energy, primarily sourced from fossil fuels.

To meet the increasing global food demand driven by population increase, there has been a notable transformation of land that was formerly covered by forests, meadows, and other natural ecosystems for agricultural utilization (Tilman et al., 2001). One additional factor within the realm of agriculture that contributes to the phenomenon of global warming is the utilization of energy in croplands for activities such as pesticide application and tillage operations. These practices necessitate substantial quantities of fossil fuel consumption, as highlighted by Lal (2004) and Huggins and Reganold (2008). The expansion of cultivated areas through intensive agricultural practices has resulted in a significant increase in the emission of greenhouse gases.

The Environmental Kuznets Curve (EKC) is attributed to Simon Kuznets, who postulated a theoretical relationship between per capita income and environmental quality. The decline in environmental quality is observed during the initial stages of per capita GDP growth; however, beyond a specific threshold, a positive trend in environmental quality emerges. The utilization of energy is closely linked to the release of different pollutants, including carbon dioxide, sulfur, and nitrogen oxides.

EKC model illustrates the interplay among energy consumption, economic development, and environmental conditions. The relationship between per capita income and environmental damages or emissions follows an inverted U-shaped pattern. EKC, first proposed by Grossman and Krueger (1991), has emerged as the prevailing method employed by economists to analyze the relationship between ambient pollution concentrations and aggregate emissions.

EKC derives its nomenclature from Simon Kuznets, who postulated the correlation between ecological integrity and individual income. In the initial phases of per capita gross domestic product expansion, ecological integrity tends to diminish; however, beyond a certain threshold, it commences an upward trajectory. The release of diverse contaminants, namely carbon dioxide, sulfur, and nitrogen oxides, is intrinsically linked to energy consumption.

The EKC model portrays the correlation connecting energy utilization, economic expansion, and the surroundings. The ecological consequences or discharges per person are a function of per capita income that takes the form of an inverted U. Ever since the inception of the EKC by Grossman and Krueger (1991), it has emerged as the prevailing method employed by economists for representing the amalgamation of ambient pollution concentrations and overall emissions.

This research aims to examine the significance of contemporary climatic changes and the environmental implications of agricultural practices, specifically

focusing on the escalating emissions of greenhouse gases resulting from animal husbandry and field crop production. The EKC in the context of agriculture can be used to analyze how agricultural practices impact the environment when economic development levels change. As agriculture develops, when a country's economy grows, there will be less reliance on traditional farming methods and more environmentally friendly farming methods will be applied. The country can thus invest in modernizing agriculture and sustainable practices, which can reduce the environmental footprint of farming activities. The EKC concept does not imply a fixed curve for all countries. With effective environmental policies and international cooperation, a shift toward sustainable agricultural practices and minimizing environmental degradation due to agriculture can be attained.

Using a panel data technique, the research empirically examines the relationship between environmental degradation and agricultural performance in 150 countries from 2000 to 2020. This paper's key contribution is to use methane emissions as a proxy for environmental degradation. The findings support the validity of agricultural EKC; an inverted U-shape relationship exists between agricultural net value added and methane emissions. The subsequent sections of this work are structured in the following manner. Section 2 elucidates the significance of the agriculture sector in the context of climate change. Section 3 of this paper comprises a comprehensive analysis and evaluation of pertinent scholarly works and literature. Section 4 of the paper provides an in-depth analysis of the data and the model employed in the study. Section 5 provides an empirical study and presents a comprehensive discussion of the findings. Section 6 serves as the final remarks.

The Role of the Agriculture Sector in Climate Change

Greenhouse gases (GHGs) have the capacity to absorb infrared radiation emitted by the sun and subsequently retain the heat within the Earth's atmosphere. This phenomenon, known as the greenhouse effect, is responsible for the occurrence of global warming and subsequent climate change. Major GHGs that are counted in international inventories are Carbon dioxide (CO_2), Methane (CH_4), Nitrous oxide (N_2O), Hydrofluorocarbons (HFCs), Perfluorocarbons (PFCs), Sulphur hexafluoride (SF_6), Nitrogen trifluoride (NF_3). According to World Development Indicators, CO_2 comprises the largest share among all GHGs, accounting for 73% in 2020. Methane is considered the second most abundant anthropogenic greenhouse gas, behind carbon dioxide (CO_2), and is responsible for around 18% of global emissions. According to the Intergovernmental Panel on Climate Change (IPCC, 2007), it has been determined that the global warming potential of methane (CH_4) and nitrous oxide (N_2O) over a span of 100 years is projected to be 21 and 310 times greater than that of carbon dioxide (CO_2), respectively.

According to 2020 estimates, agriculture accounts for around 12% of total greenhouse gas emissions that contribute to climate change. When land-use change and forestry are factored in, this rate rises to 15%. As a result, agriculture is the second-highest emitter of greenhouse gases after energy production. Direct agricultural production, animal husbandry, and the loss of wooded areas in order to improve agricultural productivity all contribute to the emission of three greenhouse gases: methane (CH_4), nitrogen oxide (N_2O), and carbon dioxide (CO_2). The shares of agricultural production in these three greenhouse gas emissions are depicted in Figure 1. As a result, agriculture accounts for around 40% of overall CH_4 emissions, 73% of N_2O emissions, and only 3% of CO_2, the greatest contributor to total greenhouse gas emissions.

According to FAO (2021), the global emissions resulting from agricultural activities, encompassing activities within the farm gate as well as land use and land use change, amounted to 9.3 billion tonnes of carbon

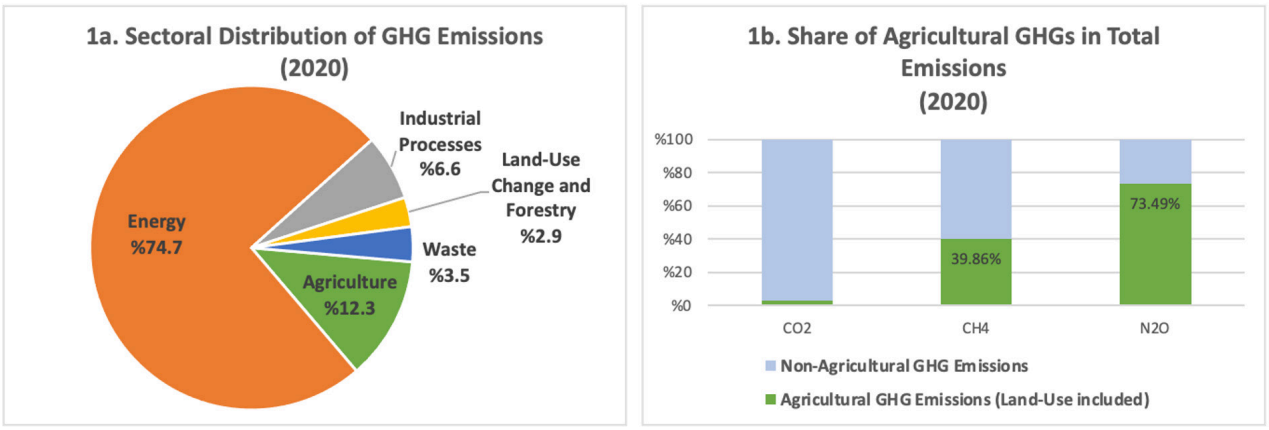

Figure 1. Sectoral Distribution of GHG Emissions and Share of Agricultural GHGs in Total Emissions
Source: (1a) Climatewatch (Link) (1b) FAOSTAT, http://www.fao.org/faostat

dioxide equivalent in 2018. The emission sources of methane and nitrous oxide deriving from crop cultivation and livestock operations accounted for 5.3 billion tonnes, reflecting a growth of 14% since the year 2000. Notably, the emissions arising from livestock production procedures such as enteric fermentation and the deposition of manure on pastures constituted the predominant portion of farm-gate emissions, yielding a total of 3 billion tonnes of carbon dioxide in 2018. Concurrently, emissions stemming from land use and land use change reached 4 billion tonnes of carbon dioxide in 2018, with deforestation being the primary cause (equating to 2.9 billion tonnes of carbon dioxide equivalent) and the incineration of organic soils through drainage burning contributing 1 billion tonnes of carbon dioxide equivalent. It is important to highlight that these land use emissions exhibited a global decrease of 20% since 2000. While emissions resulting from deforestation have shown a decline, those originating from drainage and the incineration of organic soils have witnessed an increase of nearly 35% since 2000.

The primary source of N_2O emissions can be attributed to the application of animal and synthetic fertilisers in agricultural practices, either through their incorporation into the soil or their release into the environment. According to Tilman et al. (2001), it is projected that by the year 2050, there will be a net increase of 3.5×108 hectares in worldwide agriculture. This expansion will be accompanied by a 2.4 to 2.7-fold increase in the usage of pesticides and fertilisers. Consequently, these intensified agricultural practices are expected to result in the contamination of ecosystems and the eutrophication of water sources. Specifically, the proportion of N_2O emissions originating from fertiliser residue on pastures accounts for 18% of the total greenhouse gas emissions in the agricultural sector. Additionally, within the realm of agricultural N_2O emissions, this particular source contributes to 48% of the overall emissions. The practice of tillage has the potential to contribute to the release of N_2O emissions from the soil, and this phenomenon is influenced by factors such as soil moisture, temperature, and nitrate (NO_3) concentration, as highlighted by Perdomo et al. (2009). The emission of N_2O is significantly influenced by the use of nitrogen-based fertilizers, as any surplus nitrogen that is not taken up by plants can be released into the atmosphere in the form of gaseous emissions (Smith et al., 2008).

Soil tillage is a significant factor in the generation of carbon dioxide (CO_2) emissions in agricultural areas, mostly due to the process of soil organic carbon (SOC) oxidation. The diminished soil organic carbon (SOC) reservoir has a detrimental impact on the physical and chemical characteristics of soil, as well as its fertility and productive potential (Stavi et al., 2011). Manuring is identified as a significant contributor to CO_2 emissions, primarily due to the stimulation of microbial activity (Matsumoto et al., 2008). The retention of manure decomposition rate on the soil surface has the potential to result in a decrease. Furthermore, the use of excessive fertilizers can lead to the runoff or leaching of water sources above or below the ground (De Angelo et al., 2006).

The major sources of CH_4 come from agriculture and oil and gas operations. Emissions of CH_4 derived from ruminant husbandry and rice cultivation. Enteric fermentation and paddy production are the two main sources of methane emissions from agricultural output. Enteric fermentation is the digestive process by which ruminant carbohydrates are broken down into simple molecules by microorganisms in their intestines, and methane is generated during this process. Methane is emitted by paddy grown underwater throughout the process. According to FAO data, methane gas emitted as a result of enteric fermentation accounted for 44% of all agricultural greenhouse gases in 2017, whereas it accounted for 70% of agricultural methane gas emissions.

The emission of CH_4 from ruminants is a specific consequence of the livestock industry. In order to rear livestock, it is necessary to provide the animal with an appropriate amount of food based on its body weight. The production of this feed not only involves the release of greenhouse gases through the operation of agricultural machinery and the use of fertilizers but also results in the generation of various wastes that contribute to greenhouse gas emissions (Fiala, 2009). Climate change has the potential to bring about changes in semi-natural ecosystems, and these alterations may have implications for the global livestock sector by reducing the availability of feed and pastures (Thornton & Gerber, 2010). The emergence, spread, and distribution of livestock diseases could also be influenced by climate change, as high temperatures can impact the rate of development of pathogens and parasites, potentially leading to shifts in disease patterns; consequently, there may be changes in the animal population (Randolph, 2008).

Literature Review

Commencing with the seminal contributions of Grossman and Krueger (1991) and Shafik and Bandyopadhyay (1992), numerous scholarly articles have delved into the examination of the Environmental Kuznets Curve (EKC) hypothesis, which elucidates an inverted U-shaped association between environmental pollution and economic growth. In earlier investigations, the analysis incorporated per capita GDP and per capita energy consumption as independent variables (Selden & Song, 1994; Shafik, 1994; Holtz-Eakin & Selden, 1995). Other variables that have been explored by researchers include foreign direct investment (Agvoola & Bekun, 2019), human capital (Mahmood et al., 2019), industrialization (Pata, 2018; Prastiyo et al., 2020), urbanization (Ridzuan et al., 2020), trade openness (Jebli & Youssef, 2017; Balsalobre-

Lorente et al., 2019), and economic complexity (Yılancı & Pata, 2020) in the analysis of the EKC hypothesis.

The agricultural sector was not a priority for researchers testing the EKC hypothesis, though its importance in economic development (Prastiyo et al., 2020). The findings of the limited number of studies in terms of agricultural impact on environmental pollution have been summed up in Table 1.

When a literature review is made of the existing studies, we conclude that the EKC hypothesis is validated for the impact of agriculture on environmental pollution by most of the researchers. There are some researchers who failed to validate the EKC hypothesis as Ben Youssef (2017) and Liu et al. (2017). Among 13 studies, eight of them state that agriculture accelerates carbon dioxide emissions. There are some studies stating that agriculture reduces environmental pollution, and to improve environmental quality, it is necessary to have agricultural production. (Liu et al. 2017; Zhang et al. 2019; Aziz at al. 2020; Prastiyo et al. 2020; and Ridzuan et al. 2020.)

S. Coderani and R. Esposti (2014) utilized a comprehensive panel dataset encompassing many years and focusing on a single country, specifically the Italian regions. Their objective was to examine the correlation between greenhouse gas (GHG) emissions in the agricultural sector and the rise of agricultural productivity. The panel focuses on the emissions of methane from 1951 to 2008 and N_2O from 1980 to 2008. The findings indicate that there may be a statistically significant association between greenhouse gas emissions in the agricultural sector and the rate of productivity increase. However, this link is observed to be consistently increasing or decreasing, without any fluctuations.

N. Dogan (2016) used annual data from 1968 to 2010 to experimentally examine the long-run link between agricultural performance and carbon dioxide emissions in Turkey. According to the findings, an increase in agricultural output would have the opposite effect on Turkey's carbon dioxide emissions in the long run.

E. Zafeiriou and M. Azam (2017) examine the veracity of the correlation between economic success per capita and CO_2 emissions in the agricultural sector across three Mediterranean nations, namely France, Portugal, and Spain. The validation of the Environmental Kuznets Curve (EKC) hypothesis has been observed in all nations included in the sample, as indicated by their findings.

M. B. Jebli and S. B. Youssef (2017) conducted an investigation into the dynamic causal connections between renewable energy consumption per capita, agricultural value added, carbon dioxide emissions, and real gross domestic product for a panel of five North African countries spanning the period 1980–2011. In the short term, Granger causality tests provided evidence of the existence of bidirectional causality between CO_2 emissions and agriculture. X. Liu et al. (2017) made an attempt to examine the impact of renewable energy consumption per capita and agricultural value added on carbon dioxide emissions in four selected countries of the ASEAN-4 (Indonesia, Malaysia, the Philippines, and Thailand) from 1970 to 2013. The results of their long-term estimates did not provide support for the inverted U-shaped Environmental Kuznets Curve (EKC).

K. Appiah et al. (2018) analysed the correlation between agriculture production and carbon dioxide emissions in emergent economies from 1971 to 2013. Empirical findings indicated that a 1% increase in economic growth, crop production index, and livestock production index would cause proportional increases in carbon dioxide emissions of 17%, 28%, and 28%, respectively, whereas a 1% increase in energy consumption and population would improve the environment of emerging economies.

Data and Model

The data utilized in this study was obtained from the World Development Indicators (2022) dataset, which was developed by the World Bank. The dataset encompasses information from 150 countries, covering the time period between 2000 and 2020. The selection of the study period and the national sample is contingent upon the availability of data. In the Human Development Index of 2022, it was seen that 54 of the sampled counties were categorized as having a very high level of human development, while 42 counties were classified as having a high level of human development. Additionally, 55 counties fell into the medium and poor human development categorization.

The primary focus of this study revolves around the examination of CH_4 emissions as an indicator of environmental deterioration within the agricultural sector. Chapter 4 is widely regarded as the most significant agricultural pollutant, accounting for approximately 18% of total greenhouse gas emissions. The percentage of methane emissions attributed to the agricultural sector on a global scale is 39.86%. The independent variables utilized in this study encompassed the value contributed by agriculture, forestry, and fisheries.

Given that the primary source of agricultural CH_4 emissions is enteric fermentation, it is pertinent to include the livestock production index as an additional explanatory variable in the model. This inclusion serves to provide supplementary support and enhance the explanatory power of the model. According to the World Development Indicators (WDI) for the year 2022,

Table 2 presents a summary of the descriptive statistics for the variables. There were a total of 3146 valid observations, with an average methane emission volume of 20543 thousand metric tons of CO_2 equivalent. The quantity of methane varies from 1,260 metric tons in Seychelles during the year 2012 to 502,192 metric tons in India during the year 2020. In terms of the economic

Table 1. Empirical Studies Relates Environmental Quality to Agriculture

Work	Countries	Time period	Method(s)	Variables	Agriculture-pollution nexus	A-EKC
Coderani & Esposti (2014)	Italian Regions	1951-2008 1980-2008	LSDV, LSDVC, GMM	N_2O &CH_4 | APG	Agriculture→N_2O (+) Agriculture→N_2O (+)	✓
Dogan (2016)	Türkiye	1968-2010	ARDL	CO_2 | GDP, EC, RIA	Agriculture →CO_2 (-)	✓
Zafeiriou & Azam (2017)	France, Portugal, Spain	1992-2014	ARDL	CO_2 | AGRV per capita, T	Agriculture →CO_2 (+) (France and Spain) Agriculture →CO_2 (-) (Portugal)	✓
Zafeiriou et al. (2017)	Bulgaria, Czech Republic, Hungary	1970-2014 1993-2014 (for Czech Republic)	ARDL	CO_2 | AGRV, D	Agriculture →CO_2 (+)	✓ (for Czech Rep. and Bulgaria in the LR)
Jebli &Youssef (2017)	North Africa Countries	1980-2011	Johansen-Juselius cointegration,	CO_2 | GDP, REC, NREC, TO AGRV	Agriculture →CO_2 (+)	X
Liu et al. (2017)	ASEAN-4	1970-2013	Kao panel cointegration test, OLS, DOLS and FMOLS	CO_2 | GDP, REC, NREC, AGRV	Agriculture →CO_2 (-)	X
Gokmenoglu & Taspinar (2018)	Pakistan	1971–2014	Maki cointegration, FMOLS	CO_2 | GDP, EC, AGRV	Agriculture →CO_2 (+)	✓
Appiah et al. (2018)	Selected emerging economies	1971-2013	FMOLS, DOLS	CO_2 | GDP, CP, LP, POP, EC	Agriculture →CO_2 (+)	Not tested
Agboola & Bekun (2019)	Nigeria	1981-2014	Bayer-Hanck cointegration test,	CO_2 | GDP, TO, FDI, EC, AGRR	Agriculture →CO_2 (+)	✓
Balsalobre-Lorente et al. (2019)	BRICS	1990-2014	Kao and Fisher panel cointegration tests, DOLS, FMOLS	CO_2 | GDP, ELC, MOB, TO, AGRR	Agriculture →CO_2 (+)	✓
Dogan (2019)	China	1971-2010	ARDL, FMOLS, DOLS, CCR	CO_2 | GDP, EC, AGRR	Agriculture →CO2 (+)	✓
Gokmenoglu et al. (2019)	China	1971-2014	ARDL	CO_2 | GDP, EC, AGRV	Agriculture →CO_2 (+)	✓
Qiao et al. (2019)	G20	1990-2014	Johansen-Fisher panel cointegration, FMOLS, DOLS	CO_2 | GDP, REC, AGRV	Agriculture →CO_2 (+)	✓
Zhang et al. (2019)	China	1996-2015	ARDL	CO_2 | GDP, EC, AGRV	Agriculture →CO_2 (-)	✓
Aydoğan & Vardar (2020)	E7	1990-2014	Pedroni cointegration, OLS, FMOLS and DOLS	CO_2 | GDP, REC, NREC AGRV	Agriculture →CO_2 (+)	✓
Aziz et al. (2020)	Pakistan	1990-2018	Quantile ARDL	EF | GDP, FA, REC, AGRV	Agriculture →EF (-)	✓
Prastiyo et al. (2020)	Indonesia	1970-2015	ARDL	CO_2 | GDP, IND, URB, AGRR	Agriculture →CO_2 (-)	✓

Ridzuan et al. (2020)	Malaysia	1978-2016	ARDL	CO_2 | GDP, HG, URB, CP, FP, LP	CP and FP →CO_2 (-)	✓
Selcuk et al. (2021)	N-11 Countries	1991-2019	CCEMG	CO_2 | GDP, EC, AGRV, FDI, TO	Agriculture →CO_2 (+)	Not tested
Ntim-Amo et al. (2021)	Ghana	1980-2014	ARDL, FMOLS, DOLS	CO_2 | GDP, EC, AGRV	Agriculture →CO_2 (+)	✓
Liu et al. (2021)	China, Three Gorges Reservoir Region	Not mentioned	OLS	Agricultural Chemicals | GDP, POP, Agricultural Investment	Agriculture →Agricultural Chemicals (+)	✓
Wang & Lv (2022)	China, Henan Province	2000-2019	OLS	Agricultural CO_2 | AGRV	Agriculture →Agricultural CO_2 (+)	✓
Cetin et al. (2022)	47 Developing Countries	1976-2017	DOLS, FMOLS	CO_2 | GDP, EC, AGRV	Agriculture → CO_2 (+)	✓
Atasel et al. (2022)	Top 10 Agricultural Countries	1997-2016	AMG	CO_2 | GDP, AGRV	Agriculture → CO_2 (-)	✓ (6 out of 10 countries)
Khan et al. (2023)	54 countries	1971-2017	PMG	ΔForestry | AGRV, ETR, UAG, FIND	Agriculture → ΔForestry (-)	X

Note: AGRR: Agricultural production (% of GDP), **AGRV:** Agricultural value-added, **AMG:** Augmented mean group estimator, **APG:** Agricultural production growth, **ARDL:** Autoregressive distributed lag model, **CCEMG:** Common correlated effects mean group estimator, **CCR:** Canonical cointegrating regression, **CP:** Crop production, **D:** Dummy, **DOLS:** Dynamic OLS, **EC:** Energy consumption, **EF:** Ecological footprint, **ELC:** Electricity consumption, **ETR**: Energy transition **FA:** Forest area, **FDI:** Foreign direct investment, **FIND:** Financial depth, **FMOLS:** Fully modified OLS, **FP:** Fisheries production, **HG:** Hydroelectricity generation, **IND:** Industrialization, **LP:** Livestock gross production, **MOB:** Mobile use, **NREC:** non-renewable EC, **OLS:** Ordinary least squares, **PMG:** Pooled mean group, **POP:** Population, **REC:** Renewable energy consumption, **RIA:** Real income from agriculture, **T:** Time trend, **TO:** Trade openness, **UAG:** Urban agglomeration, **URB:** Urbanization

Source: Based on Atasel et al. (2022: 34027), reviewed and updated by the author.

Table 2. Descriptive Statistics

Variable	Description	Min.	Max.	Mean	Std. Dev.
MET	Agricultural methane emissions *(thousand metric tons of CO2 equivalent)*	1,26	502192,29	20542,58	58039,45
NVA	Agriculture, forestry, and fishing, value added *(constant 2015 million US$)*	5,99	1095776,95	17504,84	71679,68
LIVESTOCK	Livestock production index *(2014-2016 = 100)*	18,47	278,17	93,13	21,58

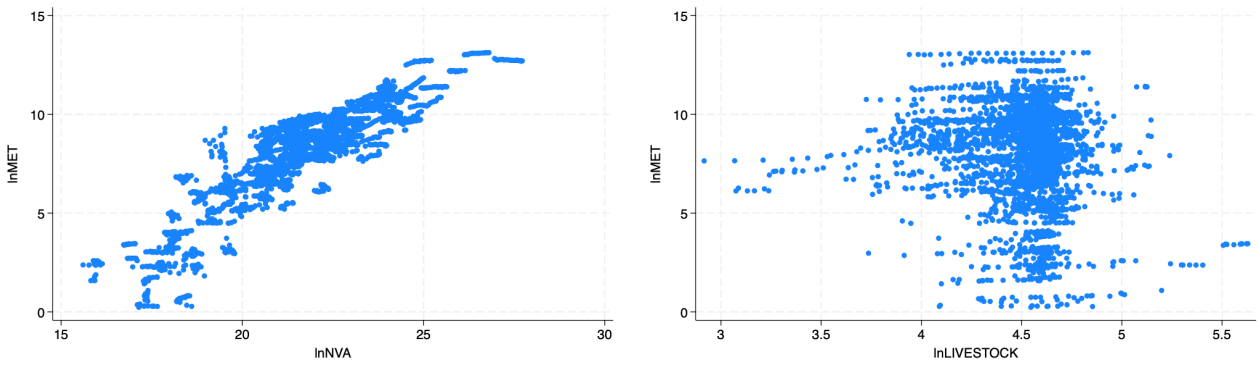

Figure 2. Scatterplots of variables

contribution of agriculture, forestry, and fisheries, China stands as the most affluent nation with a value-added of $1,095,777 million in 2020. Conversely, St. Kitts and Nevis ranks as the least prosperous country with a value-added of $5.99 million in 2000. In terms of the Livestock Production Index, Tajikistan exhibits the lowest rate of 18.47 in the year 2000, while Antigua and Barbuda showcases the greatest rate of 278.17 in 2006

Figure 2 illustrates the graphical representations of the value added for agriculture, forestry, and fishery (NVA) as well as the livestock production index in relation to agricultural methane (CH_4) emissions. As anticipated, a visual examination reveals an inverted-U-shaped correlation between NVA (non-volatile acidity) and methane emissions. However, despite the anticipation of a positive correlation between the animal production index and agricultural methane emissions, it appears challenging to ascertain this link based just on the scatterplot.

The utilization of a panel data model is recommended for this study due to the inherent advantages it offers in comparison to time series and cross-section data. One crucial aspect is that panel data analysis enables the elucidation of (i) the reasons for disparate behaviours among units (countries) and (ii) the factors contributing to variations in behaviour within a same unit (country) across different time periods. By taking into account our model, panel data is anticipated to possess a greater capacity in identifying and quantifying impacts that are not discernible in either pure cross-sectional or pure time-series data. According to Karlsson and Löthgren (2000) and Levin et al. (2002), the panel unit root test is considered to possess adequate robustness when used to panels of modest dimensions, specifically when the number of cross-sectional units (N) falls within the range of 10 to 250, and the time periods (T) range from 25 to 250. Nevertheless, when T is small, panel unit root tests exhibit limited statistical power, and there exists a possible danger of erroneously determining that the entire panel is nonstationary, even in cases where a significant fraction of the series inside the panel are stationary. In a similar vein, it is widely recognized that typical panel cointegration tests exhibit limited statistical power, particularly when applied to datasets with a small number of time periods (T) and a limited span of data (Baltagi, 2005). Hence, the researchers have opted for panel regression analysis as the appropriate method for this investigation, given the relatively limited time dimension (T=21).

The extensive body of material pertaining to the (EKC) hypothesis often operates under the assumption that the relationship between environmental degradation and income can be adequately explained by a quadratic function. The present study aims to investigate the relationship between agricultural revenue and methane emissions in the context of an agricultural Environmental Kuznets Curve (EKC). To achieve this objective, the following model will be utilized.

The vast literature on the EKC hypothesis generally assumes that environmental degradation is explained by the quadratic function of income. Since the study is interested in an agricultural EKC related to methane emissions in agricultural income, the following model will be considered:

$$lnMET_{it} = \beta_0 + \beta_1 lnNVA_{it} + \beta_2 lnNVA_{it}^2 + \beta_3 lnLIVESTOCK_{it} + \mu_i + u_{it} \quad (1)$$

where i= 1, 2, ….N for each country in the panel and t= 1, 2, …….., T refers to the period. METit refers to the methane emissions, NVAit denotes the value added for agriculture, forestry, and fishing, and lastly, LIVESTOCKit indicates the livestock production index. All variables are transformed into natural logarithms to standardize the different scales of the variables. β_0 stands for the specific country-pair effects and allows controlling for all omitted variables that are cross-sectionally specific but remain constant over time. μ_i denotes the unobservable country-specific effect, and u_{it} means the remainder disturbance. β_1 and β_2 are the coefficients of NVA and squared NVA. Under the EKC hypothesis, the signs are expected to be positive and negative, respectively. The livestock production index is assumed as a supportive indicator of methane emissions. So, it is added to the model in linear form. The coefficient of the livestock production index, β_3, is expected to be positive. On the logarithmic scale, turning points (where methane emission is maximized) for income can be calculated as;

$$NVA^* = (-\frac{\beta_1}{2\beta_2}).$$

Exp(NVA*) represents the value of the turning points.

Empirical Analysis

The initial phase of the investigation entails prioritizing the selection of a sound model. The F-test and the Breusch and Pagan LM test were employed in this investigation to determine whether the characteristics of the data can be classified as pooled or panel. The results indicate that all data pertaining to the countries are in panel format. In order to ascertain the most suitable model for the panel analysis, the Hausman specification test was utilized to compare the Fixed Effect model (FEM) and Random Effect model (REM). The Hausman test indicates the rejection of the null hypothesis (H0), hence providing evidence in favour of the fixed effects model (FEM).

Subsequently, the model underwent testing to assess the presence of heteroscedasticity, cross-sectional dependence, and serial correlation. The Modified Wald test was employed to assess group wise heteroscedasticity, resulting in the rejection of the null hypothesis that assumes homoscedasticity. The cross-sectional independence test conducted by Pesaran

Table3. Panel-data Regression Results.

lnMET	Coefficient	Driscol/Kraay Standard Errors
lnNVA	0.756728*	0.1757755
$lnNVA^2$	-0.161558*	0.0040274
lnLIVESTOCK	0.418183*	0.0220641
constant	**-2.668545**	1.978814
within R-squared	0.3247	
F (3, 149)	2385.55	
Prob > F	0.0000	
turning point for NVA	14827	

Coefficients with (*) are significant at 1%. The coefficient with bold is not significant.

revealed that the null hypothesis was rejected with a significance level of 1%. Both the Baltagi-Wu local best invariant (LBI) test and the Durbin-Watson test have indicated the presence of serial correlation. The test results are presented in the Appendix. In order to address the aforementioned concerns pertaining to heteroscedasticity, cross-sectional dependency, and serial correlation, the Driscoll-Kraay estimator was implemented. The coefficients displayed in Table 3 represent the robust estimates that have been corrected using the Driscoll-Kraay estimator.

The F-test indicates that the model is statistically significant. The coefficient of determination (R2) for the model is 0.3247, indicating that approximately 33% of the variation in agricultural methane emissions can be accounted for by the independent variables included in the model. With the exception of the constant term, all of the computed coefficients exhibit statistical significance.

The computed coefficients for the variable NVA and its squared term are determined to be statistically significant, with the expected positive and negative signs, respectively. According to Lind and Mehlum (2010), for statistical judgments regarding the presence of an inverted U-shape, it is necessary to consider not only the negative sign and significance of the second derivative but also whether the predicted extremum point falls within the range of the data. The estimated turning point of NVA for the inverted-U curve is $14827, expressed in constant 2015 million US dollars, 20 per cent of the valid observations in the sample, specifically 629 out of 3146 observations, above the threshold level. Thus, the Environmental Kuznets Curve (EKC) hypothesis is validated for the Northern Virginia area based on the data obtained from the sample. It can also be argued that a majority of the countries in the sample (2517 out of 3146 observations) exhibit a positive trend in the inverted-U-shaped graph. Therefore, the elevation of non-volatile acidity (NVA) has an adverse impact on environmental quality due to its association with increased methane emissions.

The statistical analysis reveals that the estimated coefficient of the livestock production index is both statistically significant and positive. This suggests that there is a detrimental effect of livestock production on environmental quality. Specifically, a 1% rise in the livestock production index is associated with a 0.4% increase in agricultural methane emission.

CONCLUSION

This study investigates the correlation between environmental degradation and agricultural performance across 150 nations for the period of 2000-2020, employing a panel regression model. Methane emissions are quantified as a surrogate indicator of environmental deterioration, while agricultural performance is approximated by the net value added for agriculture and the livestock production index. The findings of this study provide empirical evidence supporting the concept of the EKC in the agricultural sector. Specifically, the results demonstrate a curvilinear relationship, characterized by an inverted U shape, between agricultural net value added and methane emissions. The findings indicate that the adoption of agricultural production techniques and technology methods is imperative in order to foster a more environmentally sustainable global landscape. The peak point for the available data is determined to be $14,827. Furthermore, it is worth noting that the production of cattle has a substantial adverse effect on the release of methane emissions. Hence, the development in net value-added within the agricultural sector might have a consequential impact on the degradation of the environment. Climate change poses a substantial risk to the agricultural sector and global food security, particularly in light of the projected increase in the world's population to approximately 9-10 billion individuals by the year 2050. Agricultural activities make a significant contribution of approximately 10-14% to the overall anthropogenic greenhouse gas emissions on a global scale. This contribution principally stems from three key sources: enteric fermentation, synthetic fertilizer application, and tillage practices.

Climate change is an outcome arising from prolonged alterations in temperature patterns, predominantly influenced by anthropogenic activity, notably the combustion of fossil fuels. Consequently, the aforementioned phenomenon gives rise to the release of greenhouse gases, which subsequently ensnare solar

radiation and contribute to the escalation of ambient temperatures. Agriculture, which plays a significant role in shaping global environmental dynamics, serves as a catalyst for economic development. However, it also gives rise to greenhouse gas emissions and environmental deterioration due to activities such as deforestation, land utilization, livestock management, fertilizer application, machinery usage, and the burning of crop residue. Agricultural activities are also associated with adverse impacts such as soil degradation, water pollution, and heightened energy consumption. The model known as the EKC, which is named after Simon Kuznets, posits that there exists an inverse relationship between environmental quality and per capita GDP growth, whereas a positive association is shown between environmental quality and heightened energy use. This paper examines the significance of contemporary climatic changes and the environmental implications of agriculture, with a specific emphasis on the amplified emission of greenhouse gases resulting from animal husbandry and field crop production. EKC can be employed as a tool for examining the environmental consequences of agricultural practices in the context of varying degrees of economic growth. Previous studies have dealt with carbon dioxide as a pollutant the uniqueness of this study is that methane gas is tested as an agricultural pollutant specifically. Methane has a larger warming potential than carbon dioxide over a shorter duration. Understanding its sources and effects in agriculture is important since its emission into the atmosphere causes climate change. Enteric fermentation in ruminants like cattle produces a lot of methane. Methane emissions can reveal animal management practices and environmental impacts. Flooded rice paddies produce methane through anaerobic breakdown. Understanding and reducing methane emissions from paddy fields is essential for sustainable agriculture because rice is a staple diet for many people. Manure Management: Organic manure decomposes to release methane. Management techniques affect methane emissions, and researching them can guide sustainable agriculture. In manure lagoons and wastewater treatment systems, anaerobic digestion occurs. These conditions produce methane, therefore researching these systems can help trap or reduce methane emissions. When discharged, methane can cause ground-level ozone, impacting air quality. Understanding how agriculture emits methane is essential for environmental management.

In other words, studying methane as an agricultural pollutant is about optimizing agricultural methods for sustainability and reducing climate and air pollution As agricultural advancements occur, nations have the opportunity to allocate resources toward the modernization of agricultural methods and the adoption of sustainable approaches, thereby mitigating their environmental impact. The issue of agricultural greenhouse gas (GHG) emissions poses a greater challenge in rising and developing nations since the agricultural sector is still undergoing transformation as a result of industrialization. Therefore, the results indicate that nations ought to embrace contemporary agricultural production methods in order to foster a more ecologically sustainable global ecosystem.

COMPLIANCE WITH ETHICAL STANDARDS

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Externally peer-reviewed.

Conflict of interest

The author declares that there is no conflicts of interest.

Ethics committee approval

Ethics committee approval is not required.

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The data can be available upon the request.

Consent to participate

Not applicable.

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Not applicable.

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RESEARCH ARTICLE

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Determination of sugars and organic acids in diverse carob genotypes using HPLC techniques

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Abstract
Carob fruit is widely known for their abundance of health-boosting compounds like polyphenols, L-ascorbic acid, organic acids, and micronutrients. These compounds offer various benefits, including antioxidative, antimicrobial, antidiabetic, liver-protective, anti-inflammatory, anticancer, and heart-protective effects. In this research, High-Performance Liquid Chromatography (HPLC) methods were employed to assess sugar and organic acid levels in mature fruit pods of three distinct carob genotypes from the Kahramanmaras region in Turkey. The findings revealed that genotype-2 had the highest concentrations of glucose (1301 mg/100g) and fructose (875 mg/100g), genotype-3 exhibited the highest level of xylose (1548 mg/100g), while genotype-1 displayed the highest levels of sucrose (9183 mg/100g) and total sugars (12457 mg/100g). Regarding organic acids, genotype-1 exhibited the highest levels of oxalic acid (17.62 mg/100g), citric acid (612.50 mg/100g), and fumaric acid (8.0 mg/100g), while genotype-3 showed the highest levels of malic acid (234.92 mg/100g) and succinic acid (1089.76 mg/100g); however, genotype-2 had the highest amount of L-ascorbic acid (8.17 mg/100g). In conclusion, genotype-1 demonstrated the most favorable performance in terms of having the highest levels of total sugar and organic acids compared to the other two genotypes.

Keywords: Carob, Genotype, Sugars, Organic Acids, HPLC

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INTRODUCTION

Carob (*Ceratonia siliqua* L.) is a member of the *Leguminosae* family within the *Rosales* order and is extensively grown in the Mediterranean region (Battle et al. 1997). Turkey is recognized as one of the native habitats for both wild and cultivated carob, with its growth primarily in the Mediterranean and Aegean regions (Correia et al. 2004). The four main genetic groups of carobs are Southern Spain; South Morocco; Central Mediterranean (genotypes from Algeria, Portugal, Sicily, Sardinia, France, and the Balearic Islands; Eastern Mediterranean (Cyprus, Greece, and Lebanon) (Viruel et al., 2019). The tree grows to a height of 8-17 m and has a broad hemispherical brown crown, a thick trunk with rough bark, and a robust branch structure (Tous and Antoni, 2013). It yields fruit resembling an edible pod, commonly referred to as a grasshopper bean. The pods form clusters and remain green until they reach maturity, at which point they measure 10-25 cm in length. Variations in pod characteristics, such as size, weight, shape, density, color, and seed rate, were observed among different varieties and under various climatic conditions, as reported by Nasar-abbas et al. (2016). Carob seeds exhibit distinct features, including their brown color, significant hardness, measuring approximately 10 mm in length, and weighing roughly 0.2 grams each, with

Table 1. Global carob statistics for the years 2015 to 2018

Countries	Portugal	Italy	Morocco	Turkey	Greece	Cyprus	Algeria	Spain	World
Harvested area (ha)	13427	5600	10224	3099	2410	1004	808	2292	41593
Yield (kg/ha)	29.393	56,385	21.532	45.776	59.377	71.571	45.762	8382	32.839
Production (MT)	39.387	31.577	22.013	14.195	12.819	7.179	3.701	1.916	136.613
Production (%)	28.83	23.11	16.11	10.39	9.38	5.25	2.71	1.40	100

Source: (FAOSTAT, 2020; Brassesco et al., 2021)

their composition comprising 30-33% husk, 42-46% endosperm, and 23-25% seed material (Tous and Antoni, 2013). Between 2015 and 2018, Portugal (28.83%), Italy (23.11%), Morocco (16.11%), and Turkey (10.39%) were the primary global producers of carob pods, with their production and area harvested statistics detailed in Table 1.

It is widely used in the preparation of cold drinks, syrups, and liqueurs due to its high nutritional value (Youssef et al., 2013). Historical records indicate that ancient Egyptians fed their animals carob shells and utilized carob gum as an adhesive for mummies, while in modern Egypt, carob pods find extensive use in cookies, cakes, beverages, and different snacks. The Arabs adopted the carob seed as a measure of weight, referring to its kernel as a "carat," and the established weight of the carob seed served as the standard unit for measuring precious metals (Viruel et al., 2019). Carobs are well recognized as fruit rich in multiple health-promoting bioactive compounds, including polyphenols, ascorbic acid, organic acids, and micronutrients, which contribute to a broad range of bioactivities, such as antioxidant, antimicrobial, antidiabetic, liver protective, anti-inflammatory, anticancer, and cardioprotective activities (Goulas et al., 2016). Research has documented the presence of D-pinitol, a carbohydrate with properties resembling insulin, in carob (Bates et al., 2009). This sugar alcohol has been derived from various plant sources, including soybeans (Smith and Phillips, 1982), bougainvillea flowers (Narayanan et al., 1987), and ice plants (Vernon et al., 1993; Zunft et al., 2001). Studies have indicated that carob can have a positive impact on nutraceutical elements and blood LDL cholesterol levels, contributing to the reduction of postprandial blood sugar in individuals with Type II diabetes mellitus (Kang et al., 2006). According to Nasar-abbas et al. (2016), carob pods consist of sugar content ranging from 45% to 52%. However, up to this point, there hasn't been any prior scientific documentation concerning the analysis of various sugars and organic acids in carob pods using the HPLC method. Therefore, the primary objective of this study is to determine the sugar and organic acid levels in three distinct carob genotypes grown in the Andırın district, Kahramanmaraş region using the HPLC method. The study aims to contribute to a better understanding of carob's potential nutraceutical and related properties, as well as its potential beneficial effects.

MATERIALS AND METHODS

Plant materials

Fully ripe pods of carob fruit, weighing approximately 1.5-2 kg for each of the three carob genotypes (genotype-1, genotype-2, and genotype-3) (three replications from each genotype), were harvested from a commercial orchards in the Andırın district, Kahramanmaraş province of Turkey (Latitude: 37°34'59.99"N Longitude: 36°20'59.99"E) during the August 2020 growing season and subsequently conveyed to the Instrumental Analysis laboratory at the Department of Horticulture, Faculty of Agriculture, Çukurova University, Turkey, maintaining cold chain conditions.

Experimental procedure for sugar analysis of dry carob fruit

Alterations in the levels of glucose, fructose, sucrose, xylose, and total sugars in the homogenized carob samples were assessed using the HPLC method as described by Crisosto (1997). Prior to analysis, frozen samples were dissolved at 25 °C, and 1 g of dried fruit was mixed with 4 mL of ultrapure distilled H_2O (Millipore Corp., Bedford, MA, USA). The mixture was introduced into an ultrasonic bath and subjected to sonication at room temperature for 15 min, followed by centrifugation for 15 min at 5500 rpm. Prior to HPLC analysis, the centrifuged solution underwent filtration using Whatman nylon syringe filters with a 0.45 µm pore size and a 13 mm diameter. Sugar levels were assessed through a process involving three repetitions, utilizing HPLC equipment (Shimadzu LC-20A system, Kyoto, Japan Kyoto) with a RID (Refractive Index) detector and a Coregel-87C column (7.8 x 300mm). The separations were conducted at a temperature of 70 °C, while maintaining a 0.6 mL flow rate per min. Isocratic ultrapure water was employed in the elution process. The specific sugar was quantified based on their respective standards and presented as a percentage of the fresh weight. For the reference materials, calibration curves were created, and by referring to these calibration curves, the content was calculated.

Experimental procedure for organic acid analysis of dry carob fruit

The analysis of organic acids of carob fruit extract was conducted using an HPLC method established by Bozan et al. (1997) with some adjustments. The alterations in the levels of citric, malic, fumaric, L-ascorbic, succinic, and

oxalic acids within carob fruit samples were assessed. To extract organic acids, 1 g of working sample was combined with 4 mL metaphosphoric acid (3%) solution. The concoction was nestled in a soothing ultrasonic water ballet for a quarter-hour in ambient conditions, where it waltzed to the harmonious tune of sonication before gracefully pirouetting in the centrifuge at 5500 rpm for another 15 min. Subsequently, the concoction underwent a straining process, passing through Whatman nylon syringe filters with a 0.45 µm pore size and a 13 mm diameter. A Shimadzu LC 20A VP HPLC device from Kyoto, Japan, equipped with a UV detector, namely the Shimadzu SPD 20A VP, and an 87 H column (5 µm, 300 × 7.8 mm, Transgenomic), was employed for the analysis of organic acids. Sulphuric acid (0.05 mM) was used as a solvent. The operational parameters included a column temperature of 40 °C, an injection volume of 20 µL, detection at a wavelength of 210 nm, and a flow rate of 0.8 mL per min. Identifying organic acids and pinpointing peak values is dependent on aligning peak retention times and cross-referencing spectral data with established standards. The appropriate standard calibration curves were employed to evaluate the recognized acids.

Statistical analysis

The JMP software was employed for data analysis using analysis of variance (ANOVA) to scrutinize the results (JMP Start Statistics, 1996). The data is presented in the form of the mean ± standard deviation of the samples (n=3). The difference among the three carob fruit species is considered significant at a level of $p<0.05$.

RESULTS AND DISCUSSION

The objective of this study was to determine the sugars and organic acids content from 100 g of samples for three carob genotype mature dry pod fruit, excluding seeds. The chemical analysis of sugar compounds and organic acids for the three genotypes is presented in Table 1 and Table 2, respectively.

Sugar analysis of dry carob fruit

Genotype-1 exhibited notably greater sucrose (9183 mg/100g) and total sugar (12457 mg/100g) levels than genotype-2 and genotype-3, with the lowest levels of these sugars observed in genotype-3 pods, as indicated in Table 2. The genotype-2 exhibited notably higher glucose (1301 mg/100g) and fructose (875 mg/100g) contents compared to genotype-1 and genotype-3, with the lowest levels of glucose and fructose observed in the pods of genotype-3 (Table 2). Interestingly, genotype-3 exhibited significantly higher levels of xylose (1548 mg/100g) than genotype-1 and genotype-2, with the lowest levels of xylose (1213 mg/100g) observed in the pods of genotype-2 (Table 2). The composition of sugar compounds in carob fruit may vary depending on geographical diversity (Würsch et al., 1984; Saura-Calixto, 1988; Avallone et al., 1997). According to Karkacier and Artik (1995), carob fruits are considered ripe for harvesting when they contain 91-92% total dry matter and 62-67% total soluble solids, including 34-42% sucrose, 10-12% fructose, and 7-10% glucose. Based on prior research conducted in Turkey and other countries, sucrose, glucose, xylose, and fructose have been detected and measured in carob. Avallone et al. (1997) studied the carob genotypes containing sucrose (27-40%), glucose (3-5%) and fructose (3-8%). Ayaz et al. (2007), reported that sucrose in carob fruit is 43.73%, was the major sugar and followed by glucose and fructose. Biner et al. (2007) recorded that sucrose is a high amount of sugar in carob fruit with smaller amounts of glucose and fructose. Gubbuk et al. (2010), studied that the sugar compound of carobs significantly changed allowing the genotypes which reported sucrose (27.7-43.8%) and glucose (10.8-17.4%). Fructose (0.54-1.4%) was identified as the lowest amount of sugars in the carob and was determined to be the main sugar in carob pods.

Organic acids analysis of dry carob fruit

In terms of organic acids, genotype-1 exhibited significantly higher levels of oxalic acid (17.62 mg/100g), citric acid (612.50 mg/100g), and fumaric acid (8.0 mg/100g) compared to genotype-2 and genotype-3, while the pods of genotype-3 and genotype-2 had the lowest levels of oxalic (15.13 mg/100g) and citric acid (356.92 mg/100g), respectively (Table 3).

Genotype-3 displayed significantly higher levels of malic acid (234.92 mg/100g) and succinic acid (1089.76 mg/100g) than the other two genotypes, while genotype-1 had the lowest levels of malic acid (200.24 mg/100g) and succinic acid (662.65 mg/100g) (Table 3). Genotype-2 contained a significantly higher amount of L-ascorbic acid (8.17 mg/100g) compared to genotype-1 and genotype-3, where genotype-3 contained the lowest

Table 2. Sugar compounds are analyzed from three genotypes of the mature dry carob fruit

mg/100 g	Genotype-1	Genotype-2	Genotype-3
Sucrose	9183±560^{a}	9001±81^{b}	6894±109^{b}
Glucose	1119±84^{b}	1301±41^{a}	1025±109^{b}
Xylose	1399±122^{b}	1213±33^{c}	1548±90^{a}
Fructose	754±40^{a}	875±31^{a}	481±103^{b}
Total sugars	12457±52^{a}	12389±182^{b}	9948±195^{b}

The data is presented as Mean ± Standard deviation (n=3), and values sharing the same letter within the line indicate no statistically significant difference ($p < 0.05$).

Table 3. Organic acid compounds analyze from three genotypes of the mature dry carob fruit

mg/100 g	Genotype-1	Genotype-2	Genotype-3
Ascorbic acid	6.72±0.50[a]	8.17±1.08[a]	6.22±0.25[a]
Oxalic Acid	17.62±3.97[a]	15.98±3.60[a]	15.13±6.29[a]
Citric Acid	612.50±92.71[a]	356.94±60.85[b]	431.05±85.03[b]
Malic Acid	200.24±9.17[b]	202.16±16.33[b]	234.92±4.59[a]
Succinic Acid	662.65±14.47[b]	779.03±275.82[ab]	1089.76±85.17[a]
Fumaric Acid	8.00±0.66[a]	3.12±2.26[b]	3.12±0.07[b]

The data is presented as Mean ± Standard deviation (n=3), and values sharing the same letter within the line indicate no statistically significant difference ($p < 0.05$)

amount of L-ascorbic acid (6.22mg/ 100g) (Table 3). According to Ashoor and Knox (1982), organic acids, free sugars, and amino acids are normal constituents of fruit and vegetables, playing an important role in preserving quality and defining nutritional value. As per Ayaz et al. (2007), the fruit of the pods contained malic acid at a level of 2.4 mg/g dry weight, while citric and L-ascorbic acids were not present in detectable quantities. It can be concluded that a significant variation in the levels of sugars and organic acids was observed across distinct carob genotypes originating from Kahramanmaras, Turkey, with genotype-1 displaying notably higher concentrations of the analyzed sugars and organic acids compared to the other genotypes

CONCLUSION

In conclusion, this study has produced some significant outcomes. The results presented here indicate substantial variations in sugar and organic acid contents among carob genotypes originating from Kahramanmaras, Turkey. Based on the findings, genotype-1 displayed notably elevated levels of the examined sugars and organic acids in comparison to the other genotypes. The results of this study could prove valuable to researchers studying the nutritional content of food products and to the related industry. Further investigations should focus on exploring the potential health implications of diverse carob genotypes' nutritional profiles, particularly their impact on human metabolism and health outcomes.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, 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.

Ethics committee approval

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RESEARCH ARTICLE

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Effect of different potassium fertilizers on yield and quality of tomato (*Solanum lycopersicum* L.) under drought stress conditions

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Abstract
The experiment was carried out in open field conditions in Ciğir village, located in the Idil district of Sirnak province, during 2020 tomato growing season. The aim of the study was to investigate the effect of different potassium (potassium chloride, potassium sulfate and potassium nitrate) fertilizers on yield and quality characteristics of tomato plants (Fereng genotype and Kamenta F1 variety) grown under drought stress. The fertilizers were foliar applied at a dose of 1%. Irrigation treatments of the experiment were full irrigation (control, 100%), 66% of the full irrigation, and 33% of the full irrigation. Leaf temperature, relative water content of leaf, chlorophyll content, fresh and dry weight of green parts, membranes injury index, soluble solid content (SSC) in tomato juice, pH of tomato juice and total yield were determined. The results indicated that drought stress had a significant adverse impacts on yield and quality of both Fereng genotype and Kamenta F1 variety. The application of potassium nitrate and potassium sulfate caused an increase in the chlorophyll and water soluble solid content. Potassium chloride application resulted in a reduction in membrane damage. The effects of potassium sulfate fertilizer on yield was significantly higher than the other two potassium fertilizers.
Keywords: Drought stress, *Solanum lycopersicum*, Yield, Potassium

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INTRODUCTION

The tomato is considered a highly valued vegetable in both domestic and international markets. The tomato, used both fresh and processed, is widely cultivated and is among the most commonly produced vegetables, following the potato (Aydoner Coban et al., 2020; Agarwal et al., 2020). Turkiye is among the most important tomato producer countries of the world (Bayramoğlu et al., 2009). Based on statistics provided by the Food and Agriculture Organization (FAO) in 2020, the total tomato production in Turkiye was about 13.204.010 tons (FAO, 2020). Turkiye holds a significant role in the cultivation of processing tomatoes (Türk et al., 2019). The vegetable in question has the potential to be utilized in both its fresh and processed forms (Ertürk and Çirka, 2015). In the human health, tomatoes and tomato products provide significant protection against wide range of important significant diseases (Salehi et al., 2019). Tomatoes is known for its abundant mineral content, in addition to carbohydrates, proteins, vitamins, and antioxidants (Perveen et al., 2015; Melfi et al., 2018; Wang et al., 2022). Lycopene, a pigments present in tomatoes, has demonstrated efficacy in the prevention of chronic diseases (Przybylska, 2020). The occurrence of drought stress has a substantial impact on both the crop yield and quality. Drought poses a significant challenge to the sustainability of global agricultural production (Akhoundnejad and Dasgan, 2020). The vegetable production is

adversely affected by the increase of biotic and abiotic stressors, as well as the growing population, which has been exacerbated by climate change (Khalid et al., 2022). Water is an essential component for the production of vegetables of adequate quality (Ors et al., 2021). Drought is a significant adverse consequence of climate change that is poised to emerge as a critical concern for both our nation and the global community in the foreseeable future (Akhoundnejad et al., 2021). The drought stress reduces chlorophyll content, relative water content of leaves, growth of green part growth, plant height and fruit yield in tomato plants compared to control (Sibomana et al., 2013). The study conducted on eggplant plants under drought stress conditions revealed that relative water content of leaf, leaf membrane damage, plant growth and yield in eggplant plants have been significantly affected by the drought (Semida et al., 2021). The study on onions demonstrated that drought conditions had a negative impact on various quality characteristics, including average fruit diameter and weight, fruit firmness, and yield (Wakchaure et al., 2021). The average fruit diameter, height, weight, wall thickness and fruit yield were reported lower in drought stress conditions compared to the control in various genotypes, namely Tepeköy, Yarbaşı and Fereng (Akhoundnejad, 2020). The application of potassium fertilizer at optimal levels improves the quality attributes of plants (Kanai et al., 2007). Potassium has a positive effect on various physiological processes in tomato plants, including photosynthesis, water uptake, fruit quality and quantity (Woldmerian et al., 2018). The application of potassium nitrate fertilizer to spinach plants under drought stress conditions resulted in a significant increase in root and shoot length in the spinach plants (Bukhari et al., 2021). Wasaya et al. (2021) showed that the occurrence of drought had an adverse impact on the growth and development of maize plants. However, the application of potassium sulfate fertilizer caused an increase in the levels of chlorophyll content and relative water content of maize leaves. The application of foliar K_2SO_4 fertilizer to green pepper plants yielded positive results in fruit, stem and leaf characteristics (El-Mogy et al., 2019). Foliar application of potassium chloride, potassium nitrate and potassium sulfate resulted in improved fruit color, increased weight and firmness in apples (Solhjoo et al., 2017). The application of potassium sulfate fertilizer given to melon plants under drought stress conditions increased the chlorophyll level and relative water content of leaf, fruit yield and dry matter content in melon (Tuna et al., 2010). The objective of this study was to investigate the effect of different potassium fertilizers on yield and quality characteristics of tomato plants grown under drought-induced stress conditions.

MATERIALS AND METHODS

One tomato landrace (Fereng) and an processing tomato variety (Kamenta F1) were used in the study. The research was carried out in open field conditions in Idil district of Sirnak province. The study was carried out between April and August 2020. The laboratory analysis was completed in the laboratories of the Agricultural Faculty in Şırnak University. The layout of the experiment was randomized block design with 3 replications. Ten plants were used in each replicate. The seedlings were planted in 20 cm inter-row and 50 cm intra-row spacings. Fertilizers were applied from the leaves and application rate was 1% for potassium nitrate (KNO_3), potassium chloride (KCl) and potassium sulfate (K_2SO_4) from the leaves. Irrigation treatments of the experiment were full irrigation (control, 100%), 66% of the full irrigation (66%), and 33% of the full irrigation (33%). Fertilizer application was applied twice during the experiment, 30 days after planting and once every 21 days. Drought stress application was started 45 days after planting. The total amount of water given to tomato plants during the trial period was determined using the following formula. The total amount of water given is shown in Figure 1. Fertilizers were applied twice during the experiment, the first one was 30 days after planting and the second one was 21 days after the first fertilizer application. Drought stress application was started 45 days after planting. The total amount of water given to tomato plants during the trial period was determined using the following formula. The total amount of water given is shown in Figure 1.

$$IR= A^* E\ pan\ ^*kcp\ ^* P$$

In the equation; IR is the amount of water supplied (m^3); A is the size of a plot (da); E pan is the amount of evaporation (mm); kcp is the coefficient for tomato plant (0.80); P-mat is the vegetation (%); and P-cover is the ratio of plant crown width (cm) to row spacing (cm).

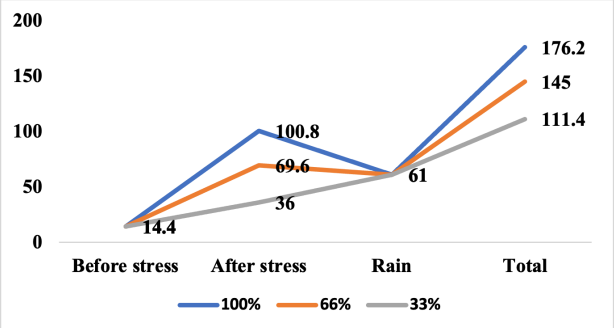

Figure 1. Total amount of water supplied (L).

(Before Stress 16.04.2020-01.06.2020, After Stress 01.06.2020-13.08.2020)

The temperature of the leaves (°C) was determined using an infrared thermometer. The procedures employed by Dlugokecka and Kacperska-Palacz (1978) and Fan and Blake (1994) were utilized to determine the membrane damage in leaf cells. The relative water content of leaves was determined by the method introduced by Türkan

et al. (2005) and Sanche et al. (2003). The chlorophyll concentration in fruit juice was measured using a SPAD 502 model instrument. The pH of fruit juice was measured using a pH meter, and the Soluble Solid Content (SSC) was determined using a refractometer. All fruits harvested during the trial were recorded. The dry matter content of the green leaf sections was measured. The substance ratios were determined by weighing the fresh and dry weights of the samples.

Statistical Analysis of Data

The significance in the irrigation treatments and fertilizer types was assessed by analysis of variance (ANOVA) test. Least Significant Difference test ($P<0.05$) was used to differentiate the means in case ANOVA denoted significant differences between the irrigation treatments or fertilizer types. in the data was evaluated using the JMP 13th statistical software.

RESULTS AND DISCUSSION

The leaf temperature of tomato plants under 66% and 33% irrigation treatments was 1.93% and 1.13% higher compared to the control (Table 1), respectively. The findings of the study indicated that the utilization of KCl (5%) fertilizer in the Kamenta F1 variety, along with a 66% irrigation treatment, yielded superior results in comparison to alternative treatments. When plants experience drought stress, they respond by closing their stomata, resulting in an absence of transpiration. This lack of transpiration leads to an increase in leaf temperatures. Fanaei et al. (2009) reported an increase in leaf temperatures of canola and mustard grass in response to drought stress conditions. Ardestani and Rad (2012) conducted a study in which they observed that drought conditions in rapeseed plants resulted in an increase of leaf temperature. However, the application of potassium and potassium sulfate fertilizers to the plants was found to mitigate this increase in leaf temperature. In general, the foliage in the plant under drought stress to balance the water loss of stomata by decreasing the water rate. They close for this purpose. This causes photosynthesis to slow down, It causes the leaf temperature to increase.

The overall mean values of the applications revealed a 0.60% rise in the proportion of plants receiving 66% irrigation, while a 0.47% decline was observed in the proportion of tomato plants receiving 33% irrigation.

The effect of several potassium fertilizers on chlorophyll content is given in Table 2. The highest chlorophyll content was obtained from KNO_3 (71.73%) application in Kamenta F1 variety under 66% irrigation. Conversely the lowest chlorophyll content (50.78%) was measured in KCl application from Kamenta F1 variety under 33% irrigation. In comparison to the control group, the overall mean chlorophyll content values of the applications revealed a 0.60% increase in 66% irrigation treatments, and 0.47% decrease in 33% irrigation treatment. The ratio of changes was the in the control (15.74%) for Kamenta F1 variety under 33% irrigation (Table 2). The study conducted by Hayat et al. (2008) indicated a reduction in the chlorophyll content of tomato plants when subjected to drought stress. Aliche et al. (2020) reported that the drought in potato plants has an adverse impact on the chlorophyll content. According to Tuna et al. (2010), the presence of drought stress circumstances led to a reduction in the chlorophyll content of melon plants. However, the application of K_2SO_4 to these plants had a beneficial impact on the chlorophyll levels. The application of KNO_3 and urea to maize plants under drought and salinity stress conditions significantly increased the chlorophyll content in plants (Saed-Moocheshi et al., 2014).

The relative water content of leaves was significantly different among the irrigation treatments (Table 3). The efficiency of control (91.28%) treatment (100% water) for Fereng genotype was higher compared to all other irrigation treatments and fertilization applications. The application of 33% irrigation increased the relative water content of leaves by 1% compared to control application. The relative water content in K_2SO_4 fertilizer application

Table 1. The effect of applied different potassium fertilizers on leaf temperature.

Application	%100 Irrigation	%66 Irrigation	%33 Irrigation	66% change compared to control in irrigation (%)	33% change compared to control in irrigation (%)
Kamenta+Control	27.70 bc	29.70 ac	30.10 a	7.22	8.66
Fereng+Control	27.60 bc	27.80 d	27.25 d	0.72	-1.27
Kamenta+KCl	30.15 ab	28.40 cd	29.95 ab	-5.80	-0.66
Fereng+KCl	30.70 a	31.00 a	30.95 a	0.98	0.81
Kamenta+K_2SO_4	30.75 a	30.70 a	30.90 a	-0.16	0.49
Fereng+K_2SO_4	27.05 c	28.40 cd	27.30 cd	4.99	0.92
Kamenta+KNO_3	29.60 ac	30.10 ab	28.65 bc	1.69	-3.21
Fereng+KNO_3	27.30 ac	28.90 bd	28.20 cd	5.86	3.30
Mean	28.85	29.37	29.16	1.93	1.13
LSD	2.99	1.57	1.37	-	-
P	0.0581	0.0046*	<.0001*	-	-

Table 2. Effect of applied different potassium fertilizers on chlorophyll content.

Application	%100 Irrigation	%66 Irrigation	%33 Irrigation	66% change compared to control in irrigation (%)	33% change compared to control in irrigation (%)
Kamenta+Control	63.27 b	65.78 b	53.31 d	3.97	-15.74
Fereng+Control	59.84 d	55.66 cd	57.36 c	-6.99	-4.14
Kamenta+KCl	53.68 e	53.03 de	50.78 e	-1.21	-5.40
Fereng+KCl	60.88 cd	69.40 a	64.98 b	13.99	6.73
Kamenta+K_2SO_4	53.18 e	50.34 e	63. 88 b	-5.34	20.12
Fereng+K_2SO_4	69.87 a	63.89 b	64.54 b	-8.56	-7.63
Kamenta+KNO_3	62.74 b	71.73 a	56.27 c	14.33	-10.31
Fereng+KNO_3	61.07 c	57.82 c	68.77 a	-5.32	12.61
Mean	60.56	60.95	59.98	0.60	-0.47
LSD	1.03	3.29	1.18	-	-
P	<.0001*	<.0001*	<.0001*	-	-

Table 3. The effect of applied different potassium fertilizers on the relative water content of leaves.

Application	%100 Irrigation	%66 Irrigation	%33 Irrigation	66% change compared to control in irrigation (%)	33% change compared to control in irrigation (%)
Kamenta+Control	74.54 e	75.76 bc	75.31 d	1.64	1.03
Fereng+Control	91.28 a	78.27 a	78.49 c	-14.25	-14.01
Kamenta+KCl	73.24 f	75.50 bc	73.21 e	3.09	-0.04
Fereng+KCl	83.89 b	75.38 bc	75.28 d	-10.14	-10.26
Kamenta+K_2SO_4	69.26 h	76.55 b	81.77 b	10.53	18.06
Fereng+K_2SO_4	79.31 c	76.57 b	78.46 c	-3.45	-1.07
Kamenta+KNO_3	72.35 g	74.59 c	77.09 c	3.10	6.55
Fereng+KNO_3	78.26 d	75.70 bc	84.74 a	-3.27	8.28
Mean	77.76	76.04	78.04	-1.59	1.07
LSD	0.60	1.20	1.47	-	-
P	<.0001*	0.0007*	<.0001*	-	-

Table 4. The effect of applied different potassium fertilizers on membrane damage in leaf cells.

Application	%66 Irrigation	%33 Irrigation
Kamenta+Control	24.48 c	25.49 b
Fereng+Control	25.89 b	28.84 a
Kamenta+KCl	25.98 b	24.45 c
Fereng+KCl	28.56 a	25.46 b
Kamenta+K_2SO_4	26.23 b	28.74 a
Fereng+K_2SO_4	26.60 b	25.19 bc
Kamenta+KNO_3	26.05 b	25.40 b
Fereng+KNO_3	26.67 b	28.33 a
Mean	26.30	26.48
LSD	0.96	0.78
P	<.0001*	<.0001*

for Kamenta F1 variety under 66% and 33% irrigation was 10% and 18% higher compared to the control (Table 3), respectively. The results revealed that application of different potassium fertilizers under drought stress improve the relative water content of tomato plant leaves. According to Soleimanzadeh et al. (2010), drought conditions reduce the relative water content of leaves. Kirnak et al. (2002) investigated the effects of drought stress on eggplant and indicated that drought stress resulted in a reduction in the relative water content of the plant leaves. Likewise, Zhou et al. (2017) showed that the relative water content of tomato plant leaves was drastically reduced under drought stress conditions. Drought stress in citrus seedlings decreased the relative

Table 5. The effect of applied different potassium fertilizers on soluble solid content (SSC) in fruit juice.

Application	%100 Irrigation	%66 Irrigation	%33 Irrigation	66% change compared to control in irrigation (%)	33% change compared to control in irrigation (%)
Kamenta+Control	4.75 bc	6.05 ab	6.51 b	27.37	37.05
Fereng+Control	5.05 ab	5.50 c	7.00 a	8.91	38.61
Kamenta+KCl	4.96 ac	5.60 bc	6.97 a	12.90	40.52
Fereng+KCl	4.97 ac	5.95 ac	7.00 a	19.72	40.85
Kamenta+K_2SO_4	4.64 c	5.90 ac	7.10 a	27.16	53.02
Fereng+K_2SO_4	4.97 ac	6.40 a	7.00 a	28.77	40.85
Kamenta+KNO_3	4.95 ac	5.95 ac	6.96 a	20.20	40.61
Fereng+KNO_3	5.28 a	5.50 c	7.10 a	4.17	34.47
Mean	4.94	5.85	6.95	18.64	40.74
LSD	0.35	0.53	0.32	-	-
P	0.1932	0.0367*	0.0384*	-	-

Table 6. The effect of different potassium fertilizers applied on the pH content of fruit juice.

Application	%100 Irrigation	%66 Irrigation	%33 Irrigation	66% change compared to control in irrigation (%)	33% change compared to control in irrigation (%)
Kamenta+Control	4.51 ab	4.65 a	4.34 cd	3.10	-3.77
Fereng+Control	4.56 ab	4.57 ab	4.37 bd	0.22	-4.17
Kamenta+KCl	4.54 ab	4.62 a	4.33 d	1.76	-4.63
Fereng+KCl	4.64 a	4.56 ab	4.43 ad	-1.72	-4.53
Kamenta+K_2SO_4	4.59 a	4.58 ab	4.50 ab	-0.22	-1.96
Fereng+K_2SO_4	4.49 ab	4.50 b	4.50 ac	0.22	0.22
Kamenta+KNO_3	4.36 b	4.31 c	4.54 a	-1.15	4.13
Fereng+KNO_3	4.62 a	4.62 a	4.51 ab	0.00	-2.38
Mean	4.53	4.55	4.44	0.30	-2.13
LSD	0.22	0.11	0.16	-	-
P	0.2460	0.0004*	0.0674	-	-

Table 7. The effect of different potassium fertilizers applied on the fresh and dry weight of tomato plants in green parts.

Application	%100 Irrigation	%66 Irrigation	%33 Irrigation	66% change compared to control in irrigation (%)	33% change compared to control in irrigation (%)
Kamenta+Control	18.76 b	20.31 ab	18.82 bc	8.26	0.32
Fereng+Control	18.31 b	18.05 d	20.72 a	-1.42	13.16
Kamenta+KCl	20.57 a	20.68 a	16.78 d	0.53	-18.42
Fereng+KCl	14.53 c	20.88 a	8.36 e	43.70	-42.46
Kamenta+K_2SO_4	18.62 b	19.60 ab	19.60 b	5.26	5.26
Fereng+K_2SO_4	19.36 ab	19.35 bc	18.15 d	-0.05	-6.25
Kamenta+KNO_3	18.75 b	16.14 e	18.88 bc	-13.92	0.69
Fereng+KNO_3	14.38 c	18.12 cd	18.85 bc	26.01	31.08
Mean	17.91	19.14	17.52	8.54	-2.07
LSD	1.35	1.29	0.98	-	-
P	<.0001*	<.0001*	<.0001*	-	-

water content of the leaves. However, the application of KNO_3 on the seedlings alleviated the drought impact and increased the relative water content of leaves (Gimeno et al., 2014).

There was no statistically significant difference observed in membrane damage in leaf cells between the various fertilizer applications under 66% irrigation, with the exception of the control application of the

Table 8. The effect of different potassium fertilizers applied on total yield.

Application	%100 Irrigation	%66 Irrigation	%33 Irrigation	66% change compared to control in irrigation (%)	33% change compared to control in irrigation (%)
Kamenta+Control	804.39 c	801.10 c	538.97 bc	-0.41	-33.00
Fereng+Control	410.90 e	450.13 e	347.00 e	9.55	-15.55
Kamenta+KCl	975.72 ab	679.59 d	605.73 b	-30.35	-37.92
Fereng+KCl	816.72 bc	767.03 cd	409.50 de	-6.08	-49.86
Kamenta+K_2SO_4	1100.83 a	1332.76 a	831.70 a	21.07	-24.45
Fereng+K_2SO_4	710.12 cd	829.88 c	471.66 cd	16.86	-33.58
Kamenta+KNO_3	1065.20 a	992.40 b	802.16 a	-6.83	-24.69
Fereng+KNO_3	636.05 cd	460.15 e	483.33 cd	-27.66	-24.01
Mean	814.99	789.13	561.25	-2.98	-30.38
LSD	158.16	105.03	121.38	-	-
P	<.0001*	<.0001*	<.0001*	-	-

Fereng genotype and Kamenta F1 variety (Table 4). The Kamenta F1 cultivar had the highest resistance (24.45%) among the several applications tested, particularly at 33% irrigation. The control group exhibited the highest level of membrane damage, with a recorded percentage of 28.84%. This was observed in the application of the Fereng genotype under 33% irrigation circumstances. Yıldızlı et al. (2018) shown that drought stress in pepper plants led to an increase in membrane damage within leaf cells. A positive correlation was recorded between the intensity of drought stress and the percentage of membrane damage in maize plants (Li-Ping et al., 2006). Zain and Ismail (2016) indicated that the application of drought stress on rice plants leads to an increased rate of membrane damage. According to Dasgan et al. (2015) the Leaf membrane damage of melon plants increased due to the abiotic conditions such as drought and salinity. However, the use of K_2SO_4 and KCl fertilizers mitigated the adverse impacts on the damage index.

The ratios of water-soluble dry matter under 100%, 66%, and 33% irrigation treatments were found to be similar. The Kamenta F1 variety exhibited the highest ratio of water soluble dry matter when treated with K_2SO_4 (7.10%) while the Fereng genotype showed the same result when treated with KNO_3 (7.10%), both under conditions of 33% irrigation. No statistically significant differences were observed among 33% irrigation treatment, except for the control application of the Kamenta F1 variety. The water soluble dry matter ratio indicated a significant increase of 18.64% and 40.74% under 33% and 66% irrigation levels, respectively, in comparison to the control group (Table 5). The increase in drought-induced stress resulted in a corresponding increase in the concentration of soluble dry matter content. According to Karam et al. (2011) an increase in the drought stress level was found to be associated with an increase in the SSC in eggplant plants. Mardanluo et al. (2018) found that the application of potassium fertilizer to chili and bell pepper plants resulted in an increase in the concentration of SSC in the fruit juice. According to Woldmeriam et al. (2018) the application of potassium fertilizer resulted in an increase in SSC of tomato juice. Wakchaure et al. (2020) showed that the application of KNO_3 and urea fertilizer during drought stress periods demonstrated the efficacy in increasing SSC in eggplant plants.

Table 6 presents the impact of several foliar potassium fertilizers on the pH levels of apple juice. Based on the obtained data, the pH levels in the various fruit juices were similar to one another (Table 6). In comparison to the control group, the experimental group demonstrated the most significant rise in pH level change under the 33% irrigation, specifically with the Kamenta F1 variety treated with KNO_3, resulting in a 4.13% increase. Conversely, the Kamenta F1 variety treated with KCl exhibited a 4.63% reduction under the similar irrigation conditions If the pH decreases, the amount of dry matter in the fruit increases, which improves the taste of the fruit. Agbemafle et al. (2014) demonstrated that the application of drought stress resulted in a significant reduction in the pH levels of tomato fruit juice. Similarly, Renquist and Reid (2001) and Coban et al. (2020) discovered that drought and salt stress conditions resulted in a reduction in the pH levels of tomato plant juice.

The use of potassium chloride (KCl) on the Kamenta F1 tomato variety with 66% irrigation led to a notable 20.68% increase in the wet and dry weight of the green components of the plants. Conversely, the application of KCl (with a ratio of increase of 8.36%) on the Fereng genotype in 33% irrigation did not yield any significant impact (Table 7). The results indicated that the mean wet and dry weight in 66% irrigation had a significant increase (8.54%) compared to the control group. Conversely, the plants subjected to 33% irrigation exhibited a decrease of 2.07% in the mean wet and dry weight. The change in wet and dry weight under different irrigation treatments relative to the control group indicated that the variability of changes was higher in the 33% irrigation treatment compared to the 66% irrigation treatment (Table 7). Zhou et al. (2017) reported that abiotic stress such as

heat and drought have a negative impact on the fresh and dry weights in green parts tomato plants. In this study, we can obtain better quality products at lower costs and under drought stress conditions. Additionally, in the future, we can test different elements in drought areas and see their performance on tomato fruits.

The highest overall yield characteristics were obtained through the use of the Kamenta F1 variety with K_2SO_4 fertilizer, resulting in a yield of 1332.76 grams per plant under 66% irrigation. Additionally, the Kamenta F1 variety with K_2SO_4 fertilizer yielded 1100.83 grams per plant at 100% irrigation. The yield (347.00 plant g^{-1}) of Fereng genotype in control under 33% irrigation was not significantly different (Table 8). In the control, the yield was significantly decreased under both 66% and 33% stress treatments. The corresponding decrease were found to be 2.98% and 30.38% for the respective treatments. The yield in 33% and 66% irrigation applications significantly decreased in all treatments, except for the Fereng genotype in control, Kamenta F1 in K_2SO_4 fertilizer, and Fereng genotype in K_2SO_4 fertilizer application under the 66% irrigation treatment (Table 8). The findings indicated a negative correlation between the intensity of drought stress and overall yield, with an observed decrease in yield as the level of stress intensified. The application of potassium sulfate fertilizer has been found to have an impact on the overall crop yield. Klunklin and Savage (2017) emphasized the significance of irrigation for obtaining optimal yields in tomato plants. According to Agbna et al. (2017) the yield of tomato fruit is negatively affected by drought-induced stress. Neseim et al. (2014) demonstrated that the production of sugar beet was adversely affected by drought stress, but the application of K_2SO_4 fertilizer proved to be efficacious to increase fruit yield. According to a study conducted by Asgharipour and Heidari (2011) the application of K_2SO_4 to sorghum plants experiencing drought stress resulted in a notable increase in fruit yield when compared to the control group.

CONCLUSION

The occurrence of drought stress resulted in an increase in leaf temperature. The use of KNO_3 and K_2SO_4 fertilizers, under 33% and 66% deficit irrigation treatments resulted in a significant increase in chlorophyll and water soluble dry matter content. The findings indicated that the application of K_2SO_4 fertilizer resulted in the highest tomato fruit yield. The use of KCl fertilizer resulted in a reduction in membrane damage observed in leaf cells. The findings of this study indicate that the application of all three fertilizers had a beneficial impact on the measured parameters in both the Kamenta F1 tomato cultivar and the Fereng tomato genotype under drought stress conditions.

COMPLIANCE WITH ETHICAL STANDARDS

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There is no conflict of interest between the authors.

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The authors contributed equally to the article.

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RESEARCH ARTICLE

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Flower morphology and sexual phenotype of *Capparis ovata* Desf.

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Abstract

It was aimed to investigate the flower morphology and sexual phenotypes of *Capparis ovata* Desf. A *C. ovata* population inhabits in Çorum Osmancık Kumbaba locality. A total of 68 flower samples were collected from the population and their morphological characteristics and sexual phenotypes were investigated. According to the morphometric data, the coefficient of variation (CV) was calculated for the examined traits. Petal width (CV:3.11-20.26%) and sepal width (CV: 3.17-20.57%) showed the highest variation range. In terms of flower phenotype, 25 out of 68 flowers (36.76%) showed perfect flower and 43 (63.24%) showed male flower phenotype. The perfect flowers are hypogeynous flowers and have campylotropous type ovules. *C. ovata* has zygomorphic flower. *C. ovata* Desf. plants were defined as andromonoecious due to both the male flower and the perfect flower are present on the same plant. In conclusion, according to morphological analysis, a remarkably high variation was observed in flower morphological structures and the ratio of male flowers were found to be more common than perfect flowers in the *C. ovata* Desf. population. During evolutionary history, the protandry feature, in which male and female reproductive organs begin to develop at different times in perfect flowers, emerged in plants to prevent the depression of self-pollination, and inbreeding.

Keywords: *Capparis* L., *Capparis ovata* Desf., flower morphology, morphological variation, andromonoecious

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INTRODUCTION

Capparis ovata Desf., defined in the *Capparis* L. section of the *Capparis* L. genus of the *Capparaceae* L. family, includes more than 250 species (Jacobs, 1965; Mabberley, 1987: Willis, 1988; Fici, 1993; Inocencio et al., 2006) growing in tropical and subtropical regions of the New and Old World. Five main species are distributed in the Mediterranean region. These were defined as *Capparis spinosa* L., *Capparis sicula* Veill., *Capparis aegyptia* (Lam.) Boiss., *Capparis orientalis* Veill., and *Capparis ovata* Desf. (Inocencio et al., 2006). There are two species (*Capparis spinosa* L. and *Capparis ovata* Desf.) and three varieties of each species; *C. spinosa* L. var. *spinosa* L, *C. spinosa* L. var. *inermis* Turra., *C. spinosa* L. var. *aegyptia* (Lam) Boiss, and *C. ovata* Desf. var. *palaestina* Zoh., *C ovata* Desf. var. *herbacea* (wild) Zoh., and *C. ovata* Desf. var. *canescens* (Coss.) Heywood. growing in Türkiye (Zohary, 1960; Davis, 1965). While it is widespread in countries such as Spain, Italy, and Sicily in the Mediterranean region, it is also widespread in Far Eastern countries such as India and China,

It grows naturally in the provinces Hakkari, Karabük, Ankara, Artvin, Denizli, Tokat (TÜBİVES), Adıyaman, Antalya, Aydın, Balıkesir, Batman, Çorum, Denizli, Diyarbakır and Şanlıurfa (Özbek & Kara 2013) in Türkiye. The caper plant has various names used by local people around the world, some of which are "kapari,

gebere otu, kapara, devedikeni, gebre, gebere, geber otu, gevil, bubu, kebere, yumuk, kemeri, menginik, keper, kepere, kedi tırnağı, şeballah" (Kara, 2012). In Türkiye, it is generally referred to as "kapari", "kebere" or "gebre otu" (Bilgin, 2004; Baytop, 1995).

C. ovata Desf. is a shrub that is shorter in height, grows procumbent and pendulous in habit (Figure 1), and can grow at higher altitudes such as 1500-2000 m (Davis, 1982). Its leaves are elliptical or broadly elliptical, rarely nearly round-shaped, with a distinct mucronate apex, and are often more or less hairy (Figures 2a, b and c). The flowers are strongly zygomorphic and andromonoecious, which is the presence of a male and a perfect flower on the same plant (Figure 3 and Figure 4). Stipules are strongly or weakly curved downwards or straight (Özcan, 1996; Kara, 2012; Özbek & Kara, 2013). The bery-like fruits, (called karpuzcuk in Turkish), rupture when they ripen and the seeds are scattered around (Figure 5 and Figure 6).

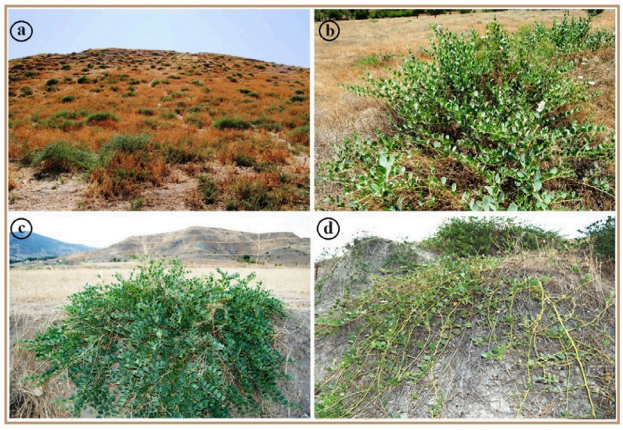

Figure 1. Types of habits in *C. ovata* Desf. a) procumbent, Diyarbakır Bismil-Üçtepe, b-c) procumbent, Çorum Osmancık, d) pendulous, Çorum Osmancık (Photo: Özlem Özbek)

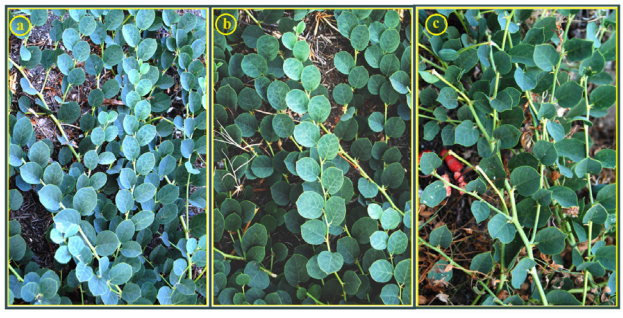

Figure 2. Leaf shapes seen on the *C. ovata* Desf. plants, in Çorum Osmancık, a-b) The leaves show an alternating arrangement, c) the mucronate structure on the apex of the leaves, and the backward and yellowish color stipules at the base of the leaves (Photo: Özlem Özbek)

Figure 3. A male flower on a *C. ovata* Desf. plant in Çorum Osmancık (Photo: Özlem Özbek)

Figure 4. A perfect (hermaphrodite) flower on a *C. ovata* Desf. plant in Çorum Osmancık (Photo: Özlem Özbek)

Figure 5. The ruptured berry-like fruit on a *C. ovata* Desf. plant in Çorum Osmancık (Photo: Özlem Özbek)

Figure 6. When the red-fleshy fruit completes its ripening, ruptured and opened to be dispersed its seeds (*C. ovata* Desf.) in Çorum Osmancık (Photo: Özlem Özbek)

Pollination is an important function in plants to ensure fertilization and development of seeds and to guarantee the continuation of generations. The adaptation of plants to different pollination factors in their evolutionary history is also observed in the capparis flower. During the evolutionary process, plants have gained plasticity in their reproductive organs to adapt to their environment. This flexibility is related not only to the flower symmetry, but also to features such as color, number of stamens, petal shape, long stamen filament, odor, time and rate of nectar production. Pollination agents have also diversified along with the evolution of plants in their evolutionary history. Pollination agents such as bees, humming birds, hawk moths and bats help pollination in capers. The existence of hermaphrodite species in which the male gamete matures before the female gamete and self-pollination is also frequently observed in hermaphrodite species in order to be protected from the depression of selfing (Kers, 2003).

Due to the very high level of dormancy observed in capparis seeds, there are not many studies on cytogenetics and karyotype of capparis. Although there is no study conducted in Türkiye, the chromosome number of *Capparis spinosa* was determined as 2n = 38 according to several previous studies (Murin & Chaudhri, 1970; Magulaev, 1979 according to Goldblatt, 1984; Al-Turki et al., 2000). According to researchers, it has been reported that caper is a polyploid and aneuploid plant, the number of chromosomes varies and the average number of chromosomes can be expressed as x = 10 (Kers, 2003).

Zhang and Tan (2009) investigated the pollen donor and pollinator attractor hypotheses of male flowers of andromonoecious plants in *C. spinosa* L. (Capparaceae). They showed that male flowers produced larger anthers, larger pollen grains, and smaller ovaries than perfect flowers, indicating that pollinators did not discriminate between flower morphs and transferred pollen grains a similar distance. They concluded that female reproductive success is likely not limited to pollen, with male flowers of *C. spinosa* L. conserving resources for female function and serving primarily to attract pollinators as pollen donors. Yang et al. (2014) examined the reproductive characteristics of *C. spinosa* L. (Capparaceae), they detected two different flowering periods in months. They stated that there were significant differences in the morphology of male flowers and perfect flowers in these two periods. The researchers concluded that variation in male to perfect flower ratio in *C. spinosa* L. plays a positive role in ensuring a constant pollen supply and controlling fruit investment. Shakarishvili and Osishvili (2013) studied the sexual phenotypes of *Capparis herbacea* Willd. They explained that *C. herbacea* Willd. also has androecium flower morphology, a hermaphrodite sexual system that produces both male and perfect flowers on the same plant. They stated that functionally male flowers have larger anthers and develop more stamens than perfect ones, while the male/perfect flower ratio varies between 0.5 and 2.6 during the flowering season. Additionally, they concluded that pollinating agents did not show any preference for flower morphs during their visit, and these results confirmed the pollen donation hypothesis regarding the role of male flowers in andromonoecious plants.

Most of the studies conducted on *Capparis* L. plants in Türkiye include on the propagation of the plant by vegetative methods and breaking seed dormancy. There are no comprehensive studies on the reproductive biology of the *Capparis* L. in Türkiye. For this reason, it was aimed to conduct a study on the investigation of flower morphology and sexual phenotypes regarding reproductive biology in *C. ovata* Desf.

MATERIALS AND METHODS

Materials

A total of 68 flower samples, including 8 plants, were collected from a *C. ovata* Desf. population located in Çorum Osmancık Kumbaba locality in June 2015.

Methods

The flowers were examined according to morphological characteristics of petal width (PW), petal length (PL), sepal width (SW), sepal length (SL), flower peduncle length (FPL), number of stamens (NS) and sexual phenotypes (male and perfect flowers). The mean and standard deviation values of morphological characters in flower structure were estimated (Table 1). Morphometrical characters for each flower were measured in cm with a ruler. Coefficients of variation (CV) were calculated based on the average morphometric values. The classical coefficient of variation (CV) is the ratio of the standard deviation calculated in a data set to the mean and can be used to compare normally distributed data based on their variability (Ospina & Marmolejo-Ramos 2019). The coefficient of variation is very useful as a statistical

tool because it allows variables to be compared independently of scale effects and is dimensionless, meaning it has no units. It allows comparison of data sets with various different units of measurement (Ospina & Marmolejo-Ramos 2019). If the CV value is equal to 1 or 100%, the standard deviation is equal to the mean. Values less than one mean the standard deviation is smaller than the mean (typical), values greater than one mean the standard deviation is larger than the mean. A CV exceeding about 30 percent is usually an indication that there are problems with the data or that the experiment is out of control. Variables with a mean less than one will also provide inaccurate results and the coefficient of variation will be very large and often insignificant. This measurement is widely used in many fields such as social science, engineering, and life science (Brown, C.E. 1998). It is calculated according to the formula below.

$$CV = \frac{Standard\ Deviation}{Mean}$$

The structures of male and female organs in the flower structure during bud development were examined and visualized under a microscope.

RESULTS AND DISCUSSION

It was determined that the *C. ovata* Desf. plants examined in this study had an andromonoecious type reproductive system. Andromonoecy is a reproductive system in flowering plants where individuals produce both perfect flowers and male flowers. It is widely distributed in thirty-three families and approximately 4000 Angiosperm species (Yampolsky & Yampolsky, 1922; Bawa & Beach, 1981; Cruden & Lloyd, 1995; Miller & Diggle, 2003; Vallejo-Mar'in & Rausher, 2007; Zhang & Tan, 2009). It has been suggested that andromonoecy evolved from hermaphroditism with the loss of female reproductive structure, which was the first step in the evolution of the plant reproductive system towards monoecy, androdioecy and dioecy (Primack & Lloyd, 1980; Bertin, 1982; Zhang & Tan, 2009). It was determined that the capparis plants examined in this study had both male and perfect flowers on the same plant. The average values of metric measurements made for the morphological characteristics of the flowers were given in Table 1. When the number of flowers collected from eight plants was considered in the study, an average of 8.5 flowers per plant were examined, with a minimum of three and a maximum of 10 flowers. Of the 68 flowers examined, 25 showed perfect flower phenotype, while 43 showed male flower phenotype. The ratio of the number of male flowers to the number of female perfect flowers was calculated as 1.72. It was found that the number of male flowers is almost as high as twice the number of perfect flowers. When the data [APW (14.37), APL (30.87), ASW (10.32) and ASL (22.92)] were examined, it was seen that the rates were greater in male flowers than perfect flowers. It was observed that the mean values for ANS (67.33) was highger in plants with male flowers.

Table 1. Mean and standard deviation values of morphological characters measured in flower structure structures of *C. ovata* plants

PN	1	2	3	4	5	6	7	8
NFS	3	10	10	10	10	5	10	10
MF/PF	2/1	6/4	6/4	4/6	4/6	3/2	10/0	8/2
SNR	65-70	50-71	46-68	46-66	50-68	55-69	54-78	51-80
APW	14.37	8.98	11.58	11.9	13.1	10.96	13.07	8.51
St.Dev.	*0.45*	*1.3*	*1.77*	*2.41*	*1.81*	*1.38*	*1.46*	*1*
APL	30.87	18.1	24.96	22.84	20.81	28.27	24.45	21.01
St.Dev.	*0.48*	*1.85*	*4.19*	*3.33*	*1.83*	*3.77*	*2.87*	*1.89*
ASW	9.92	8.13	9.51	8.69	9.67	9.15	10.32	7.28
St.Dev.	*0.31*	*0.67*	*1.31*	*1.17*	*0.71*	*1.25*	*2.12*	*0.67*
ASL	22.92	17.21	19.71	19.13	17.54	21.02	22.32	18.02
St.Dev.	*2.17*	*1.48*	*1.13*	*0.76*	*0.86*	*1.42*	*1.41*	*1.61*
AFPL	47.24	45.85	49.16	42.39	36.64	51.29	57.23	51.12
St.Dev.	*8.07*	*3.66*	*5.71*	*3.92*	*4.39*	*2.48*	*4.06*	*5.43*
ANS	67.33	60.1	56.8	57.9	59.5	63.8	61.8	58.6
St.Dev.	*2.52*	*6.4*	*7.48*	*5.7*	*7.38*	*5.26*	*6.99*	*8.75*
ANMF	66	61.17	57.67	55.75	58.75	61.67	61.8	58.13
St.Dev.	*1.41*	*2.24*	*7.74*	*2.5*	*7.46*	*5.86*	*6.99*	*9.4*
ANPF	2.5	58.5	55.5	59.33	60	67	0	60.5
St.Dev.	*1.96*	*3.61*	*8.02*	*6.98*	*8*	*2.83*	*0*	*7.78*

Abbreviations: PN: Plant Number, NFS: Number of Flower Sample, MF/PF Male:Flower / Perfect Flower Number Ratio, SNR: Stamen Number Range, APW: Average Petal Width, APL: Average Petal Length, ASW: Average Sepal Width, ASL: Average Sepal Length, AFPL: Average Flower Peduncle Length, ANS: Average Number of Stamens, ANMF: Average Number of Male Flowers, ANPF: Average Number of Perfect Flowers, St. sp.: Standard Deviation

Flowering in the capparis plants continues from May to October in nature.

According to the results of the study, it was determined that the male flower ratio of 1.72 (43 male/25 perfect flowers) was more common in flower phenotypes in plants belonging to the *C. ovata* Desf. population. This result is consistent with Yang et al. (2014), it also appears to have a higher rate, although it is consistent with their 5-month observations. Researchers stated in their studies that in the first period of flowering, the number of male flowers is more than female flowers, and in the second period, while there is no difference in the number of male flowers, there is an increase in the number of female flowers. This ensures the pollen supply of male flowers while ensuring the perfect flower's adaptation to environmental conditions to produce reproductively functional fruits and seeds. Protandry is the condition in which male and female reproductive organs develop at different times in hermaphrodite flowers in order to limit the depression of inbreeding in plants, and it is estimated that this feature might be found in *C. ovata* Desf. plants, but further studies should be performed. The results of this study seem to be consistent with previous studies on the reproductive system of *Capparis* L. (Kers, 2003; Zhang & Tan, 2009; Shakarishvili & Osishvili, 2013; Yang et al., 2014).

When the peduncles of the buds were examined under the microscope, they were observed to be covered with very dense, white, and soft velvety hairs. It was observed that there was hairiness on the parts of the sepals surrounding the flower towards the receptacle, but the density decreased towards the tips (Figure 7a). When the sepals were opened, it was seen that the petals tightly covering the reproductive organs were white and their outer surfaces were hairy (Figure 7b). When the petals were stripped off, it was seen that numerous stamens with yellowish-white stems and violet-colored anthers tightly surrounded the pistil (Figure 7c). *C. ovata* Desf. had hypogynous flower, in which the floral parts sepals and petals attached to the receptacle were beneath the ovary including many campylotropous type ovule was observed in the perfect flowers (containing female and male organs) (Figures 8 a and b). While the flower shape was observed to be zygomorphic (bilaterally symmetrical) (Figures 3 and 4), the placentation pattern in the ovary was observed to be parietal placentation (Figures 7 f and g). The color of the ovary was from yellowish to light green, and the stigma was dark, almost black. It was determined that the color of the anthers of the stamens was violet in the early development stage and had a lobed appearance. It was observed that the anthers had a swollen and lobed dorsal area and a flattened and lobed shape on the lower surface in the advancing development phase, and the reproductive organs of the flower, especially around the anthers, were covered with crystal-shaped and dense white color nectar (Figures 6 h-j). Although the pollen shapes appeared to be oval-shaped, it was observed that there were also variations. The observed pollen grains had tricloplorate aperture (they had 3 pores and 3 colpus). The shape of amb was elliptic in generally but their equatorial view was circular. Ornamentation was not observed in LM (Light Microscope) (Figure 9).

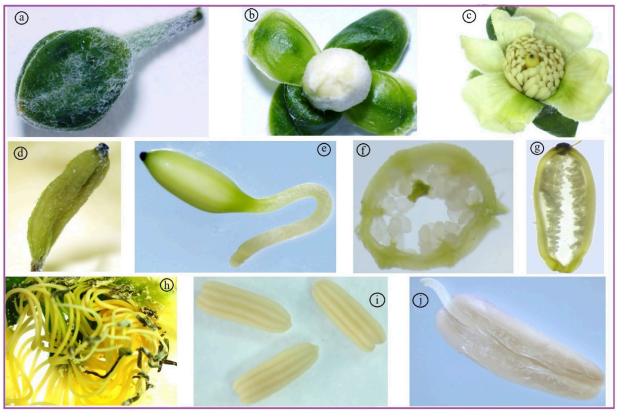

Figure 7. Flower morphology of *C. ovata* Desf. plants in Çorum Osmancık; a) flower bud, b) the flower bud with its sepals stripped off, c) the stamens surrounding the ovary in the bud, d and e) ovary, f) cross-section of an ovary, g) longitudinal section of an ovary, h) the scattered appearance of an ovarium and stamens, i-j) the unmatured anthers in Çorum Osmancık (Photo: Özlem Özbek)

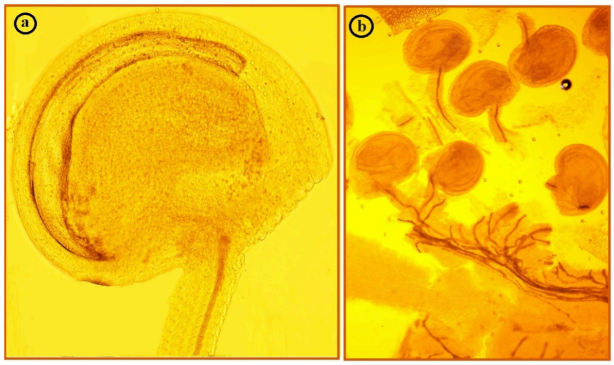

Figure 8. Morphology of the ovules in the ovary of the *C. ovata* Desf. plant in Çorum Osmancık; a) ovule, b) some ovules attached to the placenta and some was broken off (Photo: Özlem Özbek)

According to the data obtained as a result of morphometric measurements, mean, standard deviation and coefficient of variation values calculated (Table 2). PW (CV: 3.11-20.26%) and SW (CV: 3.17-20.57%) showed the highest variation range. The results showed that there is considerable variation in the morphological structures of flowers belonging to the *C. ovata* Desf. Considering the samples with high coefficient of variation, it was determined that the number of male and perfect flowers

was higher in plants that were close to each other. This is because the petal and sepal sizes in male flowers are larger than in perfect flowers, causing variation. On the other hand, variation is seen less in samples with a high number of male flowers. The fact that male flowers are larger and showy makes them appear more attractive to pollinators. This increases pollination and ensures fertilization. In addition, the pollens produced in male flowers are larger, and the vegetative cell quickly forms the pollen tube in the stylus, ensuring the completion of the fertilization process (Skogsmyr & Lankinen, 2002; Shakarishvili & Osishvili, 2013).

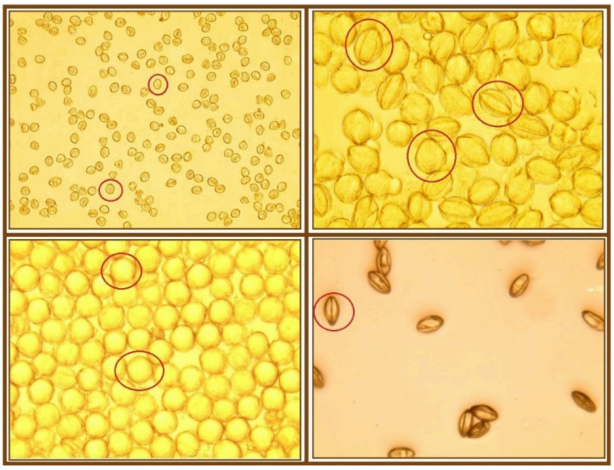

Figure 9. Images of the pollens produced in the anthers of the *C. ovata* Desf. plants in Çorum Osmancık (Photo: Özlem Özbek)

Although the formation of functional male or perfect flower sexual phenotypes on the same flower in plants is genetically determined, it may also depend on the availability of resources and other environmental conditions to ensure successful reproduction (Cao ve Kudo, 2008, Peruzzi ve diğerleri, 2012; Shakarishvili ve Osishvili, 2013). Andromonoecious, defined as the genetic mechanism responsible for the selective expression of male genes in the late stages of bud development in *C. herbacea* is not yet known. Song et al. (2012) revealed that the flower development in poplar is regulated epigenetically. Organogenesis studies have suggested that the development of a male or perfect flower in a fully developed bud may result from late epigenetic regulation of gene expression responsible for their formation (Shakarishvili ve Osishvili, 2013).

Caper plants are wild forms that grow naturally, local people collect the caper buds and make pickles, and the number of flowers in the plants that are damaged by herbivorous animals grazing is generally low. In fact, this is an important issue that could be risky for the future of natural plant populations. Personal studies and observations on capers in the study field have been continuing for about 15 years. The number of individual plants in the caper populations in the region decreased greatly in this period and the plants in the current population remained very weak and could not produce sufficient flowers and fruits. Local people unconsciously collect almost all of the plant buds, and the remaining ones cannot produce sufficient flowers, fruits and seeds due to environmental conditions and herbivores damage. In addition, a small-scale farm-like area was established in the region where there was a *C. ovata* population, and the owner of the farm stated that the caper plants were still growing even though they were removed from his yard. In order to preserve valuable genetic resources and genetic diversity, educating local people on the importance of surviving natural populations and preserving genetic diversity, and how to collect flower buds and fruits, in regions where naturally growing and economically valuable plants such as capers are grown, will make a significant contribution.

Table 2. Coefficients of variation (CV%) of morphological characters measured in the flowers of *C. ovata* Desf. plants.

PN	NFS	MF/PF	PW-CV (%)	PL-CV (%)	SW-CV (%)	SL-CV (%)	FPL-CV (%)	NS-CV (%)	STL-CV (%)	NMF-CV (%)	NPF-CV (%)
1	3	2/1	3.11	1.57	3.17	9.49	17.09	3.74	-	2	0
2	10	6/4	14.44	10.20	8.23	8.59	7.97	10.65	22.47	4	6
3	10	6/4	15.33	16.79	13.74	5.72	11.61	13.17	10.51	13	14
4	10	4/6	20.26	14.60	13.48	3.98	9.25	9.85	12.69	4	12
5	10	4/6	13.85	8.80	7.36	4.91	11.98	12.41	9.00	13	13
6	5	3/2	12.60	13.34	13.70	6.75	4.83	8.25	4.44	10	4
7	10	10/0	11.17	11.75	20.57	6.31	7.10	11.31	-	11	0
8	10	8/2	11.76	8.98	9.25	8.95	10.62	14.92	11.41	16	13

Abbreviations: Abbreviations: PN: Plant Number, NFS: Number of Flower Sample, MF/PF: Male Flower / Perfect Flower Number Ratio, PW: Petal Width, PL: Petal Length, SW: Sepal Width, SL

CONCLUSION

This study is the first attempt to reveal the andromonoecious reproductive system of *C. ovata* Desf from Türkiye. *C. ovata* Desf. has zygomorphic flowers, and hermaphrodites have hypogynous flower type

depending on the ovary position. It has campylotropous type ovules located on the parietal placenta in the ovary. The data obtained displayed that this species represents a variable sexual phenotype ratio (male flower/perfect flower = 1.72). Since the samples were collected from the natural environment, some environmental factors cannot be controlled; regular observations cannot be made on the plants throughout the flowering period from May to October. It was concluded that the studies on the reproductive biology of andromonoecious plants, especially in controlled environments such as botanical gardens, on more samples, would produce more efficient results about the reproductive biology of these species.

COMPLIANCE WITH ETHICAL STANDARDS

This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in JAEFS belongs to the author(s).

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared that for this research article, she has no actual, potential or perceived conflict of interest.

Author contribution

The author verified 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

Single authorship, therefore consent for publication is not applicable.

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RESEARCH ARTICLE

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Performance evaluation of alternate wetting and drying irrigation for rice cultivation

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Abstract

An experiment was conducted during the 2009-2010 Boro season at the Shahjalal Science and Technology University campus, Sylhet, Bangladesh, to investigate the impact of alternate wetting and drying irrigation (AWDI) on rice production. Using a randomized complete block design (RCBD), four irrigation treatments were applied to a modern variety (MV) of rice (BRRIdhan 28). One control treatment, T_0, maintained a continuous standing water depth of 1-5 cm. Water was irrigated to three AWD treatments, T_1, T_2, and T_3 when the water level dropped 10, 20, and 30 cm below ground level, respectively. In spite of the fact that treatment T0 produced the highest grain yield (4.90 t/ha), its water use efficiency was 38.64 kg/ha/cm. Compared to treatment T_1, which produced 4.68 t/ha, treatment T_1 had a water use efficiency of 41.86 kg/ha/cm. Treatments T_2 and T_3 yielded 3.96 t/ha and 3.63 t/ha, respectively. When water levels fall below 10 cm below ground level, treatment T_1 may be the best option for rice cultivation in conditions of limited water availability.

Keywords: Alternate wetting and drying, Rice, Water use efficiency, Water save, Yield

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INTRODUCTION

More than half of the world's population consumes rice as a staple food. Rice is primarily grown in irrigated fields globally. Despite this, water is becoming a more and more scarce resource. Asia's available water per capita is expected to decline by 15 to 54 percent by 2025 (Guerra et al. 1998). As urban and industrial sectors compete for water, agriculture's share of available water will decline even further. It is important to develop and implement irrigation schemes that utilize water efficiently in order to meet the needs of urban and industrial areas. As freshwater resources become increasingly scarce, irrigated agriculture will have to produce more food with less water. According to estimates, rice is the world's most irrigated crop. It is possible to save water by intermittently drying rice fields rather than continuously flooding them during irrigated rice cultivation. The water is irrigated alternately by wetting and drying (AWD). During the rice growing stage, fields are not constantly submerged but are periodically allowed to dry. While rice production has steadily increased over the years, the cost of production has not decreased in that manner, but the higher production cost has resulted in higher yields. Due to the fact that irrigation costs approximately 25% of production costs, the AWD method may be an effective means of reducing these costs. Due to the efficient use of irrigation water, higher losses of water from the field can be easily eliminated, resulting in reduced production costs. Rice is known as a water-intensive crop, and conventional continuous flooding of fields consumes a substantial amount of water. AWD reduces water usage by allowing the fields

to dry intermittently, promoting more efficient water utilization. By periodically drying the fields, farmers can significantly reduce the total water requirement for rice cultivation, helping to conserve water resources, especially in regions with water scarcity or competition for water resources. Pumping and maintaining continuous flooding require energy, typically in the form of electricity or fossil fuels. AWD reduces the energy input required for irrigation, resulting in cost savings for farmers and reducing greenhouse gas emissions associated with energy use. Continuous flooding can lead to soil problems such as reduced soil aeration, increased soil compaction, and reduced microbial activity. AWD allows the soil to dry out periodically, promoting better aeration and soil health. Enhanced soil health can lead to improved nutrient uptake by rice plants, potentially reducing the need for synthetic fertilizers and minimizing nutrient runoff into water bodies. Periodic drying of fields disrupts the life cycles of certain rice pests and weeds that thrive in submerged conditions. AWD can be used as an integrated pest management strategy, reducing the reliance on pesticides and herbicides. It also helps reduce the habitat for certain disease vectors, such as snails, which can transmit diseases like schistosomiasis. Continuous flooding of rice fields can lead to the anaerobic conditions that promote the production of methane, a potent greenhouse gas. AWD reduces methane emissions because it allows the fields to periodically dry out and aerate, inhibiting methane-producing microbes. Properly managed AWD can maintain or even increase rice yields while potentially improving grain quality. This depends on factors such as soil type, climate, and the specific rice varieties used. Enhanced grain quality can lead to higher market prices and better economic returns for farmers. AWD can help farmers adapt to changing weather patterns, including extended dry periods and irregular rainfall, which are becoming more common due to climate change. It provides a more flexible approach to rice cultivation that can better withstand erratic weather conditions. In some regions, governments and international organizations provide incentives and support for adopting AWD as part of sustainable agriculture initiatives. These programs encourage farmers to switch to more water-efficient practices. In summary, the rationale for adopting Alternate Wetting and Drying (AWD) irrigation for rice cultivation is based on its potential to conserve water, save energy, improve soil health, manage pests and diseases, reduce methane emissions, enhance yield and grain quality, adapt to climate change, and align with sustainable agriculture practices. However, the successful implementation of AWD requires careful management and monitoring to ensure that it is adapted to local conditions and effectively benefits both farmers and the environment.

MATERIALS AND METHOD

Experimental design, soil analysis, water management, crop growth monitoring, water table monitoring, yield measurement, environmental data collection, statistical analysis, economic analysis, field trials, replication, data interpretation, and etc are executed.

Experiment site

The research was conducted on the campus of Shahjalal Science and Technology University, Sylhet, Bangladesh. It was located between 24°46′ to 25°02′ North and 91°42′ to 92°00′ East.

Soil characteristics

Based on an analysis of the soil's physical properties, the soil in the experimental field was silty loam and grey. The parameters of soil fertility are presented in Table 1.

Climatic Data

Some weather data have been collected from Sylhet meteorological station for the experimental site during the experiment. Table 2 shows these data.

Field experimental design

In the field, a RCBD was used with 4 blocks and 4 irrigation treatments. Each block contained four experimental plots and represented a replication. In these plots, four irrigation treatments were randomly assigned. In total, 16 experimental plots were used.

Land preparation

The experimental field was prepared by a power tiller and a ladder. It was then fragmented into 4 major blocks. Each block was then divided into 4 experimental plots. The plots were surrounded by 25 cm wide and 20 cm high levees and separated by 1.0 m transition zones. A 1.5 m buffer zone was maintained between the blocks. The buffer zones were created to prevent seepage between adjoining plots. The dimension of an experimental plot was1.5m X 1.5m.

Soil water depletion measurement

In this experiment, some pieces of polyvinyl chloride (PVC) pipe were used to measure soil water depletion in the field. The diameter of the PVC pipes was 7.5 cm. The pipes were perforated to intake water from the saturated soil zone. The length of some of the pipes was 30 cm and that of the others was 40 cm. The 30 cm long pipes were installed in the treatments of T_1 and T_2 where the water level fell 10 and 20 cm below the ground level. Pipes 40 cm in length were installed in the treatment of T_3 where the water level fell 30 cm below the ground level. The pipe was installed in the field keeping 10 cm above the soil to check floating debris getting inside the pipe. After irrigation had been applied, water entered the pipe through small perforations and the water level inside the pipe was at the same level as that of outside.

As time went by, the water in the soil got depleted and at some moment the standing water above the ground level disappeared. But a close observation revealed that there was water inthe soil and that level was indicated by the water level inside the pipe. Thus, irrigation water was applied when the depleting water table inside the pipe reached a certain level.

Irrigation treatments

The experiment had four irrigation treatments. The treatments were:

- T_0 = 1-5 cm standing water, maintained throughout the growing season
- T_1 = Application of irrigation when the water level in the pipe fell 10 cm below the ground level
- T_2 = Application of irrigation when the water level in the pipe fell 20 cm below the ground level
- T_3 = Application of irrigation when the water level in the pipe fell 30 cm below the ground levelIn each irrigation, 5 cm ofwater was applied.

Seedlings

The specimen selected for this experiment was BRRIdhan 28. For this study, seedlings grown elsewhere were collected (Table 3).

Transplantation

The 35-day-old seedlings were collected and transplanted into the plots on the same day. Table 4 presents transplant details.

Fertilizer application

In the experimental plots, standard recommended fertilizer doses were applied. Table 5 shows the fertilizer dosesapplied to experimental plots.

Irrigation requirement Determination of effective rainfall

Effective rainfall is available in a plant's root zone, allowing it to germinate or grow. In its most basic form, effective rainfall refers to rain that is useful or usable. This study estimated effective rainfall using the United States Department of Agriculture (USDA) Soil Conservation

$$P\ effective = \frac{P\ total(125 - 0.2 \times P\ total)}{125}$$

Method (Smith, 1992). The equation is as follows:

For P total < 250 mm, and

P effective = 125 + 0.1 × P total

For P total > 250 mm

Where, P effective = effective rainfall, mmP total = total rainfall, mm

Crop water requirements estimation (WR)

Rice water requirements were calculated by adding applied irrigation water, effective rainfall during the growing season, and land preparation water (Rashid, 1997).

Harvesting activities

Each plot was harvested for the BRRIdhan 28, and 5 sample hills were chosen at random and harvested separately, Separately, the sample hills were investigated, threshed, and packed. Crops were harvested within a 1 m square (1m X 1m) plot of land to obtain yield and yield contributing parameters.

Determination of moisture content

The moisture content of the sample was determined using a moisture reader machine which was collected from the office of the Deputy Director of Department of Agriculture Extension (DAE), Sylhet.

Grain yield and straw yield

The grains were sun dried to lower the moisture content to 14 percent (weight basis) for the subsequent measurements. Similarly, the straw yield was also calculated by taking the weight of the sun-dried straw.

Table 1. Soil properties of the experimental site.

PH	Organic Matter (%)	N (%)	P (micro gram/ gm)	K (milli tullanko/ 100gm)	S (micro gram/ gm)	Zn (micro gram/gm)	Soil Texture
4.8	2.59	0.15	2	0.19	15	0.56	Silty loam

Table 2. Monthly weather data of the study area during the experimental period.

Month	Rainfall (cm)		Air temperature (°C)			Relative humidity (%)
	Total rainfall(mm)	No. ofrainydays	Maximum	Minimum	Average	
January	-	-	27.30	12.40	19.85	62
February	0.5	2	29.50	14.90	22.20	46
March	221.5	9	33.10	20.60	26.85	51
April	733.1	24	30.50	21.60	26.05	73

Table 3. Details of the seedlings collected for the experiment.

Variety	Supplying entity	Height of	Age of seedlings
BRRIdhan 28	Seedbed of Agricultural Training Institute of Sylhet	25 cm	35 days

Table 4. Information related to transplantation of seedlings.

Age of seedlings (days)	35
Hill to Hill distance (cm)	15
Row to Row distance (cm)	25
Number of seedlings per hill (nos.)	3

Table 5. Fertilizer doses kg/ha as applied to the experimental plots.

Before Transplantation		After Transplantation		
Fertilizer	Dose	Fertilizer	Days after	Dose
TSP	130	Urea	15	100
Gypsum	50			
Zink sulfate	9.52		30	180

Collection of data on yield and yield contributing parameters

Data on the following yield and yield contributing parameters were taken before threshing the grains from the plant.

- Plant height (cm)
- Number of effective tillers per hill
- Length of the panicle (cm)
- Total no. of spikelets per panical
- No. filled and unfilled grain per panicle
- The yield of unfilled grain (t/ha)
- Grain yield (t/ha)
- Straw yield (t/ha) and
- Harvest index (%)

RESULTS AND DISCUSSION

Irrigation treatments

During the first 15 days after transplantation, 5 cm of standing water was maintained in all plots to avoid weed infestation (crop establishment). Crop establishment required 27.2 cm of water. The plots were irrigated according to irrigation treatments. Treatment T_0 was considered the control and the plots under this treatment were irrigated when the surface water disappeared. Plots under the AWD treatments (T_1, T_2, and T_3) were watered when the water level in the perforated pipes dropped to specified depths below the ground surface. The depletion of water level in the perforated pipes measured from the ground surface indicated the time of water application. Table 6, shows that the maximum number of irrigations (9 nos.) was given to plots under treatment T_0 (continuous flooding). Plots under treatments T_1, T_2, and T_3 received 6, 5, and 4 irrigations. Irrigation amounts for T_0, T_1, T_2, and T_3 were 72.2, 57.2, 52.2, and 47.2 cm, respectively. One irrigation means applying 5 cm of water.

Water use efficiency

The highest water use efficiency (WUE) was found at 81.79 kg/ha/cm and was obtained in treatment T_1 and the lowest was 67.85 kg/ha/cm in treatment T_0 (Table 7). The results showed that the WUE increased in general in the AWD treatments compared to the control. However, as the water level depleted more than 10 cm below the ground surface, the WUE dropped. It could be noted that the grain yield reduced with the reduction of irrigation frequency. Thus, in situations of water scarcity, a suitable water management practice, such as AWD, could be chosen that would increase the water use efficiency at some sacrifice of crop yield.

Effect of irrigation treatments on water saving

Table 8 presents data on reductions in grain yield and the corresponding water savings in treatments.

Effect of irrigation treatments on plant height

The effect of different irrigation treatments on plant height was analyzed statistically. According to the analysis, the effect on plant height was statistically significant at a 1 percent level of probability. Statistically, there was no significant difference in plant height between treatments T_0 and T_1. It was not statistically significant to distinguish T_2 from T_3. But the plant heights in both T_0 and T_1 are significantly different from those of T_2 and T_3. Among the treatments, treatment T_0 (continuous submergence) achieved the highest average plant height (86.3 cm), while treatment T_3 (irrigation after 30 cm depletion of water below ground level) produced the lowest average (77.8 cm). The study found that increased water stress resulted in a significant decrease in plant height, while longer water stress affected plant growth and development in Table 9.

Effect of Irrigation treatments on the number of effective tillers per hill

Table 9, shows that the effect of irrigation treatments on several effective tillers was significant. The average highest number of effective tillers of 7.5 per hill was found in treatment T_0 and the average number consistently decreased in treatments T_1 (6.75), T_2 (6.25), and T_3 (5.25) in Table 9.

Effect of Irrigation treatments on panicle length

The experimental results showed that there was no effect of the treatments on panicle length in Table 9.

Table 6. Statement of water application to different irrigation treatments.

Treatment	No. of Irrigation	Water for land preparation (cm)	Water for crop establishment (cm)	Effective rainfall (cm)	The total amount of irrigation (cm)
T_0	9	20	27.2	34.6	126.8
T_1	6	20	27.2	34.6	111.8
T_2	5	20	27.2	34.6	106.8
T_3	4	20	27.2	34.6	101.8

Table 7. Water use efficiency for different treatments.

Treatment	Grain yield(t/ha)	Total water required(cm)	Water use efficiency(kg/ha/cm)
T_0	4.90	126.8	38.64
T_1	4.680	111.8	41.86
T_2	3.955	106.8	37.08
T_3	3.632	101.8	35.66

Table 8. Reduction of grain yield (%) and water saving (%) for different treatments.

Treatments	Grain yield (t/ha)	Percent of the highest yield	Grain yield reduction compared to control (%)	Total water required (cm)	Water saving compared to control (%)
T_0	4.90	100	00	126.8	To
T_1	4.68	95.51	4.49	111.8	11.83
T_2	3.96	84.51	15.49	106.8	15.77
T_3	3.63	77.61	22.39	101.8	19.72

Table 9. Statistical analysis of yield and yield contributing characters.

Treatments	Plant height (cm)	No. of effective tillers/ hill	Panicle length (cm)	No. of Spikelets/ panicle	No. of filled grains/ panicle	No. of unfilled spikelets/ panicle	1000 grain weight (g)	Grain yield (t/ha)	Straw yield (t/ha)	Biological yield (t/ha)	HI %
T_0	86.3a	7.5a	21.6a	164.5a	158.8a	5.75b	22.38a	4.90a	7.1a	12.0a	40.5a
T_1	84.8a	6.75b	21.5a	157.0ab	152.3a	4.75c	21.02a	4.68a	6.7b	11.40ab	41.48a
T_2	80.2b	6.25b	21.7a	151.0b	147.3a	3.75d	20.83a	3.95b	6.1c	10..05bc	38.92a
T_3	77.8b	5.25c	21.7a	140.8c	133.3b	7.50a	19.05a	3.62c	6.0c	9.62c	37.63a
level of significance	**	**	NS	**	**	**	NS	**	**	**	NS
CV (%)	2.5	5.34	2.8	3.5	5.3	10.73	7.33	2.34	3.4	5.86	5.36
LSD	3.3	0.55	1.0	8.6	12.4	0.93	2.52	0.23	0.4	1.12	3.11

Effect of irrigation treatments on the number of spikelets per panicle

The number of spikelets per panicle in the AWD treatments decreased from that of the control (T_0) at a 1 per cent level of significance (Table 9). The maximum average number of spikelets (164.5) came in treatment T_0 and the minimum average (140.8) in T_3 (Table 9).

Effect of irrigation treatments on the number of filled grains per panicle

The AWD treatments showed a consistent decrease in filled grains per panicle. However, in this parameter, only T_3

was significantly different from those of the control (T_0) and the other two AWD treatments (T_1 and T_2) in Table 9.

Effect of irrigation treatments on 1000-grain weight

Treatment T_0 (continuous submersion) had the highest 1000-grain average weight (22.38g), followed by treatments T_1 (21.02g), T_2 (20.83g), and T_3 (19.05g). Table 9, indicate that there is no statistically significant difference in weights between the treatments.

Effect of irrigation treatments on grain yield

The highest average grain yield (4.90 t/ha) was obtained in the control treatment T_0 (continuous submergence). The yield consistently decreased in the AWD treatments (T_1, T_2, and T_3).

Statistical analysis showed that the yields in T_0 (control) and T_1 (irrigation after 10 cm depletion of water level) were not significantly different. However, the yield obtained in T_1, T_2, and T_3 were significantly different from one another in Table 9.

Effect of irrigation treatments on straw yield

At a 1 percent probability level, straw yields were significantly different in different irrigation treatments. T_2 and T_3 do not show any statistically significant differences. Table 9, shows that the highest yield was obtained for treatment T_0 (7.2 tons/ha), followed by treatment T_1 (6.6 tons/ha), treatment T_2 (6.2 tons/ha), and treatment T_3 (6.00 tons/ha).

Effect of water stress on harvest index (HI)

The experiment showed that different levels of irrigation did not have any significant effect onthe harvest index. The highest value of harvest index (41.48%) was found for the treatment T_1 which was statistically similar to those obtained in treatments T_0 (40.5%), T_2 (38.92%), and T_3 (37.63%) in Table 9.

CONCLUSION

The alternate wetting and drying irrigation treatments significantly affected the rice yield and some other yield- contributing parameters. The results revealed that though the highest grain yield (4.90 t/ha) was found in the treatment T_0, its water use efficiency was 38.64 kg/ha/cm. Treatment T_1, on the contrary, gave a yield of 4.68 t/ha which was very close to the highest one obtained in T_0; produced the highest water efficiency of 41.86 kg/ha/cm. Both the yieldsand WUEs in treatments T_2 and T_3 were lower than the corresponding values obtained in T_1. So, the treatment T_1 appears to produce the best output. The study revealed that increasing water stress significantly reduced the plant height, number of effective tillers per hill, number of total tillers per hill, grain yield, straw yield, and biological yield. So, where water is scarce, practicing treatment T_1, when the water level goes 10 cm below the ground level would be the best choice for rice cultivation in silty loam soil.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the author to the present study is equal. The author read and approved the final manuscript.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

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RESEARCH ARTICLE

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The effect of zinc application on growth and alleviating shoot concentration of cadmium in durum wheat plant growth under conditions of salt and cadmium stress

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Abstract

A study was conducted in a greenhouse to investigate the effect of the combination of cadmium (Cd) and salinity (NaCl) stress in zinc (Zn) deficiency on growth, Cd accumulation in durum wheat (*Triticum turgidum* L. durum, cv. Balcali-2000), and micro (Zn, Fe, Cu, Mn) minerals differing in salt tolerance. The negative effects of Cd and NaCl stress on plant growth and Cd accumulation detected to alleviate Cd uptake on wheat growth increasing zinc application. The results revealed that Cd, NaCl and their combined stresses reduced shoots dry matter and Cd concentration in shoots increased compared to control pots. In increasing Cd and NaCl treatments, increasing Zn application significantly decreased the Cd concentration in shoot. In particularly, the decrease in Cd concentration was more noticeable with the improvement of Zn nutrition of plants at low doses of NaCl and Cd. The effect of increasing zinc treatments on reducing Cd accumulation decreased to slightly at high doses of Cd and NaCl. According to the results it can be suggested that Zn application to soils with low Cd content and medium salinity can be reduce Cd uptake by durum wheat.

Keywords: NaCl stressed, Cd reduced, Micro nutrient, Cd accumulation

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INTRODUCTION

Heavy metal contamination of agricultural soils has developed into an important problem for the environment. In addition to the natural decomposition heavy metals are also entering soils from anthropogenic sources due to increased human activities. Therefore, heavy metal pollution of agricultural soils creates a risk to crop production globally. (Rizvan et al., 2016). Among heavy metals, Cd is the most toxic and can be to humans, animals, and plants even at very low concentrations. Cd levels in soils are typically around 0.1 mg kg^{-1}, and the acceptable Cd content in agricultural soils is 3 mg kg^{-1}. (Alloway, 1995). It has been reported that the average Cd concentration of the world's agricultural soils is 0.53 mg kg^{-1} and Cd concentration in soils ranges from a minimum of 0.01 to a maximum of 2.7 mg kg^{-1} (Kabata-Pendias and Pendias 1992; Szolnoki, et al., 2013). Although Cd concentrations in soils are low, high Cd accumulation can be observed in cereals such as wheat, maize and rice, which play an important role in human nutrition. Toxic effects of Cd are observed in humans who regularly consume plants containing more than 3 mg kg^{-1} Cd in their tissues (Alloway, 1995). More than half of the world's population receives their basic food needs from wheat (Triticum aestivum L.), the third-most significant cereal crop in the world after rice and maize (FAO, 2012). Cereal crops can easily uptake Cd from the soil and compared to other cereals, wheat can easily uptake Cd through its roots and accumulate high concentrations in its grains even when Cd is at very

low concentrations in the soil (Jafarnejadi et al., 2011). Cd accumulated in wheat grain enters humans through the food chain and causes serious health problems (Järup et al., 1998; Du et al., 2020). Therefore, FAO/WHO (2010) reported that the maximum permissible limit of Cd in wheat grain is 0.1 mg kg^{-1}. The capacity of wheats to accumulate Cd in grain is different and it has been explained that durum wheat varieties accumulate higher Cd compared to bread wheat varieties (Gao et al.,2011; Naeem et al., 2016). In recent years, various strategies to minimize Cd risks to human health and to reduce Cd bioaccumulation have been investigated. For this, it is very important to know the factors that increase or decrease Cd transport in wheat. Cd addition to the soil solution and bioavailability depend on factors such as Cd content of the soil, different metals in the soil, cation exchange capacity (CEC), pH and soil salinity (Gallego et al., 2012). In various studies, it has been explained that soil salinity increases Cd uptake by plants (Norvell et al., 2000; Özkutlu et al., 2007). One of the most significant abiotic stresses influencing crop yield is salinity. Salinity lowers nutrient uptake and accumulation and reduces the plant's ability to absorb nutrients, according to a number of studies. (Essa, 2002; Fernández-García et al., 2004; Aydemir et al., 2023). Additionally, salinity causes a number of problems for plant growth, such as nutrient deficiencies and diseases. (Santos et al., 2002). The effect of Cd stress on nutrient uptake and distribution is related with the way it affects plant growth. There have been reports of Cd stress affecting the uptake of minerals including Fe, Zn, Cu, and Mn in crops as wheat. (Zhang et al., 2002) and barley (Wu and Zhang, 2002; Wu et al., 2003). It is widely recognized that NaCl and Cd stress together can promote Cd uptake and accumulation in plants grown in Cd-contaminated soils (Smolders et al., 1998; Ghallab and Usman, 2007; Özkutlu et al., 2007; Ondrasek, 2013; Özkutlu, 2020). Zn is an essential element in the nutrition of plants, animals, and humans while Cd, which enter soils from a variety of sources and is a significant environmental pollution, is not. Zinc (Zn) is crucial for plant and human metabolism, in addition to being a cofactor for more than 300 enzymes, including DNA and RNA polymerases. One of the sustainable strategies to reduce Cd accumulation in plants is to increase the supply of beneficial nutrients to plants (Hussain et al., 2019; Wu et al., 2019). As many researchers have shown that due to the chemically similar properties of Zn and Zn application can efficiently reduce plant Cd uptake is a good alternative (Özkutlu and Erdem, 2018; Khan et al., 2019; Rizwan et al., 2017; Wu et al., 2019). Although salinity increases Cd uptake by plants, Cd uptake may decrease with Zn application. In this research, Cd and Zn, Fe, Mn, Cu accumulation durum wheat were investigated grown under different Cd, Zn and NaCl treatments.

MATERIALS AND METHODS

Pot soil preparation

For studying, a Zn deficient soil of Central Anatolian taken from wheat field in Eskişehir-Sultanönü region origin was used in the experiment. The soil was sieved using a 4 mm sieve after initially being air dried. Table 1 shows some of the soil's chemical and physical properties.

Table 1. Physicochemical Characteristics of the Soil Used in the Experiment

Soil properties	Measurue	Methods
Sand, %	8.6	Bouyoucous, 1952
Silt, %	30.8	
Clay, %	60.6	
Texture	Clay (C)	
pH (1/2.5)	8.08	Jackson, 1959
Organic matter (%)	0.70	Jackson, 1959
$CaCO_3$ (%)	14.2	Scheibler Calcimeter
DTPA-Zn (mg kg^{-1})	0.1 0	Lindsay and Norvell, 1978
DTPA-Cd (mg kg^{-1})	0.005	Lindsay and Norvell, 1978
Total Zn (mg kg^{-1})	51	Schlichting and Blume, 1966
Total Cd (mg kg^{-1})	0.27	Schlichting and Blume, 1966
EC (mmhos/cm)	0.22	U.S. Salinity Loboratory Staff, 1954

Plant growth conditions and cadmium, salt and zinc treatments

Pots in experiment were filled with 1.65 kg of soil. Before sowing the seeds, the basic fertilizers and application doses were added to each pot as solutions and mixed thoroughly and homogeneously mixed into the soil. Basal fertilizers and Cadmium (Cd), Salt (NaCl), Zinc (Zn) doses applications: 200 mg N in the form of $Ca(NO_3)_2$ $4H_2O$; 100 mg P and 125 mg K in the form of KH_2PO_4 and as the application doses of the experiment; increasing Cd doses (0, 0.2 and 1.0 mg Cd) in the form of $(CdSO_4)_3.8H_20$; increasing NaCl doses (0, 250, 2500 mg NaCl) and increasing Zn (0, 0.05, 0.5, 5.0 mg Zn) in the form of $ZnSO_4.7H_2O$. The pots in the greenhouse were completely random during the experiment's random plots trial design, which included four pot replicates for each treatment. In plastic pots, durum wheat seeds (*Triticum turgidum* L. durum, cv. Balcali-2000) were planted. In each pot, six plants were grown. The pots were watered everyday with deionized water during the experiment. Plants were grown until differences occurred of shoot, and at harvested on the 30th day. After determining the dry weight of each plant's shoots, the concentrations of Cd, Zn, Fe, Mn, and Cu in the shoots were determined using inductively coupled argon plasma optical emission spectrometry (Jobin-Yvon, JY138-Ultrace), which was used to digest the shoot samples in 65% (w/w) nitric acid using a closed microwave system (Milestone, 1200-Mega).

Statistical analysis

All data used the means among treatments were compared with excell . Results were given in the form of mean±std.

RESULTS

Shoot Dry Matter Weights (DW)

This research was carried out to determine the effect of increasing Zn doses on the decrease in Cd uptake of durum (Balcali-2000) wheat under Cd and NaCl stress. Since the soil used in the experiment was a Zn-deficient soil, increasing Zn doses applied to the soil increased the shoot dry matter yield of the plant at different doses of both NaCl and Cd, as expected (Table 2). For example, when NaCl 0 and Cd (1.0 mg kg^{-1}) were applied, the shoot dry matter yield was at the level of 122 mg $plant^{-1}$, but with increasing Zn application, this value increased to 124, 155 and 171 mg $plant^{-1}$, respectively (Table 2). On the other hand, with increasing amounts of NaCl (0, 0.025% and 0.25% NaCl) doses application and 1.0 mg kg^{-1} of Cd, the dry matter yield of the plants decreased from 122 mg $plant^{-1}$ to 106 and 89 mg $plant^{-1}$ as 37%. These results show that dry matter yield decreases significantly with increasing NaCl stress in Cd 1.0 mg kg^{-1} contaminated soil. In the results, by applying high 5.0 mg Zn kg^{-1} dose had increased by 37% compared to the control an improving effect on shoot dry matter yield in the plant growing under 1.0 mg Cd kg^{-1} and NaCl 0.25% stress (Table 2).

Shoot Cd and Zn, Fe, Mn, Cu Concentration

In each NaCl treatment, increasing Zn application had a decreasing effect on Cd concentration in shoot. Under Zn0 and NaCl0 conditions, when 5 mg Zn kg^{-1} application, the average Cd concentration was 19.1 mg kg^{-1} in the application of 1.0 mg Cd kg^{-1}, while it decreased to 15.9 mg kg^{-1} when 5 mg Zn kg^{-1} application, control Cd concentration decreased by 20% from 19.1 mg kg^{-1} to 15.9 mg kg^{-1} (Table 3). Especially, at low doses of NaCl and Cd, shoot Cd concentration decreased more significantly with improved Zn nutrition of the plants. For example, under at the nil NaCl and Zn0 conditions, when Cd was applied at 0.2 mg kg^{-1}, the Cd concentration of the control plant was 3.8 mg kg^{-1}, whereas this value decreased by 52% to 2.5 mg kg^{-1} with 5.0 mg Zn kg^{-1} application. But, in the case of 1 mg Cd kg^{-1} and 2.5% NaCl applications at the highest doses, this reduced in shoot Cd concentration was not found (Table 3). These results showed that Zn had no significant effect on the reduction of Cd uptake at high Cd dose and high saline conditions.

Zn, Fe, Mn, Cu concentrations of shoot are given in Table 4. It was found that there were differences in Zn, Fe, Mn, Cu concentrations of durum wheat under Cd and NaCl stress. Zn concentration decreased with the increase in Cd concentration shoot. The antagonistic interaction between Cd and Zn is what causes this result.

DISCUSSION

Due to the fact that it is not an essential component of living things, cadmium is extremely hazardous to plants and animals even at very low doses. The primary means by which Cd enters humans through the food chain is through contaminated food. For wheat grain intended for human consumption, the FAO/World Health Organization (FAO, 2007) has established a concentration limit of 0.1 mg Cd kg^{-1}. Therefore, research has focused on a strategy to reduce Cd uptake and accumulation in wheat. The Cd concentration, pH, organic matter content, salinity and Zn concentration in the soil affect how much Cd the roots can take up from the soil. These distinctive characteristics of the soil not only influence the soil's chemical availability by limiting cadmium uptake in the roots or by enhancing the plant with nutrients, cadmium accumulation and toxicity in wheat can be decreased or increased. Salinity can alter Cd speciation, which can affect Cd translocation in plants. For instance, studies by Norvell et al. 2000, Özkutlu et al. (2007) and Lo'pez-Chuken et al. (2010) demonstrated examples of the relationship between elevated Cd content in food crops (wheat, maize, and sunflower) and chloride salinity in soils. According to the results of our research, the applications of Cd or NaCl, and their combination, significantly affected to decline on the plants growth. At the highest doses of Cd and NaCl %0.25 treatments, the Zn deficiency-induced reduction in shoot growth became more pronounced. Increasing Cd and low doses of NaCl 0.025% with increasing Zn applications were found to improve shoot growth. wheat plants can readily uptake Cd from the soil through their roots (Black et al., 2014). Cadmium uptake varies among wheat species and Cd uptake varies among wheat cultivars. Research has shown that, both durum wheat and bread wheat can accumulate cadmium (Cd) in their grains, but durum wheat tends to accumulate more Cd than bread wheat (Hart et al., 1998; Vergine, M., 2017). The accumulation and distribution of Cd in durum wheat plants can differ depending on the expression of genes involved in Cd uptake, and translocation from root to shoot. Another study investigated into the relationship between durum wheat's tolerance for Cd contamination and how resistant it is to NaCl salt. The results showed that the degree of salinity resistance was positively correlated with Cd accumulation in the grain (Pastuszak et al., 2020). In our research, at increasing doses of Cd and NaCl treatments in Zn deficiency, durum wheat growth decreased and Cd uptake increased. In this case, soil chloride and chelate-extractable soil cadmium can be associated with Cd uptake in durum wheat. Dahlin et al. (2016) found that chloride can mobilize Cd in soil, increasing its uptake by wheat. Our research results showed that with increasing salinity, Cd concentrations in wheat can increase, and Zn concentrations can decrease. This

Table 2. Shoot Dry Weights (DW) of Durum Wheat (*Triticum turgidum* L. *durum*, cv. Balcali-2000) Grown Under Greenhouse Condition (mg bitki^{-1}).

Treatments		Shoot Dry Matter Weight (DW) Cd Treatments, mg kg^{-1}		
NaCl	Zn	0	0.2	1.0
0%	0.00	124±8	119±36	122±8
	0.05	136±4	142±3.3	124±31
	0.50	179±15	164±33	155±25
	5.00	191±13	179±6.3	171±17
	mean	158	151	143
0.025%	0.00	121±26	124±18	106±12
	0.05	124±5	140±4	116±1
	0.50	167±9	156±13	137±14
	5.00	197±19	171±7	148±11
	mean	152	148	127
0.25%	0.00	115±6	119±8	89±9
	0.05	125±13	121±8	101±11
	0.50	127±7	123±4	134±4
	5.00	122±22	129±3	122±15
	mean	122	123	111

Table 3. Shoot Cd Concentration of Durum Wheat (*Triticum turgidum L. durum*, cv. Balcali-2000)) Grown Under Greenhouse Conditions.

Treatments		Shoot Cd Concentration, mg kg^{-1} Cd treatments, mg kg^{-1}		
NaCl	Zn, mg kg^{-1}	0	0.2	1.0
0%	0.00	0.25±0.12	3.8±0.8	19.1±1.1
	0.05	0.28±0.06	3.8±0.5	20.2±1.2
	0.50	0.20±0.01	3.6±0.0	18.4±0.7
	5.00	0.14±0.02	2.5±0.5	15.9±1.8
	mean	0.22	3.4	18.4
0.025%	0.00	0.31±0.03	4.5±0.6	21.5±3.5
	0.05	0.29±0.06	5.4±0.4	22.2±1.6
	0.50	0.21±0.03	4.8±0.4	23.2±1.5
	5.00	0.17±0.05	4.0±1.6	19.5±1.2
	mean	0.24	4.7	21.6
0.25%	0.00	0.34±0.02	6.3±1.0	26.2±2.9
	0.05	0.32±0.14	7.9±2.2	27.2±0.8
	0.50	0.36±0.19	6.2±0.5	24.8±3.3
	5.00	0.20±0.16	5.6±0.2	26.6±2.5
	mean	0.30	6.5	26.2

Table4. Shoot Zn, Fe, Mn and Cu Concentration of Durum Wheat (*Triticum turgidum* L. *durum*, cv. Balcali-2000) Grown Under Greenhouse Condition (mg kg^{-1}).

Treatments		Zn			Fe			Mn			Cu		
		Cd treatments, mg kg^{-1}											
NaCl	Zn,mg kg^{-1}	0	0.2	1.0	0	0.2	1.0	0	0.2	1.0	0	0.2	1.0
0%	0.00	8.5	6.5	6.1	60	68	70	121	111	115	6.9	6.4	7.2
	0.05	8.8	6.6	6.2	63	71	69	115	120	109	7.4	6.8	6.8
	0.50	20	19	19	62	69	66	127	133	127	7.9	7.4	7.8
	5.00	72	68	63	66	66	61	108	109	111	8.7	7.7	7.4
	mean	27.3	25.0	23.6	63	69	67	118	118	116	7.7	7.1	7.3
0.025%	0.00	7.6	5.80	7.1	64	63	66	132	114	111	6.8	6.7	6.8
	0.05	7.1	6.00	7.7	60	62	61	127	124	117	7.2	6.5	5.9
	0.50	17	19.00	17	59	66	58	124	126	114	7.5	6.4	6.2
	5.00	75	75.00	72	61	72	64	126	133	122	7.9	6.2	6.6
	mean	26.7	26.5	26.0	61	66	62	127	124	116	7.4	6.5	6.4
0.25%	0.00	7.5	7.5	7.6	55	58	64	117	114	124	8.4	6.1	5.7
	0.05	8.2	8.2	7.7	58	55	66	124	125	125	9.4	5.9	5.8
	0.50	19	19	18	64	54	68	127	114	127	9.7	6	6.4
	5.00	84	77	68	69	53	62	133	136	134	9.5	6.2	5.3
	mean	29.7	27.9	25.3	62	55	65	125	122	128	9.3	6.1	5.8

effect zinc a micronutrient for plants that competes with Cd for binding sites on root surfaces and in the soil due to its physical and chemical similarities. In accordance with several research (Hart et al., 2005; Liu et al., 2007; Zhao et al., 2011; Erdem et al., 2012; Li and Zhou, 2012; Singh and Shivay, 2013), Zn treatment decreased wheat's Cd concentration. Due to their physical and chemical similarities, zinc and cadmium may compete for binding sites on the root surfaces of plants as well as in the soil. In agreement with several research (Liu et al., 2007, Zhao et al., 2011, Erdem et al., 2012, Singh and Shivay, 2013, and Özkutlu and Kara., 2018), Zn application reduced the concentration of Cd in wheat. Increased Cd and NaCl and their combined stresses had no significant effect on the concentrations of micronutrients Cu, Fe and Mn.

CONCLUSION

The shoot growth of durum wheat plant were negatively affected by increasing of Cd and NaCl. Cadmium accumulation in shoots of durum increased with increasing soil Cd concentrations. Increased Cd and NaCl combined stress in Zn deficiency, Cd concentration in shoot increased more with the effect of salt. In increasing NaCl applications, increasing Zn application had a decreasing effect on Cd concentration in shoot. At low doses of NaCl and Cd, Cd concentration of shoot decreased more significantly with improved Zn nutrition of the durum wheat. But it was determined that Zn had no significant effect on Cd uptake at high Cd dose and under high saline conditions. As a result, Cd concentration in wheat can be reduced by improving Zn nutrition of plants in low saline soils.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

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.

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Ethics committee approval is not required.

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RESEARCH ARTICLE

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Herbal food supplements usage awareness of university students: Example of Echinacea and St. John's Wort

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Abstract

In recent years, usage of food supplements (Fs) has increased in order to maintain healthy living, have well-being, and be protected from the diseases. There are many medicinal plants used as herbal food supplements (HFs). Within the scope of this study, Echinacea and St. John's Wort were selected among the plants that are frequently encountered. This descriptive, cross-sectional study was conducted through completing a self-administered online questionnaire by health students. The questionnaire was filled out by 211 students studying at the faculties of Medicine, Dentistry, Pharmacy and Health Sciences at Istanbul Medipol University. The greatest participation was achieved with Pharmacy students (47.4%), whereas the lowest participation was reached with Dentistry students (5.7%). The most commonly used products as Fs were vitamin D (21.3%) and multivitamins (16.1%), while the use of HFs was 8.5%. St. John's Wort is commonly preferred for wound and burn treatment and Echinacea is used to boost immunity. In parallel with this use, St. John's Wort is preferred as olive oil maceration and Echinacea as herbal infusion. While the use of HFs was 58.3%, that of the Fs were 44.5%. Echinacea use was found to be 14.4% and St. John's Wort was 31.3%. The relationship between the presence of chronic disease and the use of Fs or HFs was not statistically significant. This study is significant to detect the opinions and knowledge levels of health students about Fs, especially HFs, St. John's Wort and Echinacea, which are available in the market.

Keywords: Herbal food supplements, Food supplements, Pharmacy students, Echinacea, St. John's Wort

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INTRODUCTION

The food supplements (Fs) are studied in a wide range of aspects in terms of quality and resource. The plant-based products, the beneficial substances such as vitamins, minerals, fatty acids and the probiotics are examples of product types on the market (Lam et al., 2022). Especially the plants or the plant-based products are often included in the food supplement products (Bardia et al., 2007; Thakkar et al., 2020). In recent years, the use of Fs has increased in order to maintain healthy living, have well-being, and be protected from the diseases (Arslan et al., 2021). The reason behind the increase in the use of Fs is having fewer regulatory requirements for safety and efficacy compared to drugs and the Fs becoming more common (Bardia et al., 2007). Marketing claims such as providing overall health, improving physical and cognitive performance, boosting energy, losing excess weight, and reducing pain are included in the marketing strategies of the food supplements (Knapik et al., 2021). Although food supplements are subjected to different regulations in many countries, the interest and demand for them are increasing day by day (Biagi et al., 2016; Welz et al., 2019; Xiong et

al., 2021).

In Türkiye, Fs are sold over the counter with the approval of The Republic of Türkiye Ministry of Agriculture and Forestry. As of August 2023, more than 18000 approved supplementary foods are sold in Türkiye (Gıda Güvenliği Bilgi Sistemi-Food Safety Information System, n.d.) In the Ministry's "List of Restricted Substances Used in Supplementary Foods", the minimum and maximum daily doses of more than 250 components from fish oil to Vitamin B1 and Vitamin B12, which are limited to be used in the supplementary foods for children aged 4-10 years and adult individuals are provided (Gıda Güvenliği Bilgi Sistemi-Food Safety Information System, n.d.). Although there is no full consistency in the legal status of the food supplements across the EU countries, a regulatory framework has been established by the "Food Supplements Directive 2002/46/EC" (Food Supplements, n.d.). In the US., it is regulated at the federal level by the Food and Drug Administration (FDA), the Federal Trade Commission (FTC), and government agencies in all 50 states. The FDA has regulatory authority under the Federal Food, Drug, and Cosmetic Act. (Regulatory Information | FDA, n.d.).

The increasing interest in Fs might be attributed to various reasons such as self-medication due to pandemic conditions, ease of access to information, increasing healthy living, protection from diseases and motivation to create a routine for exercise and nutrition (Erarslan & Kültür, 2021; Gardiner et al., 2007; Neuhouser et al., 1999). However, various risks exist such as food supplements interacting with prescription medicine used or not used in the appropriate dose (Khampang et al., 2022).

There are many medicinal plants used as herbal food supplements (HFs). Within the scope of this study, Echinacea and St. John's Wort were selected from the plants are frequently encountered. *Echinacea* is a genus of Asteraceae family. Principally *E. angustifolia, E. pallida* and *E. purpurea* have been used in traditional medicine for centuries to treat respiratory tract infections and inflammatory conditions, including the common cold, coughs, bronchitis, and inflammation of the mouth and pharynx, upper respiratory infections, and some additional inflammatory conditions (Catanzaro et al., 2018; Percival, 2000). *Hypericum* is a genus of Hypericaceae family. *Hypericum* species have been used in the traditional medicine for antidepressant, sedative, diuretic, antiphlogistic and analgesic purposes (Galeotti, 2017; Schepetkin et al., 2021). The well-known and the most studied species of the genus is *H. perforatum* (also known as St. John's Wort). *H. perforatum* is known for its antimicrobial, pain relief and antioxidant effects (Schepetkin et al., 2021; Yılmaz et al., 2019; Zhang et al., 2020).

E. purpurea and *H. perforatum* are two medicinal plants often used as HFs (Agbabiaka et al., 2017; Smith et al. 2022; Thakkar et al., 2020). However, interactions of these two species with drugs, especially through CYP 450 enzymes, are common (Freeman & Spelman, 2008; Mannel, 2004).

The aim of this study is to evaluate the use, attitude and awareness of HFs and other Fs among students studying in the faculties of Pharmacy, Medicine, Dentistry and Health Sciences based on *H. perforatum* and *E. purpurea* species. In addition, the participant's personal use and preferred dosage forms were also investigated.

MATERIALS AND METHODS

This descriptive, cross-sectional study was conducted by completing a self-administered online questionnaire during October 2019-April 2020 by Medicine, Pharmacy, Dentistry and Health Science (Nursing, Nutrition and Dietetics, Physical therapy and Rehabilitation, Healthcare Management) students. This group was selected to obtain an overview of the level HFs knowledge of students studying health.

With the 53-question questionnaire prepared, essential health, demographic information, lifestyle, drug and Fs use and information of the two selected plants were collected. The questionnaire includes yes/no, open-ended, numbered evaluation scale and multiple choice questions. The preparation and configuration of the questionnaire were in accordance with the existing literature on the subject with some modifications (Karelakis et al., 2020; Nakhal et al., 2020; Niva & Mäkelä, 2007). This study was approved by the Ethics Committee of Istanbul Medipol University. The survey was provided to 211 randomly selected participants.

Data analysis

The results and significance values were analyzed with Statistical Package for the Social Science (SPSS) version 22.0. The 53-question survey is prepared under five headings: 1) sociodemographic information such as gender, age, lifestyle (smoking/drinking alcohol); 2) general health status and usage of prescription or over-the-counter medications; 3) usage of food supplements; 4) usage of herbal food supplements and the level of knowledge; 5) yes/no, open ended, numbered evaluation scale, multiple choice questions that evaluate the level of knowledge in Echinacea and St. John's Wort. The data were analysed with Chi-square test. Differences between values at $P < 0.05$ levels were considered significant.

RESULTS AND DISCUSSION

The online questionnaires were filled out by 211 students studying at the faculties of Medicine, Dentistry, Pharmacy and Health Sciences at Istanbul Medipol University. The greatest participation was achieved with Pharmacy students (47.4%), whereas the lowest participation was reached with Dentistry students (5.7%) (Table 1).

Majority of the participants (71.5%) described their health status as good; 26.5% as moderate; 1.4% as bad

Table 1. Sociodemographic characteristics of the participants

Sociodemographic Characteristics (n=211)											
Gender	**n**	**%**	**Age**	**n**	**%**	**Faculty**	**n**	**%**	**Academic Year**	**n**	**%**
Female	192	91	19-20	26	12.3	Pharmacy	100	<u>47.4</u>	1st Year	16	7.6
			21-22	105	<u>49.8</u>	Nutrition and Dietetic	49	23.2	2nd Year	30	14.2
			23-24	68	32.2	Medicine	22	10.4	3rd Year	79	<u>37.4</u>
Male	19	9	25 and +	12	5.7	Dentistry	12	5.7	4th Year	50	23.7
						Other	28	13.3	5th Year	36	17.1

* The highest values are underlined.

and 0.48% as very bad. 17.5% of the participants reported having a chronic disease. 37.8% of the participants were seen by a doctor regularly. 44.5% of the participants indicated using food supplements. The most commonly used products as Fs were vitamin D (21.3%) and multivitamins (16.1%), while the use of HFs was found to be 8.5%. In the question of food supplement use, participants were able to select more than one option (Table 2). The most preferred places to obtain Fs were pharmacies. Among the reasons for preferring HFs, the first place was to use it for weight loss and the second place was to boost immunity. The herbal infusion was the most preferred form (43.7%). As a result of using the HFs, 86.9% of the participants reported positive effects. Of the two plants examined in this study, St. John's Wort was used for the treatment of wounds and burns, and Echinacea was preferred to boost immunity. In parallel with this use, St. John's Wort was preferred as olive oil maceration and Echinacea as herbal infusion.

Table 2. Food supplement preferences

Food supplement (n=211)	n	%
Vitamin D	45	21,3
Multivitamin	33	16,1
Fish oil/ Omega 3	31	15,6
Vitamin C	28	13,3
Vitamin B	21	10
Coenzyme Q10	19	2,8
Probiotic / Prebiotic	19	9
Magnesium	18	8,5
Iron	17	8,1
Herbal food supplement	17	8,5
Zinc	16	7,6

* Higher values are taken. More than one option can be selected.

Over-the-counter drug use was found to be 15.6%. It was stated that the participants received the majority of the over-the-counter drug recommendations from doctors (41.4%) and pharmacists (20.7%), which were followed by non-recommendation usage (17.2%), family-friends (10.3%) and their own research (10.3%). The most common reason for using over-the-counter drugs was "Failure to get the desired result from previously used prescription medicines" (61.5%).

While the use of HFs was 58.3%, that of the Fs were 44.5%. Echinacea use was found to be 14.4% and St. John's Wort was 31.3%. The most commonly used herbal food supplement was green tea, which was preferred for weight loss. The least used ones were Ginseng and Passiflora. When we investigate the participants who use Fs and have chronic diseases, we revealed that 37 of participants had chronic diseases, 19 people (44.5%) of them were given Fs and 22 people (58.3%) of them were using HFs. 50.7% of the smokers were taking Fs and 65.7% of them were on HFs. The relationship between the presence of chronic disease and the use of Fs or HFs was not statistically significant. In addition, no relationship has been detected between having a chronic disease and the use of St. John's Wort and Echinacea. The connection between smoking or drinking alcohol and using Fs or HFs was also not significant.

The participants were asked "How adequate do you find the sources of information given in the options below about the herbal food supplements?". The medical students replied that the doctors were more adequate, whereas the other students indicated that pharmacists were more competent. The least adequate information source was "tv-radio".

In the evaluation of the level of personal knowledge about the plants, the participants were requested to score between 0-5 (0: the least, 5: the most), about the purpose of use, preparation, dose, side effect, drug, plant and food interactions of the plant and the average score was found as 2.3. The highest mean score was the purpose of use (2.9) and the scores with lower means were drug, plant and food interactions (1.8).

In a study by Tuğut et al., 77.5% of the participants described their health status as "good" and 0.6% of them as "very bad" (Tuğut & Bekar, 2008). The studies conducted on university students from different countries revealed that chronic disease status was found to be Slovakia 26.1% (Klemenc-Ketis et al., 2011), USA 27% (Herts et al., 2014) and Serbia 16.5% (Gazibara et al., 2018).

The most preferred Fs in this study were vitamins/ multivitamins similar to previous studies (Dickinson et al., 2014; Knapik et al., 2021; Knudsen et al., 2002; Serdarevic et al., 2019). Although there are some differences, the most preferred Fs were found in products such as multivitamins and fish oil, which is in accordance with our study (Knapik et al., 2021; Knudsen et al., 2002; Nakhal et al., 2020).

In our study, those who had chronic diseases and used

HFs despite not using Fs were also identified. For this reason, the outlook on HFs might be considered as more positive compared to other Fs. Our findings are the first to demonstrate the relationship between chronic diseases and Fs use among university students in Türkiye. In general, there are limited studies on the use of food supplements, especially HFs, by university students, and the number of studies on students of health departments is very low (Axon et al., 2017; Bukic et al., 2018; Nakhal et al., 2020; Stanojević-Ristić et al., 2017).

Since the departments of the Faculty of Health Sciences and the Faculties of Pharmacy in Türkiye predominantly have female students, the results were not classified based on gender. Although there were differences between the groups, these differences were not statistically significant because the sample size was small. Use of Fs varies depending on many different factors such as age, gender and socioeconomic status. People use Fs as a form of self-medication (Knudsen et al., 2002).

In a previous study (n=6666), it was determined that 17% of the participants used HFs and 26% were smokers, 24% were regular alcohol users. 21% of smokers and 24% of those who consumed alcohol used HFs (Gardiner et al., 2007).

Echinacea one of the two plants included in our study, is one of the well-known immunomodulatory plants that has gained popularity in recent years (Kim & Calderón, 2022; Lam et al., 2022). St. John's Wort is known to interact with different groups of drugs (Chrubasik-Hausmann et al., 2019). The preferred form of the product changes the amount of the active compounds. Side effects and drug interactions occur according to the amount of the active compounds as well. Therefore, it should be ensured that people who use these types of products are informed about the side effects and drug interactions by health professionals.

It is thought that the differences in opinion among the students of medicine, pharmacy and other departments regarding the information sources of the HFs are due to the courses taken by the students and the course contents (Aina & Ojedokun, 2014; Shahwan & Al Abdin, 2018).

The National Health and Nutrition Examination Survey (NHANES) is a face to face interview survey held in the United States since the 1970s and handed to 5,000 people who monitor the health and nutrition status of the public. For years, regular Fs usage was monitored this way (Moore et al., 2020). Similar surveys can be carried out regularly in different countries.

In the evaluation of the level of knowledge about Echinacea and St. John's Wort (0: the least, 5: the most), the lowest score in St. John's Wort was found as "oil preparation" with "2", the highest score was found as "storage conditions" with "3.75", the lowest score in Echinacea was found as "side effects" with "1.62" and the highest score was found as "tea preparation" with "2.53" (Table 3-4). Given that the participants are in different classes and departments, their level of knowledge about plants might be less. The Pharmacy students were educated about the medicinal plants and Fs, whereas the Nutrition and Dietetics students took courses related to Fs. However, there were no related courses in other departments, which might explain their level of knowledge. In order to enhance the depth of knowledge, it would be advantageous to consider incorporating courses pertaining to the utilization of food supplements within various departments in the future.

Table 3. The level of knowledge on St. John's Wort

St. John's Wort		
N=71	**Mean**	**Standard Deviation**
Active compounds	2.69	1.7
Part of the plant used	2.76	1.7
Factors affecting quality	2.81	1.8
Drug and food interaction	2.29	1.8
Side effects	2.37	1.6
Tea preparation	2.86	1.7
Oil preparation	2.00	1.7
Conditions to be considered when using oil	3.62	1.8
Storage conditions	3.75	1.8

*The highest values are underlined.

Table 4. The level of knowledge on Echinaceae

Echinacea		
N=34	**Mean**	**Standard Deviation**
Active compounds	2.18	1.9
Part of the plant used	2.41	1.8
Factors affecting quality	2.03	1.9
Drug and food interaction	1.62	1.8
Side effects	1.97	1.6
Tea preparation	2.53	1.8

*The highest values are underlined.

The questionnaire used in this study was not validated. The data collection tool lacks validity and reliability. However, such research on food supplements, particularly herbal food supplements, is scarce. Use of Fs is a rising trend with the concept of healthy living and wellness. This descriptive study will be beneficial as it will provide information for future research.

CONCLUSION

Food supplements whether of plant base or not, are not an alternative to a prescribed medicine. It should be used as a supportive or complementary treatment. The approach of health professionals such as doctors, pharmacists and dietitians to these products is very important for the compliance of patients with treatment. For this reason, health professionals play an important role in ensuring that patients reach the right and reliable

product. This study is important in terms of determining the opinions and knowledge levels of health students about the Fs, especially the HFs, St. John's Wort and Echinacea, which are also available in the market. The increase in the knowledge levels of the students on medicinal plants and food supplements, especially the health students other than pharmacy students, will prevent the unconscious consumption of these products.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author claims that there is no conflict of interest.

Author contribution

The process of reviewing the study, preparing it as an article and submitting was completed by the author.

Ethics committee approval

Ethics committee approval was obtained from the NonInterventional Clinical Research Ethics Committee of Istanbul Medipol University (Decision No. 04/03/2020/227).

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Arginine: a useful treatment to delay enzymatic browning of fresh-cut pear and apple

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Abstract
This study examined the effect of arginine treatments on the prevention of enzymatic browning in fresh-cut apples and pears. For this, 0, 25, 50, 75, and 100 mM, and 0-, 50-, 100-, and 200-mM arginine solutions were prepared for apples and pears, respectively. Slices of both fruits were dipped in these solutions for 5 min and dried for 20 min. Then, they were packaged and stored at 5±1°C temperature and 80-90% relative humidity for 18 days for pears and 12 days for apples. Browning index, color values, weight losses, firmness of slices, and total soluble solids were examined at three-day intervals during storage. The results showed that arginine treatment retarded the enzymatic browning of both apple and pear slices. While arginine suppressed browning at increasing doses, especially at 200 mM in pears, it retarded browning in apple slices at all concentrations. In addition, the fact that the L values were higher than the control and water control applications showed that both apple and pear slices remained lighter in color. Hue values supported both the L and browning index results. Arginine treatment decreased the weight loss; however, it did not affect the firmness of the slices. In addition, arginine treatments did not have a significant effect on the total soluble solid content of apple and pear slices.
Keywords: Arginine, Browning, Fresh cut, Apple, Pear, Color

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INTRODUCTION

Fresh-cut products are fruit and vegetables whose shape physically changed, washed, peeled, cut, sliced, chopped, etc, and are 100% usable. The quality components of fresh-cut products are color, appearance, taste, flavor, texture, and nutritional value. Metabolic changes occurring in fresh-cut products and affecting the quality are the increase in ethylene production and respiration rate, discoloration, enzymatic browning, and microbial contamination. Although the nutritional quality and freshness of these fresh-cut products are high, the quality, especially color quality, is quickly reduced due to the removal of the protective layer during minimal processing. (Erbay and Demir, 2006; Kasım and Kasım, 2016). Browning of the cut surface is a crucial factor in reducing the quality of fresh-cut fruit and vegetables. The polyphenol oxidase, peroxidase, and other enzymes released from the cut cell unite in fruits and vegetables containing phenolic compounds with the oxygen molecule in the air, forming brown pigments called melanin. This reaction is called enzymatic browning, and different treatments can delay this. One of these applications is arginine treatment.

Arginine is an amino acid, which was found in previous studies that post-harvest arginine treatments improved tolerance to diseases, delayed enzymatic browning, reduced ethylene production, and therefore, lengthened storage life

and maintain the quality of fruit and vegetables (Wang et al., 2017; Babalar et al., 2018; Hasan et al., 2019; Shu et al., 2020). Arginine has recently studied for its inhibiting effect on enzymatic browning in fresh-cut fruits and vegetables. In a study, it was found that 50 ppm arginine treatment effectively delayed enzymatic browning symptoms in fresh-cut red cabbage compared with the control and 100 ppm (Nilprapruck, 2020). Also, in another study 50 mM arginine showed a similar effect in fresh-cut apples, whereas 100 mM was more successful in fresh-cut lettuce slices (Wills and Li, 2016). Furthermore, Prabasari et al. (2020), showed that L arginine treatments inhibited the synthesis of phenolics that caused browning in fresh-cut salacca.

However, there are almost no studies on the effect of arginine on the inhibition of browning in fresh-cut apples and pears. Therefore, this research aimed to investigate the effect of different doses of arginine on reducing browning in fresh-cut apple and pear slices.

MATERIALS AND METHODS

Plant material

The 'Starking' apple and 'Deveci' pear fruits, used in the experiment were bought from the Kocaeli Wholesaler Marketplace. The fruits are in the extra quality class with 60-65 mm diameter for apples and 60 mm for pears. The fruits were immediately transferred to the Postharvest Laboratory of the Agricultural Faculty, Horticulture Department.

Preparation of fruits for dipping treatments

The fruits were washed first for both surface disinfection and cooling. The washed fruits were cut with a sharp knife into eight slices without peeling the skin.

Arginine treatment and drying

Arginine solutions were prepared at 0, 25, 50, 75, and 100 mM, and 0, 50, 100, and 200 mM for apples and pears, respectively. Seventy-two pear or apple slices from each treatment group were dipped into a 3-liter solution for 5 min separately. Two controls were used in the study. In the dry control (C), slices of fruits did not dip into any solution, and in the second control (CW), fruit slices were dipped only in tap water. The arginine-treated fruit slices were left to dry on filter paper for 20 min to prevent decay during storage.

Packaging and storage conditions

Four slices of apples and pears in each treatment were packaged separately into a plastic box with a lid, 50 mm in height, 115 mm in width, and 125 mm in length. These packaged fruits were held in cold storage at 5±1°C temperature and 80-90% relative humidity for 18 days for pears and 12 days for apples.

Color measurements

The color measurement of fruit slices was conducted using a Minolta CR-400 chroma meter with a D65 lamp, on three points of each fruit slice in each replicate. The device was calibrated using a standard white calibration plate. Color values were measured as L*, a*, and b*. The color L* value in the chroma meter shows the brightness or whiteness of the fruit slices. In addition, the browning index (BI) and hue values were calculated from a* and b* values using the following formulas:

$$BI = [100\ (X\text{-}0.31)]/0.17, \text{ where } x=(a^*+1.75L^*)/(5.645L^*+a^*\text{-}0.3012b^*)$$

$$h^o = tan^{-1}\left(\frac{b^*}{a^*}\right) when\ a^* > 0\ and\ b^* > 0, or\ h^o = 180 + tan^{-1}\left(\frac{b^*}{a^*}\right) when\ a^* < 0\ and\ b^* > 0$$

Weight loss (%)

The three packages from each treatment group and each fruit were labeled and weighed each analysis period for determining weight loss of fresh-cut fruit slices. Next the weight loss was calculates as:

Weight loss (%) = ((Initial weight-the weight at each analysis period) x100)/ Initial weight.

Total soluble solids (TSS, %)

TSS from fruit juice was detected using an Atago DR-A1 digital refractometer (Atago Co. Ltd. Japan). Fruit juice from four fruit slices from each replicate was used for measurements. The TSS measurement was conducted in three replicate.

Fruit firmness (N)

Fruit firmness was measured from three fresh-cut fruit slices in each replicate using a Shimadzu EZ-LX texture analyzer.

Statistical analysis

The experiments were conducted using a completely randomized experimental design. The study was conducted with three replicates and five fruit slices in each replicate for both apple and pear. The data were processed using SPSS 16 software. The differences in treatments were compared using Duncan's multiple range tests, at $p<0.05$ error limit.

RESULTS AND DISCUSSION

Browning index

The browning index (BI) of pear slices was 9.79 at the beginning of the study and increased from the third day and ranged from 12.49 to 18.84 (Fig.1). However, all arginine treatments suppressed the increase in BI until the 12th day, and 200 mM arginine treatment continued this effect during 18 days of storage (Fig.2).

A similar situation was observed in apple slices, and all arginine treatments prevented an increase in BI during storage, particularly at increasing doses (Fig.3). Previous studies have found that arginine treatments delayed or inhibited enzymatic browning in fresh-cut fruit and vegetables (Wang et al., 2017; Shu et al., 2020). In

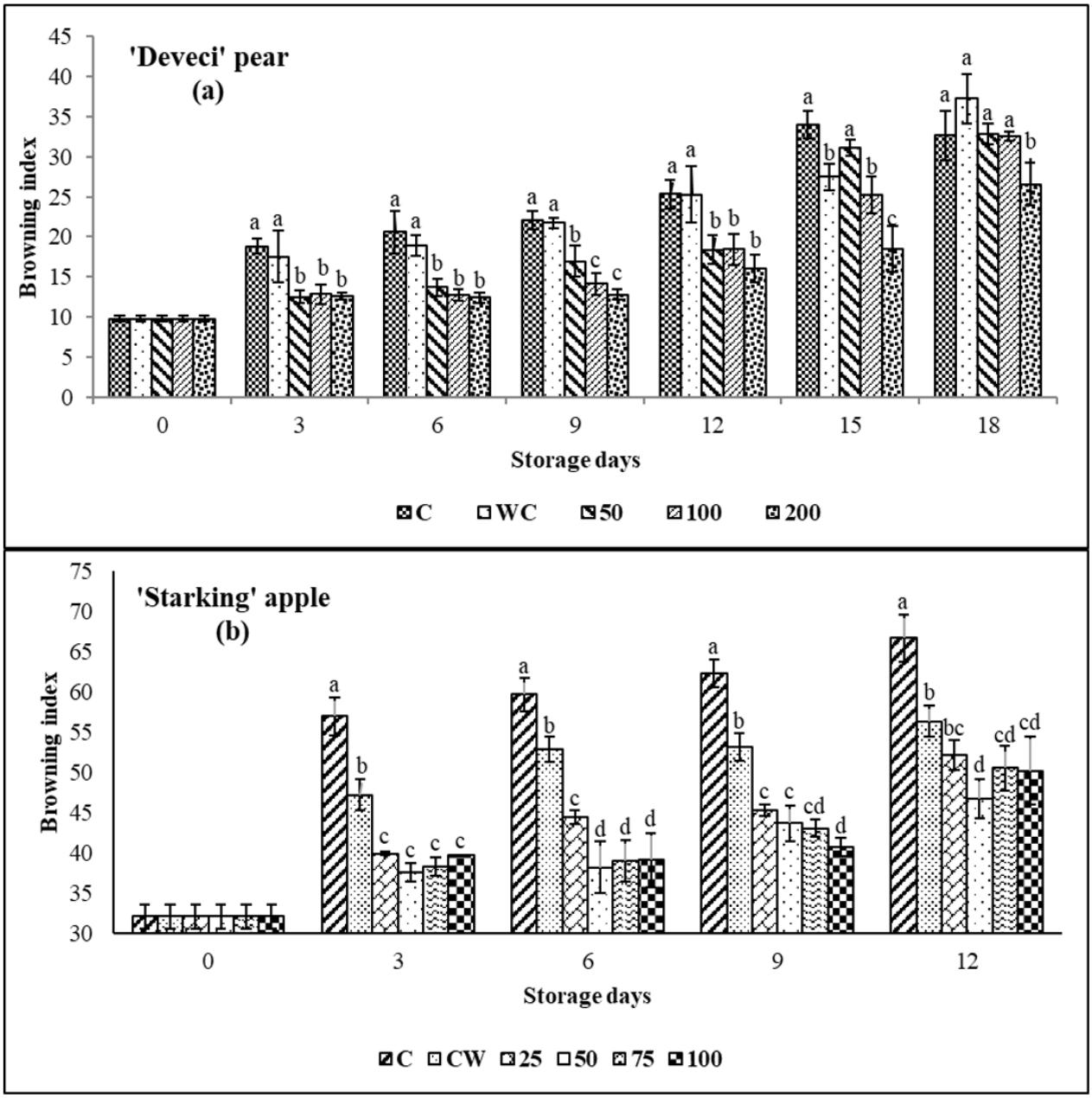

Figure 1. Browning values of fresh-cut pear (a) and apple (b) slices during storage. The letters above the bar represent differences among the treatments at $p<0.05$.

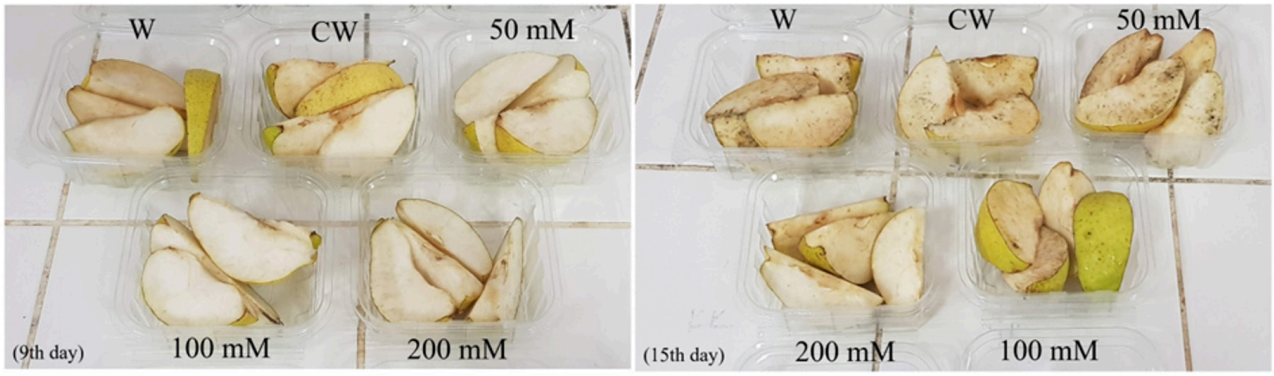

Figure 2. Appearance of pear slices after the ninth and fifteenth days of storage.

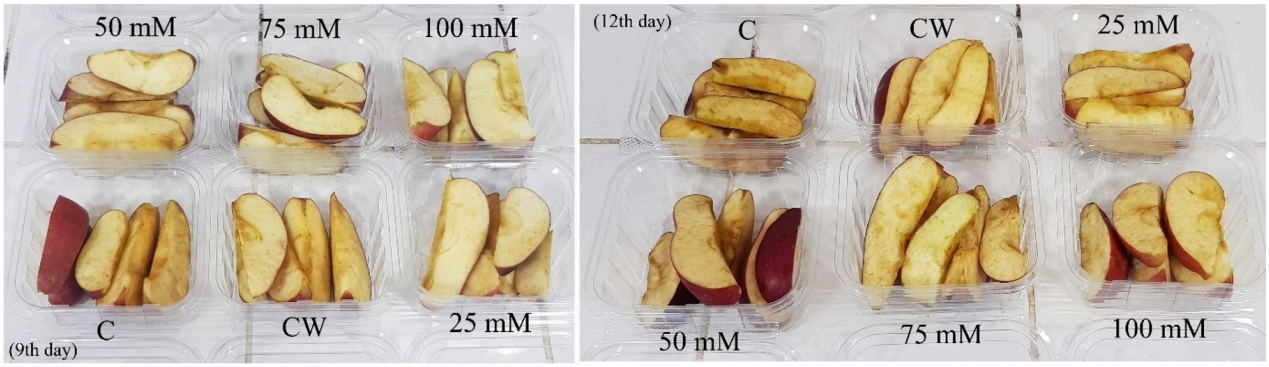

Figure 3. Fresh-cut apple slices after the ninth and twelfth days of storage

addition, Nilprapruck (2020) stated that 50 mM arginine treatment retarded enzymatic browning, and Wills and Li (2016) 50 ppm for fresh-cut apples and 100 ppm for fresh-cut lettuce successfully delayed enzymatic browning. All arginine treatments delayed an increase in BI, an indicator of enzymatic browning, particularly the highest dose as in previous studies. Furthermore, the L values of arginine-treated pear slices were higher than those of C and CW, and this has shown that the result arginine prevents an increase in browning.

L values

L values of fresh-cut pear and apple fruit were higher at all arginine treatments for 12 days in pears and nine days in apples compared with C and CW treatments. The L values of pear slices treated with 100- and 200-mM arginine remain high during the 18 days of storage (Fig 4). The results showed that the arginine treatments were quite efficient on L values or whiteness of fruit slices (Fig. 5). L values of fresh-cut fruit slices decreased on the third day in all treatments compared with initial levels. However, the L values of arginine-treated slices were higher than those of C and CW during storage. High L levels indicate that the color of slices is more bright or white, while low ones mean indicate darker or black. Besides, the browning index values of slices confirm these findings because the BI values of arginine-treated slices are lower than those of C and CW.

Hue values

The hue angle values of fresh-cut pear slices decreased from 94.29 to 89.05 and 89.23 in the C and CW treatments on day three, whereas it was close to or higher than the initial values in arginine treatments, and it changed to 94.16, 94.3, and 94.70 in 50 mM, 100 mM, and 200 mM arginine treatments, respectively. Moreover, the hue values of arginine-treated pear slices remained high compared with C and CW throughout storage. In other words, arginine applications ensured that the color of the pear slices was preserved without changing much compared with the initial color during storage (Fig. 6).

Weight losses

In the study, the lowest weight loss of pear slices was observed with CW and 50 mM arginine treatment, and the differences between these treatments and C were significant ($p<0.05$). The other arginine treatments, however, did not show a decreasing effect on weight loss (Table 1). Arginine treatments, except for 50 mM, slowed the weight loss increase in fresh-cut apples, but the differences among treatments were not found to be significant (Table 1). The fresh-cut process, i.e., the cutting of fruits or vegetables removes the protective layer above the cells; tissue becomes vulnerable to environmental conditions. This phenomenon leads to increased weight loss, due to increased respiration and transpiration during storage. Increased weight loss causes decreased freshness and loss of visual quality. Weight losses of pear slices increased with increasing storage duration, whereas these losses were low and ranged from 0.034% to 0.308% during storage. In addition, the weight loss increased during storage as expected, up to 0.318% at the end of storage, but this loss did not affect the appearance of the fresh-cut apples. Wang et al. (2023) stated that the weight loss of blueberry fruits increased during the 10-day storage period in control and arginine treatments. In the present study, in line with researchers, the weight loss of fruit slices of pear and apple in all treatment groups was increased during storage. In addition, the authors expressed the fruit WL was significantly lower in the 1-mM arginine-treated group than in the control group. Similarly, in the study, the weight loss of all arginine-treated pear slices was lower than in the control; the same trend was observed in apple slices except for the 50 mM treatment, in which the weight loss was the highest. Wang et al. (2017) reached the same findings in green asparagus spears to which they applied arginine.

Firmness of the slices

The firmness of the slices is an important quality criterion for consumer choices. The firmness of both pear and apple (Table 2) slices fluctuated during storage. The firmness of pear slices was the highest in C and apple slices at 50 mM. However, the arginine treatments did not cause significant differences in fruit firmness in pear or apple slices. Actually, arginine is a polyamine that is strongly bonded to pectin in the middle lamella

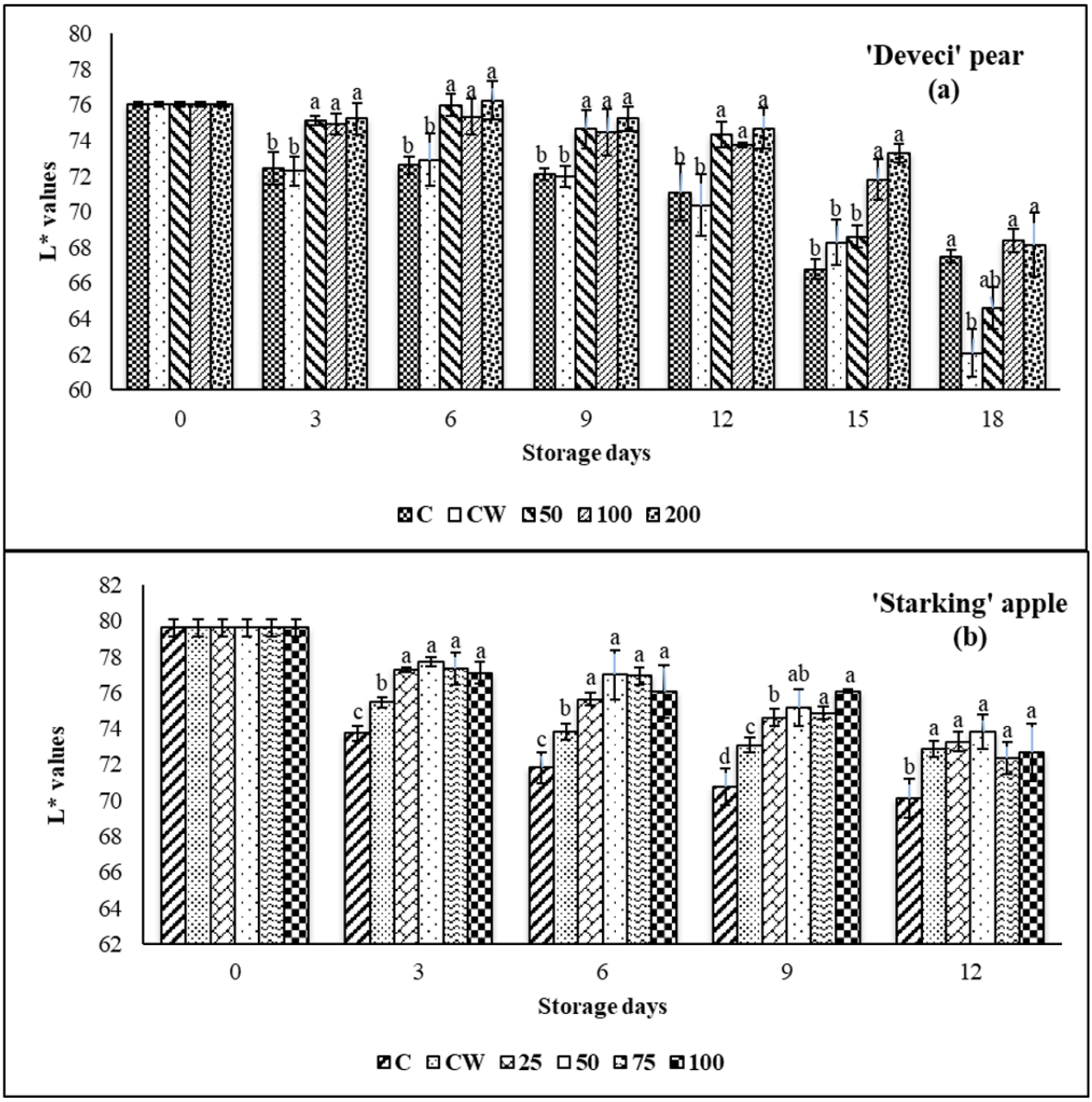

Figure 4. L values of fresh-cut pear (a) and apple (b) slices during storage. The letters above the bar represent differences among the treatments at $p<0.05$.

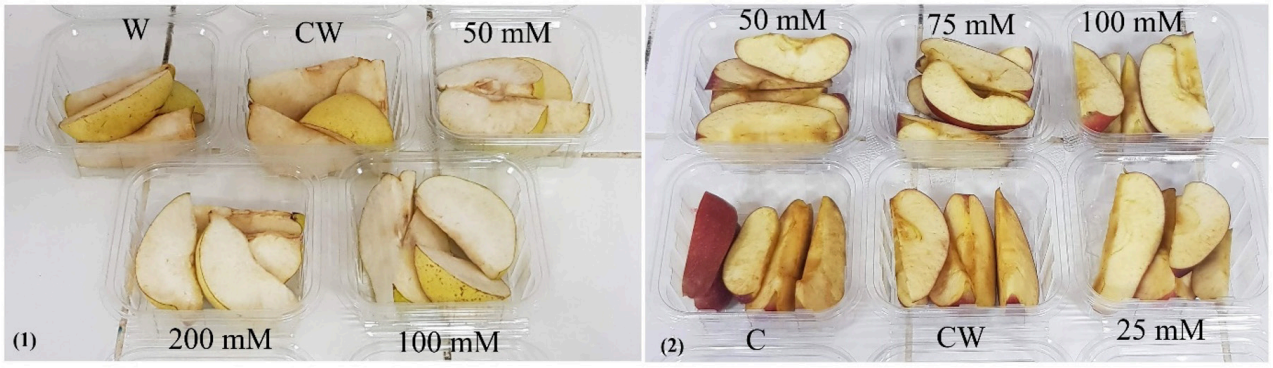

Figure 5. The appearance of fresh-cut and arginine-treated pear and apple slices. Pear slices on day 12 (1), and apple slices on day 9 of storage (2). C: Control, CW: Water control.

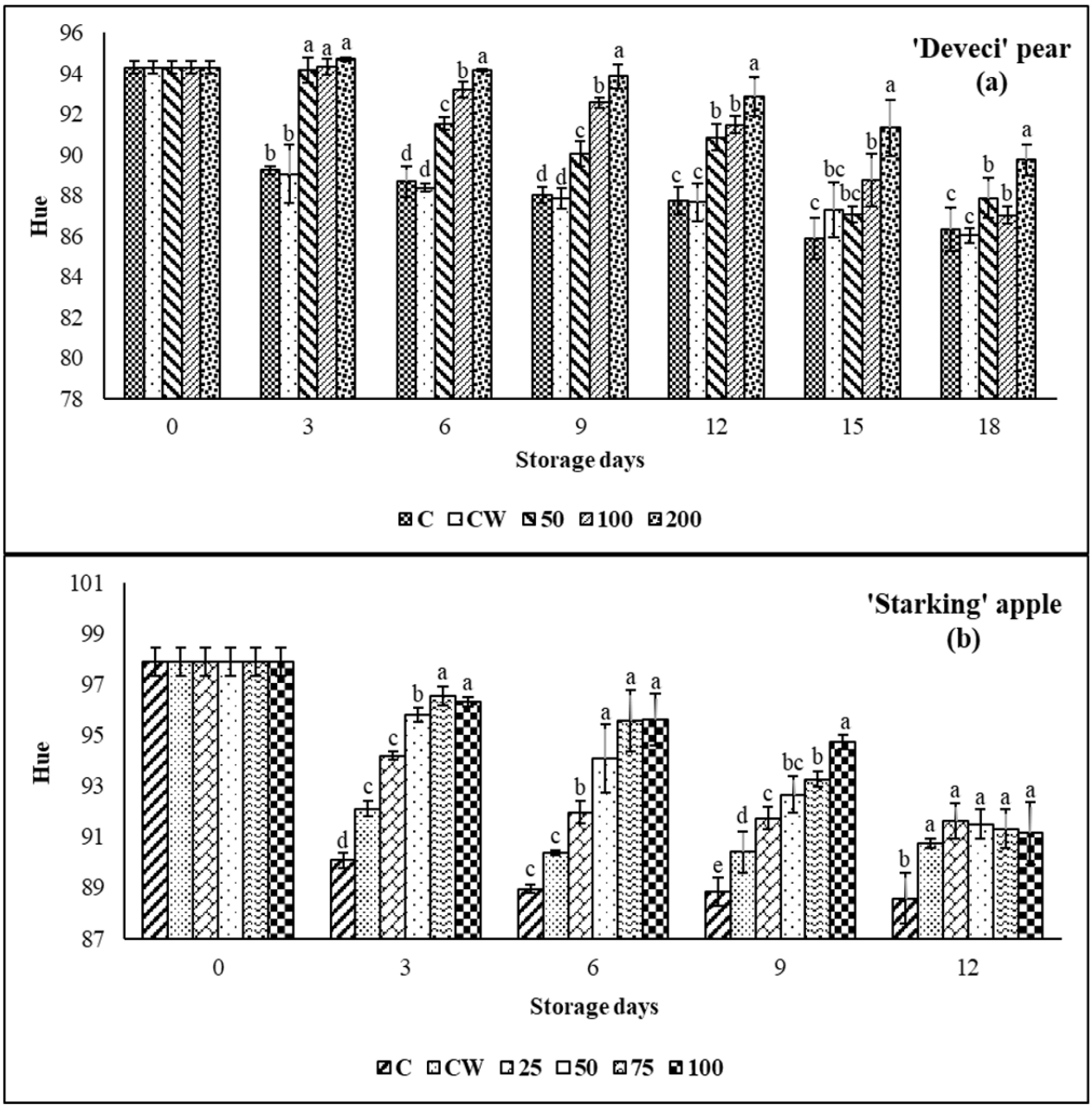

Figure 6. Hue values of fresh-cut pear (a) and apple (b) slices during storage. The letters above the bar represent differences among the treatments at $p<0.05$.

and increases the strength of the cell wall (Valero et al., 1998; Shan et al., 2007). Therefore, it is expected that the arginine treatments increased the firmness of fresh-cut pears and apples. The arginine treatment, however, did not increase the firmness of the slices. Wang et al. (2023) declared that the fruit firmness of both in 1 mM arginine-treated blueberry fruit and control groups decreased and remained at a lower level than the initial value during the whole storage period. On the other hand, Li et al. (2019) stated that treatment with a 10-mM L-arginine maintained the tissue firmness of mushrooms. In the present study, however, the fruit firmness of both arginine treatment and C and CW in pear and apple slices were higher compared with the initial value and at the end of the storage. In addition, the authors found that the fruit firmness of arginine-treated blueberry fruit was higher than in control. However, in this study, the arginine treatments were unsuccessful in maintaining firmness compared with C and CW.

Total soluble solids

The total soluble solids of pear slices were 14.6% initially but decreased in all treatments, however, it was lowest in 50 mM arginine treatment compared with the control, but the differences among the arginine treatments were insignificant (Table 3). Total soluble solids of apple slices increased in the C and 50 mM treatments, while decreased in the other treatments, 12th days of storage,

Table 1. Weight losses of 'Deveci' pear and 'Starking' apple during storage

Treatments	Weight loss (%), pears							Treatment avg.
	Days							
	0	3	6	9	12	15	18	
C	0	0.04±0.009	0.11±0.019	1.17±0.027	0.22±0.036	0.28±0.044	0.33±0.044	***0.164 a***
CW	0	0.03±0.002	0.10±0.004	0.15±0.008	0.20±0.008	0.25±0.009	0.29±0.009	***0.145 b***
50	0	0.03±0.008	0.09±0.010	0.14±0.013	0.19±0.023	0.26±0.032	0.30±0.032	***0.145 b***
100	0	0.04±0.010	0.10±0.003	0.15±0.004	0.21±0.004	0.27±0.002	0.31±0.002	***0.154 ab***
200	0	0.03±0.005	0.11±0.001	0.16±0.003	0.21±0.008	0.26±0.003	0.31±0.003	***0.154 ab***
Time avg.	***0 g****	***0.034.f***	***0.101 e***	***0.153 d***	***0.206 c***	***0.264 s***	***0.308 a***	
	Weight loss (%), apples							
	Days							
	0	3	6	9	12			
C	0	0.09±0.008	0.14±0.011	0.21±0.013	0.32±0.022			***0.152 ab***
CW	0	0.07±0.003	0.13±0.003	0.19±0.007	0.29±0.014			***0.136 b***
25	0	0.07±0.008	0.12±0.007	0.18±0.017	0.28±0.020			***0.128 b***
50	0	0.07±0.004	0.17±0.054	0.28±0.143	0.45±0.252			***0.193 a***
75	0	0.07±0.017	0.13±0.019	0.19±0.027	0.29±0.040			***0.134 b***
100	0	0.07±0.025	0.12±0.026	0.18±0.031	0.28±0.036			***0.132 b***
Time avg.	***0 e****	***0.074 d***	***0.133 c***	***0.204 b***	***0.318 a***			

*The interaction of treatment x time was insignificant at p<0,05 level.

Table 2. Fruit firmness of 'Deveci' pear and 'Starking' apple during storage

Treatments	Firmness of slices (N), pears							Treatment avg.
	Days							
	0	3	6	9	12	15	18	
C	69.57±9.15	79.49±5.88	77.32±1.12	75.50±5.41	78.28±8.89	75.00±8.24	80.71±3.76	***76.5****
CW	69.57±9.15	72.18±9.71	71.23±5.76	74.25±2.42	73.78±5.99	74.37±3.52	75.1±2.54	***72.93***
50	69.57±9.15	76.27±8.36	72.78±4.28	73.12±5.10	77.30±8.50	74.56±3.05	81.62±6.03	***75.03***
100	69.57±9.15	80.46±7.60	72.93±4.41	77.92±13.66	83.76±4.87	76.20±5.63	68.45±7.13	***75.61***
200	69.57±9.15	75.7±5.90	77.07±3.61	77.98±7.35	75.23±2.30	70.73±12.72	78.3±5.87	***74.9***
Time avg.	***69.57 b***	***76.82 a***	***74.27 ab***	***75.75 a***	***77.67 a***	***74.17 ab***	***76.84 a***	
	Firmness of slices (N), apples							
	Days							
	0	3	6	9	12			
C	31.25±1.55	38.00±2.85	41.92±4.15	34.39±6.32	36.12±3.37			***36.34****
CW	31.25±1.55	34.58±3.94	32.60±1.43	33.60±6.54	37.65±6.56			***33.94***
25	31.25±1.55	39.12±6.18	37.24±1.61	38.18±2.73	35.71±5.64			***36.30***
50	31.25±1.55	36.53±11.13	39.40±4.46	31.24±3.45	34.40±2.13			***34.56***
75	31.25±1.55	34.44±2.31	33.86±2.78	31.81±1.43	32.28±4.12			***32.73***
100	31.25±1.55	32.80±8.42	33.59±8.68	39.09±10.16	27.80±2,05			***32.90***
Time avg.	***31.25 a***	***35.91 b***	***36.43 a***	***34.72 a***	***33.99 ab***			

*The interaction of treatment x time was insignificant at p<0,05 level.

**Differences between treatments was non-significant.

compared with the initial. While the 50 mM treatment was prominent from this point, differences between this treatment and C and 25 mM were insignificant. In addition, it was observed that the CW and 75- and 100-mM arginine treatments have a reducing effect on total soluble solids compared with 50 mM. In the present study, the TSS of both pear and apple slices was lower than C at the first three days, but at six days, the TSS of apple slices treated with 50 mM arginine, and pear slices treated with 100 and 200 mM was higher compared with the other treatments. After that, 25- and 50 ppm arginine treatment increased the TSS of apple slices and the TSS of 100- and 200 ppm arginine-treated pear slices were higher than 50 ppm but lower in C and CW. Shu et al. (2020) showed that the TSS of arginine-treated strawberry fruits increased and then decreased. Besides, they found that the TSS of 1 mM arginine-treated fruits was significantly higher than that of control, 0.5 and 5 mM. In this study, the arginine doses used were higher than those used by Shu et al. (2020), and the TSS contents of 200 mM arginine-treated slices in pear and 50 mM in apple remained high.

Table 3. Total soluble solids of 'Deveci' pear and 'Starking' apple during storage

Treatments	Total soluble solids (%), pears							
	Days							Treatment avg.
	0	3	6	9	12	15	18	
C	14.60±0.40	14.53±0.67	14.20±0.62	14.57±1.10	14.80±0.89	14.67±1.01	14.53±1.29	***14.56 a****
CW	14.60±0.40	14.13±0.68	14.17±1.00	14.33±0.78	14.70±0.85	14.00±0.26	14.43±0.12	***14.34 ab***
50	14.60±0.40	14.17±0.49	13.97±0.60	13.73±1.08	13.53±0.47	13.13±0.64	13.93±0.57	***13.87 b***
100	14.60±0.40	14.13±0.49	14.87±0.80	14.37±0.40	14.40±0.72	13.33±1.00	14.03±0.38	***14.25 ab***
200	14.60±0.40	13.83±0.55	14.70±0.61	14.70±0.46	14.50±0.98	14.03±1.43	14.13±1.00	***14.36 ab***
Time avg.	14.60 a	***14.16 ab***	***14.38 ab***	***14.34 ab***	***14.39 ab***	***13.83 b***	***14.21 ab***	
	Total soluble solids (%), apples							
	Days							
	0	**3**	**6**	**9**	**12**			
C	13.40±0.35	13.80±0.26 **a****	12.80±0.60 **b**	13.47±0.75 **a**	13.60±0.85 **ab**			***13.41***
CW	13.40±0.35	12.60±0.66 **bc**	12.80±0.44 **b**	13.03±0.23 **ab**	12.43±0.15 **c**			***12.85***
25	13.40±0.35	13.60±0.61 **ab**	12.60±0.10 **b**	13.87±0.40 **a**	13.30±0.53 **abc**			***13.35***
50	13.40±0.35	13.60±0.36 **ab**	13.70±0.26 **a**	13.70±0.46 **a**	13.87±0.06 **a**			***13.65***
75	13.40±0.35	12.47±0.71 **c**	12.77±0.21 **b**	12.97±0.55 **ab**	12.63±0.21 **bc**			***12.85***
100	13.40±0.35	13.50±0.60 **abc**	12.90±0.50 **b**	12.37±0.47 **c**	12.63±0.75 **bc**			***12.96***
Time avg.	***13.4***	***13.26***	***12.93***	***13.23***	***13.08***			

*The interaction of treatment x time was insignificant at p<0,05 level. **The interaction of treatment x time was significant at p<0,05 level.

CONCLUSIONS

The effect of arginine treatment on the browning index and other quality parameters of fresh-cut pears and apples during storage were studied. The 200 mM arginine treatment was the most effective in retarding browning in pear slices, whereas all arginine concentrations delayed browning in apples. Therefore, we suggest that arginine treatments can delay/prevent enzymatic browning of fresh-cut pears and apples. Also, we found that the arginine treatments had a positive effect on weight loss but did not affect fruit firmness and total soluble solids.

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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Not applicable

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RESEARCH ARTICLE

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Research on the characteristics of model meat systems with emulsion gels including different legume flours

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Abstract

In this study, it was aimed to decrease the animal fat content of the meat products without changing the quality of the products. To this end, emulsion gels prepared with sunflower oil and legume (pea, lentil, bean and chickpea) flours were utilized in place of 50% and 75% animal fat in the model meat system. The moisture (%) of the control was 71.28, but in the treatments the values were between 72.84 and 74.27. The protein amounts of the samples containing emulsion gels were in the range of 69.30-72.28 g /100 g dw, whilst the amount of control was 65.63 g /100 g dw. According to these results the moisture and protein amounts of the samples containing emulsion gels were similar to each other ($p>0.05$), but higher than the control ($p<0.05$). The fat content lowered in the experimental samples as expected ($p<0.05$). The pH values of the samples were 6.27-6.41 and similar to control in most of the samples ($p>0.05$). No significant difference was determined among the color (L * and b *) values and the water holding capacity (WHC) of the samples. The texture values (hardness, binding, flexibility, chewiness) of the products were similar to the control ($p>0.05$). NMR studies showed that there were differences in T_2 relaxation times which is related to free moisture in the product ($p<0.05$). Morphological images of the treatments were observed by Scanning Electron Microscope (SEM). In general, substitution of animal fat with emulsion gels prepared with vegetable oil and legume flours at these amounts improved the nutritional properties of the products by increasing the protein amount and decreasing the fat content. Moreover, no undesirable effect was observed in the products such as water and oil leakage.

Keywords: Emulsion gel, Model meat system, Low fat meat product, Legume flour

INTRODUCTION

Recently, the relationship between nutrition, food and health has gained prominence. Remarkably, recent studies have focalized on daily fat intake, and its strong association with coronary heart diseases, obesity and some types of cancer (Phillips et al., 2012; Yang et al., 2017; Jiao et al., 2018; Bhupathi et al., 2020). The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) emphasized that 15-30% of total daily energy intake should be met with fats, and at most 10% of this should be saturated fats (WHO, 2003; FAO, 2010; Hooper et al., 2015). Moreover, a maximum of 300 mg daily cholesterol intake is recommended (Gray and Griffin, 2009). It is known that consuming more than the specified percentages of saturated fats and cholesterol increases the amount of LDL (Low-density lipoprotein), also known as "bad cholesterol", in the body. In addition, the relationship between high LDL levels and the saturated fat ratio increases coronary heart diseases

(Mensink et al., 2003; Joris and Mensink, 2016). These health concerns have influenced purchasing habits, consumer perception and created a substantial demand for products with reduced fat content. In this case, meat products with high nutritional value and protein sources pose a significant health risk due to their saturated fat content. This situation reveals the necessity of fat reduction studies in meat products as in various foods. Therefore, the enrichment of the unsaturated fatty acid profile by reducing saturated fat and cholesterol in emulsified meat products without changing their physical and chemical properties have been conducted in many studies (Pintado et al., 2016; Herrero et al., 2017; de Souza Paglarini et al., 2019). Using vegetable oil (hazelnut, soybean, sunflower, olive oil, etc.) as an animal fat replacer can be considered one strategy; however, due to its distinctive texture, mouthfeel, and flavor, the replacement of animal fat is very challenging. Also, using 30-40% saturated fats in emulsified meat products is essential to achieve the desired texture, rheological structure, technological and sensory properties. However, recent studies showed that integrating vegetable oils in the form of emulsion gel can reduce the animal fat amount without quality problems (Serdaroğlu and İpek, 2019). For instance, a study conducted on frankfurter type sausages showed that replacing animal fat with emulsion gels prepared by olive oil, fish oil, flaxseed oil, soy protein isolate, sodium caseinate, and microbial transglutaminase did not affect the shelf life and quality of the products adversely (Delgado-Pando et al., 2010). Moreover, in the study in which an oil/water emulsion gel prepared using flaxseed oil and carrageenan was used in salami, it was stated that the sensory properties did not change, and a product rich in unsaturated fatty acids was obtained (Poyato et al., 2014). Therefore, emulsion gels prepared with vegetable oils to enrich the product composition of mono and polyunsaturated fatty acids have gained popularity.

Legumes are among the most important food groups in the world due to their carbohydrate, protein, vitamin and mineral content. Legumes are low in fats and do not contain cholesterol. They are known as low glycemic index foods. With these features, it has very important benefits in the treatment of diabetes through nutrition and on the proper functioning of lipid and sugar metabolism. It has been observed that legumes are significantly therapeutic on diabetes, cardiovascular diseases, obesity, and some skeletal cancer types (Tharanathan and Mahadevamma 2003, Hera et al. 2012). Today, legume flour is used as a food ingredient due to its high protein content and functional properties (Singh et al. 2017, 2020). The performance of legume flour as a food ingredient is dependent on functional properties that contribute to the final product, such as foaming, emulsification, gelling, water and oil absorption capacities, and viscosity (Adebowale and Lawal 2004).

Model meat systems provide to make meat and meat products in a more convenient and economical way. In model meat systems, industrial meat products are imitated particularly using their basic ingredients in a laboratory scale. There are many studies on model meat systems in the literature such as Cofrades et al. (2013), Schmiele et al. (2015), Han and Bertram (2017), Câmara et al. (2020), Öztürk-Kerimoğlu et al. (2021). In our study, model meat products were prepared using the fundamental ingredients of a typical frankfurter type sausage.

It is quite difficult to produce meat products which vegetable oils are used instead of animal fat, because vegetable oils have a low melting point and lead to leakage. Also, replacing the animal based proteins with plant proteins probably cause to poor texture problems in the product. This study aimed to investigate the use of vegetable oil (sunflower oil) and legume flours (pea, lentil, navy bean and chickpea) in emulsion based product formulation. By that way, products with higher protein content and lower animal fat could be obtained without leading to any undesirable effect such as water/oil leakage or unacceptable appearance and texture.

MATERIALS AND METHODS

Materials

Ground beef and tail fat used in the research were obtained from Ankara Meat and Milk Board. Sunflower oil (Yudum) was purchased from a local supermarket. Gluten-free pea flour, gluten-containing bean flour, lentil flour (Değirmencibaşı, Smart Kimya, Türkiye) and gluten-free raw chickpea flour (Vegrano, Kimbiotek, Türkiye) were purchased from the market. Soy lecithin was purchased from Alfosol, Istanbul.

Preparation of emulsion gels

Emulsion gels were prepared according to the method described by Alejandre et al (2016) with some modifications. In the preliminary trials, emulsions were formed at 10%, 15%, 20% (Sunflower oil) oil ratios by using 1% and 2% lecithin. Following several preliminary trials, emulsions were prepared with 20% sunflower oil + 12% legume flour + 67% water and 1% lecithin on a dry basis. Later, emulsions were kept in a water bath at 80˚C for 30 minutes and then placed in an ice bath to obtain gel formation. The selection of the appropriate gel formulation to be used in the study was determined by evaluating the data obtained from particle size and texture (hardness) analysis.

Model Meat System Preparation

To prepare model meat systems, three different formulations were pre-tested without using emulsion gel (control). The pretested formulations were:
Formulation A: 70% meat, 10% animal fat and 20% water
Formulation B: 75% meat, 10% animal fat and 15% water
Formulation C: 33% meat, 52% animal fat and 15% water.

These formulations were prepared as 200 g batches in a blender. After mixing in the blender and obtaining an emulsion structure, they were stuffed in a 50 ml Falcon tube. The mixture was cooked in a hot water bath at 80˚C for 30 minutes. The formulation of the control samples to be used in the study was decided based on the naked eye appearance and textural properties of the samples. Accordingly, the composition of the control sample was determined as 75% meat, 10% animal fat and 15% water (Formulation B). This formulation was chosen because it gave the best appearance and structure. Afterwards, model meat systems were created by adding 25%, 50%, 75% and 100% vegetable oil in place of animal fat. Considering the appearance and texture values obtained, it was decided to use 50% and 75% emulsion gels in place of animal fat in the samples. Model meat systems prepared according to the trial design given in Table 1 were cooked in a hot water bath at 80˚C for 30 minutes as in control (with an internal temperature of 72˚C measured by using thermocouples).

Table 1. Formulation of model meat systems

Sample	Legume Flour	Meat (%)	Fat (%)		Water (%)
			Animal Fat	Emulsion Gel	
C	-	75	10	-	15
P50	Pea	75	5	5	15
P75	Pea	75	2.5	7.5	15
L50	Lentil	75	5	5	15
L75	Lentil	75	2.5	7.5	15
B50	Navy Bean	75	5	5	15
B75	Navy Bean	75	2.5	7.5	15
CP50	Chickpea	75	5	5	15
CP75	Chickpea	75	2.5	7.5	15

Analyzes of Emulsion Gels

Particle size of emulsions

The particle sizes of the prepared ungelled emulsions were measured using equipment working with the laser diffraction principle (Malvern Instruments, mastersizer 3000, Malvern, UK).

Parameters used: Mixer speed: 2500 rpm, refractive index (Sunflower oil): 1.464-1.474, absorption index: 0.01, olive oil density 0.924 g/cm^3, acceptance range (5-15). The prepared gel was added slowly to the mixer and reading was conducted between the limit values (Pocan et al., 2019).

Texture analysis of emulsion gels

The texture values of the samples were determined with a texture analyzer (TA.HD Plus Texture Analyzer Texture Technologies Corp., Hamilton, MA, USA). Compression was applied to the samples with a cylinder probe. Trigger load and test speed were set as 0.1 N and 0.50 mm/s, respectively. A cylinder probe (TA10) with a length of 13.93 mm and a diameter of 14.11 mm was used for measurements.

Analyzes in Cooked Model Meat Systems

Proximate analyzes

Moisture, fat and protein contents of model meat systems were determined following AACC Methods (AACC, 2000). The moisture content was measured by drying the samples in an oven at 105˚C. Measurements were conducted in quadruplicate for each treatment. Then, these dried samples were used to carry out the fat and protein analysis by the Soxhlet extraction method and the modified Kjeldahl method, respectively.

Analysis of pH

The pH-meter was calibrated with buffer solutions prior to analyses. In pH measurements, 1 g of sample was homogenized with 9 ml of distilled water and reading was performed by immersing the pH-meter electrode into this mixture (Pintado and Cofrades, 2020).

Water Holding Capacity (WHC)

To determine the water holding capacity of the sample (Bowker and Zhuang, 2015), 15 ml of 0.6 M NaCl solution was added to 10 g of meat and mixed for 1 minute. After the sample was kept at 4 °C for 15 minutes, it was centrifuged at 4000 rpm for 25 minutes, and the volume of the supernatant was measured. The following equation was applied to calculate water holding capacity (WHC) (%):

$$WHC\ (\%) = \frac{V_2 - V_1}{V_1} x\ 100$$

where V1 and V2 are the volumes of NaCl solution before and after centrifuge, respectively.

Color analysis

The color analysis of samples was conducted by the CIELAB method (Ilhan et al., 2020). The L* (lightness), a* (red-green), and b* (yellow-blue) values were recorded via Konica Minolta spectrophotometer (CM-5, Tokyo,

Japan).

Texture Profile Analysis

The texture values of meat samples were determined with a texture analyzer (TA.HD Plus Connect, Texture Technologies Corp., MA, USA). Compression tests were applied to the samples with a cylinder probe. Trigger load and test speed were set to 0.1 N and 0.50 mm/s, respectively, and a cylinder probe (TA10) with a length of 13.93 mm and a diameter of 14.11 mm was used to measure hardness, cohesiveness, springiness, and chewiness (Hjelm et al., 2019).

Time-Domain Nuclear Magnetic Resonance (TD-NMR) Relaxometry

T2 relaxation times of control and samples were measured via a 0.5 T (20.34 MHz) benchtop TD-NMR system (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany). CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence for T2 measurements and saturation recovery pulse sequence with appropriate acquisition parameters were applied. Mono-exponential fitting was performed by MATLAB (R2019b, The MathWorks Inc., USA) to calculate the relaxation times (Bitik et al., 2019).

Field Emission Scanning Electron Microscopy (FE-SEM)

The morphologic analysis was carried out by a field emission scanning electron microscope (FE-SEM) (QUANTA 400F, Field Electron and Ion Company, OR, USA). The surface morphology of the samples was analyzed at 20 kV, and the images were examined in 1000x and 2000x magnitudes (Claver et al. 2010).

Statistical Analysis

In the study, analyses were performed in four replicates in moisture (%), pH, water holding capacity, color, texture, NMR analyzes and the average value of the data for each sample was presented. It was studied as three replicates in fat analysis and two replicates in protein analysis, and the averages of the values were taken. Statistical evaluations were carried out in Minitab (Version 19, Minitab Inc., Coventry, UK) package program. One-way analysis of variances (ANOVA) method was used in the statistical evaluation of the differences between the samples. Primarily, it was examined whether the obtained data provided a normal distribution. Then the equality of variance analysis was performed and its suitability for ANOVA was tested. Tukey's multiple comparison test was applied at 95% confidence interval to determine the significant differences between the samples.

RESULTS AND DISCUSSION

Moisture, Fat and Protein Contents

The moisture, fat and protein values of the control sample and the samples containing emulsion gel formed with pea, lentil, bean and chickpea flour are presented in Table 2. When the moisture contents of the samples were examined, the values of control and CP50 were found to be lower than the other samples ($p<0.05$). This is thought to be due to the water content of the emulsion gels. Considering that 10% animal fat was replaced with 50% and 75% emulsion gel in the model meat system in the study, it is an expected result that emulsion gel with high moisture content entering the structure instead of oil causes this situation. Except for CP50, there was no significant difference between the moisture values of all samples containing emulsion gel ($p>0.05$).

Table 2. The moisture, fat, and protein contents (%) of the control and emulsion gel containing samples

Sample	Moisture Content (%)	Fat (%) (g /100 g dw)	Protein (%) (g /100 g dw)
C	71.28 ± 0.57 ^{b}	27.77 ± 0.30 ^{a}	65.63 ± 1.11 ^{b}
P50	73.47 ± 0.27 ^{a}	21.89 ± 0.51 ^{c}	69.39 ± 0.50 ^{a}
P75	74.09 ± 0.59 ^{a}	19.72 ± 0.06 ^{d}	71.09 ± 0.06 ^{a}
L50	73.55 ± 0.95 ^{a}	22.62 ± 0.29 ^{c}	71.93 ± 0.62 ^{a}
L75	73.56 ± 0.16 ^{a}	18.86 ± 0.22 ^{d}	72.28 ± 0.50 ^{a}
B50	74.27 ± 0.30 ^{a}	24.68 ± 0.28 ^{b}	70.92 ± 0.93 ^{a}
B75	73.74 ± 0.53 ^{a}	19.54 ± 0.16 ^{d}	71.40 ± 1.61 ^{a}
CP50	72.84 ± 0.73 ^{b}	22.81 ± 0.50 ^{c}	69.30 ± 0.62 ^{a}
CP75	73.97 ± 0.62 ^{a}	19.61 ± 0.36 ^{d}	71.05 ± 0.62 ^{a}

Values are expressed as mean ± SE (n=3). In each column different letters represent significant differences ($p < 0.05$).

Pintado and colleagues reported that the moisture content of the reduced-fat sausages in which the animal fat was replaced with emulsion gels containing chia flour, oat bran and olive oil was higher than that of the control sample (Pintado et al., 2018). In another study where amorphous cellulose fiber was substituted to reduce the fat content in model meat systems, the moisture content was found to be high due to introducing a high amount of water into the formulations than control samples (Schmiele et al., 2015).

In the fat analysis, the oil content of the control sample was higher than the other samples ($p<0.05$). It can also be explained by replacing fat with emulsion gels in model meat systems. Significant differences were found between the fat contents of the samples containing

Table 3. pH, L*, a* and b* values of the control and emulsion gel containing samples

Sample	pH	L*	a*	b*
C	6.36 ± 0.03 ^{ab}	47.13 ± 0.83 ^{a}	9.7 ± 0.91 ^{ab}	20.48 ± 0.52 ^{a}
P50	6.41 ± 0.02 ^{a}	49.03 ± 1.67 ^{a}	7.53 ± 0.05 ^{d}	19.83 ± 0.44 ^{a}
P75	6.37 ± 0.03 ^{ab}	50.15 ± 1.82 ^{a}	8.00 ± 1.14 ^{d}	20.58 ± 1.28 ^{a}
L50	6.31 ± 0.06 ^{bc}	49.45 ± 2.07 ^{a}	9.87 ± 1.48 ^{ab}	19.60 ± 0.90 ^{a}
L75	6.34 ± 0.02 ^{abc}	46.37 ± 1.46 ^{a}	8.57 ± 0.64 ^{cd}	18.97 ± 1.18 ^{a}
B50	6.27 ± 0.05 ^{c}	49.18 ± 2.35 ^{a}	9.33 ± 1.57 ^{bc}	19.75 ± 1.12 ^{a}
B75	6.36 ± 0.03 ^{ab}	47.85 ± 2.39 ^{a}	10.47 ± 1.10 ^{a}	19.40 ± 1.15 ^{a}
CP50	6.27 ± 0.04 ^{c}	50.18 ± 2.07 ^{a}	5.80 ± 2.25 ^{e}	18.28 ± 1.93 ^{a}
CP75	6.29 ± 0.02 ^{bc}	48.03 ± 0.73 ^{a}	9.18 ± 0.56 ^{bc}	19.35 ± 0.59 ^{a}

Values are expressed as mean ± SE (n=3). In each column different letters represent significant differences ($p < 0.05$).

emulsion gels ($p<0.05$). While the lowest fat content was found in L75, P75, B75 and CP75 samples, the highest fat value was observed in B50 after the control ($p<0.05$). A significant similarity was found between the oil values of the other samples (P50, L50 and CP50) ($p>0.05$). Pintado and Cofrades (2020) indicated that oleo gel and emulsion gel induced fermented sausages and had higher moisture and low-fat content than control.

Protein contents of the legume flours which were used in this study had been determined in a previous study (Tas et al., 2022). The protein contents of pea, lentil, navy bean and chickpea flours were 25.03, 27.03, 23.19 and 23.67 g/100 g dw, respectively.

Protein analysis of the meat samples was also performed on dried samples. According to the results obtained, the protein content of the control sample was found to be lower than the other samples ($p<0.05$), but no significant difference was observed between the other samples ($p>0.05$). This was due to the high protein content in pea, lentil, navy bean and chickpea flours used in the formulation. Therefore, substituting emulsion gels containing legume flours can be considered effective in increasing the protein amount in model meat systems. Pintado et al. (2018) stated that the protein values of the reduced-fat sausages to which they added emulsion gels prepared with chia flour, oat bran and olive oil instead of animal fat were similar to those of the sausages prepared with animal fat and without reduced fat ($p<0.05$). It was also mentioned in another study that the protein content increased when emulsion gels containing using chia flour, olive oil and alginate was utilized in frankfurters instead of animal fat (Herrero et al., 2017). Salcedo-Sandoval et al. (2013) stated that the moisture and protein content of the sausages increased, while the fat content decreased ($p<0.05$), in the study where they replaced animal fat by emulsion gels containing olive oil, flaxseed oil, fish oil and konga flour ($p<0.05$).

pH and Color Analysis

As given in Table 3, the pH values of the samples ranged from 6.27 to 6.41. Although statistical differences were observed in some samples, all the pH values were consistent with products of this kind (Salcedo-Sandoval et al., 2013; Scapin et al., 2015; Herrero et al., 2017; Pintado et al., 2016, 2018).

The color (L*, a*, b*) values of the samples are presented in Table 3. No significant difference was observed in color values L* and b* ($p>0.05$), but a* values were significantly different ($p<0.05$). L*, a* and b* values vary between 46.37-50.18, 5.80-10.47 and 18.28-20.58, respectively. In general, model meat systems containing emulsion gels were less red than the control except for B75 sample. Less redness might be due to the substitution of fat by emulsion gels (Jiménez-Colmenero et al., 2012; Pintado et al., 2018). The most suitable color can be easily obtained by several strategies which focuses on modulating the color of the ingredients in emulsion gels.

Water Interactions

No significant difference was observed between the water holding capacity (WHC) values of the samples ($p>0.05$) (Table 4). Although the moisture values of the samples containing emulsion gel were found to be higher than the control ($p<0.05$), the use of emulsion gel instead of oil caused only minor numerical differences in the water holding capacity of the samples but did not cause a statistical difference ($p>0.05$). This may be due to the relatively low content of legume flours in the whole mass of the model meat system. Moreover, the pH values could influence the WHC of the samples. At the isoelectric point of the muscle proteins (5.8 for beef), WHC is expected to be minimum, and studies showed that as pH increases, WHC also increases (Nacak et al., 2021). However, according to a study that focuses on cooked pork and beef sausages (Puolanne et al., 2001), the effect of a further increase in pH after 6.1 was trivial, and the pH values of cooked sausages tend to be between 6.0 and 6.5 (Korkeala and Johanna Björkroth, 1997). Therefore, the WHC of the samples in this study was expected to be similar due to the slight differences in pH values (pH range: 6.27 to 6.41).

T2 relaxation times of the samples are given in Table 4. Based on results, significant differences were found between the values ($p<0.05$). The lowest T2 relaxation time was observed in control sample while the highest was observed in the B75 sample ($p<0.05$). As the amount

Table 4. Water holding capacity (WHC) (%) and T_2 relaxation times of the control and emulsion gel containing samples

Sample	WHC (%)	T_2 relaxation time (ms)
C	65.33 ± 1.22 [a]	141.82 ± 7.75 [d]
P50	65.83 ± 1.48 [a]	160.66 ± 11.61 [bcd]
P75	65.33 ± 1.22 [a]	187.42 ± 10.46 [b]
L50	64.50 ± 0.33 [a]	165.15 ± 0.81 [bcd]
L75	66.67 ± 0.00 [a]	165.73 ± 13.68 [bcd]
B50	65.00 ± 0.39 [a]	173.46 ± 21.05 [bc]
B75	66.00 ± 1.44 [a]	237.17 ± 13.12 [a]
CP50	66.33 ± 0.39 [a]	152.85 ± 9.25 [cd]
CP75	65.33 ± 1.33 [a]	169.17 ± 11.32 [bcd]

Values are expressed as mean ± SE (n=3). In each column different letters represent significant differences ($p < 0.05$).

of free water in the sample increases, T2 time is expected to increase. The model meat systems had complex and heterogeneous structures containing different components such as fat and protein along with water, thus components other than water may also have an impact on relaxation times. Since the samples containing emulsion gels contain more water molecule than the control as can be seen from the moisture content results, observing the lowest T2 relaxation time in control was expected.

Textural Properties

When the texture values of the samples given in Table 5 were analyzed, it was found that the hardness and chewiness values of the samples were similar to those of control samples ($p>0.05$). The highest hardness and chewiness values were observed in P75, and C75 samples, whereas the lowest value was observed in the L50 sample. Also, the cohesiveness and springiness values of all samples were statistically indifferent ($p>0.05$). Considering similar formulations of model meat systems, the difference in the hardness and chewiness of samples would appear to be due to emulsion gels fortified as a fat replacer (de Souza Paglarini et al., 2019). During cooking, a gel matrix that retains the components (e.g. additives, polysaccharides) inside is created from the meat protein. The emulsion gel components (sunflower oil, animal fat and plant protein) are also expected to be trapped in this matrix, causing an increase in the hardness and chewiness of the samples (Gao et al., 2015). Furthermore, the better dispersion of sunflower oil droplets and the protein-polysaccharide interaction in legume flours could affect the textural properties of samples (Matsumura et al., 1993; Mcclements et al., 1993; Dickinson, 2013; Pintado et al., 2015; Herrero et al., 2017). Several studies complied with the results and showed that the model systems in which emulsion gels were utilized as a fat replacer had higher hardness and chewiness than the control (Pietrasik and Janz, 2010; Sanjeewa et al., 2010; Shahiri Tabarestani and Mazaheri Tehrani, 2014).

Microstructural Properties

SEM images of the samples captured at 20 kV with magnifications of 1000x and 2000x are presented in Figures 1 and 2. In Figure 1, the typical morphology of a meat product having a three-dimensional cooked gel network could clearly be observed in control samples. The main characteristic of this product consisted of the formation of several cavities and a rough and coarse structure where irregular shapes could be seen due to the expansion of fat, air and water constituents (Ayadi et al., 2009; Salcedo-Sandoval et al., 2013; Nacak et al., 2021). On the other hand, compared to control samples, P50 and B50 had a smoother gel network containing more small cavities. Sample L50 showed a more fringed structure. CP50 seemed more compact with smaller cavities. These images indicated the formation of new connections along with the already existed gel network due to the crosslinking reaction between polysaccharides, proteins, and water molecules (Ayadi et al., 2009).

In Figure 2, P75 had smaller cavities than those of control. B75 exhibited the smoothest image of all. L75 sample

Table 5. Texture values of the control and emulsion gel containing samples

Sample	Hardness (N)	Cohesiveness	Springiness (mm)	Chewiness (g.cm)
C	10.71 ± 0.67 [ab]	0.63 ± 0.01 [a]	8.43 ± 0.64 [a]	577.00 ± 78.48 [ab]
P50	8.44 ± 0.35 [ab]	0.67 ± 0.04 [a]	8.45 ± 1.71 [a]	482.00 ± 49.50 [ab]
P75	12.67 ± 2.18 [a]	0.66 ± 0.05 [a]	8.56 ± 0.49 [a]	738.33 ± 183.63 [ab]
L50	7.03 ± 0.37 [b]	0.66 ± 0.04 [a]	8.40 ± 0.18 [a]	393.00 ± 7.07 [b]
L75	10.04 ± 1.42 [ab]	0.66 ± 0.11 [a]	8.96 ± 0.91 [a]	614.67 ± 150.41 [ab]
B50	11.89 ± 2.01 [ab]	0.69 ± 0.08 [a]	8.08 ± 0.48 [a]	671.33 ± 90.67 [ab]
B75	10.16 ± 2.24 [ab]	0.64 ± 0.06 [a]	8.06 ± 0.38 [a]	527.67 ± 70.87 [ab]
CP50	12.70 ± 0.40 [ab]	0.67 ± 0.04 [a]	8.84 ± 0.73 [a]	774.50 ± 136.47 [ab]
CP75	13.55 ± 2.26 [a]	0.68 ± 0.06 [a]	8.46 ± 0.78 [a]	791.00 ± 121.51 [a]

Values are expressed as mean ± SE (n=3). In each column different letters represent significant differences ($p < 0.05$).

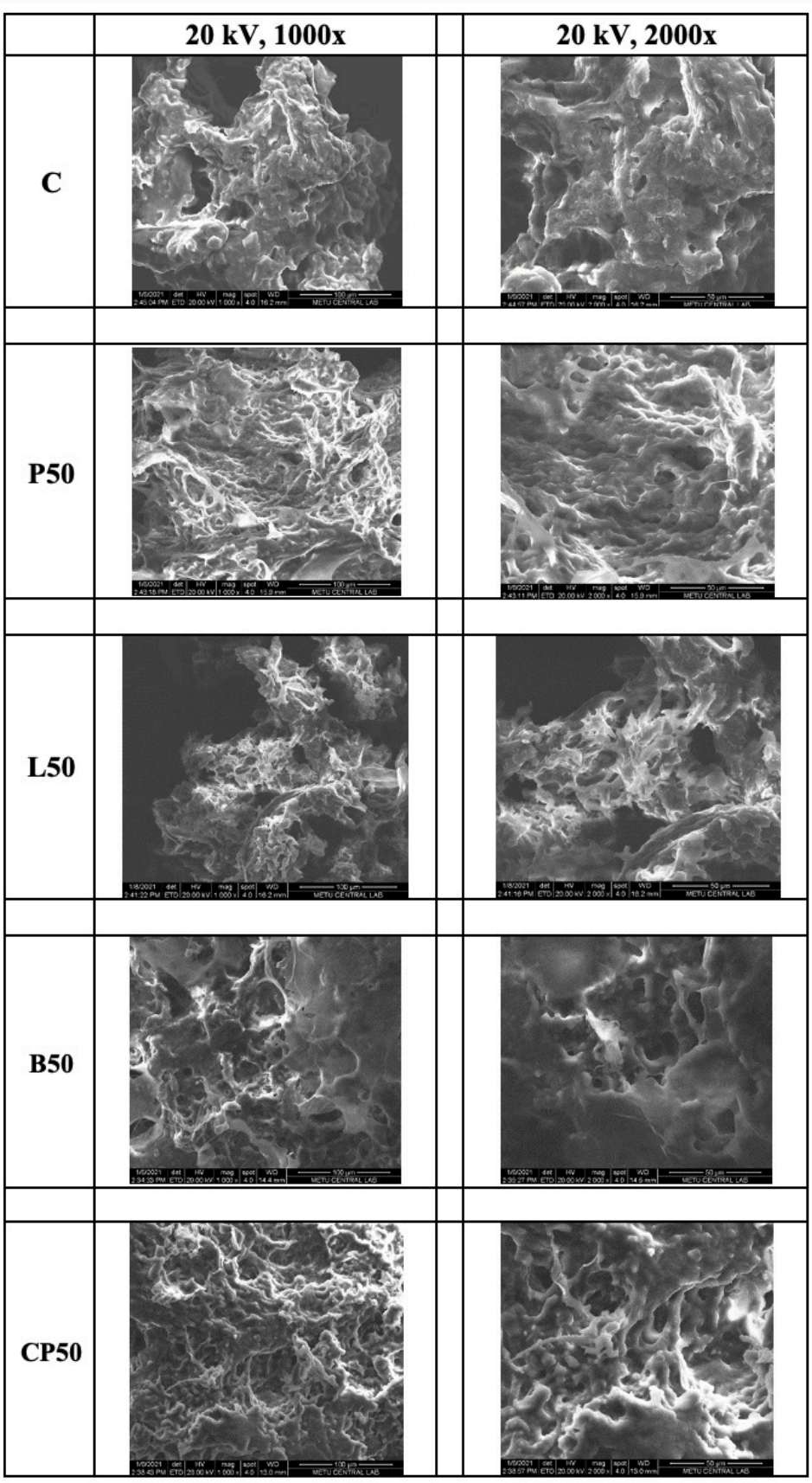

Figure 1. Scanning electron microscopy (SEM) images of control, P50, L50, B50 and CP50 samples captured at 1000× and 2000x magnification

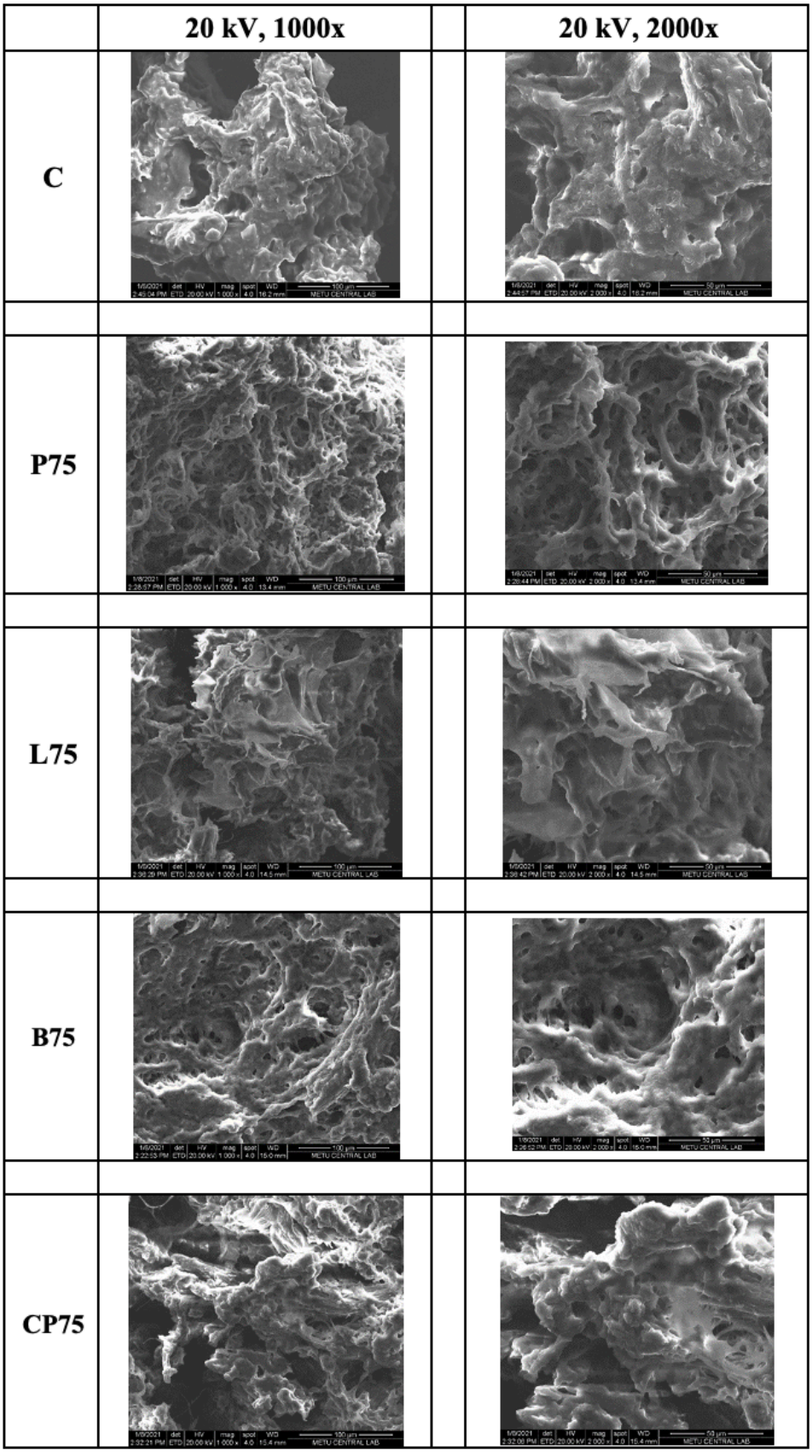

Figure 2. Scanning electron microscopy (SEM) images of control, P75, L75, B75 and CP75 samples captured at 1000× and 2000x magnification

seemed to be fringy without round cavities. Sample containing emulsion gels prepared with chickpea flour (CP75) presented the most irregular and indented structure.

In general, it can be deduced that a stable and homogenous structure similar or better than control may also be obtained by emulsion gels as seen in literature (Ayadi et al., 2009; Afoakwah et al., 2015; Wang et al., 2018).

CONCLUSION

Addition of vegetable oils into meat products usually cause some technological problems such as oil leakage. The basic aim of this study was to solidify the oils by forming emulsion gels. Emulsion gels were prepared using sunflower oil and different leguminous flours (pea, lentil, bean and chickpea flours) and the prepared gels were used in place of animal fat at the amounts of 50% and 75% in the model meat system. The secondary aim was to increase the protein content of the whole product by legume proteins. The moisture content (%) of the majority of the samples to which emulsion gel was added was higher than the control sample ($p<0.05$). Considering that emulsion gels contain a high percentage of water, this was a predicted outcome. It was determined that the amount of oil in the samples including emulsion gel was significantly less than the control sample, but on the contrary, the protein amounts were higher than the control ($p<0.05$). Although there were significant differences in the pH values of the products, extreme changes were not observed. The water holding capacity values of all products were found to be similar ($p>0.05$) despite the high water content of the emulsion gels. Emulsion gels and leguminous proteins are likely have an effect on this situation and no adverse effect on the product such as water release was observed. However, there were significant differences ($p<0.05$) in the relaxation times of T_2 by NMR analysis. No significant changes were found in the L* and b* values among all the samples ($p>0.05$). According to texture analysis, the hardness, cohesiveness, springiness and chewiness values were similar to the control ($p>0.05$). With the morphological images obtained by SEM analysis, the effects of different emulsion gels on the internal microstructure of the products were examined. In conclusion, addition of emulsion gels prepared by using different leguminous flours did not change the quality properties properties of the products according to the results of the conducted analyses. Moreover, products with low animal fat and high protein content were obtained. In the future, utilization of emulsion gels in meat products would be an attractive application for the industry. Many products based on new formulations can be obtained using different organic polymers and gelling agents.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

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

Author contribution

This study is the part of the master thesis of DD. She (DD) conducted experiments, data analysis. EBO created and designed the study, supervised DD and wrote the manuscript. UE and OT assisted and led DD while doing the experiments and wrote the manuscript. MHO supervised DD. All the authors reviewed the manuscript.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Evaluation of energy and nutrient content of fruit juices and similar beverages in Türkiye and their investigation in terms of sustainability

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Abstract
This study aims to evaluate the contents of fruit juices and similar beverages in the Turkish market and to examine them in line with the Sustainable Development Goals. Within the scope of the study, the researchers examined the label information of 187 fruit juices and similar beverages from 26 different brands in four different product types. It was determined that all fruit nectars (100%) and almost all fruit and flavored beverages contain added sugar (92.8% for fruit beverages and 70.0% for flavored beverages). It was further detected that 17.5% of fruit nectars and 20.3% of fruit beverages contain sweeteners. Moreover, more than 100% of the daily sodium requirement is met by consuming 100 ml of fruit juice, nectar, or beverages. It was determined that orange and apple juices, apricot, peach, and cherry fruit nectars, and peach, sour cherry, and orange fruit beverages contain more sugar than freshly squeezed fruit juices ($p<0.05$). The high-fructose syrup manufacturing industry produces large volumes of waste liquid containing various waste carbohydrates, which has a significant environmental impact. Greenhouse gas emission sources are also released to the environment in sugar production processes. On the other hand, it was determined that most of the products examined were packaged in Tetra Pak® packaging. More sustainable product supply chains are those that optimize the use of materials, water, and energy throughout their lifecycle while minimizing waste from products and used packaging. In light of the data obtained from this study, it would be useful for the fruit juice industry to make regulations to ensure sustainable production and consumption patterns in order to ensure a healthy and quality life at all ages.
Keywords: Fruit Juice, Fruit Nectar, Food Labelling, Sustainable Development

INTRODUCTION

Malnutrition continues to affect millions of people globally, particularly women, children, and other vulnerable populations (Lopez de Romaña et al., 2021). Unsustainable food production is one of the leading causes of malnutrition (Grosso et al., 2020). The consumption of healthy foods produced through sustainable food systems is a fundamental approach not only for malnutrition but also for the solution to climate change, loss of biodiversity, and environmental pollution (Pekcan, 2023; UN Nutrition, 2022). United Nations member states adopted 17 Sustainable Development Goals (SDGs) to end poverty, protect the planet, and improve the lives and futures of all by 2030. Nutrition directly affects two of the Sustainable Development Goals (SDG 2: "End hunger, achieve food security and improved nutrition and promote sustainable agriculture" and SDG 3: "Ensure healthy lives and promote well-being for all at all ages"). It also indirectly affects many of the other development goals. In turn, nearly all Sustainable

Development Goals affect nutrition (Lopez de Romaña et al., 2021).

Food production and consumption significantly impact environmental objectives (SDG 6, 7, 9, 12, 13, 14, and 15). Food production is responsible for a significant portion of global greenhouse gas emissions. In addition, food production is responsible for the overuse of fresh water and agricultural land and the loss of biodiversity (Smetana et al., 2019). Considering the environmental sustainability and health consequences of food production and consumption, the necessity of a global food system transformation is discussed to achieve significant and ambitious changes in sustainability (Grosso et al., 2020). In this context, there is a growing need to evaluate the fruit juice industry, an essential economic activity in the world, regarding sustainability (Esturo et al., 2023).

When the guidelines on fruit juice consumption worldwide are examined, it is seen that the guidelines of numerous countries include the necessity of preferring whole fruits over fruit juice and place limitations on the amount of fruit juice consumption (Herforth et al., 2019). For example, the Dietary Guidelines for Americans emphasize that the majority of fruits consumed should be whole fruits rather than fruit juice and that when fruit juice is consumed, it should be 100% fruit juice (United States Department of Agriculture, 2016). Furthermore, several countries convey clearly negative messages about juice and similar beverages (Herforth et al., 2019). The Turkish Dietary Guidelines (2022) state that fruit juices contribute to daily energy intake but are insufficient in terms of dietary fiber, may cause an increase in body weight depending on the amount of consumption, and frequent consumption may cause tooth erosion because they are generally acidic. It is recommended that the daily consumption of fruit juice should be 125 mL and should not exceed 120 mL/day for 1–3-year-olds, 180 mL/day for 4–6-year-olds, 240 mL/day for 7–18-year-olds and 240 mL/day for adults (T.R. Ministry of Health, 2022). A study evaluating fruit juice consumption with 569,000 participants from 46 countries determined that adults around the world consumed an average of 0.16 servings of fruit juice per day in 2010 (Singh et al., 2015). In the sector report of the Fruit Juice Industry Association, it is stated that the consumption amount of fruit juice and similar products per capita reaches approximately 12 liters per year, and approximately 9 liters of this amount consists of 100% fruit juice and fruit nectar consumption (Akdağ, 2011).

The fruit juice sector faces various challenges to meeting the Sustainable Development Goals, such as providing healthy and safe working conditions (related to SDG 8), reducing waste from production (related to SDG 12), protecting the ecosystem (related to SDGs 14 and 15) and rational use of natural resources. Environmental sustainability and the impact of food production on the planet have come to the fore through increased consumer awareness of the nutritional benefits of fruit juices without added sugar, sweeteners, and preservatives for a healthy and quality lifestyle (Esturo et al., 2023).

This study aims to evaluate the energy and nutritional content of fruit juices and similar beverages available in the Turkish market and to examine them in line with the Sustainable Development Goals.

MATERIALS AND METHODS

This study examined the labels of fruit juices and similar beverages offered for sale in the Turkish market from supermarkets and virtual markets in Kırıkkale and Ankara provinces between August and December 2022. As a result of the market research, 187 products from 26 brands were reached. Fruit juices, fruit nectars, fruit beverages, and flavored beverages were included in the study based on the Turkish Food Codex Communiqué on Fruit Juice and Similar Products (T.R. Official Gazette, 6 August 2014). These are products produced without gas by adding water and/or other ingredients, with or without sugar. The study did not include fruit-flavored acidic beverages, fruit-flavored soda, mineral waters, and fruit-flavored beverage powders.

As a data collection tool, an evaluation form was used that provides information about the brand name, product name, fruit type, fruit ratio, ingredients, the status of added sugar, the type of added sugar, the status of sweetener content, the sweetener type of fruit juice and similar beverages, energy and nutrient content, package size, package type, and product technology. This form was created by examining the Turkish Food Codex Communiqué on Soft Drinks (Communiqué No:2007/26) (T.R. Official Gazette, 15 June 2007), the Turkish Food Codex Communiqué on Sugar (Communiqué No:2006/40) (T.R. Official Gazette, 23 August 2006), and the Turkish Food Codex Communiqué on Fruit Juice and Similar Products (Communiqué No:2014/34) (T.R. Official Gazette, 6 August 2014). The energy and nutrient contents obtained from the examination of the products were compared with the dietary reference intake levels of an average adult, and the coverage percentages were calculated (National Institutes of Health, 2023). In addition, the carbohydrate and sugar contents of the products based on the fruit type were evaluated by comparing them with freshly squeezed fruit juices (unpackaged) in the National Food Composition Database (Türkomp, 2023).

Features of fruit juices and similar drinks such as energy and nutritional content, added sugar status, added sugar type, sweetener content, sweetener type, fruit type, production technologies and packaging were examined in terms of sustainability based on the articles "to ensure a healthy and quality life at all ages" (SDGs 3) and "to provide sustainable production and consumption patterns" (SDGs12).

Statistical analysis

The data obtained from the study were evaluated with the SPSS 22.0 statistical package program. The conformity of the variables to the normal distribution was examined by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Qualitative variables are expressed as numbers (n) and percentages (%), and quantitative variables as lower and upper values, mean, and standard deviation ($\bar{X} \pm SD$). Pearson's Chi-square test was used to compare categorical variables, and Fisher's Exact Chi-square test was used when the number of samples in the crosstab was insufficient, and the assumption could not be met. The comparison of two groups independent of a numerical variable in parametrically distributed data was performed with the help of a one-sample t-test. For data showing a non-parametric distribution, the Wilcoxon one-sample signed-rank test was used, and the deviation values from the reference were given. In all statistical tests, the confidence interval was accepted as 95.0%, and statistical significance was accepted as $p<0.05$.

RESULTS AND DISCUSSION

Within the scope of the study, the label information was examined for 187 fruit juices and similar beverages from 26 different brands in four different product types. These products consist of six kinds of fruit juices from 12 brands, 10 kinds of fruit nectars from 16 brands, 15 kinds of fruit beverages from 17 brands, and six kinds of flavored beverages from five brands (Table 1).

Table 1. Number of brands, types, and products of fruit juice and similar beverages included in the study

Product Type	Number of Brands (n)	Number of Fruit Types (n)	Number of Products (n)
Fruit Juices	12	6	51
Fruit Nectars	16	10	57
Fruit Beverages	17	15	69
Flavored Beverages	5	6	10

Table 2 presents information on the type of fruit, fruit ratios, the status of added sugar content and type, the status of sweetener content and type, product technology, and packaging type of 187 fruit juices and similar beverages examined within the scope of the study. The majority of the products consisted of mixed fruit types. It was determined that the products with the highest fruit rate were fruit juices and fruit nectars. All fruit nectars (100%) and almost all fruit and flavored beverages contain added sugar (92.8% for fruit beverages and 70.0% for flavored beverages). The most common type of added sugar is sucrose. Fruit juices and flavored beverages do not contain sweeteners, while 17.5% of fruit nectars and 20.3% of fruit beverages contain sweeteners. Furthermore, 70.0% of fruit nectars containing sweeteners, and 50.0% of fruit beverages containing sweeteners contain sucralose. It was determined that most of the products examined were packaged in Tetra Pak® packaging.

Table 3 presents the comparison of the energy and some nutrient contents of 100 ml of fruit juice and similar beverages with the dietary reference intake levels of an average adult and the percentages of coverage. It was determined that fruit juice has the highest coverage percentage in general in terms of meeting the needs of an adult individual. The consumption of 100 ml of fruit juice fulfills 2.53% of the daily energy requirement, 9.21% of the carbohydrate need, and 0.66–0.80% of the protein need. Fruit juice consumption meets 21.62% of free sugar intake, fruit nectar consumption meets 21.84%; fruit beverage consumption meets 19.64%, and flavored beverage consumption 19.46%. It has been found that more than 100% of the daily sodium requirement is met with the consumption of 100 ml of fruit juice, fruit nectar, or fruit beverages.

When the carbohydrate and sugar content of fruit juices and similar beverages were compared with freshly squeezed fruit juices according to the National Food Composition Database (Türkomp), it was determined that orange-type fruit juices contained fewer carbohydrates and more sugar compared to freshly-squeezed orange juice, and apple-type fruit juices contained more carbohydrates and sugar compared to freshly-squeezed apple juice ($p<0.05$). Apricot- and peach-type fruit nectars contained fewer carbohydrates and more sugar than freshly-squeezed apricot and peach juices, respectively ($p<0.05$). Sour cherry fruit nectars contained more sugar than freshly-squeezed cherry juice ($p<0.05$). Peach- and cherry-type fruit drinks contained fewer carbohydrates and more sugar compared to freshly-squeezed peach and cherry juices, respectively. Finally, orange-type fruit drinks contained more sugar than freshly-squeezed orange juice ($p<0.05$) (Table 4).

Sustainable development aims to provide economic and social development and environmental protection. These aspects of sustainable development are crucial to securing the future of the juice industry. For this reason, fruit juice and similar beverages should be produced within the limits of sustainability (Esturo et al., 2023). From this point of view, it has become essential to focus on the sugar contents, sugar amounts, fructose-glucose syrup contents, and production and packaging techniques of fruit juice and similar beverages that all age groups can reach. This study hence evaluated the energy and nutritional content of fruit juices and similar beverages in Türkiye regarding the sustainability from different aspects.

The Sustainable Development Goals and Indicators booklet published by the Presidency of the Republic of Türkiye Strategy and Budget Department in 2019 includes the articles "to ensure a healthy and quality

Table 2. Evaluation of fruit juice and similar beverages in terms of sustainability

	Fruit Juices (n=51)		Fruit Nectars (n=57)		Fruit Beverages (n=69)		Flavored Beverages (n=10)		
	N	%	n	%	n	%	n	%	p
Fruit Type									
Mixed	31	60.8	18	31.5	23	33.3	2	20.0	
Apricot	-	-	14	24.5	3	4.3	1	10.0	
Peach	-	-	13	22.8	5	7.2	-	-	
Cherry	-	-	5	8.8	10	14.5	-	-	
Orange	6	11.8	2	3.5	5	7.2	-	-	
Apple	8	15.7	-	-	-	-	2	20.0	0.00[a*]
Lemon	-	-	-	-	8	11.7	1	10.0	
Pineapple	-	-	1	1.8	6	8.7	1	10.0	
Pomegranate	4	7.8	1	1.8	-	-	-	-	
Mango	-	-	1	1.8	1	1.4	3	30.0	
Others (**)	2	3.9	2	3.5	8	11.7	-	-	
Fruit Ratio									
%100	51	100.0	-	-	-	-	-	-	
%25-99	-	-	57	100.0	9	13.0	-	-	0.00[a*]
%10-24	-	-	-	-	58	84.1	-	-	
<%10	-	-	-	-	2	2.9	7	70.0	
Not specified	-	-	-	-	-	-	3	30.0	
Condition of Containing Added Sugar									
Yes	-	-	57	100.0	64	92.8	7	70.0	0.00[b*]
No	51	100.0	-	-	5	7.2	3	30.0	
Type of Added Sugar									
Sucrose	-	-	34	59.6	34	53.1	6	85.7	
Fructose-glucose syrup	-	-	11	19.4	5	7.8	-	-	0.133[a]
Sucrose+Fructose-glucose syrup	-	-	6	10.5	8	12.5	-	-	
Not specified	-	-	6	10.5	17	26.6	1	14.3	
Sweetener Content Status									0.001[a*]
Yes	-	-	10	17.5	14	20.3	-	-	
No	51	100.0	47	82.5	55	79.7	10	100.0	
Sweetener Type									
Sucralose	-	-	7	70.0	7	50.0	-	-	
Acesulfame K+Sucralose	-	-	2	20.0	3	21.5	-	-	
Aspartame+Acesulfame K	-	-	-	-	2	14.3	-	-	0.717[a]
Aspartame+Acesulfame K+Sucralose	-	-	-	-	1	7.1	-	-	
Acesulfame K+Sodium Cyclamate+Sucralose	-	-	-	-	1	7.1	-	-	
Not specified	-	-	1	10.0	-	-	-	-	
Product Technology									
Pasteurization	36	70.6	36	63.2	45	65.2	3	30.0	
Flash Pasteurization	4	7.8	-	-	-	-	-	-	0.017[a*]
Heating+Cooling	-	-	1	1.7	1	1.5	-	-	
Not specified	11	21.6	20	35.1	23	33.3	7	70.0	
Packaging Type									
Tetra Pak	34	66.7	48	84.2	41	59.4	6	60.0	
PET	4	7.8	-	-	18	26.1	-	-	
Glass	12	23.5	1	1.8	-	-	3	30.0	
Tetra Rex	-	-	3	5.3	6	8.7	-	-	0.00[a*]
Sunpack	1	2.0	4	6.9	-	-	-	-	
Tin box	-	-	1	1.8	2	2.9	-	-	
Pouch partners	-	-	-	-	2	2.9	-	-	
Polypropylene	-	-	-	-	-	-	1	10.0	

* p<0.05, ** Mandarin, Guava, Grape, Crane, Passion Fruit, Blackcurrant, Pear, Strawberry, Grapefruit, Black Mulberry, [a] Fisher test, [b] Chi-square test, PET: Polyethylene terephthalate

Table 3. Evaluation of energy and some nutritional content of fruit juices and similar beverages according to references (100 ml)

Energy and Nutrients	Fruit Juices (n=51)		Fruit Nectars (n=57)		Fruit Beverages (n=69)		Flavored Beverages (n=10)	
	$\bar{X}$±SD Min-Max	Coverage Percentage	$\bar{X}$±SD Min-Max	Coverage Percentage	$\bar{X}$±SD Min-Max	Coverage Percentage	$\bar{X}$±SD Min-Max	Coverage Percentage
Energy (kcal)	50.67 ± 8.44 20.00-72.00	%2.53[a]	47.01 ± 8.58 28.00-65.00	%2.35[a]	41.81 ± 11.36 4.00-59.00	%2.09[a]	39.95 ± 15.33 17.50-64.00	%1.99[a]
Carbohydrate (g)	11.98 ± 2.12 4.00-16.30	%9.21*	11.29 ± 2.14 5.00-15.50	%8.68*	10.13 ± 2.82 0.20-14.00	%7.79*	10.06 ± 3.88 4.30-16.00	%7.73*
Sugar (g)	10.81 ± 2.40 2.10-16.30	%21.62[b]	10.92 ± 2.27 4.80-15.50	%21.84[b]	9.82 ± 2.93 0.00-14.00	%19.64[b]	9.73 ± 3.65 4.10-14.00	%19.46[b]
Fat (g)	0.02 ± 0.06 0.00-0.30	-	0.02 ± 0.09 0.00-0.50	-	0.03 ± 0.12 0.00-0.50	-	0.00 ± 0.00 0.00-0.00	-
Saturated fat (g)	0.00 ± 0.00 0.00-0.00	-	0.0036 ± 0.018 0.00-0.10	-	0.0079 ± 0.032 0.00-0.20	-	0.00 ± 0.00 0.00-0.00	-
Protein (g)	0.37 ± 0.72 0.00-5.00	%0.66-0.80*	0.08 ± 0.19 0.00-1.00	%0.14-0.17*	0.046 ± 0.12 0.00-5.00	%0.08-0.1*	0.00 ± 0.00 0.00-0.00	%0*
Sodium (g)	2.20 ± 3.47 0.00-8.00	%146.66**	1.64 ± 3.15 0.00-7.50	%109.33**	1.60 ± 3.13 0.00-7.50	%106.66**	0.00 ± 0.00 0.00-0.00	%0**
Salt (g)	0.0032 ± 0.014 0.00-0.10	%0.064[c]	0.0045 ± 0.019 0.00-0.10	%0.09[c]	0.0063 ± 0.016 0.00-0.10	%0.126[c]	0.032 ± 0.056 0.00-0.13	%0.64[c]
Fiber (g)	0.22 ± 0.37 0.00-1.60	%0.57-0.88**	0.38 ± 0.73 0.00-3.30	%1.0-1.52**	0.08 ± 0.24 0.00-1.00	%0.21-0.32**	0.00 ± 0.00 0.00-0.00	%0**

SD: Standard deviation [a] The percentage of coverage was calculated from the energy intake of 2000 kcal for an average adult. [b] According to the World Health Organization (WHO) recommendation to reduce total energy intake for free sugar intake to less than 10%, free sugar intake is equivalent to a maximum of 50 grams of sugar per day. [c] The percentage of coverage was calculated from WHO recommendations (<5 g/day salt). * The percentage of coverage is calculated from Recommended Dietary Allowances (RDAs). ** The coverage percentage is calculated with Adequate Intakes (AIs).-: Not determined.

Table 4. Comparison of carbohydrate and sugar content of fruit juices and similar beverages according to fruit type with freshly squeezed fruit juices in the National Food Composition Database (Türkomp)

Fruit Type		Türkomp	Fruit Juices (n=51)		Fruit Nectars (n=57)		Fruit Beverages (n=69)		Flavored Beverages (n=10)	
		Reference Values (g)	Deviation from Reference	p	Deviation from Reference	p	Deviation from Reference	p	Deviation from Reference	p
Apricot	Carbohydrate	14.28	-	-	-2.93 (n=14)	0.002*	-3.6467 (n=3)	0.109	-9.98 (n=1)	-
	Sugar	6.82	-	-	+4.1338 (n=14)	0.004*	+3.78 (n=3)	0.109	-2.62 (n=1)	-
Cherry	Carbohydrate	12.69	-	-	-0.07 (n=5)	0.893	-2.34 (n=10)	0.016*	-	-
	Sugar	5.91	-	-	+6.59 (n=5)	0.043*	+4.22 (n=10)	0.007*	-	-
Peach	Carbohydrate	14.23	-	-	-2.8685 (n=13)	0.003*	-4.284 (n=5)	0.042*	-	-
	Sugar	5.61	-	-	+5.165 (n=13)	0.002*	+4.336 (n=5)	0.042*	-	-
Orange	Carbohydrate	14.64	-4.0733 (n=6)	0.028*	-4.34 (n=2)	0.180	-3.56 (n=5)	0.500	-	-
	Sugar	4.6	+4.66 (n=6)	0.028*	+5.66 (n=2)	0.180	+6.42 (n=5)	0.043*	-	-
Pomegranate	Carbohydrate	13.03	+1.195 (n=4)	0.144	+0.07 (n=1)	-	-	-	-	-
	Sugar	4.84	+6.435 (n=4)	0.068	+7.26 (n=1)	-	-	-	-	-
Apple	Carbohydrate	9.95	+1.8875 (n=8)	0.011*	-	-	-	-	-0.95 (n=2)	0.157
	Sugar	3.25	+8.1625 (n=8)	0.012*	-	-	-	-	+5.75 (n=2)	0.157
Grape	Carbohydrate	13.45	+2.85 (n=1)	-	-	-	-	-	-	-
	Sugar	6.68	+9.62 (n=1)	-	-	-	-	-	-	-
Strawberry	Carbohydrate	11.90	-	-	-	-	-0.5 (n=1)	-	-	-
	Sugar	-	-	-	-	-	-	-	-	-

* p<0.05, Wilcoxon one-sample sign rank test

life at all ages (SDGs 3)" and "to provide sustainable production and consumption patterns (SDGs 12)" (T.R. Strategy and Budget Department, June 2020). When fruit juice and similar beverages are evaluated in terms of ensuring a healthy and quality life at all ages, it is seen that these products in the Turkish market generally have a high sugar and sodium content, beverages other than fruit juice contain added sugar, and fruit nectar and

fruit beverages contain sweeteners. It was determined that all of the fruit nectars, the contents of which were examined, and almost all of the fruit beverages and flavored beverages contained added sugar. The use of sucrose and fructose-glucose syrup is common in fruit juices and similar beverages with added sugar in the Turkish market.

Nutrition labels are of great importance when making the right food choice in order to protect and improve health and reduce the risk of disease (Erem et al., 2018). However, according to the observations, the product labels did not specify the type of added sugar in 10.5% of fruit nectars, 26.6% of fruit beverages, and 14.3% of flavored beverages. In the study where the compliance of 16 fruit juice samples of apple, pomegranate, orange and grape varieties offered for sale in the Turkish market was investigated with the Turkish Food Codex and fruit juice standards, it was determined that some samples did not comply with the standards in terms of formol number, sorbic acid and benzoic acid amounts and Hydroxymethylfurfural (HMF) content. It was determined that fruit juice samples, which should not contain preservatives, contained preservatives, contrary to the relevant standards. In addition, the lead content in two of the apple juice samples, all of the pomegranate and grape juice samples, and three of the orange juice samples was found to be higher than the lead levels allowed in the communiqué (Tüfekci and Fenercioğlu, 2010). On the other hand, it is stated in the Turkish Dietary Guidelines (2022) that fruit juices should always be made of 100% fruit, with pasteurization, and without added sugar, and may be diluted with water (T.R. Ministry of Health, Ankara 2022). The study findings show that the fruit juices evaluated in the study are generally suitable, but the sodium content of even 100 ml of beverage is above the daily requirement of an adult individual (146.66%). A similar situation was observed in fruit nectars and fruit beverages. In addition to this finding, it was seen that free sugar intake was quite high, with the consumption of 100 ml of fruit juice and similar beverages. As a matter of fact, orange and apple juices, apricot, peach, and cherry fruit nectars, and peach, sour cherry, and orange fruit beverages contain more sugar than freshly-squeezed fruit juices ($p<0.05$). Health care costs are high for chronic diseases (such as type 2 diabetes, obesity, hypertension, metabolic syndrome, and kidney disease) that have an increased risk of occurring as a result of frequent and excessive consumption of sugar, fructose or glucose syrup (Bray, 2013; Hayran, 2019; T.R. Strategy and Budget Department, 2020). When evaluated from another aspect, it was determined that 17.5% of fruit nectars and 20.3% of fruit drinks contained sweeteners. Moreover, 70.0% of fruit nectars contained sweeteners, and 50.0% of fruit drinks contained sucralose. Sweeteners are risky compounds when consumed in excessive amounts and frequently, and it should not be ignored that many health hazards are associated with excessive consumption of artificial sweeteners (Singh et al., 2020). It is possible to reduce the global burden of chronic diseases by reducing the consumption of sweetened beverages. When all these findings are evaluated together, it becomes clear that the content of fruit juices and similar beverages in the Turkish market has deficiencies in terms of their suitability for sustainable development goals (SDGs 2 and 3). In this context, it is thought that it is necessary to focus on initiatives that will reduce health expenditures and eliminate the emergence of diseases in order to improve the health of society and improve the quality of life in parallel with the goals of sustainable development (Hayran, 2019; T.R. Strategy and Budget Department, 2020).

When the fruit juice industry is assessed in terms of providing sustainable production and consumption patterns (SDGs 12), it is seen that there is a negative result similar to the third objective of the Sustainable Development Goals. Fruit juices and fruit juice concentrates may contain naturally occurring free sugars and added (additional) sugars (such as sucrose and fructose-glucose syrup) (World Health Organization, 2023). Although sucrose is a natural product extracted from sugar cane and sugar beet pulp, it is not natural because the fructose in the fructose syrup contains a modified structure (Yılmaz and Nurcan, 2015). Fructose syrup, which is found in many packaged foods, especially fruit juices and carbonated drinks, is a food additive preferred by manufacturers instead of sucrose because of its advantages, including being a stronger sweetener than sucrose, being cheaper and having osmotic stability, providing a long shelf life as it does not crystallize quickly, and having organoleptic effects (Arslan and Şanlıer, 2016; Aşıcı et al., 2020). Within the scope of the efforts to limit the use of starch-based sugars in foods and to promote healthy nutrition, the press release of the T.R. Ministry of Health on the report of the Science Board (Effects of Starch-Based Sugars on Health) recommends supporting the industry in minimizing the use of high fructose corn syrup by reformulation (T.R. Ministry of Health, 2018). When purchasing fruit juices and similar beverages, the sugar content and type on the label should be read, and the nutritional choice should be made carefully. Products with the phrase "does not contain added sugar" on the label should be preferred (T.R. Ministry of Health, 2022).

On the other hand, the high-fructose syrup manufacturing industry produces large volumes of waste liquid containing various waste carbohydrates, which has a significant environmental impact (Gao et al., 2021). Greenhouse gas emission sources are also released to the environment in sugar production processes, and this situation is a matter of national concern (de Figueiredo et al., 2010). According to Türkiye's Water Footprint report prepared in cooperation with the World Wide Fund for Nature (WWF- Türkiye) and the T.R. Ministry of Forestry and Water Management, beet sugar production, since

Türkiye has the highest blue water footprint among the leading sugar beet producer countries, beet sugar production, which requires intensive water use, cannot be considered as an example of sustainable production. As the demand for water resources increases, the amount of blue water used for sugar beet will pose a greater risk (WWF-Turkey, 2014). It is predicted that using plant-based packaging made of polyethylene, a renewable polyethylene obtained from sugar cane, and, more importantly, reducing sugar consumption will contribute to the global goals for protecting human health, climate, and biodiversity. In this context, it is essential to evaluate the effectiveness of policies on reducing national sugar consumption and to expand their scope, to renew, strengthen and effectively implement environmental policies (de Andrade et al., 2020).

When the production technologies are examined, it is seen that the pasteurization method is frequently used. The food industry is responsible for approximately 30% of the world's total energy consumption and 22% of the total greenhouse gas emissions. Energy consumption depends on processing time and temperature, and the primary energy consumption in juice bottling plants emerges from juice pasteurization, bottle cleaning, and cooling. Water footprints between 0.6 and 1.48 L per liter of juice are reported in the juice industry (Esturo et al., 2023). Some processing methods can reduce water consumption by recycling or reusing water at different stages of production. It is thought that using these methods can reduce the blue water footprint.

In a study investigating greenhouse gas emissions from fruit production, the carbon footprint of fruit production in China was found to be 0.24, 0.27, 0.14, 0.37, and 0.18 kg CO2 -eq/kg for apples, bananas, oranges, peaches, and pears, respectively (Yan et al., 2016). In different studies conducted in Türkiye, the greenhouse gas rate for orange production was determined as 0.08 kg CO2 -eq/kg (Saltuk et al., 2022), and for apple, grape, pomegranate, and strawberry as 0.09, 0.10, 0.15, and 0.78 kg CO2 -eq/kg, respectively (Eren et al., 2019). On the other hand, the waste generated during fruit juice production from citrus fruits corresponds to approximately half of the whole fruit weight and 10–30% of the fruit weight for apple juice production (Esturo et al., 2023). Sour cherries, pomegranates, and strawberries are other industrial fruits that generate high amounts of waste. In light of this information, it is thought that peach, which constitutes 9.6% of the products in the Turkish market examined in this study, and strawberry, which constitutes 0.5%, may pose a disadvantage in terms of carbon footprint. Citrus fruits, which constitute 13.4% of the products in the Turkish market, sour cherries, which constitute 8%, and pomegranate, which constitutes 2.7%, may pose a disadvantage in terms of food waste.

More sustainable product supply chains are those that optimize the use of materials, water, and energy throughout their lifecycle while minimizing waste from products and used packaging (Russell, 2014). However, in a study involving participants with different roles in juice production from 20 countries, it was reported that only 44.11% of companies implemented measurable targets for reducing, reusing, and recycling packaging materials, in addition to systems to reduce the use of energy and materials (Esturo et al., 2023). Most fruit juices and similar beverages examined in this study had Tetra Pak, glass, and PET-type packaging. The amount of waste originating from packaging used to store beverages, such as Tetra Pak, is constantly increasing, and most of this waste is treated as garbage by landfilling and incineration. This causes a massive waste of resources and environmental pollution problems. For this reason, converting Tetra Pak wastes into valuable chemicals or fuels is thought to benefit the economy and the environment. In addition, solutions continue to be developed for the difficulties of recycling Tetra Pak wastes (Ma, 2018). Most food packaging, such as plastic, glass, and tin, can be recycled. Bottle-to-bottle recycling provides a significant reduction in environmental burdens. PET, a polyester plastic, is one of the most widely used packaging materials for beverages. A PET bottle is more easily recycled due to its single-layer and single-material composition. Thus, glass bottles have performance advantages not found in alternative packaging options such as aluminum cans, cardboard boxes, and other plastics. Compared to glass, PET bottle is lighter and has a lower carbon footprint in production and transportation (Benyathiar et al., 2022). Another thing to consider when choosing packaging is the acidic content of soft drinks, which makes the metals in the cans more soluble. In a study investigating exposure to potentially toxic elements through the consumption of non-alcoholic beverages such as flavored beverages and energy drinks offered for sale in Türkiye, it was found that in some samples, at least one of the cadmium, nickel, iron and manganese contents were measured above the threshold values determined by official authorities. Before a particular material is used as packaging, the possibility of contamination of contaminants from the packaging to the food must be evaluated through tests (Yüksel et al., 2023). On the other hand, essential and non-essential element concentrations of fruit juices produced by some commercial brands in Türkiye were determined and a health risk assessment was made. When risk analysis was performed by calculating the hazard indexes of non-essential, trace and ultra trace elements and the target carcinogenic risks, it was determined that the HI and TR values of the samples were less than 1 and 1x10−4, respectively. As a result, all samples were evaluated in the low risk group. (Demir et al., 2020). The packaging of a product is linked to SDGs 12 as well as SDGs 3. In this sense, active packaging, eco-design packaging, transportation options, and systems based on returnable glass bottles or easily recyclable materials are foreseen as improvements that can be planned by the fruit juice

industry in the future (Esturo et al., 2023).

The limitations of the study are that fruit-flavored acidic beverages, fruit-flavored sodas and mineral waters, fruit-flavored beverage powders were not included in the study.

CONCLUSION

It has been determined that fruit juices and similar beverages in the Turkish market are inadequate in terms of sustainability in terms of ensuring a healthy and quality life at all ages as they have a high sugar and sodium content, beverages other than fruit juice have added sugar content, and fruit nectars and fruit beverages contain sweeteners. One possible way to balance the health benefits and harms of these products is to consume them in moderation and to choose beverages with a lower energy density and higher nutritional value that do not contain added sugar or artificial sweeteners.

In this study, considering the environmental effects of the sugar beet and fructose syrup production industry, it was observed that the addition of sucrose obtained from sugar cane and sugar beet and the addition of fructose syrup to fruit juices and similar beverages contradict some of the sustainable development goals. It is also clear that the fruit juice industry has deficiencies in terms of sustainability, as sustainable product supply chains that optimize the use of materials, water and energy, and technologies that will minimize waste from products and used packaging have not yet been provided. In light of the data obtained from this study, it is thought that it would be beneficial for the fruit juice industry to make regulations to ensure sustainable production and consumption patterns. For the sustainability of the fruit juice industry, it is of great importance for fruit juice producers to adopt innovative technologies that will reduce water use, sustainable agricultural practices, waste management strategies such as the reuse and recycling of packaging materials, and post-production food waste. It is also foreseen that reducing the chemicals used in the production process of fruit juices and similar beverages and using alternative, natural and biological solutions will also be effective in terms of sustainability. Fruit juice producers should take a sensitive approach to human health by producing fruit juice with clean content and reduced sugar content. At the same time, producers should support the sustainability of food systems in terms of nutrition with 100% sustainable fruit juices by producing solutions against environmental problems such as decreasing water resources, consumed energy, climate change and reducing food residues, and meeting consumer demands. For the sustainability of the fruit juice industry, not only the producers but also the consumers must take responsibility. Consumers should prefer organic, local, and sustainable fruit juices, primarily seasonal fruits, in order to support sustainable fruit juice production.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors have no conflict of interest to disclose.

Author contribution

Berk G. and Kayhan B. collected the study data and contributed significantly to the content. Kaçar M. contributed significantly to the content and revised it critically for important intellectual content, and approved the final content of the manuscript. Özenir Ç. designed and drafted the work and revised it critically for important intellectual content and final approval of the version to be published. All authors have read and agreed to the published version of the manuscript.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

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Not applicable.

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RESEARCH ARTICLE

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Determination of some quality parameters in early maturing tomato lines

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Abstract
In recent years, tomato breeding research, as in many vegetable species, has focused on improving the intrinsic quality of the fruit. To identify the superior lines in terms of biochemical properties, 20 early maturing tomato lines were evaluated. Yields of the lines used in this work ranged from 2.5 to 9.2 kg per plant, with average fruit weights between 86 and 246 g. Consequently, L* values for tomato fruit varied from 30.87 to 45.35, a* values from 8.36 to 21.48 and b* values from 15.28 to 42.17. The values of titratable acidity, pH, brix, total carotene, total xanthophyll, ascorbic acid, and lycopene in tomato fruits changed from 0.27 to 0.40%, from 3.75 to 4.95, from 2.60 to 6.30%, from 80.2 to 197.5 mg/100 g, from 115.3 to 256.6 mg/100 g, from 10.50 to 28.78 mg/100 g, and from 1.6 to 4.09 mg/100 g, respectively. The contents of soluble and reducing sugars ranged from 7.31 to 17.51 mg/g and 2.46 to 6.57 mg/g respectively. According to these values, the lines with the highest biochemical properties were L7, L17 and L3. This data could then be used as a genetic resource in breeding programmes for the development of new varieties.
Keywords: Tomato, Breeding, Quality, Biochemical, *Solanum esculentum*

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INTRODUCTION

Tomato is one of the most produced, consumed, and traded vegetable in the world. It is consumed as fresh or used as processing tomato, which is becoming more significant on a global scale to produce tomato products such as tomato paste, juice, sauce, puree, soup, dried tomatoes, ketchup, and tomato powder (Silva et al., 2019). The total world production of tomato, the most cultivated vegetable in the world, is 189,133,955 tons according to 2021 data, and it is produced in every part of the world except Antarctica. Asia supplies 63.0%, America 12.5%, Europe 12.9%, and Africa 11.3% of the world tomato production. Shares of the continents in tomato production, also show that tomato is a product that can be offered to all world markets. In terms of tomato production, Türkiye ranks 3rd in the world with 13,095,258 tons of production after China (67,636,725 tons) and India (21,181,000 tons). Tomato is the most exported fresh vegetable in the world. Among the countries exporting fresh tomatoes, Mexico ranks first with 1,903,779 tons, while Türkiye ranks 5th with 606,583 tons (FAO, 2023).

Tomato has important effects on human health and nutrition. It is very rich in micronutrients and antioxidants that are important for human nutrition (Carli et al., 2011). Tomato with its low calorie and low fat content, is characterized as a healthy vegetable with lycopene, β-carotene, niacin, vitamins A, B, C, and K, and its mineral substances, such as potassium, calcium, and iron (Willcox et al., 2010; Yahia and Brecht, 2012). It is very important for health, with its powerful antioxidant content (Khalil et al., 2022) such as vitamins A, C, and E, which help to

reduce the risk of cancer. It is also a rich source of lycopene, which gained importance with the development and application of lycopene in food, medicine, cosmetics, and other fields (Xie et al., 2022), and consumption of tomatoes is effective in reducing the risk of cancer death (Mazidi et al., 2020). The ratio of protein and fat in tomatoes is lower than that of carbohydrates indicates that tomatoes are an important source of dietary fiber (Gölükçü et al., 2016), especially in the prevention of common obesity disease (Zhu et al., 2020). The most prevalent phenolic components in tomatoes are quercetin, kaempferol, naringenin, caffeic acid, and lutein. Several of these substances are beneficial in defending the body against various oxidative stress-related disorders and contain antioxidant properties. Tomato consumption reduces oxidative stress by increasing the body's antioxidant levels, trapping reactive oxygen species, and reducing oxidative damage to important macromolecules like DNA, enzymes, proteins, and membrane lipids (Ali et al., 2021).

Until today, phenotypic properties, such as disease resistance, productivity, shelf life for transportation and marketing, fruit color, and fruit size have been emphasized in breeding studies (Şimşek, 2013). Breeding studies on tomatoes have been largely limited to these parameters. Most of the breeding studies have been focused on producers, seed producers and retailers. Quality parameters, such as flavor, aroma, taste, and healthy ingredients (biochemicals compounds), which are the direct focus of the consumer, were not taken into account (Heuvelink, 2005). On the other hand, taste and aroma substances in tomatoes are among the criteria consumers consider (Dorais et al., 2001). Although tomato is the most produced and consumed vegetable, it is necessary to study to improve fruit quality, such as nutritional content, aroma, and functional compounds (Rodrigues et al., 2022; Ruiz-Cisneros et al., 2022). Therefore, the present study aimed to determine superior tomato lines in terms of some fruit quality properties.

MATERIALS AND METHODS

In the study, 20 purified semi-determinate tomato lines showing early maturing characteristics were selected and their general characteristics are given in Table 1. The study was planned according to the randomized plots experimental design with three replications and 10 plants in each replicate. The spacing between plants was planned as 40 cm, between narrow rows as 50 cm, and between wide rows as 150 cm.

The experiment was conducted in the plastic greenhouse (36° 57′ 6″ N, 30° 57′ 42″ E; 16 m above the sea level) of Enza Zaden vegetable breeding station (Antalya, Türkiye), in 2017. Average temperature (20.3°C) and relative humidity (73.1%) values were obtained for 4 months in 2017, where the experiment was conducted (Figure 1).

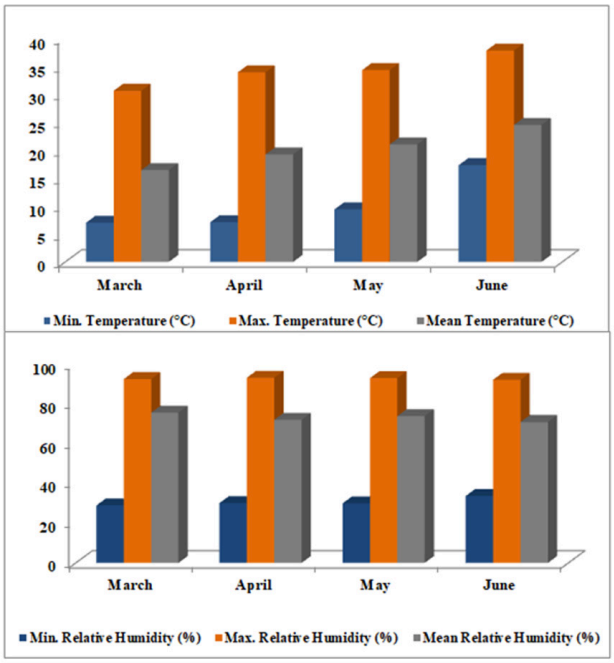

Figure 1. Meteorological data of the research area

The soil texture of the study area was clay-loam structure and its physicochemical properties were given in Table 2.

The physical and chemical analyses of the tomato fruits were carried out in the Horticulture Laboratory of Isparta University of Applied Sciences.

Color determination

L^* (brightness), a^* (redness), and b^* (yellowness) values of fruits were determined by measuring two opposite surfaces in the equatorial region of the fruits with a CR 400 Model Minolta colorimeter (Japan). The values of chroma (C^*) and hue angle (h°) were calculated according to equation 1 and equation 2, respectively (McGuire, 1992).

$$C^* = \sqrt{(a^*2+b^*2)} \quad (1)$$

$$h^\circ = \arctan(b^*/a^*) \quad (2)$$

Determination of pH, titratable acidity (TA) and brix

For the determination of titratable acidity, 10 mL of the prepared fruit juice samples were taken and titrated with 0.1 N NaOH solution until pH reached 8.1. Results were given in % of citric acid (Cemeroğlu, 1992). The pH value was determined by dipping the digital pH meter probe (Hanna HI 2211, Romania) into the prepared fruit juice. Brix values of tomato fruits were determined as % (brix°) using a digital refractometer (Hanna HI96801, Romania).

Determination of total soluble and reducing sugars

For the extraction of sugars, samples (5 g) were first homogenized in 80% ethanol, incubated overnight at -20°C and then centrifuged at 2000 x g for 5 min. The supernatant was used for the determination of both total soluble and reducing sugars. The amount of reducing sugars was measured according to Honda et al. (1980),

Table 1. General properties of early maturing tomato lines

LINES	Generation	Yield per Plant (kg)	Average Fruit Weight (g)	Disease Resistance
Line-1	F10	9.2	186	Clad, Fol, Forl, ToMV, TSWV, Vd
Line-2	F10	5.6	201	Clad, Fol, Forl, ToMV, Vd
Line-3	F17	3.1	86	Fol, Mi, Vd
Line-4	F6	8.1	202	Fol, Forl, Vd
Line-5	F6	5.9	171	Clad, Fol, Vd
Line-6	F7	5.4	135	Clad, Fol, ToMV
Line-7	F8	5.1	170	Clad, Fol, Forl, Mi, ToMV
Line-8	F6	5.2	218	Clad, Fol, Forl, ToMV
Line-9	F6	6.2	124	Clad, Fol
Line-10	F6	6.1	136	Clad, Fol, Forl
Line-11	F7	4.5	190	Clad, Fol, Vd
Line-12	F6	7.0	160	Fol, Forl
Line-13	F6	5.9	153	Clad, Fol, Forl
Line-14	F8	8.7	218	Fol, TYLCV, Vd
Line-15	F7	3.5	175	Fol, TSWV, TYLCV, Vd
Line-16	F7	2.5	235	Fol, TSWV
Line-17	F7	7.9	166	TSWV, TYLCV, Vd
Line-18	F8	2.9	145	Vd
Line-19	F7	3.9	246	Fol, Mi, Vd
Line-20	F5	7.0	195	Fol, Vd

Clad: ***Cladosporium fulvum*** **Fol:** ***Fusarium oxysporum*** f. sp. ***lycopersici*** **Forl:** ***F. oxysporum*** f. sp. ***radicis lycopersici*** **Mi:** *Melodigyne incognita* nematode **ToMV:** Tomato Mosaic Virus **TSWV:** Tomato Spotted Wilt Virus **TYLCV:** Tomato Yellow Leaf Curl Virus **Vd:** ***Verticillium dahliae***

Table 2. Physicochemical properties of the soil of experimental area

Soil Properties	Results	Unit
pH	7.7	-
Electrical conductivity (EC)	1099	µs/cm
Organic matter	1.31	%
Total N	0.103	%
Available P	26.74	kg P_2O_5/da
Available K	114.7	kg K_2O/da
Ca	1774.9	kg CaO/da
Mg	191.5	kg MgO/da
Na	49.20	ppm
Fe	28.10	ppm
Mn	14.96	ppm
Zn	0.70	ppm
Cu	3.61	ppm

and the amount of total soluble sugars was determined by the phenol sulfuric acid method (DuBois et al., 1956). Both assays were conducted using glucose as a standard, which varied at concentrations of 40, 80, 120, and 200 g/ml.

Determination of total carotene and xanthophyll

To extract carotenes and xanthophylls, 1 g of fruit flesh was homogenized with 10 ml of acetone: hexane (4:6) mixture. Samples mixed with vortex for 30 seconds were shaken on a shaker at 200 rpm for 15 min. The upper phase was removed and an equal amount of 20% NaCl solution was added and mixed. Then, the upper phase was taken again and an equal amount of 20% NaCl solution was added and mixed. Readings were performed at wavelengths of 436 nm to detect carotenes in the samples and 474 nm for xanthophylls. Total carotene and xanthophyll content was calculated according to AOAC (1984).

Estimation of total soluble phenolics

Fruit samples (5 g) were homogenized in 10 mL 95% ethanol for 2.5 min and the resulting mixture was boiled for 10 min and then centrifuged at 8000 rpm 10 min. Samples were filtered through filter paper. 10 mL of 80% ethanol was added and boiled for 10 minutes. After boiling, the supernatant was made up to 100 mL with 80% ethanol. Total phenol content analysis was then

performed according to Coseteng and Lee (1987) using the Folin-Ciocalteu reagent. Absorbance values of the samples were read at 760 nm with a spectrophotometer and the results were reported as mg/g.

Determination of ascorbic acid

A homogeneous mixture was obtained by adding an equal amount of 6% metaphosphoric acid solution to 250 g sample. Twenty five grams of slurry was placed in a graduated cylinder and volume was brought to 100 mL with a 3% metaphosphoric acid solution. After shaking the samples, they were filtered and 10 mL of the filtered samples were titrated with 2.6 dichlorophenolindophenol solution. The amount of ascorbic acid in the samples was calculated using the equation specified in Cemeroğlu (1992).

Ascorbic acid (mg/100 g)= (V x Fx 100)/W

V: Volume of 2,6-dichlorophenolindophenol solution used for titration (ml)
F: Factor of 2,6% dichlorophenolindophenol solution
W: Amount of sample in filtrate used for titration (g)

Determination of lycopene and ß-Carotene

Fruit samples were first homogenized in acetone: hexane mixture (4:6) for extraction. Then, readings were made in the spectrophotometer at different wavelengths (663, 645, 505, and 453 nm). Lycopene and ß-Carotene amounts were calculated according to the formulas specified in Nagata and Yamashita (1992), and the results were expressed as mg/100g.

Statistical analysis

Statistical analysis was performed in triplicate using the Minitab (17) Inc. package program. One-way analysis of variance (ANOVA) was used to analyse the data. Significance of means were compared with Tukey's multiple range test at $P < 0.05$ level of significance. Principal component analysis (PCA) was performed using version 4.3.1 of the R statistical package.

RESULTS AND DISCUSSION

In terms of color values, the difference between 20 tomato lines was significant ($P < 0.05$) (Table 3). The L^* values obtained in the lines ranged from 30.87 to 45.35. Lines L3 (45.35), L1 (43.63), L13 (43.42), L10 (42.49), L16 (42.33), and L11 (42.27) came to the fore in terms of L^* values. The lowest L^* values were obtained from the L19, L5, and L17 at 30.87, 31.21, and 31.17, respectively. It was reported that L^* values vary between 32.0-38.6 (Bhandari et al., 2016a), 36.95-45.68 (Borghesi et al., 2011), 40.56-45.07 (Gözükara and Kaplan, 2017). Our findings are in agreement with these reports. The range of chroma values was 16.14-45.88. The highest chroma value was determined from L3 (45.88), followed by L7 (40.39), L10 (39.25), and L16 (38.90), while the lowest chroma values were L19 (16.14), L5 (16.41), and L9 (17.96). According to Viskelis et al. (2015) and Gözükara and Kaplan (2017), the chroma values in various tomato cultivars ranged from 39.20 to 47.23 and 27.36 to 32.81, respectively. The lines' color angles were assessed and it was found that L1 had the highest (69.72) and L17 had the lowest value (45.86). Sacks and Francis (2001) found hue values ranging from 45.8 to 59.7. a^* value represents the red color, the lowest a^* value among the lines was 8.36 (L19) and the highest a^* value was 21.48 (L7) followed by 19.05 (L10). In different studies, a^* values varied between 21.1-25.0 (Hernandez et al., 2007) and 24.70-34.29 (Viskelis et al., 2015). The reason for low a^* values observed in the present study is that the selection criteria of the lines included orange fruit colour. The b^* value was found to be the lowest in L5 (13.59), and the highest in L3 (42.17) followed by L16 and L10. The lowest b^* value was obtained from the L5, L19, and L17. Bhandari et al. (2016a) found that the b^* values ranged from 13.8 to 27.0 in 7 tomato breedin lines; Renna et al. (2018) found that the b^* values for the Regina tomato variety ranged from 30.3 to 41.0.

Table 4 shows that there is a significant difference for pH and titratable acidity values among the tomato lines ($P < 0.05$). The pH range for tomato lines was from 3.75 to 4.95. The pH values of tomato ranged from 3.75 to 4.50 (Acharya et al., 2018), from 3.8 to 4.5 among Kenyan tomato germplasms (Agong et al., 2001), from 4.19 to 4.49 among Tunisian tomato germplasm (Aoun et al., 2013). Different studies also placed pH value of tomato fruits from 3.41 to 5.46 (Dar et al., 2012; Frusciante et al., 2007; Figueiredo et al., 2017; Kumar et al., 2016; Liu et al., 2017; Peixoto et al., 2018; Turhan et al., 2011). In the present study, L6 had the highest pH value (4.95), followed by L16 and L4, whereas L13 (3.75), L9 (3.96) and L8 (4.11) had the lowest pH values, which are consistent with previous reports. Titratable acidity values among the tomato lines were between 0.27 to 0.40%. Line L13 (0.40%) had the highest acidity value followed by L9 (0.38%), L20 (0.36%), and L18 (0.36%). Titratable acidity values were between 0.28 and 0.49% among 40 tomato genotypes (Kumar et al., 2016). Other researchers reported values ranging from 0.35% to 0.46% (Stommel et al., 2005), from 0.27% to 0.75% (Ruggieri et al., 2014), from 0.27 to 0.37% (Sio et al., 2018), and our results were within the reported ranges.

The difference between brix values was also significant at $P < 0.05$ level of signifance. As seen in Table 4, the brix values of the lines have changed between 2.60% and 6.30%. L7 (6.30%), L17 (5.10%), and L2 (4.75%) had the highest brix values, whereas L5 (2.60%) and L20 (2.85%) had the lowest brix values. Brix values of different tomato genotypes ranged from 3.12% to 6.03% (Al-Aysh et al., 2012; Giorio et al., 2007; Pal et al., 2018; Raj et al., 2018). However, Hanson et al (2004) found that brix values ranged from 3.6 to 8.6% among *L. esculentum*, *L. pipinellifolium* genotypes.

The difference in total soluble sugar content between

Table 3. Fruit skin color of tomato lines (L*, C*, h°, a*, b*)

Lines	L*	Chroma (C*)	Hue (h°)	a*	b*
L-1	43.63 a*	36.08 bcd	69.72 a	12.49 c-g	33.84 bc
L-2	39.68 abc	29.79 de	65.79 abcd	12.23 c-g	27.10 de
L-3	45.35 a	45.88 a	66.96 abc	17.97 abc	42.17 a
L-4	34.75 b-f	21.79 g	52.03 ef	13.41 b-g	17.10 f
L-5	31.21 f	16.41 g	55.99 cdef	9.18 efg	13.59 f
L-6	39.41 a-d	32.92 cde	57.66 b-f	17.59 abcd	27.83 de
L-7	39.13 a-e	40.39 ab	57.92 a-e	21.48 a	34.17 bc
L-8	39.07 a-e	30.15 de	59.64 a-e	15.28 a-e	25.98 e
L-9	33.10 def	17.96 g	59.68 a-e	9.06 efg	15.28 f
L-10	42.49 a	39.25 bc	60.86 a-e	19.05 ab	34.23 bc
L-11	42.27 a	32.82 cde	65.68 abcd	13.53 b-g	29.83 cde
L-12	39.08 a-e	29.06 ef	58.99 a-e	14.99 b-e	24.89 e
L-13	43.42 a	34.75 bcde	68.03 ab	12.98 b-g	32.23 bcd
L-14	33.91 c-f	18.99 g	62.87 a-e	8.62 fg	16.91 f
L-15	40.55 ab	33.27 cde	59.60 a-e	16.83 abcd	28.64 cde
L-16	42.33 a	38.90 bc	67.94 ab	14.74 b-f	35.98 b
L-17	31.17 f	20.41 g	45.86 f	14.13 b-g	14.66 f
L-18	35.35 b-f	22.53 fg	54.98 def	12.96 b-g	18.42 f
L-19	30.87 f	16.14 g	58.87 a-e	8.36 g	13.79 f
L-20	32.81 e-f	19.09 g	53.56 ef	11.36 d-g	15.34 f

*Average values with different letters in the same column differ significantly by Tukey test at P < 0.05.

Table 4. pH, titratable acidity, brix, total soluble sugars and reducing sugars values of tomato lines.

Lines	pH	Titratable Acidity (%)	Brix (%)	Total Soluble Sugars (mg/g)	Reducing Sugars (mg/g)
L-1	4.57 bcd*	0.27 fg	4.00 efg	11.59 b-f	5.46 bc
L-2	4.35 cde	0.30 ef	4.75 bc	12.51 bcd	5.85 ab
L-3	4.42 bcde	0.30 def	4.35 cde	11.45 b-f	4.75 cd
L-4	4.66 abc	0.27 fg	3.05 jkl	8.80 efg	3.41 gh
L-5	4.40 cde	0.31 de	2.60 l	8.65 efg	3.32 hı
L-6	4.95 a	0.24 g	4.60 cd	14.16 ab	5.51 bc
L-7	4.45 bcd	0.30 def	6.30 a	17.51 a	6.57 a
L-8	4.11 ef	0.34 cd	4.20 def	11.90 bcde	4.47 d
L-9	3.96 fg	0.38 ab	3.95 efgh	8.75 efg	3.31 hı
L-10	4.48 bcd	0.29 ef	3.50 hıj	10.42 c-g	4.29 def
L-11	4.52 bcd	0.28 ef	3.40 ijk	8.16 fg	3.29 hı
L-12	4.51 bcd	0.29 ef	4.25 def	11.09 b-f	4.50 d
L-13	3.75 g	0.40 a	2.95 kl	7.31 g	2.46 ı
L-14	4.47 bcd	0.30 def	3.60 ghı	9.48 defg	3.37 h
L-15	4.51 bcd	0.29 ef	4.50 cd	12.05 bcde	4.48 d
L-16	4.75 ab	0.27 fg	4.50 cd	12.93 bcd	4.48 d
L-17	4.61 abcd	0.28 ef	5.10 b	13.16 bc	4.46 de
L-18	4.34 cde	0.36 bc	3.80 fghı	10.77 b-g	4.25 defg
L-19	4.48 bcd	0.30 ef	3.45 ij	10.74 b-g	3.46 fgh
L-20	4.30 def	0.36 abc	2.85 l	8.46 efg	3.59 efgh

*Average values with different letters in the same column differ significantly by Tukey test at P < 0.05.

the lines was significant (P < 0.05) (Table 4). The highest value of 17.51 mg/g found in L7, and the lowest value was found in L13 with 7.31 mg/g. Al-Aysh et al. (2012) evaluated 14 different tomato genotypes for yield and quality properties. They reported that the total sugar content of the tomato genotypes ranged from 2.62% to 3.25%. In other studies, total soluble sugar content of tomato genotypes ranged from 2.01% to 3.96% (Kumar et al., 2016), from 1.67% to 3.73% (Turhan and Şeniz, 2009). Table 4 shows a significant difference (P < 0.05) in the amount of reducing sugars among the tomato lines. The range of reducing sugar content was from 2.46 to 6.57 mg/g. Lines L7 (6.57 mg/g) followed by L2 (5.85 mg/g) and L6 (5.51 mg/g) had the highest reducing sugar concentration while L13 (2.46 mg/g) had the lowest amount of reducing sugars, followed by L11

Table 5. Total carotene, xanthophyll, soluble phenolics, ascorbic acid, lycopene and β-carotene content of the tomato lines

Lines	Carotene (mg/100 g)	Xanthophyll (mg/100 g)	Soluble Phenolics (mg/g)	Ascorbic Acid (mg/100 g)	Lycopene (mg/100 g)	β-carotene (mg/100 g)
L-1	80.2 ^{g*}	115.3 ^{h}	0.46 ^{de}	27.47 ^{a}	2.21 ^{hı}	1.24 ^{cde}
L-2	93.6 ^{fg}	145.5 ^{fgh}	0.38 ^{e}	16.61 ^{efg}	2.30 ^{h}	1.22 ^{cde}
L-3	124.6 $^{c-g}$	195.8 $^{b-g}$	0.31 ^{e}	22.47 ^{bc}	4.09 ^{a}	1.41 $^{a-e}$
L-4	97.4 ^{efg}	153.4 ^{efgh}	0.69 ^{cd}	13.99 ^{ghij}	3.54 ^{de}	1.29 ^{bcde}
L-5	130.3 ^{cdef}	203.6 $^{a-f}$	0.83 ^{abc}	19.60 ^{cde}	1.82 ^{j}	1.69 ^{abc}
L-6	121.6 $^{c-g}$	199.8 $^{a-g}$	0.71 ^{bcd}	25.46 ^{ab}	3.69 ^{cd}	1.20 ^{cde}
L-7	144.8 ^{bcde}	232.7 ^{abc}	0.93 ^{abc}	27.13 ^{a}	3.89 ^{b}	1.86 ^{a}
L-8	149.8 ^{abcd}	224.6 ^{abcd}	0.72 ^{bc}	10.50 ^{j}	2.50 ^{g}	1.18 ^{de}
L-9	113.4 ^{defg}	190.4 $^{c-g}$	0.78 ^{abc}	11.79 ^{ij}	2.05 ^{ı}	1.07 ^{e}
L-10	127.6 $^{c-g}$	208.5 $^{a-e}$	0.82 ^{abc}	12.09 ^{hij}	3.79 ^{bc}	1.29 ^{bcde}
L-11	89.6 ^{fg}	140.9 ^{gh}	0.81 ^{abc}	16.02 ^{efg}	2.61 ^{g}	1.23 ^{cde}
L-12	168.8 ^{abc}	241.0 ^{abc}	0.79 ^{abc}	14.78 ^{ghı}	2.57 ^{g}	1.47 $^{a-e}$
L-13	113.8 ^{defg}	186.5 $^{c-g}$	0.86 ^{abc}	19.60 ^{cde}	2.26 ^{h}	1.77 ^{ab}
L-14	144.6 ^{bcde}	219.1 ^{abcd}	0.95 ^{ab}	21.82 ^{cd}	1.6 ^{k}	1.48 $^{a-e}$
L-15	112.4 ^{defg}	171.4 $^{d-h}$	0.92 ^{abc}	15.46 ^{fgh}	3.71 ^{bcd}	1.90 ^{a}
L-16	114.8 ^{defg}	189.5 $^{c-g}$	0.88 ^{abc}	18.51 ^{def}	2.13 ^{hı}	1.31 ^{bcde}
L-17	197.5 ^{a}	256.6 ^{a}	0.97 ^{a}	28.78 ^{a}	3.47 ^{e}	1.54 $^{a-e}$
L-18	164.7 ^{abc}	236.3 ^{abc}	0.95 ^{ab}	21.24 ^{cd}	3.05 ^{f}	1.64 ^{abcd}
L-19	187.5 ^{ab}	251.8 ^{ab}	0.87 ^{abc}	14.40 ^{ghı}	1.85 ^{j}	1.58 ^{abcd}
L-20	150.0 ^{abcd}	235.8 ^{abc}	0.84 ^{abc}	20.74 ^{cd}	2.93 ^{f}	1.16 ^{de}

*: Average values with different letters in the same column differ significantly by Tukey test at $P < 0.05$.

(3.29 mg/g) and L9 (3.31 mg/g) (Table 4). According to a study carried out on four varieties of tomato, the levels of Reducing sugars content could be between 0.64% to 3.86% (Adedeji et al., 2006; Kumar et al., 2016).

There were significant differences for the biochemical indices presented in Table 5 ($P < 0.05$). Tomato line L17 was found to have the highest total carotene concentration with a value of 197.5 mg/100 g. As can be seen, the lines with the highest levels of total carotene are after line L17 were L12, L18 and L19. The lowest total carotene content was found in L1, L11 and L2. Bhandari et al. (2016b) reported that total carotene content of tomato varieties ranged from 76.87 to 110.27 mg/100 g, and carotene content of three high lycopene tomato cultivars from 105-278 mg/kg (Ilahy et al., 2011). Kavitha et al. (2013) investigated ascorbic acid, total phenols, total flavonoids, total carotenes and lycopene levels and total carotene concentrations ranged from 90.4 to 220.8 mg/kg.

Total xanthophyll levels of the tomato lines range from 115.3 to 256.6 mg/100 g. L17 (256.6 mg/100 g) and L19 (251.8 mg/100 g) had the highest xanthophyll levels, while L1 (115.3 mg/100 g), L11 (140.9 mg/100 g) and L2 (145.5 mg/100 g) had the lowest levels of xanthophyll. Schweiggert et al. (2017) investigated different parameters, such as lutein, beta-carotene, lycopene, total carotenoids and xanthophylls, and reported that xanthophyll content ranged from 2.9 to 10.7 g/g among the tomato genotypes.

Total soluble phenolics content of the lines was between 0.31 to 0.97 mg/g, L17, L18 and L14 had the highest total phenolics content among the tomato lines. The lines with the lowest total phenolics content were L3 (0.31 mg/g) and L2 (0.38 mg/g). Pal et al. (2018) found that the total phenolics content of 22 tomato selections ranged from 0.60 to 1.14 mg/g, from 0.11 and 0.31 mg/g (Ilahy et al., 2011) and from 0.20 and 1.34 mg/g (Kavitha et al., 2013).

The highest ascorbic acid content of the tomato lines were 28.78 mg/100 g (L17), 27.47 mg/100 g (L1) and 27.13 mg/100 g (L7). The line with the lowest concentration of ascorbic acid was L8 (10.50 mg/100 g). Ascorbic acid content of green house grown tomates varied between 8.26-22.54 mg/100 g (Bhandari et al., 2016a). Other research also reported different levels of ascorbic acid for different tomato genotypes ranging from 19.77 to 33.41 mg/100 g (Dar and Sharma, 2011), from 8.0-15.6 mg/100 g (Frusciante et al., 2007), and from 11.6-39.7 mg/100 g (Hanson et al., 2004). These reports are consistent with our findings.

Lycopene content of the tomato lines ranged from 1.60 to 4.09 mg/100 g. Results revealed that L3 (4.09 mg/100 g) had the highest lycopene value among the lines, followed by L7 and L10, with values of 3.89 and 3.79 mg/100 g; respectively. The results also showed that L14 had the lowest lycopene content (1.6 mg/100 g), followed by L5 (1.82 mg/100 g) and H19 (1.85 mg/100 g). D'Ambrosio et al. (2004) reported that the lycopene content of tomato genotypes ranged from 1.0 to 4.5 mg/g. Lycopene values were found to vary between 0.95-5.12 mg/100g (Bhandari et al., 2016a), 1.98-4.62 mg/100

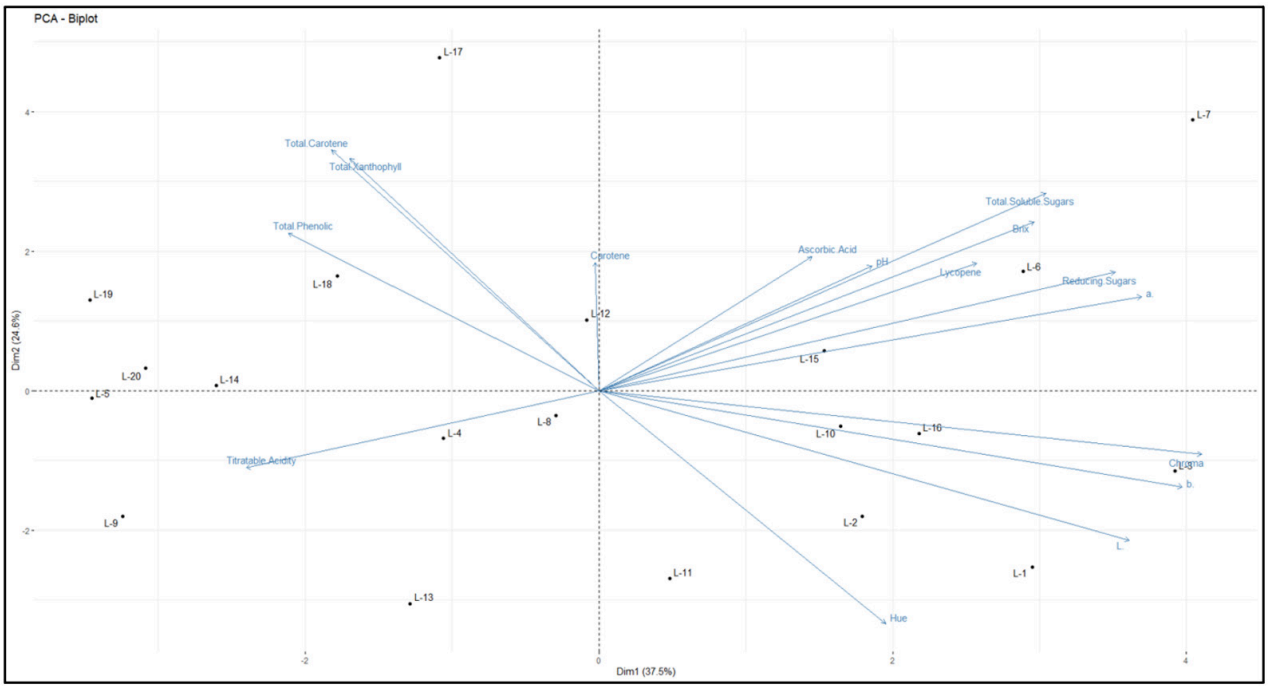

Figure 2. Principal component analysis (PCA) of some biochemical properties of 20 early maturing tomato lines

g (Dar and Sharma, 2011), 0.20-1.85 mg/100 g (Gautam et al., 2018), and 2.84-9.83 mg/100 g (Pal et al., 2018). Our results are in agreement with these literature reports.

The lines with the highest β-carotene levels were L15 and L7, with values of 1.90 mg/100 g and 1.86 mg/100 g, respectively. After the L15 and L7, the highest β-carotene value was found in L13 (1.77 mg/100 g). L9 had the lowest β-carotene value with 1.07 mg/100 g, while L20 and L8 both had low β-carotene values with 1.16 mg/100 g and 1.18 mg/100 g, respectively. β-carotene values of 60 tomato genotypes ranged between 1.09-2.53 mg/100 g (Dar and Sharma, 2011). Tomlekova et al (2007) investigated the lycopene and β-carotene levels of 7 tomato genotypes. They reported that the β-carotene levels of these genotypes varied between 1.28-2.84 mg/100 g.

Principal component analysis (PCA) was performed to minimise the dimensionality of the datasets and to visually identify the differences and similarities between the tomato lines (Figure 2). The biochemical parameters that determine the positions of these lines in the PCA plane are indicated by arrows. While these values were high in the lines in the directions where the biochemical parameters were shown, the parameters in the opposite direction were low. L7, L17 and L3 lines showed high positive collinearity, while L9 and L13 lines showed negative collinearity by clustering on the opposite negative side of the PCA graph. The PCA graph, in which the measured parameters and the tomato lines are plotted together, supports our statistical results.

CONCLUSION

In this study, L7 line was found to be the richest line in terms of redness of skin colour with the highest a* value, brix, total and reducing sugars content. These characteristics of the line will be particularly important in the flavour studies to be carried out. L17 line had the highest values for total carotene, xanthophyll, vitamin C and total soluble phenolics content. Recently, with the growing importance of the relationship between food and health, interest in functional foods has increased. It could be surmised that L17 will have a high antioxidant property and be beneficial in quality parameters. In addition, L3 with high values for L*, a*, b*, chroma and lycopene content, it could be concluded that it is a line that should be particularly evaluated in colour studies.

As a result of the study, it was found that there was a wide variation between the lines for all the parameters studied. The wide variation of lines is very important in terms of being a source of breeding studies for the desired characters of new varieties to be realized. All over the world, the number of studies on the improvement of quality traits in breeding is increasing. To this end, it is hoped that the present study will be useful in breeding studies where quality criteria are prioritized.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

All of the authors declare that they have no conflicts of interest.

Author contribution

All authors contributed equally to this study. All authors read and approved the final manuscript. All authors confirm that the text and tables are original and have not been published previously.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

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Not applicable.

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RESEARCH ARTICLE

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Effect of molybdenum application in pepper (*Capsicum annuum* L.) under cold stress conditions

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Abstract

Cold stress (low temperature stress) is one of the abiotic stress factors. It causes many morphological and physiological problems in plants. One of the applications to eliminate and alleviate these negative effects is molybdenum application. The aim of this study was to determine the effect of molybdenum application on cold stress in commercial variety Mazamort pepper. In the experiment, control, 25 ppm molybdenum concentration, 72 hours cold stress and 25 ppm molybdenum +72 hours cold stress applications were included. Pots of 2 L were used to grow the plants and the growing medium was a mixture of peat and perlite in a ratio of 2:1 by volume. Climatic chamber conditions were set to 24±1°C during the day and 18±1°C at night with 16/8 h light/dark photoperiodicity for control conditions and 24±1°C during the day and 5±1°C at night with photoperiodicity for cold stress conditions. The experiment was planned according to the random plots factorial design with 3 replications and 6 plants in each replicate. At the end of the study, plant height, stem diameter, number of leaves, plant fresh and dry weight, SPAD, wet basis moisture content, leaf proportional water content and ion leakage parameters were analysed. The highest plant height of Mazamort pepper variety was determined in 25 ppm molybdenum+72 hours cold stress application (44.51 cm). Application of 25 ppm molybdenum was effective in alleviating the negative effect of cold stress on plant stem diameter, plant fresh-dry weight and turgor potential. Moisture content wet basis was lowest in 25 ppm molybdenum +72 hours cold stress application. SPAD value in pepper plants decreased under cold stress conditions. It was observed that 25 ppm molybdenum application was ineffective and the decrease increased under cold stress conditions. Ion leakage in Mazamort pepper variety was highest under 72 hours cold stress and 25 ppm molybdenum +72 hours cold stress conditions. Under cold stress conditions, 25 ppm molybdenum application was ineffective. Molybdenum application under cold stress conditions was found to have positive effects on some parameters in general. In future studies, we believe that the application of different molybdenum concentrations and different cold stress periods will reveal the effects of molybdenum more clearly.

Keywords: Pepper, Molybdenum, Cold stress, Low temperature, Abiotic stress

Introduction

Pepper (*Capsicum annuum* L.), a member of the Solanaceae family, is one of the most important vegetable crops grown worldwide, with economic value as a spice, medicine, vegetable and biopesticide (Lim et al., 2018b; Bea et al., 2021). It is important in terms of consumption after tomatoes and onions worldwide

(Ocharo et al., 2017). Although the demand for chillies is increasing worldwide, their productivity can be limited to varying degrees due to unfavourable environmental conditions such as dehydration, high salinity, low and high temperatures. To address this problem, numerous studies have focused on defence mechanisms activated in response to such environmental stresses (Chen et al., 2014; Guo et al., 2014; Park et al., 2016; Lim et al., 2018a, 2020; Kang et al., 2020; Wu et al., 2020). Pepper is a species native to tropical and subtropical regions. The optimum temperature required for germination and plant development is between 25°C and 30°C, and temperatures below 15°C are detrimental to germination and fruit set (Lorenz and Maynard, 1988). Chilling temperatures, which are often encountered in unheated greenhouses during autumn or winter production, can adversely affect fruit set of marketable fruits due to poor pollination and delay ripening and earliness in production (Sharaf-Eldin et al., 2022). In addition, the growth and development of pepper, especially in the reproductive stage, is affected by cold stress in early spring.

In many parts of the world, cold stress is one of the most important problems in agricultural production. Approximately 25% of the terrestrial area of the world consists of regions that do not drop below 15 °C and are safe from freeze damage, while the remaining 75% consists of regions where the temperature drops below 0 °C during certain periods. In these regions, sensitive plants can be damaged (Gözen and Kuşvuran, 2021; Aslantaş et al., 2010; Peşkircioğlu et al., 2016). Cold stress (low temperature stress) is one of the environmental stress factors that economically limit plant growth, crop productivity and quality, and post-harvest life. Cold stress occurs at temperatures above 0°C (usually 0-15°C). In low temperature stress, it causes damage to plant tissues without forming ice crystals. Most tropical and subtropical plants belong to this group. The damage to the plant is defined as chilling or cold damage (Gözen and Kuşvuran, 2021; Chen, 1994; Hasanuzzaman et al., 2013; Kumar et al., 2018). Symptoms of damage vary depending on factors such as the temperature and duration of exposure, genotype, developmental stage and light intensity of the environment (Gözen and Kuşvuran, 2021).

Each plant species has different optimum temperature limits. As a result of cold stress, cellular changes such as changes in the structure and composition of membranes, decreased protoplasmic flow, electrolyte leakage and plasmolysis occur in cold-sensitive plants. Depending on the severity of stress, metabolic changes such as increased or decreased respiration, production of unusual metabolites due to anaerobic state also occur (Kumar et al., 2018; Gözen and Kuşvuran, 2021). This situation shows that metabolic and physiological events are negatively affected and enzymatic activity decreases in sensitive plants under low temperature stress. Furthermore, osmolytes (such as amino acids, sugars and K^+) and products of photosynthesis leak out through the plasma membranes (Guy et al., 1992). Physiological damage to many plant tissues below 15°C and above the freezing point is called 'chilling injury'. Chilling damage is seen in plants as the formation of surface lesions, tissue water absorption, water loss, drying or shrinkage, internal discolouration, tissue degradation, accelerated senescence and ethylene production, changes in cell integrity due to leakage of plant metabolites, reduced growth, wilting and increased putrefaction (Lukatkin et al., 2012; Gözen and Kuşvuran 2021). In the study to identify lines resistant to low temperature stress in tomato genotypes, three pure lines tolerant to cold were identified in the results of electrolyte leakage and dry matter yield parameters (Tepe et al., 2022). It was reported that electrolyte leakage increased in tomato exposed to cold stress (4°C) for 3 days. Cold stress tolerant tomato lines have been reported to show low electrolyte leakage (Cao et al., 2015).

In recent years, different applications have been made to reduce the negative effects of cold stress. One of these applications is chemical applications. Molybdenum (Mo) is one of the chemicals used to reduce the negative effects of cold stress. Mo is a very important and essential micronutrient for plants, animals and bacteria (Rana et al., 2020a; Ismael et al., 2018). It has a major role within the plant system although only required in small amounts. Molybdenum application beneficial to increase plant growth (Müftüoğlu et al. 2021). Mo uptake is low in acidic media, so foliar Mo application was important (Bambara and Ndakidemi, 2010). Mo is one of the components of nitrate reductase and nitrogenase in nitrogen metabolism in plants (Zhang et al., 2012). The amount of molybdenum in the soil and its uptake by the plant directly affect symbiotic N fixation in legumes (Gök, 1993; Haktanır and Arcak, 1997; Durrant, 2001; Ferreira et al., 2002). Mo is utilized by certain plant enzymes in the process of reduction and oxidative reactions (Mendel and Hansch, 2002). An integral part of an organic pterin complex is called a molybdenum co-factor (Moco). Most higher plants have molybdoenzymes (enzymes that require molybdenum) and bind to Moco plants (Zimmer and Mendel, 1999; Kaiser et al., 2005; Mendel and Kruse, 2012; Bittner, 2014; Kovács et al., 2015). Mo is known to be involved in phosphorus and sulphur metabolism (Mendel and Hansch, 2002; Liu et al., 2010; Zhang et al., 2012). Mo also plays an important role in resistance to many abiotic stresses in plants. Winter wheat under cold stress has been shown to benefit from Mo application in terms of photosynthetic rates and products (Yaneva et al., 1996). When winter wheat was under drought stress, Mo application had a positive impact on photosynthetic rates and products (Zakhurul et al. 2000). By enhancing the activities of antioxidant enzymes, Mo also improved the cold tolerance of turf grasses (Yu et al., 2005). In the

study conducted by Sun et al. (2009), it was reported that application of Mo increased the resistance of winter wheat to cold stress. It was reported that application of three amounts of Mo (0, 0.15, 0.3 mg kg^{-1}) to Chinese cabbage under salt stress significantly increased fresh weight; significantly improved the activities of antioxidant enzymes such as peroxidase, superoxide dismutase and catalase; significantly increased the content of non-enzymatic antioxidants such as glutathione, carotenoids and ascorbic acid. A significant increase in osmotic adjustment products such as soluble low molecular weight sugar, soluble protein and proline was also observed. In addition, Mo was reported to significantly increase the level of potassium ions (K^+) and improve the K^+/Na^+ ratio by decreasing the level of sodium ions (Na+). At the end of the study, Chinese cabbage was reported to improve its tolerance to salt stress by increasing its capacity to eliminate active oxygen and its osmotic adaptability (Zhang et al., 2014).

However, there is no report on whether Mo fertiliser application creates resistance to cold stress, especially in pepper plants. Therefore, the aim of our study was to reveal some physiological and morphological effects of Mo application in Mazamort pepper variety under cold stress conditions.

MATERIALS AND METHODS

This study was carried out in the climate chamber and laboratory of Siirt University Faculty of Agriculture. Mazamort pepper variety was used as plant material and this variety was purchased from Sunagri Seed Company. This variety is a widely used commercial variety. Seeds are not hybrid. They are local vegetable seeds collected as a result of research from various regions of Anatolia. It is a variety which is cultivated under greenhouse and also suitable for open field cultivation. Fruits are 10-12 cm long, crisp, sweet, smooth shaped, dark green coloured and have three tips. It is used for edible. For the sowing of pepper seeds, peat and perlite were mixed in 2:1 ratio and sown. After the seeds were sown and irrigation was done. One month pepper seedlings (4-5 leaf stage) were transferred to plastic pots in a volume of 2 liters. The application was started 15 days after the pepper seedlings were transplanted. In the study, control, 25 ppm Mo concentration, 72 hours cold stress and 25 ppm Mo+72 hours cold stress applications were applied. Molybdenum is a brand of Alfa Aesar and was purchased from BigMed. Molybdenum concentration was determined by conducting preliminary studies. In our previous preliminary studies, cold stress was applied for 12 hours and 24 hours with 25 ppm, 50 ppm and 75 ppm molybdenum doses on different species and cultivars. The climate chamber conditions were set at an average humidity of 60–65% and a light intensity of 8000 lux. Pots of 2 L were used to grow the plants and the growing medium was a mixture of peat and perlite in a ratio of 2:1 by volume. Climatic chamber (19 m^2) conditions were set to 24±1°C during the day and 18±1°C at night with 16/8 h light/dark photoperiodicity for control conditions and 24±1°C during the day and 5±1°C at night with photoperiodicity for cold stress conditions. In pepper, the application was started before the flowering stage. Molybdenum application was applied as a spray every other day at the same time. Molybdenum application was performed 9 times. Control plants were sprayed with distilled water at application times.

The experiment was planned according to the random plots factorial design with 3 replications and 6 plants in each replicate. It was planned as 1 plant in each pot. Plants were irrigated with standard nutrient solution during the experiment. Sample pots were kept in order to determine the amount and time of irrigation in order to prevent the water holding capacity to be different in the pots. Pot plates were placed in the pots of each application and irrigation was made to reach the saturation point and the amount of irrigation was calculated by considering the amount of water drained. The ratio of "drained solution/applied solution" was taken as basis in irrigation (Schröder and Lieth, 2002). Drainage levels were determined and this ratio was adjusted to approximately 30-32% during the experiment. The pH and EC of the drained water were measured irrigation at times.

Nutrient content was prepared using Hoagland nutrient solution. The pH of the nutrient solution was kept between 6.0-6.5 and EC between 1.5-2.5 dS m^{-1}. Plant measurements were made 54 days after sowing. Plant height, stem diameter, number of leaves, plant fresh and dry weight, SPAD measurement, wet base moisture content, leaf proportional water content and ion leakage parameters were analysed in at least 4 plants.

Determination of Plant Height

At the end of the experiment, the plant was measured from the root collar to the growing tip with a metre and recorded in centimetre (cm).

Determination of Plant Stem Diameter

Plant stem diameter was measured using compass and recorded in millimetre (mm). Plant stem diameter were made 54 days after sowing.

Determination of the Number of Leaves

All leaves on the plant were counted at the end of the study and the number of leaves was determined as number/plant.

Plant Fresh Weight

All green parts were weighed on a precision balance and recorded in grams (g).

Plant Dry Weight

After the plant fresh weights were taken, the plant samples were dried in an oven at 75°C for 48 hours and

recorded in g (Arshadullar and Zaidi, 2007).

Measurement with SPAD Meter for Chlorophyll

Readings were taken with a Minolta SPAD meter to determine the tone of green in young, middle-aged and young leaves of pepper plants depending on the amount of chlorophyll (Daşgan et al., 2010).

Wet Basis Moisture Content (%)

Wet basis moisture content was determined by using fresh weight and dry weight of the plants according to the following formula (Koksal et al., 2016).

MCwb = ((FW – DW)/FW)*100

MCwb: Moisture content wet basis (%), FW: Plant fresh weight (g), DW: Plant dry weight (g)

Leaf Proportional Water Content (RWC)

Five pieces of 1 cm discs were taken from fresh plant leaves and weighed and recorded. After the leaves were kept in pure water for 24 hours, the leaves were removed from the water, dried and their turgor weights were determined. The leaves whose turgor weights were determined were kept in an oven at 70°C for 24 hours. After drying, the dry weight was taken in grams. Leaf proportional water content (%) was calculated by placing the values in the following formula (Van Laere et al., 2011).

RWC= (FW-DW)/(TW-DW)*100

FW: Fresh Weight DR: Dry Weight TW: Turgor Weight

Ion Leakage

The 3rd leaves of the plant from the growth tip were used for this purpose. For this purpose, 1 cm diameter leaf discs were kept in de-ionised water for 5 hours and then EC was measured (EC1), the same discs were kept at 75°C for 24 hours and then the EC value of the solution (EC2) was measured again. Ion leakage was calculated as % using the formula (Arora et al., 1998).

Ion leakage= (EC1/ EC2)*100

Statistical Analysis

The significance between control, molybdenum application, cold stress and molybdenum+cold stress application was evaluated by analysis of variance (ANOVA) test. In case ANOVA showed significant differences between control, molybdenum application, cold stress and molybdenum+cold stress application, Least Significant Difference (LSD) test (P≤0.05) was used to compare the means. Differences in the data were evaluated using JMP 8th statistical software (Steel et al., 1997).

RESULTS AND DISCUSSION

Molybdenum is a rare element that is essential for plant growth and can be obtained from soil (Kaiser et al., 2005). However, at high concentrations Mo is known to have a negative effect on plant growth (Rihan et al., 2014). Many studies have reported that Mo application may have ameliorative effect against frost (Du et al., 1994; Li et al., 2001) and cold stress (Sun et al., 2006; Al-Issawi et al., 2013) damage.

The difference among applications in terms of plant height was found statistically significant (p≤0.05). Plant height values are shown in Figure 1. Among the applications, the highest plant height was determined in 25 ppm Mo+72 hours cold stress application with 44.51 cm and the lowest plant height was determined in 72 hours cold stress with 38,75 cm. It was determined that plant height increased in 25 ppm Mo application under 72 hours cold stress conditions. Plant height was higher in 25 ppm Mo application and 25 ppm Mo+72 h cold stress application compared to control. The highest plant height of Mazamort pepper variety was determined in 25 ppm Mo+72 hours cold stress application (44.51 cm). Molybdenum application was effective in alleviating the negative effect of cold stress on plant height. Molybdenum application under control conditions positively affected plant height. It has been reported that prolonged cold stress conditions cause a decrease in plant height (Hassan et al., 2021).

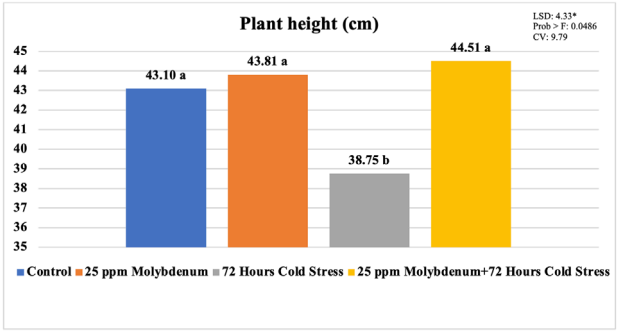

Figure 1. Effect of applications on plant height

The values of the applications in terms of plant diameter are shown in Figure 2. Plant diameter values of the applications were not statistically significant (p≤0.05). The highest plant diameter among the applications was in the control application. The lowest plant diameter was 6.14 mm in 72 hours cold stress application. Plant diameter was 6.98 mm in 25 ppm Mo application under 72 hours cold stress. Plant diameter decreased in other applications compared to the control, but the least decrease was determined in 25 ppm Mo+72 hours cold stress application. Cold stress decreased the plant diameter. The negative effect of cold stress was alleviated by 25 ppm Mo application under cold stress conditions.

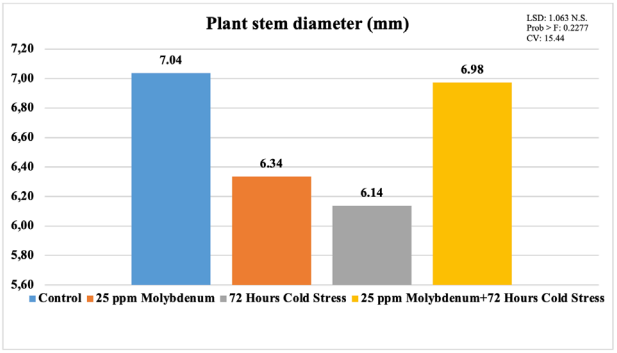

Figure 2. Effect of applications on plant diameter

The number of leaves was found to be statistically insignificant ($p \leq 0.05$). The number of plant leaves is shown in Figure 3. The number of leaves varied between 25.75 and 29.75 number/plant. The highest number of leaves (29.75 number/plant) was obtained in the control application. The lowest number of leaves was in Mo application with 25.75 number /plant. While the number of leaves was 27.25 number/plant under cold stress, it was 27.00 number/plant in 25 ppm Mo application under cold stress. The number of leaves of Mazamort pepper variety differed among applications. The lowest number of leaves was in Mo application compared to the control. It was determined that cold stress decreased the number of leaves and 25 ppm Mo application did not stop this decrease. It was determined that 30 ppm application of Mo decreased micro shoot growth. It was also reported that Mo application increased the average plant weight under low temperature stress (Rihan et al., 2014).

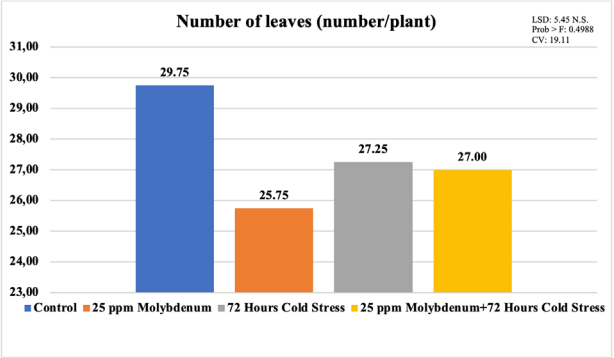

Figure 3. Effect of applications on the number of leaves

It was found that plant fresh weight of Mazamort pepper variety differed between applications, but this difference was not statistically significant ($p \leq 0.05$). The plant fresh weight obtained as a result of the applications was shown in Figure 4. Plant fresh weight varied between 22.78 g and 27.48 g. The highest plant fresh weight was in the control application (27.48 g) and the lowest was in the 72 hours cold stress application (22.78 g). Higher plant fresh weight was obtained in Mo application than 72 hours cold stress and 25 ppm Mo+72 hours cold stress application. Plant fresh weight decreased under cold stress conditions. Application of 25 ppm Mo was effective in alleviating the negative effect of cold stress on plant fresh weight. It has been reported that leaf size, leaf area and shoot biomass are reduced under cold stress (Valluru et al., 2012). It was reported that the combined application of cold and freezing stress caused chlorosis and a decrease in shoot biomass compared to the control (Hassan et al., 2021). It was reported that Mo application increased the micro shoot weight (Rihan et al., 2014).

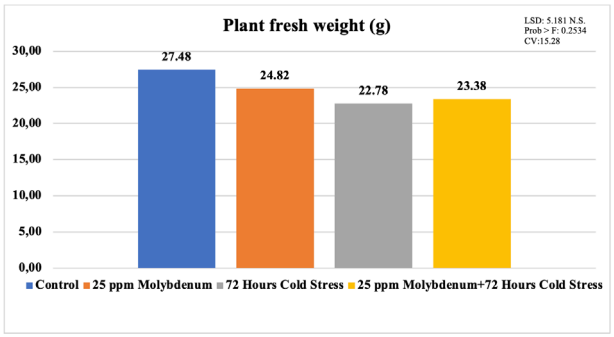

Figure 4. Effect of applications on plant fresh weight

It was determined that the applications had no statistically significant effect on plant dry weight ($p \leq 0.05$). Plant dry weight obtained as a result of the applications is shown in Figure 5. The highest plant dry weight was 2.57 g in the control application and the lowest was 2.22 g in the 72 hours cold stress application. While the plant dry weight was 2.22 under cold stress conditions, 25 ppm Mo+72 hours cold stress application increased the plant dry weight to 243 g. The lowest plant dry weight of Mazamort pepper variety was found under cold stress. It was found that 25 ppm Mo application was effective under cold stress conditions and increased plant dry weight under cold stress. Molybdenum application also had a negative effect on plant dry weight in control conditions. Mo application was reported to increase plant dry weight (Imran et al., 2019).

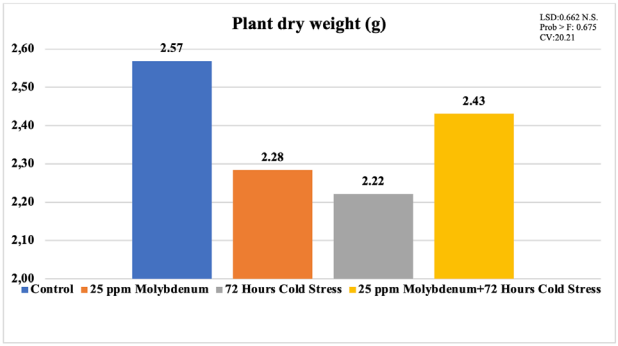

Figure 5. Effect of applications on plant dry weight

Pepper moisture content wet basis was shown in Figure 6. The moisture content of Mazamort pepper variety differed among the applications, but it was not statistically significant ($p \leq 0.05$). The highest moisture

content was in 25 ppm Mo application with 90.81% and the lowest was in 25 ppm Mo+72 hours cold stress application with 89.53%. Moisture content wet basis was lowest in 25 ppm+72 hours cold stress application. It was observed that cold stress decreased the moisture content wet basis and 25 ppm Mo application was ineffective in alleviating this decrease. Under control conditions, Mo application increased the moisture content wet basis.

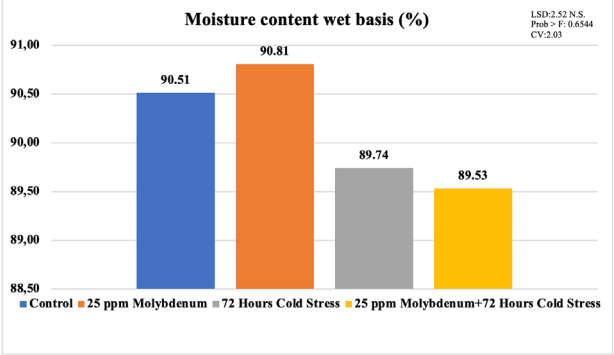

Figure 6. Effect of applications on moisture content of wet basis

SPAD value of Mazamort pepper variety showed a difference between applications and this difference was found statistically significant ($p \leq 0.0001$). SPAD value is shown in Figure 7. The highest SPAD value was in control application with 34.78 and the lowest was in 72 hours cold stress+25 ppm Mo application with 29.46. SPAD value decreased in other applications compared to control. SPAD value, which is important in determining the amount of chlorophyll in the plant, was the highest under control conditions. SPAD value in pepper plants decreased under cold stress conditions. It was observed that 25 ppm Mo application was ineffective and the decrease increased under cold stress conditions. The main damage site of cold stress is the chloroplast and photosynthesis. Tolerance in these aspects is expressed in native vegetation adapted to cold conditions (Sanghera et al., 2011). Yield losses occurring under cold stress conditions have been reported to be associated with low leaf area and reduced photosynthetic capacity. It has been stated that prolonged cold stress conditions cause leaf chlorosis (Hassan et al., 2021). Cold stress conditions affect cellular function due to changes in the photosynthetic apparatus (Manasa et al., 2022).

The difference in turgor potential of Mazamort pepper variety between applications was found statistically significant ($p \leq 0.0001$). RWC value is shown in Figure 8. RWC value varied between 93.80% and 96.31%. We think that some chemical applications can be effective in increasing plant growth and content, therefore, 25 ppm Mo application increased RWC. RWC decreased in 72 h cold stress and 25 ppm Mo+72 h cold stress application compared to the control. While RWC was 93.80% in cold stress, RWC increased to 94.36% in 25 ppm Mo application under cold stress conditions. Under control conditions, 25 ppm Mo application increased RWC. Water and nutrient relations have been stated to deteriorate in plants exposed to prolonged cold stress conditions (Hassan et al., 2021). The turgor potential was the lowest in 72 hours cold application. In alleviating the negative effect of cold stress on turgor potential, 25 ppm Mo application was effective under cold stress conditions. In control conditions, Mo application had a positive effect on turgor potential and increased it. It was reported that root length was more sensitive to cold stress conditions than dry weight. It has been reported that root length decreases under cold stress conditions and this disrupts the balance of water and nutrient uptake (Hussain et al., 2018).

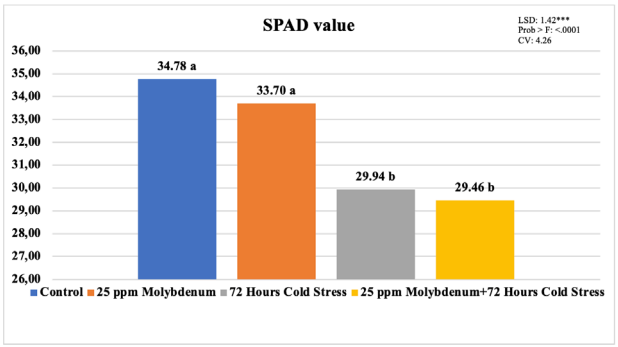

Figure 7. Effect of applications on SPAD value

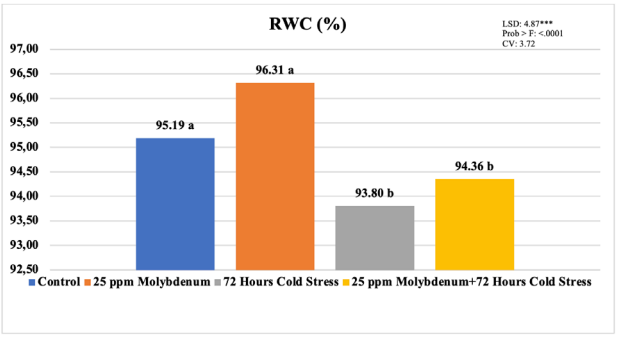

Figure 8. Effect of applications on RWC value

The difference among applications in terms of ion leakage was found to be statistically insignificant ($p \leq 0.05$). The ion leakage of Mazamort pepper variety as a result of the applications was shown in Figure 9. Ion leakage increased in other applications compared to the control. It was determined that 25 ppm Mo application did not reduce ion leakage under cold stress conditions and had the same rate of ion leakage with cold stress. The lowest ion leakage was in the control application with 17.32% and the highest was in the 72 hours cold stress and 25 ppm Mo+72 hours cold stress application with 17.88%. Changes in membrane fluidity occur during temperature stresses. This is a consequence of temperature stress damage and represents a potential sensing and/or damage zone (Horvath et al., 1998; Orvar

et al., 2000). Ion leakage is an important parameter in determining the effect of stress in stress applications. Ion leakage in Mazamort pepper variety was highest under 72 hours cold stress and 25 ppm Mo +72 hours cold stress conditions. Under cold stress conditions, 25 ppm Mo application was ineffective. It has been shown that the primary site of freezing damage in plants is the membrane systems of the cell (Steponkus, 1984; Levitt, 1980). It is well known that freeze-induced membrane damage is initially caused by severe dehydration related with freezing (Steponkus, 1984; Steponkus et al., 1993). At non-freezing low temperatures, many species of tropical or subtropical origin are known to be damaged or killed. Various symptoms of chilling damage such as chlorosis, necrosis or growth retardation have also been reported. However, it has been reported that cold stress tolerant species continue to grow under cold conditions. Therefore, it is important to stabilise membranes in tolerance to cold stress (Sanghera et al., 2011). Under cold stress conditions, it affects cellular function due to changes in electron flow (Manasa et al., 2022). It was reported that Mo application was effective in reducing the negative effect of cold stress on ion leakage under cold stress conditions (Rihan et al., 2014).

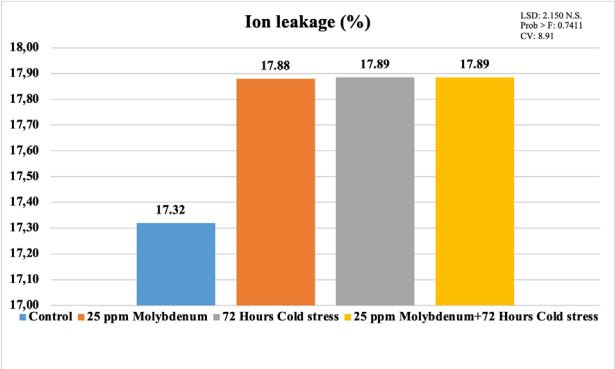

Figure 9. Effect of applications on ion leakage

CONCLUSION

The highest plant height of Mazamort pepper variety was determined in 25 ppm Mo+72 hours cold stress application. Application of 25 ppm Mo was effective in alleviating the negative effect of cold stress on plant stem diameter, plant fresh-dry weight and turgor potential. Moisture content wet basis was lowest in 25 ppm Mo+72 hours cold stress application. SPAD value in pepper plants decreased under cold stress conditions. It was observed that 25 ppm Mo application was ineffective and the decrease increased under cold stress conditions. Ion leakage in Mazamort pepper variety was highest under 72 hours cold stress and 25 ppm+72 hours cold stress conditions. Under cold stress conditions, 25 ppm Mo application was ineffective. Mo application under cold stress conditions was found to have positive effects on some parameters in general. We believe that the application of different Mo concentrations and different cold stress periods in future studies will reveal the effects of Mo more clearly.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The author of the article declares that there is no conflict of interest since he/she is the sole author

Ethical committee approval

Ethics committee approval is not required.

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Data availability

Not applicable

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Determination of fuel and power requirement of a branch shredder for different vineyard pruning wastes

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Abstract

The aim of the study was to determine the fuel consumption, torque and power requirement of a shredder machine used in the shredding of vineyard pruning wastes of different grape varieties. In the study, pruning wastes of Boğazkere, Öküzgözü and Şire grape cultivars, which are widely grown in Diyarbakır region, were used for tests. The experimens were carried out at three different rotation speeds of the engine (1500, 2000, 2500 rpm) and feeding rates (kg/h). Depending on these parameters fuel consumption, moment values and power were measured. The branch shredder machine has a 15 HP powerful. There are three chopper blades on the machine. The blades are driven by gasoline engine with the a belt and pulley system. According to obtained test results, per hour fuel consumption, power requirement and moment values increased for all three cultivars depending on the increase in the number of rotation of the blades, both at unloaded and under load. The highest fuel consumption was observed in Boğazkere cultivar, followed by Öküzgözü and Şire varieties, respectively. While the highest fuel consumption value was found as 1.535 kg/h in Boğazkere cultivar, the lowest value was obtained as 0.918 kg/h in Şire cultivar. A similar situation was obtained for power values. The power also increased with the increase in the number of blade revolutions While the highest power consumption value was obtained as 5.560 kW in Boğazkere cultivar, the lowest value was obtained as 3.326 kW in Şire cultivar. Here, there was no statistical difference between Boğazkere and Öküzgözü cultivars, but there was a significant difference between these cultivars and Şire cultivar. When the moment values of the cultivars were examined, the difference between cultivars was found to be very significant ($p<0.01$). The highest value was obtained as 26.26 Nm in Boğazkere variety, the variety with the highest Power and Fuel consumption value, while the lowest value was obtained as 15.65 Nm in Şire variety, which also had low fuel and power requirements.

Keywords: Shredder, Vineyard, Pruning waste, Fuel consumption, Power

INTRODUCTION

Viticulture is one of Turkey's most important agricultural activities. In the vineyard areas where grape cultivation is carried out, a large amount of pruning wastes occur after annual maintenance operations such as shoot pruning and training at different times. Failure to remove and evaluate the pruning wastes remaining in the vineyards creates major problems. This process is tiring, costly and time consuming. In addition, the energy requirement is high.

Vineyard pruning wastes are usually left in the vineyard and burned, or left on the walls forming the border of the vineyard and used for hedge purposes. This creates a basis for both environmental pollution and the formation and

proliferation of diseases and pests. These problems require an effective solution. One of the solutions is to decompose the pruning wastes using any machine/equipment and to bring them into the soil as organic residue. Evaluation in this way is important in terms of preventing environmental pollution and reintroducing the wastes to the soil. Thus, it will be of great benefit both economically and in terms of the implementation of recycling activities in agriculture. At the same time, sustainability in agriculture will be achieved by re-evaluating the vine wastes ground in the branch shredder. As a result of the use of pruned branches as organic waste, the use of chemical fertilizers will also decrease.

In order to break up the pruning wastes in the vineyards and orchards and make them useful, machines driven by the tractor PTO in large areas and stationary branch shredders are used in small areas. Various studies are carried out on this subject. In the study of Çanakçı et al. (2018) developed a self-propelled shredding machine prototype that can be used to break down the wastes generated in horticultural activities and return them to the soil as organic matter. Similar studies were conducted by Dereli (2009), Şeflek et al. (2006), Recchia et al. (2009), Spinelli et al (2014), Adamchuk et al. (2016). Pavankumar et al. (2018) designed and manufactured a portable organic waste chopping machine that shreds grape vine and fruit tree pruning wastes in order to demonstrate the importance of organic fertilization. As a result of the experiments of the study, it was stated that the vineyard rods were broken down well with the machine manufactured and these wastes could be converted into organic fertilizers and that the fragmented wastes could be used as biogas and feed as well as meeting the fertilizer needs of the farmer. Similar studies were conducted by Sucipto ve ark. (2020) ve Spinelli ve ark. (2010). Spinelli et al. (2012), Bilandzija et al. (2012), Magagnotti et al (2013), Nasser et al. (2014), Picchi et al. (2018) and Margaritis et al. (2020) stated that solid biomass fuels derived from agricultural wastes and other waste types are in excess for sustainable energy production. They stated that vine pruning wastes are an important fuel source as well as being used as fertilizer. Spinelli et al. (2014) developed and tested a new baling system designed to recover pruning wastes from vineyards inaccessible to conventional tractors as an alternative to on-site burning of pruning wastes from mountain vineyards. Çanakçı et al. (2019) stated that grinding is a critical process in recycling pruning wastes in different ways and choosing the right blades in the machines used for this purpose will contribute positively to obtaining suitable particles and reducing operating costs.

The main purpose of this study is to make some changes in accordance with the structure of the vine rods on a machine used in horticulture, which is currently produced for shredding branches in our country. Thus, it will be ensured that the vineyard pruning wastes are broken down and the fragmented wastes are left on the soil surface, making the wastes more useful. Another purpose is to determine the machine's performance depending on various parameters in working with the machine. With this study, various suggestions are made for both grape producers and machine manufacturers, thus contributing to the widespread use of machinery and thus ensuring sustainability in viticulture.

For these purposes, for small vineyard areas, the shredding of pruning wastes was tested with a branch shredder, which is driven by a thermic motor.

MATERIALS AND METHODS

Materials

Vine branches of Boğazkere (wine), Öküzgözü (wine) and Şire (table) (Vitis vinifera L.) grape cultivars belonging to the region were used as plant material in the study. Pruning branches were obtained from the vineyards of the grape producers in Diyarbakır. The pruned branches were turned into bundles in the vineyard. It was transported to Dicle University Faculty of Agriculture, Department of Agricultural Machinery and Technology Engineering to be tested later, and was kept under a covered porch. In the shredding of the vineyard pruning wastes, a branch shredder machine manufactured by a private company, which has a 15 HP powerful, 4-stroke gasoline engine (starter-battery powered), the chimney system can rotate 360^0, is both fixed and capable of moving in the vineyard by one person is used. There are three chopper blades on the machine. The blades are driven by three replications gasoline engine with the a belt and pulley system (Figure 1).

Figure 1. Branch shredding machine used in trials

The tests were carried out at 1500, 2000 and 2500 rpm chopper blade rotation speeds. The number of revolutions was adjusted with the engine accelerator pedal, and a DT-2236 revolution tachometer device was used in the measurements (Figure 2). From the moment the weighed material was fed to the machine, the time was measured with a chronometer. Dikomsan type balance (Figure 2).with a capacity of 15 kilograms (kg) was used for the weigth of test materal during the study. BMI brand digital caliper was used to determine the diameters of the vineyard pruning wastes. Also, precise weighing processes required to determine the moisture content of the product were made with a 0.1 precision VIBRA brand electronic balance. Oven drying method was used to determine the moisture content of the branches. For this, NUVE FN 500 brand drying oven (Figure 2) was used to determine the moisture content. In order to determine the moisture content of the branches during shredding, five samples were taken from each cultivar and weighed with precision scales and kept in a drying oven at 105 ºC for 24 hours. At the end of this period, the samples were weighed again. At the end of drying, the moisture content of the shoots was determined as % according to the wet base (ASABE, 2006). Moisture contents of pruning branches were measured as 38.10% for Boğazkere cultivar, 38.80% for Öküzgözü cultivar and 38.30% for Şire cultivar.

Figure 2. Balances, drying cabinet (oven), caliper and speed measuring device

In order to measure the amount of fuel consumed at different rotation speeds, number of blades and feed rates, ISOLAB brand 500 milliliter (ml) graduated separation funnel made of glass material was used (Figure 3). For fuel measurement, the fuel tank on the machine is disabled and the fuel is directly entered into the engine through a 500 ml graduated glass funnel (Figure 3). Feeding time has been taken into account for the calculated fuel values. Trials were carried out at constant feeding amounts (kg/h). For each trial, the branches, which were weighed with scales beforehand, were tried to be fed to the machine in the same time as possible. Fuel consumption was determined by measuring the amount of fuel that decreases depending on the number of revolutions in each trial. Experiments were made in three replications. The mean values of three replications were used.

Figure 3. Graduated glass funnel

Methods

During the trials, fuel measurements were made during the unloaded operation of the machine as well as all grape varieties and blades rotations. Experiments were made in three replications. Specific fuel consumption, which is the amount of fuel consumed for each kilowatt (kW) power unit as a result of one hour of engine operation, is calculated using the equation given below (Dinçer, 1981; Sabancı, 1993; Georging ve Hansen 2004; Srivastava ve ark. 2006; Sessiz ve ark. 2020).

be= 3600/(Hu.Ⅱ)

Where;

be : Specific fuel consumption (kg/kW.h) (be =0.276)

Hu : Lower calorific value of fuel (kj/kg) (constant: 43.472 kj/kg)

Ⅱ : Total efficiency of the motor % 30 (constant: 0.3)

The power values were calculated by proportioning the fuel consumption value measured during the trials to the specific fuel consumption (kg/kWh) and using the equation given below (Dinçer, 1981; Georging and Hansen 2004). Fuel consumption measured in L/s depending on time. It was then converted to (kg/h) to calculate hourly fuel consumption. Density (kg/l) = 0.7475 was taken into account in the calculations.

Pe= B/be

Where;

Pe : Power drawn by the shredder, kW

B : Per hour fuel consumption of the engine (kg/h)

be : Spesific fuel consumption (kg/kWh)

Moment values were also calculated using the equation given below (Dinçer, 1981; Sabancı, 1998; Saral et al.,2008; Sessiz et al. 2020).

Md=(9550.Pe)/n

Where;

Pe : Power drawn by the shredder, kW

Md : Moment, Nm

n : Number of shredder rotations, min^{-1}

For statistical comparison between data, JMP, 13. Version, package program was used. Trials were planned according to random plot design using analysis of variance (ANOVA). Comparisons were made according to the LSD test and 5% and 1% significance.

RESULTS AND DISCUSSION

In the calculations, density (kg/l) = 0.7475, Hu (kj/kg) = 43.472 (heating value of fuel), total efficiency = 0.3, Be (kg/kWh) = 0.276 (specific fuel consumption) were kept constant (Georgin et al. 2005).

The LSD test results obtained according to the variance analysis results based on the variety are given in Tables 1 and 2. When Table 1 and Figure 3 were examined together, the difference between the cultivar was found to be significant in terms of fuel consumption ($p<0.05$). While there was no difference between Boğazkere and Öküzgözü cultivars, the difference between these cultivars and Şire cultivar was significant. While the highest fuel consumption value was found as 1.535 kg/h in Boğazkere cultivar, the lowest value was obtained as 0.918 kg/h in Şire cultivar.

A similar situation was obtained for power values. While the highest power consumption value was obtained as 5.560 kW in Boğazkere cultivar, the lowest value was obtained as 3.326 kW in Şire cultivar. Here, there was no statistical difference between Boğazkere and Öküzgözü cultivars, but there was a significant difference between these cultivars and Şire cultivar (Table 1 and Figure 4). When the moment values of the cultivars were examined, the difference between cultivars was found to be very significant ($p<0.01$). The highest value was obtained as

26.26 Nm in Boğazkere variety, the variety with the highest Power and Fuel consumption value, while the lowest value was obtained as 15.65 Nm in Şire variety, which also had low fuel and power requirements (Table 1 and Figure 2).

Table 1. LSD test results for average Fuel Consumption, Power and Moment values measured depending on the grape variety *

Cultivar	Fuel Consumption Kg/h	Power kW	Moment Nm
Unloaded	0.666	2.391	11.330
Boğazkere	1.535a	5.560 a	26.26 a
Öküzgözü	1.246 a	4.513 a	21.22 b
Şire	0.918 b	3.326 b	15.65 c
LSD	0.1095	0.396	1.628

* There is no difference at the 1% significance level between the means denoted by the same letter.

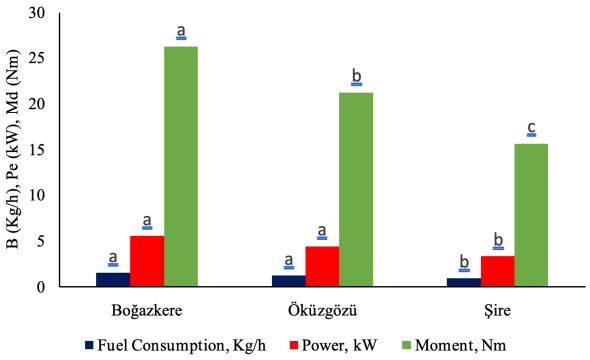

Figure 4. Average Fuel Consumption, Power and Moment values measured depending on the variety.

Average LSD values of fuel, power and moment depending on blade rotation speed are given in Table 2 and Figure 5. As can be seen in Table 2, per hour fuel consumption, power requirement and moment values increased for all three cultivars depending on the increase in the number of rotation of the blades, both at unloaded and under load. The difference between the rotation speed in all three cultivars was found significant ($p<0.01$). While the highest fuel consumption was obtained as 1.614 kg/h at 2500 rpm, the lowest fuel consumption was obtained as 0.822 kg/h at 1500 rpm.

The difference between the revolutions was found to be significant in the power consumption values. Similarly, the power requirement was highest at 2500 rpm and was 5.847 kW. The lowest power requirement was obtained as 2.976 kW at 1500 rpm.

When the moment values are examined depending

on the number of revolutions, there is no statistically significant difference. The highest moment value was 22.333 Nm at 2500 rpm and the lowest moment value was 18.942 Nm at 1500 rpm.

Table 2. LSD test results for average Fuel Consumption, Power and Moment values measured based on blade rotation speed*

Blade rotation speed	Fuel Consumption Kg/h	Power kW	Moment Nm
rpm			
1500	0.822 c	2.976 a	18.942 a
2000	1.263 b	4.577 b	21.859 a
2500	1.614 a	5.847 ab	22.333 a
LSD	0.10668	0.3865	0.483

* There is no difference at the 1% significance level between the means denoted by the same letter.

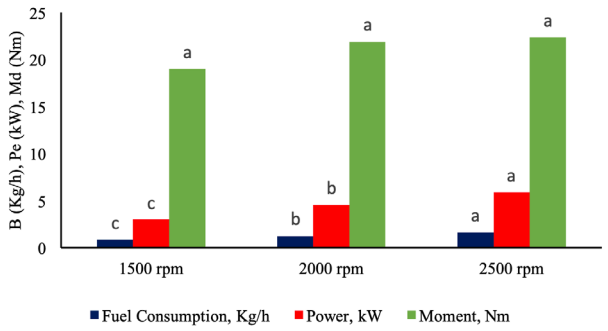

Figure 5. Average Fuel Consumption, Power and Moment values measured based on blade revolution

CONCLUSION

The results obtained depending on independent parameters at the end of the tests carried out in this study for the shredding of pruning wastes are given below.

Per hour fuel consumption increased for all three cultivars in unloaded and loaded conditions due to the increase in the number of revolutions of the chopper blades. Hourly fuel consumption value at unloaded changed between 0.554 L/h (0.414 kg/h) and 1.170 L/h (0.875 kg/h). The difference between all cultivars in the loading condition was significant. The highest fuel consumption was observed in Boğazkere cultivar, followed by Öküzgözü and Şire varieties, respectively. The power also increased with the increase in the number of revolutions. The lowest value for Boğazkere variety was 3,628 kW at 1500 rpm, while the highest value was 6,962 kW at 2500 rpm. During the disintegration of the pruning shoots of Öküzgözü cultivar, 2,938 kW was obtained at the lowest revolution speed and 5,917 kW at the highest revolution. The situation in the cultivar of Şire; While the lowest power value was 2.362 kW at 1500 rpm, the highest power value was 4.666 kW at 2500 rpm. The moment values also increased as the number of blade revolutions increased. This increase varied according to the number of revolution and the cultivar. Moment values according to three different revolutions were between 23,098 and 29.080 Nm in Boğazkere cultivar and between 18.708 and 22.601 Nm in Öküzgözü cultivar, while it was recorded between 14.106 Nm and 17.802 Nm in Şire cultivar.

COMPLIANCE WITH ETHICAL STANDARDS

This research study complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, 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.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

All relevant data is inside the article.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Economic efficiency of smallholder okra (*Abelmoschus species*) production in Kaduna State, Nigeria: Implication for poverty alleviation

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Abstract
This study evaluated economic efficiency of smallholder okra (Abelmoschus species) production in Kaduna State, Nigeria: Implication for poverty alleviation. A multi-stage sampling technique was used to select 120 smallholder okra farmers. Primary sources of data were used. Data were analyzed using farm budgetary technique, financial analysis, stochastic production frontier model, allocative efficiency model, economic efficiency model, Tobit dichotomous regression model, and principal component model. The results show that the mean age of smallholder okra farmers was 43 years. Averagely, okra farmers had 1.8 hectares of farm land. The gross margin and net farm income of okra production per hectare was estimated at 619,325.77 Naira and 559, 194.76 Naira respectively. This signifies that the smallholder okra production was profitable. The mean technical, economic, and allocative efficiency scores were 0.7918, 0.5338, and 0.8345 respectively. The socio-economic factors influencing economic efficiency of smallholder okra production include: age ($P<0.01$), educational level ($P< 0.10$), marital status ($P< 0.01$), household size ($P <0.01$), farm size ($P<0.01$), and member of cooperative organization ($P<0.05$). The major constraints encountered by okra producers were lack of farm inputs, lack of credit facilities, and high cost of labour. The study recommended that farmers should be provided with improved variety of seeds, chemicals, credit facilities, and fertilizer inputs in order to increase their productivity and efficiency.
Keywords: Economic Efficiency, Smallholder, Okra Production, Kaduna State, Nigeria

INTRODUCTION

Vegetables are a staple food whose production has increased continually in most countries of the world, including Nigeria. The major vegetables grown in Nigeria include onion, tomato, okra, pepper, *amaranthus*, carrot, and melon etc. They are the most lucrative agricultural enterprises for small and marginal farmers, as it forms the main source of farm income for small and resource-poor farmers (FAO, 2015 and FAO, 2016). There is an increase in demand for the vegetable crop. Okra (*Abelmoschus esculentus* L. Moench) was domesticated in West and Central Africa but is now widely cultivated throughout the tropics, primarily for local consumption (Schipper, 2000). Okra production ranks third in Nigeria after tomato and pepper in terms of consumption and production area. The economic importance of okra cannot be over- emphasized. Its rich source of carbohydrates, proteins, and vitamin C, as well as amino acids in high quantity (Law-Ogbomo *et al.,* 2013; Ijoyah & Dzer, 2012). Hence, it plays a vital role in the human diet (Farinde *et al.,* 2007). In addition, okra has attributes that are used for other purposes and

its leaves, buds, and flowers are edible. Also, its seeds, when dried, can be used to produce oil, vegetable curd, and coffee additives or substitutes, among other things (Adeboye *et al.,* 2009). The farming households who are the bedrock of agricultural production are the ones most affected by food insecurity and poverty in Nigeria (Kurwornu *et. al.,* 2013). West Africa Insight (2010) reported that over 53 million Nigerians lived in hunger and they represent about 30% of the country's total population of roughly 150 million. Poverty entails inadequate income and absence of basic necessities such as education, health service, food, clean water and sanitation that are necessary for human survival and dignity (World Bank, 2007b). It denies its victims the most basic needs (food, water, clothing and shelter) for survival. World Bank (2012) viewed a poor person as one who is under-nourished and cannot care for himself. Also, National Bureau of Statistics (NBS, 2012) reported that 60.9% of the population was living in absolute poverty, and about 70% of Nigerians lived below the Poverty line of $1.25/day. This according to World Bank (2007[a]) is the minimum cash and non-cash expenditure needed to be made by a person or household in order to be able to consume the minimum number of calories (food) plus a small number of essential non-food items such as housing, clothing and health care. Food security on the other hand is reported to be a situation where all people, at all times, have access to sufficient, safe and nutritious food to meet dietary needs and food preference for an active and healthy life (FAO, 2007). The bulk of Agricultural production in Nigeria takes place in the rural areas and ironically, the level and incidence of poverty and food insecurity is very pronounced in the areas (NPC, 2004). With the recognition by the Nigerian government of the multi-sectoral and multi-dimensional nature of poverty, which causes hunger, malnutrition, illiteracy, disease, life of misery and squalor, low life expectancy, socio political instability and bribery and corruption, a number of coordinated programs and policies have been formulated to combat poverty in all ramifications. The Federal Government of Nigeria has also taken a number measures to reduce the level or incidence of poverty in Nigeria. Some of these measures and programs include the National Economic Empowerment and Development Strategy (NEEDS) and National Poverty Eradication Program (NAPEP). Increased agricultural efficiency could be achieved by improving input application techniques for a given production technology (Ogundari & Ojo 2007). There are two aspects to efficiency measurement: Firstly, the technical efficiency, and secondly, the allocative efficiency. The technical efficiency defines the capacity of the farmers to engage productive resources to achieve the maximum output obtainable (Ali & Khan 2014). Allocative efficiency defines the farmers' ability to optimize the use of individual inputs – where the ratio of the marginal value product (MVP) equates with the unit price of a particular input (Ogundari 2008). It is explained whether the farmer is involved in wasteful/ inadequate application of resources or not. The product of these two (2) efficiency components defines the economic efficiency. The concepts of both the technical and allocative efficiencies relate with the fact that production variables such as the land, seed, labour and chemicals (fertilizer, herbicides, and insecticides), feed, fingerlings, etc., as well as the soil and edaphic factors alone do not define the levels of output. Certain human factors also have effects on the production level of the farm firm. Given the same level of inputs and environment, a technically more efficient farmer's output is closer to the production frontier than a technically less efficient one. In the same manner, a more allocative efficient farmer combining of resources and minimizes wastage/ under-utilization better and is therefore farther away from the cost frontier than a less allocative efficient one. That is, the closer to the frontier (which has maximum value of 1 in the case of technical efficiency), the better the farmer. On the other hand, the closer a farmer's cost efficiency estimate is to the minimum estimate of one (1), the more efficient he or she is, where allocative efficiency is assumed. Technical and allocative efficiencies are key variables in poverty estimation and consideration since production output closer to the frontier yields more output (and consequently more income). Allocative efficiency leads to minimization of resource wastage and liberates resources for expansion of production base or consumption. The nexus between efficiency levels and income poverty among Okra farmers in Kaduna state, Northwestern Nigeria has not received much attention in the literature.

Objectives of the Study

The broad objective is to evaluate economic efficiency of smallholder okra (*Abelmoschus species*) production in Kaduna State, Nigeria: Implication for poverty alleviation. The specific objectives were to:

(i) determine the socio-economic profiles of smallholder okra farmers,

(ii) analyze the cost, returns and profitability of smallholder okra production,

(iii) determine the technical (TE), economic (EE) and allocative efficiency (AE) scores of smallholder okra production,

(iv) evaluate the socio-economic factors influencing economic efficiency (EE) of smallholder okra

production, and

(v) determine the constraints facing smallholder okra farmers in the study area.

Methodology

This research study was conducted in Kaduna State, Nigeria. The state occupies between Longitudes 06^0 15$^|$ and 08^0 50$^|$ East and Latitudes 09^0 02$^|$ and 09^0 02$^|$ North

of the equator. The State has total land area of 4.5 million hectares. The state vegetation is divided into 2, they are: - the Northern guinea savanna and the Southern guinea savanna. There are 2 seasons in the State, they are: the dry season and the wet seasons, the wet season starts from April to October, and the dry season is between October to March, in between the dry and wet seasons is the brief harmattan period which span from November to February. The mean rainfall stood at 1,482mm, the temperature of the state ranges from 35^{0}C - 36^{0}C, which can be as low as 10^{0}C to 23^{0}C during the harmattan period. The population of the State in 2021 stood at 8.9 million people. They are involved in farming, crops grown include: pepper, okra, maize, sorghum, ginger, rice, yam, millet, cassava, and tomatoes. Animal reared include: goats, cattle, sheep, poultry and rabbit. A multi-stage method of sampling was used. About 120 smallholder okra farmers were selected. Data obtained were of primary sources and were collected using a well-designed and also a well-structured questionnaire. The questionnaire was administered to smallholder okra producers using well trained enumerators.

Research Design

A descriptive and cross-sectional research design was employed with the aim of describing the socio-economic profiles of okra producers, and to evaluate technical (TE), economic (EE), allocative efficiency (AE) scores and socio-economic factors influencing economic efficiency of okra production.

Sampling Techniques and Sample Size

A multi-stage sampling technique was adopted for this study. In the 1st stage, purposive sampling procedure was used to select Kaduna State based of the numerous numbers and concentration of okra producers in the area. The 2nd stage involved random selection of 4 area councils using ballot box method. In the 3rd stage, 3 villages were selected randomly from each local government area based on the intensity of okra producers. In the 4th stage, from sampling frame of 171 okra farmers, proportionate and simple random sampling technique was used to select the desired sample size of 120 okra farmers. This study employed the formula advanced by Yamane (1967) in the determination or estimation of the sample size. The formula is stated thus:

$$n = \frac{N}{1+N(e^2)} = 120 \ldots\ldots\ldots (1)$$

Where,

n = Desired Sample Size

N = Finite Size of the Population

e =Maximum Acceptable Margin of Error as Determined by the Researcher

Methods of Data Collection

The data for this study was collected through the use of a well-designed and well-structured questionnaire. The data collected were cross sectional data from primary source, the data collected from the smallholder okra producers were socio-economic profiles of the farmers, prices of production inputs, quantity of inputs used and constraints faced by farmers in the course of okra production in the study area. Data were analyze using the following descriptive and inferential statistics:

Descriptive Statistics

Data collected from field survey on smallholder okra farmers were summarized through the use of mean, frequency distributions, and percentages. Descriptive statistics was used to summarize the socio-economic profiles of smallholder okra farmers as stated in specific objective one (i)

Farm Budgetary Technique

Gross margin (GM) and net farm income (NFI) analysis of okra production was estimated using the following models:

$$GM = TR - TVC \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$
$$GM = \sum_{i=1}^{n} P_i Q_i - \sum_{j=1}^{m} P_j X_j \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$
$$NFI = TR - TC \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$
$$NFI = \sum_{i=1}^{n} P_i Q_i - \left[\sum_{j=1}^{m} P_j X_j + \sum_{k=1}^{k} GK\right] \ldots\ldots\ldots (5)$$

Where;

P_i = Price of Okra ($\frac{₦}{Kg}$),

Q_i = Quantity of Okra (Kg),

P_j = Price of Variable Inputs ($\frac{₦}{Unit}$),

X_j = Quantity of Variable Inputs (Units),

TR = Total Revenue obtained from Sales from Okra (₦),

TVC = Total Variable Cost (₦),

GK = Cost of all Fixed Inputs (Naira)

NFI = Net Farm Income (Naira)

The farm budgetary technique was used to analyze the profitability of smallholder okra production as specifically stated in objective 2 (ii).

Financial Analysis

According to Alabi *et al*. (2020), gross margin ratio is defined as:

$$Gross\ Margin\ Ratio = \frac{Gross\ Margin}{Total\ Revenue} \ldots\ldots\ldots\ldots (6)$$

According to Olukosi & Erhabor (2015), operating ratio (OR) is defined as:

$$Operating\ Ratio = \frac{TVC}{GI} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Where,

TVC = Total Variable Cost (Naira),

GI = Gross Income (Naira),

The financial analysis was used to analyze the profitability of okra production as stated specifically in objective 2 (ii).

Stochastic Production Frontier Model

According to Alabi *et al*. (2022), the stochastic production frontier model is stated as follows:

$$Y_i = f(X_i, \beta_i)e^{v_i - u_i} \ldots\ldots\ldots\ldots\ldots\ldots(8)$$

The stochastic production frontier model was used to estimate the technical, economic and allocative efficiency scores as stated specifically in objectives 3 (iii).

Allocative Efficiency Model

Allocative Efficiency (AE) is computed as follows:

$$\mathrm{AE} = \frac{1}{\mathrm{CE}} \ldots\ldots\ldots\ldots\ldots\ldots (9)$$

$$\mathrm{AE} = \frac{\mathrm{EE}}{\mathrm{TE}} \ldots\ldots\ldots\ldots\ldots\ldots(10)$$

$$\mathrm{EE} = \mathrm{AE} \times \mathrm{TE} \ldots\ldots\ldots\ldots\ldots\ldots(11)$$

Where,

AE=Allocative Efficiency

TE=Technical Efficiency

EE=Economic Efficiency

CE=Cost Efficiency

Tobit Dichotomous Regression Model

The dichotomous response model is defined as follows:

$$Y_i^* = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + U_i \ldots\ldots (12)$$

$$Y_i = \begin{cases} 1 \; if \; Y_i^* \geq 1 \\ Y_i^* \; if \; 0 < Y_i^* < 1 \\ 0 \; if \; Y_i^* \leq 0 \end{cases}$$

Y_i^* = Latent or Unobserved Variable

Y_i = Efficiency Score Representing Economic Efficiency (Number)

X_1 = Age (Years),

X_2 = Educational Level (Years),

X_3 = Marital Status (1, Married; 0, Otherwise)

X_4 = Farming Experience (Years)

X_5 = Household Size (Number)

X_6 = Access to Credit Facilities (Naira)

X_7 = Farm Size (Hectares)

X_8 = Member of Cooperatives Organizations (1, Member; 0, Otherwise)

U_i = Error Term,

$\beta_1 - \beta_8$ = Regression Coefficients,

β_0 = Constant Term,

This was used to achieve specifically objective 4 (iv) which is to evaluate socio-economic factors influencing economic efficiency (EE) of smallholder okra production.

Principal Component Analysis

The constraints facing small-scale okra farmers and militating against okra production were subjected to principal component analysis. This was used to achieve specifically objective 5 (v).

RESULTS AND DISCUSSION

Socio-Economic Characteristics of Smallholder Okra Farmers

The results in Table 1 indicate that 36% of Okra farmers were female, while 64% were males. This is an indication that okra farming was a male dominated business. This may not be unconnected with the limited access of women to productive resources in many cultures and traditions. This is in consonance with the findings of Haruna *et al.* (2007). Table 1 also shows that 29% of Okra farmers were single, 10% were divorced, and 61% were married. Simonyan & Omolehin (2012) observed in their study on gender differentials in technical efficiency among maize farmers that marital status had positive coefficient and was significant in influencing the productivity of the male farmers. Table 1 also revealed that 28% of the respondents were between the age ranges of 31 to 40years, 18% were between the age ranges of 41 to 50 years, while 23% were between the age ranges of 51 to 60 years. The mean age okra producer was 43 years, the role of age of farmers is very critical in agricultural production. In their estimation of technical and allocative efficiency analysis of Nigerian rural farmers, Asongwa *et al*. (2011) reported that the age of farmers had a positive effect on technical inefficiency effects. The result further explained that 68% of the okra producers had one form of formal education or the other, while 32% had no formal education. According to Imonikhe (2004), education would significantly enhance farmers‟ ability to make accurate and meaningful management decisions, it could also enhance the knowledge of improved techniques such as how to read and interpret recommended practices and packages. The result in Table 1 also shows that 58% of the okra producers had households' range of 1-5 persons, 21% of the farmers had household size between the ranges of 6 to10 persons, while 21% of the respondents had household size between the ranges of 11 to15 persons, in addition, the mean household size was 6 persons. The implication of this is that farming households have a good source of family labour for farm business by providing the needed cheap and available manpower all-round the year. Amos (2007) in his study of productivity and technical efficiency of smallholder cocoa farmers in Nigeria reported that family size was a significant variable which greatly influence the technical efficiency of farmers. The result further indicates that 57% of the okra producers had extension contacts, while 43% of the respondents had no extension contacts. According

to Umar *et al.* (2007), higher extensions contact was reported to increase the adoption of improved farm production technologies. They further observed that the frequency of extension contact is very essential as it guides the farmers from awareness to the adoption stage. The result in Table 1further shows that 27% of the respondents had farming experience of 1-5 Years, 23% had farming experience of 6-10 years, 33% had 11-15 years' experience, while16% had 16-20 years farming experience. Adebayo (2006) in the study of resource use efficiency of pastoralists in Adamawa state observed that the longer a person stays on a particular job, the better the job performance tends to be. The result also indicates that 67% of the respondents were members of cooperative organizations, while 33% of the respondents were not members of cooperative organizations. The membership of a cooperative organization enables farmers to interact with one another, share their experiences and assist themselves in bulk purchase of inputs. Similarly, Gashaw *et al.* (2013) and Folorunso & Bayo (2020) reported that membership of cooperatives enhances efficiency by easing access to productive inputs and facilitating extension linkage compared to those who were not members. Also, the result in Table 1 shows that 56% of the respondents had farm size range of 1.1 – 2.0 ha, 28% had farm size range of 2.1-3.0 ha and 17% of the respondents had farm size range of 3.1 – 4.0 ha. Based on Olayide (1980) classification of farms; 0.1 - 5.0 hectares (small-scale); 5.1 - 10 hectares (medium-scale); and 10 hectares and above (large-scale). Since the majority of respondents had farm holdings between 0.1 and 5.0 hectares, it means that they are smallholder, smallscale farmers. This is consistent with the findings of Onuche & Oladipo (2020) whose findings revealed the bulk of the farm households that majority of the respondents operated on farmland sizes between 1–2 ha suggesting the smallholder nature of agriculture in the area.

Profitability of Okra Production of Smallholder Farmers

The result in Table 2 shows the profitability analysis of okra production. The result indicates that the total cost of production (TCP) incurred per hectare was ₦145,674.25. The variable cost includes: cost of seeds (₦5,674.56) representing 3.8% of the TCP, fertilizer input (₦24,783.45) representing 17.9% of the TCP, insecticides(₦10,567.00) representing 7.2% of the TCP, herbicides (₦15,675.87) representing 10.8% of the TCP and labour costs(₦68,842.36) (land clearing and preparation, planting, weeding, fertilizer application, chemicals application, harvesting, transportation, and loading and offloading) representing 47.3% of the total cost of production. Table 2 also indicated that the total revenue (TR) generated per hectare was ₦765,000. The result also indicated that the total variable cost (TVC) was ₦125,543.24 per hectare representing 86.2% of the TCP. Finally, the budgetary analysis per hectare indicated that okra farming was profitable as shown by gross margin (₦145,674.25) per ha and NFI of (₦550,194.76) per ha. The GMR and OR were 0.81 and 0.16 respectively, indicating that the 81% of the gross revenue accruing to okra production constituted the GM, while 16% of the gross income was committed to the TVC of okra production. The operating ratio was less than unity, lower OR was preferable. This report is similar to the findings of Busari & Okanlawon (2015) and Folorunso *et al.* (2023). The implication of this on the poverty status of okra farmers is that increased and sustained profitability of this enterprise will enable farming households have economic access to basic amenities and thereby aid in poverty alleviation.

Farm Level Technical, Allocative and Economic Efficiency of Smallholder Okra farmers

The frequency distribution of the allocative efficiency (AE), technical efficiency (TE), and economic efficiency (EE) estimates of smallholder okra farmers as obtained from the stochastic frontier analysis is presented in Table 3. The frequencies of occurrences of the predicted TE, AE and EE in decile range indicate that the highest number of okra farmers had TE, AE and EE between 0.81 – 1.00. The sample frequency distribution indicates a clustering of TE, AE and EE in the region of 0.81 – 1.00 efficiency ranges, representing 48.3%, 66.7% and 31.7% respectively. The implication of this is that the farmers were technical inefficiency, allocative efficient and inefficient economically. That is, the farmers were inefficient in deriving maximum output from input, given the available resources. The minimum TE, AE and EE of the okra farmers as found in Table 3 are 0.0265, 0.01583 and 0.08401 respectively, while the maximum TE, AE and EE of the respondents are 0.98912, 1.00 and 0.9563 respectively. This means that on the minimum, smallholder okra farmers were 8% economically efficient, while on the maximum, the okra farmers were 96% economically efficient. The result of the Cobb-Douglas production frontier further indicate that technical efficiency varied widely among the sampled okra farmers, with minimum and maximum values of 0.01583 and 0.98912 respectively. The wide variations in technical efficiency estimates is an indication that most of the okra farmers were still using their resources inefficiently in the production process and there still exists wide opportunities for improving on their current level of TE. This result suggests that the farmers were not utilizing their production resources efficiently, indicating that they were not obtaining maximum output from their given quantities of inputs. On the other hand, the predicted allocative efficiency varied widely among the sampled farmers, with minimum and maximum values of 0.0265 and 1.00 respectively. The wide variations in allocative efficiency estimates is an indication that most of the farmers still allocate their resources inefficiently in the production process and there

Table 1. Socio-Economic Profiles of Smallholder Okra Producers

Variables	Frequency	Percentage	Mean
Gender			
Male	77	64.2	
Female	43	35.8	
Marital Status			
Single	35	29.2	
Divorced	12	10.0	
Married	73	60.8	
Age (Years)			**43**
31 – 40	33	27.5	
41 – 50	22	18.3	
51 – 60	27	22.5	
Level of Education			
Non-Formal	38	31.7	
Tertiary	40	33.3	
Secondary	20	16.7	
Primary	22	18.3	
Household Size (Units)			**6**
1 – 5	70	58.3	
6 – 10	25	20.8	
11 – 15	25	20.9	
Extension Contact			
Yes	69	57.5	
No	51	42.5	
Farming Experience (Years)			
1 – 5	32	26.7	
6 – 10	28	23.3	
11 – 15	40	33.3	
16 – 20	20	16.7	
Memberships of Cooperative			
Yes	80	66.7	
No	40	33.3	
Farm Size (Hectares)			**1.8**
Less than 1.0	67	55.8	
1.1 - 2.0	33	27.5	
2.1 – 3.0	20	16.7	
3.1 – 4.0			
Total	**120**	**100.00**	

Source: Field Survey (2022)

still exists opportunities for improving on their current level of allocative efficiency. This result suggests that the farmers were not minimizing production costs, thus indicating that they were utilized the inputs in the wrong proportions, given the input prices. Also, the EE varied widely among the sampled farmers, with minimum and maximum values of 0.008401 and 0.9563 respectively. This wide variation in EE estimates is an indication that most of okra farmers were still economically inefficient in the use of resources for production and there still exists opportunities for improving on their current level of EE. This result further suggests that the farmers were not maximizing profit. The implication of this findings is that the more economically inefficient the okra farmers, the more the likelihood of the increased poverty status of the farmers. This is consistent with the findings of Onuche & Oladipo (2020) and Asogwa *et al.* (2011) who in their findings concluded that TE, AE and EE of small-scale farmers in Nigeria varied widely between minimum and maximum values and was an indication of their inefficiencies. Furthermore, the study revealed that for the minimum TE, AE and EE Okra farmers to become the most TE, AE and EE, they will need to realize about 98% output level closer to the production frontier (i.e. his or her output is closer to the maximum output obtainable from resources combined), 97% minimum wastage/ underutilization of resources to be closer to the frontier, and 92% output and minimization of resource wastage/ underutilization of resources in okra production to be able to achieve EE in okra production.

Relationship between Economic Efficiency and Farmers' Socio-Economic Factors

The relationship between EE and farmers' socio-economic factors was determined using Tobit dichotomous regression model, the result is shown in Table 4. The likelihood function was positive (6713.0616), while Chi-squared value (14029.12) is positive and significant at

Table 2. Profitability Analysis of Smallholder Okra Production per Hectare

Items	Amount (Naira)	% of Total Cost
Total Revenue	**765,000**	
Gross Income		
Variable Cost		
Seeds	5,674.56	3.8
Fertilizer Input	24,783.45	17.9
Insecticides	10,567.00	7.2
Herbicides	15,675.87	10.8
Labour Cost:		
(i) Land Clearing and Preparation	12,456.98	
(ii) Planting	7,765.90	
(iii) Weeding	13,674.76	
(iv) Fertilizer Application	8,574.65	
(v) Chemical Application	5000.00	
(vi) Harvesting	14,567.23	
(vii) Transportation	4,567.34	
(viii) Loading and Offloading	2235.50	
Total Labour Cost	**68,842.36**	**47.3**
Total Variable Cost	**125,543.24**	**86.2**
Fixed Cost		
Estimated Depreciation Value on Tools (Hoes, Machetes)	2,785.34	1.9
Rent on Land	17,34**5.67**	**11.9**
Total Fixed Cost	**20,131.01**	13.8
Total Cost	**145,674.25**	
Gross Margin (GM)	**619,325.77**	
Gross Margin Ratio (GMR)	**0.81**	
Net Farm Income (NFI)	**559,194.76**	
Operating Ratio (OR)	0.16	

Source: Field Survey (2022)

Table 3. Summary Statistics of Technical, Economic and Allocative Efficiency Scores

	Allocative Efficiency		Economic Efficiency		Technical Efficiency	
Efficiency Score	**Frequency**	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
0.00 – 0.20	5	4.2	21	17.5	7	5.8
0.21 – 0.40	12	10.0	25	20.8	16	13.3
0.41 – 0.60	18	15.6	19	15.8	22	18.3
0.61 – 0.80	5	4.2	17	14.2	17	14.2
0.81 – 1.00	80	66.7	38	31.7	58	48.3
Mean	0.7918		0.53388		0.83450	
Standard Deviation	28355		0.30451		0.26183	
Minimum	0.0265		0.08401		0.01583	
Maximum	1.00		0.9563		0.98912	

Source: Field Survey (2022)

1% probability level, the pseudo R^2 is 66% implying an absolute relationship between the explanatory variables and EE and this signifies that 66% of the variations in the predictor variables was explained by the model. From the Table 4, variables; age of household, educational level, farming experience, marital status, household size, farm size and membership of cooperative organizations were statistically significant and would increase the likelihood of household being economically efficient in okra production.

Age

The age of the farming households was found to have a positive coefficient (22.16075) and significant at 1% level of probability and consistent with apriori expectation. This implies that an increase in age of respondents would increase the probability of an increase in EE by 22%. It is well-known that in general, the older the farmers the more the experience they have in the production process. This finding is consistent with Kolawole & Ojo (2007) who in their study of small-scale oat growers in Nigeria found age to be positively related to inefficiency.

Educational Level

The coefficient (0.5308782) of this variable was found to be positive and significant at 1% level and is consistent

with apriori expectation. This means that an increase in educational status will increase the probability of EE by 53%. Education enhances the acquisition and utilization of new technologies by farmers Dey *et al.* (2001); Nwaru (2004); Effiong (2005); Onyenweaku *et al.* (2005). This implies that the greater number of years' people spent in school, the more likely will be the increase in their ability to produce to maximize their profit.

Marital Status

The coefficient of this variable was found to be positive (0.2466856) and significant at 1% level of probability. This means that marital status is an important variable in the probability of the farmers being able to maximize their profit. A change in marital status of the respondents will increase the probability of the respondents being able to maximize profit by 24%.

Farming Experience

The farming experience of okra farming households was found to have a positive coefficient (0.511709) and significant at 10% probability level. The sign of the variable is consistent with the apriori expectation. This means that an increase in the farming experience of okra farmers will result likelihood increase in the probability of profit maximization. This finding is in consistent with that of Onu *et al.* (2000) whose result showed a negative relationship farming experience and TE in cotton production in Nigeria.

Household Size

The coefficient of household size (32.08233) was found to be positive as expected and significant at 1% probability level. Household size determines the availability of family labour or large household size demands large amount of production to feed its members, that is as household size increases the demand for food increases. Increased in family size necessitates increase in household expenditures on food and other necessities/ utilities which ultimately increase food insecurity. This implies that farming households have a good source of family labour for the farm business. This is a positive indication that there would be more availability of family labour for farm work. In his study of productivity and TE of smallholder cocoa farmers in Nigeria, Amos (2007) found that family size was a significant variable which greatly influenced the TE of farmers.

Farm Size

Small farm size is an impediment to agricultural mechanization because using farm machineries like tractors to control weeds will be difficult. The size of farm cultivated by farmers is a function of population pressure, family size, labour productivity, financial background and experience of the farmers (Imonikhe, 2004). The coefficient of farm size (0.3245578) was found to be positive as expected and significant at 1% level of probability. Farm size determines the availability of supply to the markets. Therefore, increase in farm size will increase the probability of an increase EE of okra production.

Membership of Cooperative Organization

The coefficient of membership of cooperative organization (0.443748) was found to be positive and significant at 5% level of probability. This means that cooperative membership is positively related with level of respondents' EE of okra production. This implies as okra farmers becomes member of cooperative memberships will lead to probably of an increase in the respondents' EE in okra production. Cooperatives provides a cheap and an alternative means of raising the required capital for farm operation and expansion, which will have a positive impact on the EE of the respondents. Memberships of cooperative organization increases the chances of low interest credit and bulk purchase of inputs as well as training which reduce the cost of production and hence increase EE of the okra farmers (Gashaw *et al.,* 2013).

Table 4. Maximum Likelihood Results of the Tobit Dichotomous Regression Model

Variables	Parameters	Coefficient	Standard Error	t-Value
Constant	β_0	6.651348***	1.733975	3.84
Age	β_1	22.16075***	6.972824	3.18
Educational Level	β_2	0.5308782*	0.2988978	1.78
Marital Status	β_3	0.2466856***	0.0354053	6.97
Farming Experience	β_4	0.511709*	0.2998081	1.71
Household Size	β_5	32.08233***	9.071002	3.54
Access to Credit Facilities	β_6	0.012775	0.018464	0.69
Farm Size	β_7	0.3245578***	0.033339	9.74
Member of Cooperative Organization	β_8	0.443748**	0.185651	2.39
Sigma	4.24e-15			
LR Chi^2	14029.12			
Pseudo R^2	0.6572			
Log Likelihood	6713.0616			

Source: Data Analysis (2022) *Significant at (P<0.10)., **Significant at (P<0.05), ***Significant at (P<0.01).

Table 5. Principal Component Model of Constraints Encountered by Okra Producers

Constraints	Eigen-Value	Difference	Proportion	Cumulative
Lack of Farm Input	4.02962	2.09571	0.3100	0.3100
Lack of Credit Facilities	1.93391	.480707	0.1488	0.4587
High Cost of Labour	1.4532	.297931	0.1118	0.5705
Lack of Extension Agents	1.15527	.143099	0.0889	0.6594
Bad Road Infrastructures	1.01217	.270745	0.0779	0.7372
Pest and Disease Infestations	1.00142	.0565095	0.0570	0.7943
Lack of Chemicals	1.00119	.0982798	0.0527	0.8470
Lack of Fertilizers	1.00063	.153186	0.0451	0.8921
Bartlett Test of Sphericity				
Chi Square	234.56			
KMO	0.87			
Rho	1.0000			

Source: Field Survey (2022)

Principal Component Analysis of Constraints Facing Smallholder Okra Farmers

Table 5 shows the results of the constraints faced by smallholder okra farmers, PCA is a statistical package that transform interrelated data with many variables into few numbers of uncorrelated variables. From the result the number of principal components retained using the Kaiser Meyer criterion are eight (8) based on the Eigen value greater than 1. The retained components explained 89.21% of the variations of the component included in the model. The Kaiser-Meyer- Olkin measures of sampling adequacy (KMO) of 0.87 and Bartlett test of sphericity of 234.56 was significant at 1 % level of probability and demonstrated the feasibility of using the data set for principal component analysis. Lack of farm inputs had an Eigen value of 4.02962 and it was ranked 1st in the order of importance based on perceptions of the smallholder okra farmers. Lack of credit facilities and high cost of labour with Eigen values of 1.93391 and 1.4532 respectively were ranked 2nd and 3rd respectively in the order of occurrence based on the perceptions of the smallholder okra farmers as the major constraints facing okra production. Lack of extension agent, bad road infrastructures and pest and disease infestations with Eigen values of 1.15527, 1.01217 and 1.00142 were ranked 4th, 5th and 6th respectively in order of their occurrence and importance respectively based on the perceptions of smallholder okra farmers.

Conclusion and Recommendations

Based on these findings, it is concluded that okra production was profitable going the both profitability and financial indices. Similarly, the wide variations in the minimum and maximum values of technical, allocative and economic efficiencies were indicative of the inefficiencies of okra farmers, while lack of farm inputs, credit facilities, high cost of labour, lack of extension agents, bad road infrastructure, pests and disease infestations, lack of chemicals and lack of fertilizers were the identified constraints to okra production. It is therefore recommended that: - [1] Farmers should increase their farm size in order to increase their profitability, [2] Farmers should be educated through extension agencies in order to improve their technical, allocative and economic efficiencies for optimum profitability, and [3] Farm inputs like improved seeds, fertilizer input, tractors, chemicals, credit facilities should be made available to okra farmers to increase productivity and efficiency.

COMPLIANCE WITH ETHICAL STANDARDS

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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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RESEARCH ARTICLE

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Influences of grape seed substitution on the bioactive and sensory properties of brewed coffee

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Abstract

In this research, ground coffee beans were replaced with grape seed powder in different proportions. Thus, it was aimed to benefit from the health benefits of grape seeds and develop a new coffee formulation that is functional, low in caffeine and has a different taste and odor. For this purpose, the moisture, ash and crude fiber contents, antioxidant activity and total phenolic compounds of Besni karası (*Vitis vinifera* L.) grape seeds were examined. Grape seeds were ground and included in the coffee formulation in different proportions (0, 25, 40, 55%) with the same particle size. Physicochemical, bioactive and sensory properties of the produced coffee grounds were examined. As a result of the analyses, as the grape seed powder concentration increased in the coffee composition, the acidity decreased ($p<0.05$) and the antioxidant activity and total phenolic compounds increased ($p<0.05$). The brightness (L*) of the samples increased depending on the grape seed powder composition and usage rate. As a result of the sensory analysis, it was determined that the samples with 25% grape seed powder added received the closest score to the control group, and the samples with 40-55% grape seed powder had the similar scores with the control group in terms of roughness. In our study, the nutritional composition of grape seeds, which are food waste in the industry, has been revealed that it can be used as a substitute product in coffee and will contribute to sustainability.

Keywords: Functional food, Fortification, Antioxidant activity, Quality parameters

INTRODUCTION

Coffee is a type of beverage that is prepared according to the grinding of raw coffee seeds and different brewing methods and made ready for consumption (Arslan, 2019). Coffee, which is in the category of the most consumed beverage group in the world, especially after water and tea, is the second most traded commodity after petroleum (Atabani et al., 2019). Coffee is very rich in components that have healing effects on health, including polyphenols, compounds with antioxidant activity (chlorogenic acids), macro (carbohydrates, nitrogenous compounds, lipids) and micro (vitamins and minerals) nutrients (Nieber, 2017). The type of coffee bean, brewing conditions (temperature-time) and consumption frequency are factors that affect human health. Studies have shown that coffee consumption generally has a protective effect on cardiovascular diseases, hypertension, cancer, type 2 diabetes, Parkinson's disease, Alzheimer's and cognitive functions (Higdon and Frei, 2006; Bae et al., 2014).

Coffee culture, spanning health, social, and commercial realms, enjoys broad appeal across age groups (Hung, 2012). Consumption patterns are influenced

by factors like gender, age, diet, occupation, and income, with coffee accessible in diverse settings (Samoggia et al., 2020). Recent shifts in coffee habits have spurred an uptick in producers, prompting a focus on novel coffee varieties to meet evolving demand (Czarniecka-Skubina et al., 2021). In this context, there are various coffee brewing methods (filtration, boiling, pressure) (Stanek et al., 2021), aromatic components (hazelnut, vanilla, chocolate etc.) (Andrzejewski et al., 2004) and different coffee preparing methods to reveal the characteristic aroma and taste of coffee. Different types of coffee such as espresso, americano, latte macchiato or filter coffee are offered to consumers using various coffee beans (Banu et al., 2020; Wu et al., 2022). In order to increase the aromatic and nutritional value of coffee, seeds from different plant species can be used. There are some studies using different seeds such as durian seed (Natania and Wijaya, 2022), baobab seed (Ismail et al., 2022), pedada seed (Wulandari et al., 2021), palm seed (Fikry et al., 2019), salak seed (Suastitu et al., 2019) as substitutes for coffee. In general, one of the common goals of this type of research is to reduce the caffeine content of coffees. However, the plants used in such research are quite local and their availability is very limited.

Grape seed, which is called a by-product of grape processing in the food industry, is a rich source of vitamins, minerals and phytochemicals (polyphenols, aromatic acids, phenolic acids, flavonoids) (Makris et al., 2007). Due to its nutritive quality and high antioxidant capacity, its popularity is gradually increasing and it attracts the attention of both the producer and the consumer (Duba et al., 2015). It is a valuable product included in bread, biscuit, yogurt, and various meat product formulations to add functional properties (Antonic et al., 2020; Ayoubi et al., 2022; Elkatry et al., 2022). However, it has been determined that the study using grape seeds in the composition of coffee with a high consumption potential is limited in the literature. Only one study was found where grape seed powder was used as a coffee substitute. Ülger (2022) conducted the study in which grape seeds were used as a coffee substitute and 0-15% grape seed powder (30% Boğazkere and 70% Öküzgözü) were added to Turkish coffee. In the study, it was revealed that the total amount of phenolic compound 41.52-41.84 mg GAE/g DM, antioxidant activity value of 258.98-311.14 mmol trolox/g DM, grape seed addition improved the bioactive properties of Turkish coffee. The low concentration of grape seed powder used in the samples in the research and the limited global effect of Turkish coffee are different from the current study.

Caffeine content may vary depending on the brewing method applied, the type and amount of coffee beans used, the amount of water, brewing process conditions (pressure, temperature, time) and roasting conditions. In this case, the caffeine content of espresso type coffees obtained with the coffee machine was reported as 4.20 g/L (Olechno et al., 2021). The average caffeine content in grape seeds was reported as 0.96 mg/mL (Kim et al., 2006). In the current research, a new product with reduced caffeine content was obtained by reducing the amount of coffee and using grape seed powder as a substitute.

In this study, it was aimed to develop sustainable, functional coffee with high added value in terms of dietary fiber and bioactive properties and with less caffeine content by using Besni Karası grape seeds (in Figure 1), which are food waste in the industry.

Figure 1. Besni Karası Grape Seed

MATERIALS AND METHODS

Raw materials

Medium roasted Arabica whole coffee beans (*Coffea arabica* L.) grown in Latin American countries were procured from the Tchibo (Hamburg, Germany) sales point. The dried seeds of Besni Karası grapes grown in the Adıyaman region of Türkiye were obtained from a local spice shop (Güneş Baharat, Mersin, Türkiye). Tap water was used in the coffee production.

Ground coffee and grape seed powder production

Whole coffee beans and grape seeds were ground in a laboratory mill (Kiwi KSPG-4812, Istanbul, Türkiye) using 150 W power. Then, they were sieved (Model VE 50, Retsch, Germany) with a pore diameter of 50 µm.

Coffee production

Some preliminary experiments were carried out to determine the appropriate coffee production method. For this purpose, three different coffee brewing methods (filtration (French-press), pressure and boiling methods) were tried. In each method, coffee was replaced by grape seed by 55%. As a result of the sensory analyses made by 15 trained panelists (age range 20-36, non-smokers) the production was carried out with the most creditable method. The method used for sensory

analyses is explained in detail in the "coffee analysis" section. According to the sensory analysis results, the roughness caused by the insoluble of the ground grape seeds in the coffee obtained by boiling and filtration methods was not appreciated by the panelists. For this reason, it was decided that the appropriate method to be used in production was the pressure method. However, due to the fact that Espresso and many Espresso-based coffees can be produced with the pressure method, two different coffees, Espresso and Americano, were presented to the panelists. As a result, Americano was the more appreciated coffee type.

Americano-type coffee samples were made with a fully automatic espresso machine (Philips 3200 Series, Cluj-Napoca, Romania) working with a pressure of 15 bar and water temperature of 70-82°C as declared by the manufacturer. Americano coffee brew was prepared as described by Liu et al. (2017). Americano can simply be defined as a diluted espresso. When preparing single-shot espresso, 7 g of ground coffee and hot distilled water are used. Americano is made by adding 200 mL of boiling distilled water to single-shot espresso. In the study, the control sample was prepared according to the standard Americano production technique. In the other samples, 25%, 40% and 55% of the ground coffee was replaced with grape seed powder. Coffees prepared with grape seed substitute were coded as C208 (25%), C967 (40%) and C580 (55%), while the control sample was coded as C157. Coffee samples that were produced are presented at Figure 2. While the coffees prepared for use in sensory analyses were served hot, the coffees used in other analyses were stored in the refrigerator (UES 273 D2K 208 LT A++, Uğur, İzmir, Türkiye) at +4 °C for further use. Production, processes and all analysis on raw materials and coffees were carried out in the Toros University Food Technology laboratory.

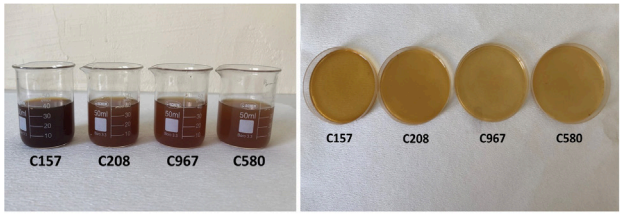

Figure 2. Americano coffee samples without and with grape seed powder
C157: Control, C208: Coffee with 25% grape seed powder, C967: Coffee with 40% grape seed powder, C580: Coffee with 55% grape seed powder

Raw material analysis

The moisture content of the samples was found by using the gravimetric method, by drying the coffee and grape seeds in a laboratory oven (Nuve EN400, Türkiye) at 105°C until they reach a constant weight, and then calculating the percent moisture content (AACC, 2010).

While calculating the amount of ash, the samples were weighed into porcelain crucibles and burned in a furnace (Thermnevo, Nevola, Türkiye) at 550±5 °C until they turned white. The residue was reweighed and the results expressed as a percentage (AOAC, 1990).

The crude fiber ratios in the raw material were determined using the gravimetric method (Özdemir et al., 2022), and for this purpose, 10 g of ground and sieved sample was kept in 300 mL of distilled water for 20 minutes. The mixture was filtered through coarse filter paper. Washing with distilled water was continued until the residue remaining on the paper reached a constant weight, and then dried in a laboratory oven (Nuve EN400, Türkiye) at 105°C. The dried residue was weighed and the amount of insoluble fiber and, accordingly, the % crude fiber content were calculated.

The total amount of phenolic substances was determined according to the Folin–Ciocalteau method (Singleton and Rossi, 1965). Solutions consisting of a mixture of samples and 80% methanol (1:25 w/v) were centrifuged (Nuve NF800 R, Türkiye) at 4500 rpm for 15 minutes. After 0.2 mL of the supernatant was taken, it was mixed with 1.5 mL of Folin-Ciocalteu reagent (reagent:water mixture, 1:10 v/v) and left in the dark for 5 minutes. Then, 1.5 mL of 7.5% sodium carbonate solution was added and after 90 minutes of resting in the dark, their absorbance at 765 nm was recorded with a UV–Vis spectrophotometer (UV-1601, Rayleigh, BFRL, China). The results were evaluated by calculating the phenolic content over the gallic acid equivalent.

Antioxidant activities of raw materials and coffee samples were measured according to the method specified by Brand-Williams et al. (1995). For this purpose, 5 g of sample and 50 mL of 80% methanol aqueous solution were mixed for 30 minutes. The mixture was filtered through coarse filter paper and the filtrate was centrifuged (Nuve NF800 R, Türkiye) at 4500 rpm for 15 minutes. Then, 100 µL of the extracts were taken into a cuvette and 3900 µL of DPPH (1,1-diphenyl-2-picrylhydrazil radical) solution (3.94 mg/100 mL methanol) was added and kept in the dark for 30 min. The absorbances of the samples were measured at 515 nm with a UV–Vis spectrophotometer (UV-1601, Rayleigh, BFRL, China). Trolox calibration was used to determine the antioxidant activity.

Coffee analysis

pH measurements were determined directly using the WTW 3110 brand pH meter (WTW, Germany) (Hannon et al., 2003). Titratable acidity was determined by titration of 1g aliquot of coffee brew with 0.1N NaOH at 22°C to a pH of 6.0 and a pH of 8.0 (Rao and Fuller, 2018). The results were expressed in mL of (0.1 N) NaOH. Total phenolic compounds and antioxidant activity were determined as specified in the raw material analysis section. Color analysis was performed using Chroma Meter (Minolta, CR300, Japan). Results were expressed in parameters

L*, a* and b*. Before measurements, calibration was performed with white and black calibration plates as references. The results were the average of at least three measurements from different quadrants of each sample (Martley and Michel, 2001). Sensory evaluation of coffee samples obtained in preliminary trials and final productions were performed according to the method specified by Stone and Sidel (2004). A trained panelist group of 15 consisting of Toros University faculty members and graduate students participated in the analyses. Necessary training was given to the panelists before the analysis and random codes were given to the samples. Sensory evaluation was performed immediately after the coffees were produced, while the samples were still warm (70±5 °C). Coffee samples were evaluated using a 5-point linear hedonic scale (1:extremely dislike to 5:extremely like); rated for color, odor, taste and flavor, fluidity, roughness, mouthfeel and overall acceptability.

Statistical analysis

Coffee production was carried out as four different samples at three replications. The experimental data were evaluated by variance analysis (ANOVA) to detect the significant differences ($p<0.05$). SPSS (Version 20, IBM, USA) was used to determine the Duncan correlation coefficients with 95% confidence level (Efe et al., 2000).

RESULTS AND DISCUSSION

Compositional properties of coffee bean and grape seed

The compositional properties of ground coffee bean and grape seed powder are given in Table 1. The moisture and ash contents of the coffee bean and grape seed powder were 7.65-6.98% and 5.39-2.39%, respectively. The coffee bean moisture content should be below 12.5% (Kyaw and Budiastra, 2020). Moisture content above this value causes microbial deterioration in the coffee bean and causes off-flavors (Adnan et al., 2017). When previous studies were examined, it was determined that the moisture content of granule coffee beans varied in the range of 5.52-13% and was consistent with the results of our study (Mazzafera, 1999; Murthy and Manonmani, 2009). Elkatry et al (2020) determined the moisture and ash content of grape seed powder as 7.16%-3.54%, respectively. The difference in results with our study is related to the type of grape seed, cultivation area and soil composition. Total fiber content of grape seed powder was found higher than that of ground coffee beans (1.5 times higher). Therefore, coffee enriched with grape seed powder has been of superior quality in terms of fiber. When the previous studies were examined, it was determined that the crude fiber content of the coffee bean and the grape seed varied between 2.30-24.70% and 32.21-83.01%, respectively and this situation was due to the raw material composition (Maman and Yu, 2019; Grzelczyk et al., 2022; Oprea et al., 2022). Vazquez-Sanchez et al (2018) reported that the change in the chemical composition of polysaccharides in coffee, depending on the roasting process, affects the fiber content. When the total phenolic content of ground coffee beans and grape seed powder used in making Americano coffee are examined (Table 1), it was observed that the total phenolic content of the coffee bean was 435.84 mgGAE/100g and the grape seed powder was 989.52 mgGAE/100g. Similarly, the antioxidant activity of grape seed powder was higher than that of coffee beans (145.32 and 139.11 µmolTE/100g, respectively). As a result, it was determined that grape seed powder had 2.26 times more phenolic substances and 1.04 times more antioxidant activity than coffee beans. In the current study, the higher phenolic component content of grape seed powder (catechin, epicatechin, epicatechin gallate, dimeric, trimeric) compared to granulated coffee might be associated with the diversity of phenolic species. Grape seeds and coffee beans contain different phenolic compounds. Grape seeds are rich in proanthocyanidins (OPCs), a type of phenolic compound known for its potent antioxidant properties. They also contain flavonoids such as quercetin and kaempferol, as well as phenolic acids like gallic acid and caffeic acid. On the other hand, coffee beans are abundant in chlorogenic acids, which contribute to the taste and antioxidant properties of coffee. They also contain caffeine, providing a stimulating effect, and trigonelline, another important alkaloid. Both seeds provide significant antioxidants, but their phenolic profiles are distinct, suggesting different potential health benefits (Rababah et al. 2004; Bucić-Kojić et al., 2009).

When the studies are examined, it has been determined that the antioxidant activity of the Arabica coffee bean is in the range of 511.66-1150.50 µM TE/g (de Souza et al., 2020) and the grape seed powder is in the range of 88.60-3068.00 µM TE/g dry matter (Li et al., 2008). The higher antioxidant capacity of some species of grape seed powder is associated with the presence of polymeric and monomeric compounds (catechin and epicatechin) (Peng et al., 2010). It was reported that the total phenolic substance amount of Arabica coffee was in the range of 14.92-16.55 mg GAE/g (Alnsour et al., 2022), and the phenolic substance amount of grape seeds was in the range of 79.06–111.22 mg GAE/g (Krasteva et al., 2023). As a result, other studies have also shown that the phenolic substance content of grape seeds is higher than roasted coffee.

pH and titratable acidity of coffee

The pH value and titratable acidity of the coffee samples are presented in Figure 3. When the graph is examined, it is seen that the pH value of the control sample is lower than the pH values of all coffees using grape seed ($p<0.05$). As the addition of grape seed powder increased, the pH values of the coffees decreased and statistically significant differences were detected between pH values of all coffee samples ($p<0.05$). Supporting these results, the titratable acidity of the grape seed powder added

Table 1. Compositional properties of coffee bean and grape seed (n=3)

Composition	Ground coffee beans (*Coffea arabica* L.)	Grape seed powder (Besni Karası, *Vitis vinifera* L.)
Moisture (%)	7.65±0.24	6.98±0.31
Ash (%)	5.39±0.08	2.39±0.03
Crude fiber (%)	27.22±1.56	48.65±2.67
Antioxidant activity (mmol trolox/100g)	139.11±9.73	145.32±8.22
Total phenolic compound (mg GAE/100g	435.84±6.45	989.52±8.71

samples was also found lower than the control sample ($p<0.05$). The lowest acidity level was determined in coffees with 25% grape seed powder substitute. While there was no statistical difference between the acidity of 40% and 55% grape seed powder substituted coffees ($p>0.05$), the acidity of these samples was found significantly higher than that of 25% grape seed powder substituted coffee ($p<0.05$).

The acid content of coffee is an important factor that determines the aroma, flavor and content of bioactive components of coffee. Chlorogenic acid is responsible for the bitterness of coffee and is the main component that creates the acidity of coffee, and it provides the decomposition of phenolic components during the roasting process of coffee. Many low molecular weight compounds with water-soluble properties such as citric, malic, quinic, succinic and gluconic acids are responsible for the acidity of coffee (Yıldız, 2022). Maier et al. (1983) stated that pH values in the perception of bitterness of coffee provide a better correlation with bitter taste than titratable acidity value. In this study, it was observed that the high acidity of coffee decreased with the addition of grape seeds. It is known that grape seed releases potassium and potassium causes precipitation in acid fractions (Pascual et al., 2016). It is thought that the decrease in the acidity and the increase in the pH may be related to the potassium released from the grape seed.

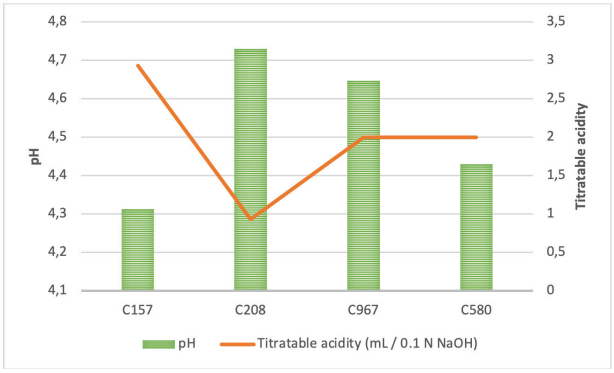

Figure 3. pH and titratable acidity of Americano coffee with grape seed powder (n=3)

C157: Control, C208: Coffee with 25% grape seed powder, C967: Coffee with 40% grape seed powder, C580: Coffee with 55% grape seed powder

Antioxidant activity and total phenolic compound of coffee

Antioxidant activity and total phenolic compounds of coffee samples are given in Table 2. The antioxidant activity depending on the grape seed powder in coffee formulations varies in the range of 140.43 (control)-179.03 (55%) mmol trolox/100g and this data shows a gradual increase as the use of grape seed powder ($p<0.05$). The increase is associated with the rich phenolic compounds of grape seeds. Additionally, it has been shown that high temperature application in the coffee roasting process affects the chemical composition of the coffee bean. Polyphenol levels of coffee decrease due to polymerization, auto-oxidation and degradation that occur during the roasting process (Cheong et al., 2013). The grape seeds used in our study were not subjected to the roasting process, but were directly ground and included in the product formulation. Thus, the effectiveness of compounds with antioxidative properties (chlorogenic acid) was preserved (Priftis et al., 2015).

Coffee is an important source of bioactive compounds, in particularly phenolic compounds (chlorogenic, coumaric, caffeic acid) (Ismail et al., 2022). In our study, it was determined that the total phenolic compound of coffee samples varies between 203.26 (control)-304.75 (55% grape seed powder) mg GAE/100 g. As with antioxidant activity, the total amount of phenolic substances in coffees increased proportionally with the increase in the use of grape seed powder. It is thought that the type-origin and usage amount of grape seeds may be effective in this increase. Ülger (2022) found that the total phenolic substance increased significantly due to the use of grape seed in Turkish coffee. It was stated that this situation is related to grape seed type, brewing temperature and particle size. It has been reported that the total phenolic substance and antioxidant capacity increases with the use of grape seed powder, especially in bakery products such as dough (Aghamirzaei et al., 2015), bread (Hoye and Ross, 2011), muffins (Yalçın et al., 2022) and cookies (Acun and Gül, 2014).

Color properties of coffee

The L*, a*, b* color values of Americano coffee with grape seed powder describing brightness (L*), redness (a*) and yellowness (b*) (Seçilmiş et al., 2015), and the color characteristics of the coffee samples are given in Table 3. It was observed that the L* values of the coffees increased

Table 2. Antioxidant activity and total phenolic compound of Americano coffee with grape seed powder (n=3)

Coffee samples	Grape seed powder (%)	Antioxidant activity (mmol trolox/100g)	Total phenolic compound (mg GAE/100g)
C157	-	140.43±0.79d	203.26±2.77d
C208	25	152.22±1.00c	225.27±2.80c
C967	40	165.79±0.52b	246.38±3.13b
C580	55	179.03±0.45a	304.75±2.25a

a, b, c, d Values shown with different exponential letters in the same column differ from each other at the $p < 0.05$ level

significantly as the grape seed powder concentration in the coffees increased ($p<0.05$). In other words, the addition of grape seeds resulted in lighter and brighter coffees. While the L* values of the 25% and 40% grape seed powder added coffees were statistically equivalent to each other ($p>0.05$), they were significantly different from the other samples ($p<0.05$). The a* values of the coffees decreased due to the increase in the grape seed concentration, that was, a higher amount of redness was detected in the control sample ($p<0.05$). Coffees made with grape seed substitutes were less intense in terms of redness ($p<0.05$). When the b* value of the coffees was examined, it was seen that the coffees made with 25% grape seed powder substitute had a similar level of yellowness with the control sample ($p>0.05$). However, when the grape seed powder was substituted at 40% and 55%, the b* value increased significantly ($p<0.05$). There was no statistically difference between C967 and C580 samples ($p>0.05$).

was seen that grape seeds contain higher levels of gallic acid than coffee. In addition, it was reported that the tannin content (3.12 mg/g to 8.82 mg/g) (Ju et al., 2021) and sinapic acid content (0.169-0.291 mg/g) (Salem et al., 2022) of grape seeds are higher than coffee (0.7-0.9 mg/g and 0.07-0.16 µmol/g, respectively) (Monente et al., 2015; Choi and Koh, 2017). Under these conditions, it can be expected that the a* values of the coffees made with grape seed substitute are higher than the control sample, but on the contrary, it is seen that the a* value of the control sample was higher. The reason for this was that the roasting process was not applied in the grape seeds. As mentioned before, high roasting temperature is an important criterion in obtaining red color. In addition, when the acidity and pH values of the samples were examined, it was seen that the control sample had higher acidity than the grape seed substituted samples. This was another factor explaining the highest a* value of the control sample.

Table 3. Color properties of Americano coffee with grape seed powder (n=3)

Coffee samples	Grape seed powder (%)	L*	a*	b*
C157	-	10.11±1.76c	11.51±0.20a	8.86±0.41b
C208	25	19.23±0.57b	8.72±0.33b	9.05±0.51b
C967	40	20.62±0.43b	6.92±0.24c	20.75±1.76a
C580	55	23.92±1.14a	4.07±0.14d	20.10±2.07a

a, b, c, d Values shown with different exponential letters in the same column differ from each other at the $p < 0.05$ level

The caramelization and Maillard reactions that produce the melanoidins responsible for browning the coffee take place during the roasting of the coffee. Therefore, the color of roasted coffee darkens (Ismail et al., 2022). Since the grape seeds were not roasted, no browning reaction took place. This was due to the fact that the L* and b* values of the control sample were lower than the coffees with the addition of grape seed powder. In a study where durian seeds were used as a coffee substitute, it was reported that the L* values of coffees produced with durian seeds were higher than coffees produced from Arabica and Robusta coffee beans (Natania and Wijaya, 2022). According to Hutami et al. (2018), the red color increases when tannins interact with enzymes or acids such as gallic acid, sinapic acid, which give the red color pigment under conditions of high roasting temperatures. Therefore, tannin, gallic acid, sinapic acid amounts, pH and acidity levels and heat treatment conditions have critical importance on red color. When the levels of total phenolic compounds were examined, it

Sensory properties of coffee

The sensory properties of Americano type coffees produced with the addition of grape seed powder at different rates were evaluated in terms of color, odor, taste and flavor, fluidity, roughness, mouthfeel and overall acceptability, and the results are presented in Figure 4. As a result of the evaluation, significant differences between the samples were observed in all sensory parameters except roughness ($p<0.05$). It was determined that the color, odor, taste and flavor, fluidity, mouthfeel and overall acceptability of the control sample and the coffees with 25% grape seed powder (C208) added were statistically equivalent to each other ($p>0.05$). However, the addition of grape seed powder at the rates of 40% and 55% significantly reduced the scores given to these characteristics of the coffees ($p<0.05$). As the concentration of grape seed powder increased, the sour taste and odor perceived in the coffee samples were not liked. For the same reason,

the C967 and C580 samples also scored low in terms of mouthfeel. The high level of polyphenols contained in grape seeds can cause sourness (Axten et al., 2008). It has been reported that the addition of grape seed powder causes sour taste and odor in bread samples (Pecivova et al., 2014). Considering the pH and titratable acidity results, the unique bitterness of the coffee decreased due to the fact that the addition of grape seed reduced the acidity, and accordingly, the taste, odor and mouthfeel scores decreased. In addition, the color of the coffees with high grape seed powder concentration was lighter, so samples C967 and C580 were not liked in terms of color when compared to the control. The water solubility of grape seed powder is not as high as that of coffee. Therefore, the viscosity of the coffee samples differed, and the C967 and C580 samples with less coffee got low scores in terms of fluidity. However, the panelists did not observe any difference in the roughness characteristics of the samples. In terms of overall acceptability, it was detected that the coffee with 25% grape seed powder got similar scores with the control sample ($p>0.05$), but the 40% and 55% grape seed powder addition was not acceptable ($p<0.05$).

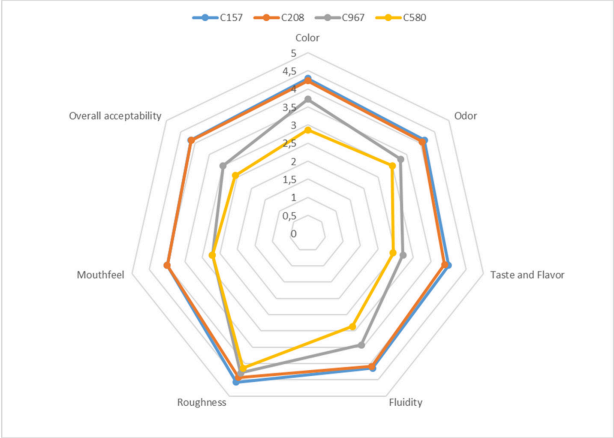

Figure 4. Sensory properties of Americano coffee with grape seed powder

C157: Control, C208: Coffee with 25% grape seed powder, C967: Coffee with 40% grape seed powder, C580: Coffee with 55% grape seed powder

In the research conducted by Ülger (2022), different proportions of grape seeds (Öküzgözü and Boğazkere) and pine hull were used in the production of Turkish coffee, and the sensory properties of the coffees were examined. The researcher stated that the grape seed substitute creates fruity and fermented odors in coffees, and oily and sour taste was perceived. The author reported that the coffees using grape seed were high-grained and high in roughness. The author also stated that in terms of taste, the coffee with 10% grape seed addition was the most demanded coffee. In studies where fruit seeds such as date (Fikry et al., 2019), baobab (Ismail et al., 2022), and durian (Natania and Wijaya, 2022) were used as coffee substitutes, it was stated that the fruity taste and odors that were generally perceived in coffees were liked.

CONCLUSION

As a result of this research, which aims to provide functional properties to coffee, one of the most consumed beverages in the world, a coffee variety with different taste, texture and flavor, low in caffeine and high in bioactive properties has been obtained. The raw materials used in the research were examined and it was determined that grape seeds were richer than coffee in terms of crude fiber, antioxidant activity and total phenolic substance amount. For this reason, using grape seeds, which mostly appear as food waste, in food products not only gives functional properties to the products, but also provides gains in sustainability, environmental health and financial terms. In coffees obtained by substituting grape seed powder in different amounts, as the amount of grape seeds increased, the amounts of antioxidants and phenolic substances also increased. Since grape seeds release potassium into the media, they reduced the total acidity of the coffees and increased the pH. As the amount of grape seed powder increased, a lighter color was observed in the coffees. Additionally, brightness and yellowness increased, while redness decreased. It was determined that 25% grape seed addition was equivalent to the control sample in terms of all sensory properties, while 40 and 55% were not acceptable. The use of grape seeds increased the sourness in coffees and reduced the desired bitterness. As a result, 25% grape seed substitution can be applied in coffee production. Due to the high water content of Americano type coffee, the use of high amounts of grape seeds may have caused a further decrease in sensory properties. Therefore, in coffees with more intense coffee content, such as espresso, the evaluation of substances with rich composition, which are considered waste, can be tried in different studies.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

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.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Yield prediction of wheat at different sowing dates and irrigation regimes using the AquaCrop model

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Abstract

Water efficiency models are playing an increasingly important role in helping agricultural activities adapt to climate change. AquaCrop is one of the models that can accurately correlate water-plant-climate parameters. In this study, the effects of irrigation strategies (I_1; rainfed, I_2; irrigation at Germination (G)+Tillering (T)+Heading (H) stages, I_3; irrigation at G+H stages, I_4; irrigation at G+T stages) and sowing dates (SD_1; normal sowing date, SD_2; late sowing date) on winter wheat yield and soil water conditions were investigated in semi-arid climate conditions. Biomass, grain yield, soil water content and crop canopy cover values observed in field conditions and simulated by AquaCrop. According to results SD_1 did not have a negative effect on grain yield and biomass however SD_2 would significantly reduce grain yield and biomass amount. Considering the biomass and grain yields in terms of irrigation, the highest yield was obtained in the irrigation water applied during the I_2SD_1 treatment. The yield reduction was 39% in rainfed treatments, 22% when irrigated in G+T periods, and 5% when irrigated in G+H stages. The model predicted 2-year grain yield and biomass values more accurately in SD_1 than in SD_2. The model predicted yield, biomass, soil moisture content and canopy cover values with an acceptable accuracy.

Keywords: Yield prediction, AquaCrop, Winter wheat, Sowing date, Canopy cover, Irrigation regimes

INTRODUCTION

The pressure on fresh water resources to meet food demand of the growing population is increasing because of the rapid growth in world population. In order to produce enough food to meet the demand, it is possible with irrigated agriculture. Therefore, agriculture is the largest water consumer sector with a usage share of 75% (Aouade et al., 2016; Tan et al., 2017; Zhuo and Hoekstra, 2017). Especially in arid and semi-arid regions, water is the main limiting factor. The water used in irrigation constitutes 73% of the total water resources in Türkiye (Gokalp and Cakmak, 2016). Ankara is located in center part of Central Anatolia Region in Türkiye. Drought and water scarcity is an iterative climate phenomenon in this region which leaves many socioeconomic and ecological challenges (Delju et al., 2013). Because of that, assignation sustainable methods to increase water use efficiency is becoming the aim of many studies (Debaeke and Aboudrare, 2004). The most widespread approach for overcoming current and future water-related challenges is to focus firstly on improving agricultural water productivity by applying irrigation water -saving strategies. If considered in more detail, these difficulties can be solved owing to efficient agronomic planning, including appropriate irrigation scheduling with considering such as

deficit irrigation and suitable sowing dates, which would allow the same amount of agricultural production with less water (Davarpanah and Ahmadi 2021).

The global wheat production came to about 778.6 million tons in 2021-2022 growing season (Shahbandeh, 2022). Wheat are main cultivated crops in Türkiye where wheat cultivation area has a value of 2.4% in the world as of 2020-2021 production season (USDA, 2021). However, production occurs highly variable from year to year depending on climatic variability (TUIK, 2021). In order to prevent this fluctuation in wheat production, which is also affected by climate change, it should be a priority to increase water efficiency in agriculture.

In different climatic conditions, it is very important to monitor plant development, estimate yield, choose appropriate planting dates and develop crop management strategies. The effects of environmental factors and agricultural inputs on crop production are generally tested by conducting field studies. However, these studies take a long time, are expensive, and the application method is quite complex when more than one variable is involved. For this reason, computer simulation models that empirically formulate the ecosystem environment have been developed. It is assumed that this mathematically formulated system will respond to different environmental factors like a real plant system.

FAO's AquaCrop water efficiency model is developed to predict the effects of different irrigation practices and all parameters affecting plant growth on crop yield (Steduto et al., 2012). The model has been tested to simulate the yield response to water for most of the major field crop such as the forage plants, vegetables, cereals, fruits, root and tuber crops grown worldwide (Hsiao et al., 2009; Raes et al., 2009a; Steduto et al., 2009; Kale Celik et al. 2018). Simulation results of the model have shown high accuracy (Salemi et al., 2011; Zhang et al 2013; Tavakoli et al., 2015; Kale Celik et al. 2018).

In this study, calibration and validation of the AquaCrop model was performed using experimental field data under various irrigation strategies and different winter wheat planting dates. By using the model simulation results it will be possible; i) to estimate the yield at the regional level under semi-arid climate conditions ii) to determine the changes in yield, water use efficiency and the moisture levels of soil profile under several irrigation strategies and different planting dates. This model was used in this study to simulate the effects of different irrigation strategies and different seed planting dates on winter wheat yield and biomass. Thus, it will be possible to make management strategies to prevent yield losses that may occur due to climate change, such as changing the irrigation regime or planting date.

MATERIAL AND METHODS

Materials

Experimental Area

The field experiment was carried out in the 2015-2016 and 2016-2017 growing seasons at the İkizce/Haymana Research Station of the Central Research Institute of Field Crops. The study area is in the Haymana plain, which is located in the central of Türkiye, Ankara province, extended between 39°12′ and 43°6′ northern latitudes, and 35°58′ and 37°44′ eastern longitudes (Figure 1).

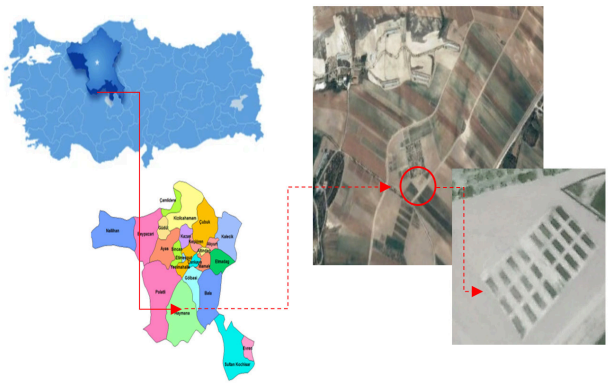

Figure 1. Experimental area.

Climatic data including rainfall, average air temperature and relative humidity for the period of 2015-2017, were obtained from the Meteorological station of Haymana plain (Table 1). Average rainfall, air temperature and relative humidity for simulation years were 259.2 mm, 10.7 °C and 60.9% respectively (TSMS, 2018).

Silty clay at 0-30 cm of soil depth and clay at 60-90 cm of soil depth is the most dominant soil texture in the experimental area. Average volumetric field capacity, volumetric permanent wilting point and bulk density are 36%, 17%, and 1.19 gr cm^{-3} respectively. The soil saturation hydraulic conductivity is about 0.75 cm h^{-1}.

"Konya 2000" winter wheat variety was used in this research. This variety has high yield potential (400-750 kg da^{-1}) and is recommended for irrigable areas of Central Anatolia Regions. This variety, which is sensitive to drought, has high tolerance to winter and cold (Aydoğan and Soylu 2017).

AquaCrop, simulation model developed by the United Nations Food and Agriculture Organization (FAO), is mostly used to simulate the response of plants to water and the impact of meteorological risks on plant development. In this study, version 5.0 of the AquaCrop model was used (Raes et al., 2016).

AquaCrop Model organized at several modules which are climate, soil, plant and agricultural activities. Model inputs;

- Climate input file (daily rainfall, minimum and

Table 1. Climate data on Haymana experimental station

Months	Rainfall (mm)			Average air temperature (°C)			Average Relative Humidity (%)		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
January	42.2	87.3	20.2	-1.4	-0.7	-4.6	88.6	67.1	76.1
February	4.5	24.9	10.0	0.8	5.9	0.9	82.1	67.4	63.9
Mart	16.3	5.4	35.7	4.7	5.7	6.5	72.7	76.1	63.1
April	12.8	3.2	14.8	9.5	12.1	9.1	64.7	52.1	54.3
May	45.3	22.8	27.8	14.3	13.1	13	61.9	62.2	56.9
June	1.2	1.5	25.2	22.8	19.3	17.4	56.4	48.0	57.4
July	15.5	0.0	0.4	22.6	22.1	22.6	48.6	40.1	42.3
August	16.2	18.9	26.2	17.7	23.0	22.1	47.2	46.8	48.5
September	13.4	64.2	30.2	18.6	17.5	20.0	47.2	51.8	39.3
October	7.0	10.8	15.9	11.1	12.1	9.8	78.0	55.7	59.8
November	7.2	30.5	31.5	5.4	5.0	5.4	68.5	55.7	73.5
December	43.6	9.9	35.1	0.8	-1.9	2.9	75.8	69.5	72.6

maximum air temperature, wind speed, relative humidity, CO_2 amount (by default AquaCrop obtains the atmospheric CO_2 concentration for a particular year), sunshine hours, reference evapotranspiration). In this study, daily climate data were obtained from Haymana Meteorological Station of the General Directorate of Meteorology. ETo was calculated by FAO Penman-Monteith Equation (Allen et al., 1998) in the AquaCrop model.

- Soil input file (field capacity and permanent wilting point and saturated hydraulic conductivity, soil texture etc.)
- Crop input file (emergence, maximum root depth, time and duration of flowering, canopy senescence, maximum canopy cover and maturity),
- Field management input file (irrigation schedule, irrigation water quality, management practices) and
- Initial condition input (soil water content, soil salinity).

Methods

The study was carried out through two growing seasons from 2015 to 2017. 2015-2016 growing season values were used for calibration and 2016- 2017 for validation processes. The experiment consists of 4 irrigation regimes and two different sowing date with 3 replications. Field treatments are given at Table 2.

The experimental design was as a complete randomized block design with a split plot layout. Plot dimensions were taken 17.5 m^2 (5 m x 3.5 m). There was 2 m distance between all plots. The plots have almost zero slope and were surrounded about 0.30 m high soil bunds (Figure 2). Before irrigation, soil moisture content was measured by the gravimetric method. Irrigation water was applied until the soil moisture reached the field capacity with surface irrigation method. Water meter was used to measure applied irrigation water amount. Water table depth around 4 m. Tensiometers were used to control deep seepage.

The irrigation treatments consisted of water application at different stages of the plant growth. Irrigation water was applied one time at the beginning of the growing stages (according to treatments which were given in Table 2) until the soil water content was reached to field capacity in 90 cm soil depth. The total irrigation amount (without rainfall) for irrigation treatments were I_1: 0 mm, I_2: 275 mm (G:30 mm, T; 70 mm and H:145 mm), I_3: 175 mm (G:30 mm and H:145 mm), I_4:100 mm (G:30 mm and T; 70 mm). Total rainfall has been 337.2 mm during the

Table 2. Irrigation treatments and sowing date of the experiment

Sowing dates	Irrigation treatments	Wheat growing stages		
		Germination (G)	Tillering (T)	Heading (H)
SD_1 (Normal sowing date)	I_1 (rainfed - no irrigation)	-	-	-
	I_2 (irrigate at germination, tillering and heading stage)	x	x	x
	I_3 (irrigation at germination and heading stage)	x	-	x
	I_4 (irrigation at germination and tillering stage)	x	x	-
SD_2 (Late sowing date)	I_1 (rainfed - no irrigation)	-	-	-
	I_2 (irrigate at germination, tillering and heading stage)	x	x	x
	I_3 (irrigation at germination and heading stage)	x	-	x
	I_4 (irrigation at germination and tillering stage)	x	x	-

(-); no irrigation, (x); irrigation

growing season.

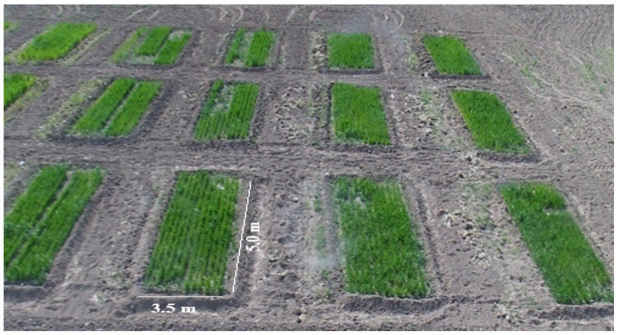

Figure 2. Field experimental design.

In the model the Crop file inputs consist of conservative (crop development, crop transpiration, biomass production and stresses which is not change with management and time) and non-conservative parameters (sowing rate, plant density, time from germination to maturity etc.) (Raes et al., 2009b). The AquaCrop model was run in the basis of the growing degree day (GDD). GDD was calculated by Equation 1.

$$GDD = \left(\frac{T_{max} + T_{min}}{2}\right) - T_b \quad [1]$$

T_{max} and T_{min} is daily maximum and minimum air temperature. The base temperature (T_b) is the cool temperature at which a plant does not develop. Crop inputs for wheat used in the AquaCrop model were given in Table 3.

Calibration and validation of the model

Calibration and validation steps of the models are the basic required steps to increase the accuracy and validity of simulations. The AquaCrop model was calibrated during 2015-2016 cropping season using measured data set of grain and biomass yield (GY, BY) and canopy cover (CC) and validated during the 2016-2017 cropping season using measured data set.

The statistical evaluation of the validity of the model was carried out by comparing the measured and estimated grain and yield biomass and canopy cover percentages. Determination coefficient (R^2), root mean square error (RMSE), normalized root mean square error (NRMSE) and model performance coefficient or model efficiency (EF) were used for determining the relationship between measured and estimated values. Those statistical parameters were calculated using equation 2, 3, 4 and 5 (Nash and Sutcliffe, 1970; Lyman, 1993; Janssen and Heuberger, 1995).

where n is the total number of observations, O_i and S_i are observed and simulated values respectively, O_{avg} and S_{avg} are average values of O_i and S_i (i from 1 to n) respectively. Coefficient of determination (R^2) ranges from 0 to 1, with values close to 1 indicating a good agreement and typically values greater than 0.5 are considered acceptable simulation (Moriasi et al., 2007).

$$R^2 = \left(\frac{\sum_{i=1}^{n}(o_i - o_{avg})(s_i - s_{avg})}{\sqrt{\sum_{i=1}^{n}(o_i - o_{avg})^2}\sqrt{\sum_{i=1}^{n}(s_i - s_{avg})^2}}\right)^2 \quad [2]$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(S_i - O_i)^2}{n}} \quad [3]$$

$$NRMSE = \frac{RSME}{O_{avg}} x100 \quad [4]$$

$$EF = 1 - \frac{\sum_{i=1}^{n}(O_i - S_i)^2}{\sum_{i=1}^{n}(O_i - O_{avg})^2} \quad [5]$$

The RMSE has the same unit as that of studied simulation variable. The closer RMSE value is to zero show the better match between the model simulation and field observation. According to Raes et al. (2015), the interpretation of the indices of NRMSE is lower than 5% shows model has been calibrated excellent, between 6% to 15% good, 16% to 25% moderately good, 26% to 35% moderately poor, 36% to 45% poor and more than 45% very poor.

The value EF is from - ∞ to 1. The EF value is close to 1 indicates that there is a perfect fit between the model and the observation values, and if it is close to 0, the model should not be used.

Determination of soil moisture values

Soil moisture was measured to gravimetric method before irrigation events. Soil samples were taken 30 cm increments from 0-120 cm for moisture analysis. According to the gravimetric approach, the amount of moisture in the soil has been determined after it has been dried at 105°C until soil moisture is constant (24 or 48 hours).

Biomass measurements

During the growing period of the plant, biomass measurements were made by taking into account the above-ground vegetative part within a 50x50 cm frame at several times. The plants were cut from the soil surface and dried in an oven at 75°C until constant weight. Dry matter was determined by weighing the dry plants (Todd et al. 1998).

Determination of Plant Green Cover Percentage (CC)

Digital photographs were taken from the fixed level with a high-resolution digital camera from an area of 0.25 m^2 (with 50x50 cm frame) at each plot from April to June (between 11:00 and 15:00). The percentage of green cover (vegetation) was determined by automatic classification using the Greencrop Tracer program. This program is a histogram-based program developed in

Table 3. Crop inputs for wheat used in the AquaCrop model

Defining	Values	Remarks
Conservative input parameters		
Base temperature (^{0}C)	0	Local experience
Upper temperature (^{0}C)	26	Local experience
Canopy cover per seedling at 90% emergence CC_0 (%)	7.16	Calibrated
Canopy growth coefficient (CGC) % in each GDD	2.4	Calibrated
Canopy decline coefficient at senescence % in each GDD	0.39	Calibrated
Maximum canopy cover percentage, CC_x (%)	95	Calibrated
Upper threshold for canopy expansion	0.20	Default (Steduto et al. 2012)
Lower threshold for canopy expansion	0.65	Default (Steduto et al. 2012)
Leaf expansion stress coefficient curve shape	5.0	Default (Steduto et al. 2012)
Upper threshold for stomatal closure	0.65	Default (Steduto et al. 2012)
Stomata stress coefficient curve shape	2.5	Default (Steduto et al. 2012)
Canopy senescence stress coefficient	0.70	Default (Steduto et al. 2012)
Senescence stress coefficient curve shape	2.5	Default (Steduto et al. 2012)
Reference harvest index, HI (%)	38	Local experience
Normalized crop water productivity, g m^{-2}	15	Default (Steduto et al. 2012)
Non-Conservative input parameters		
Sowing rate kg seed ha^{-1}	180	Measured
1000 seed mass g	43.7	Measured
Plant density plant m^{-2}	477	Measured
Canopy cover per seeding (cm^2 $plant^{-1}$)	1.5	Measured
Germination rate %	98	Measured
Sowing date (for SD_1 and SD_2 sowing date)	October 12th/ November 8rd	
Time from sowing to emergence date (GDD)	86 / 300	
Time from sowing to maximum root depth date (GDD)	866 / 903	
Time to reach flowering date (GDD)	1384 / 1409	
Duration of flowering stage	7 days	
Time to reach max canopy cover date	1239 / 1384	
Time to start senescence date	1815 / 1798	
Time from sowing to reach maturity date	2210 / 2210	
Minimum effective root depth m	1.2 Local experience	
Maximum effective root depth m	0.3 Local experience	

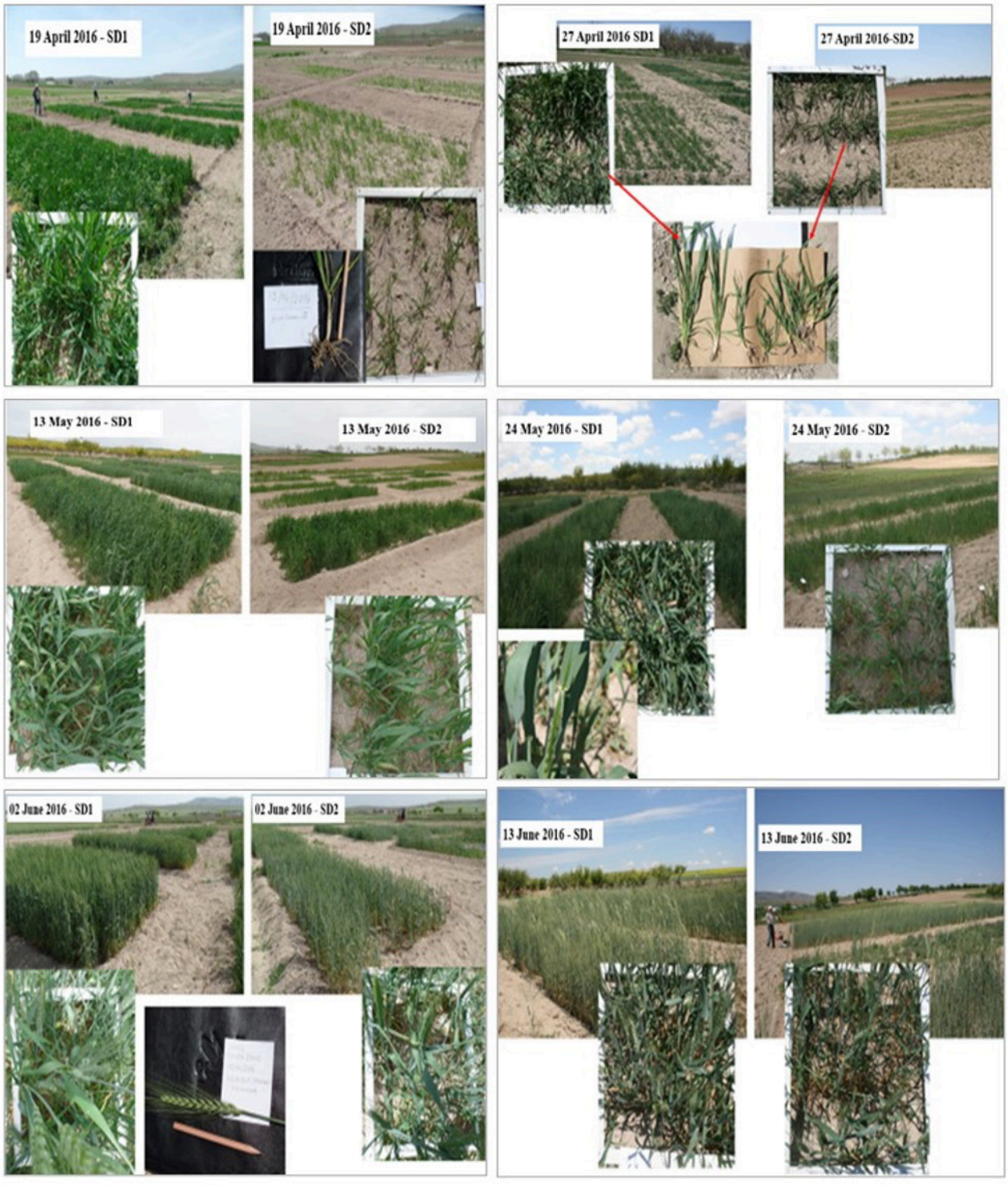

Figure 3. Canopy cover measurement during 2015-2016 growing season.

Canada (Liu and Pattey, 2010; Liu et al., 2013). Canopy cover measurement during calibration stage in 2015-2016 growing season were presented at Figure 3.

RESULTS AND DISCUSSION

Model calibration

The calibration is based on a trial-and-error method in which simulated and observed grain yields, biomass yields and canopy cover of plants are compared.

Grain and biomass yields

The observed grain yield varied from 3.81 t ha^{-1} to 5.78 t ha^{-1} for SD_1 and from 1.84 t ha^{-1} to 4.71 t ha^{-1} for SD_2 while simulated grain yield had been found in the range from 4.11 t ha^{-1} to 6.20 t ha^{-1} for SD_1 and from 2.07t ha^{-1} to 5.14 t ha^{-1} for SD_2 during the growing seasons in 2015–2016. As expected, the highest grain yield and biomass were measured at full irrigation treatment (irrigated at G+T+H stage). The highest biomass value observed in the field (10.25 t ha^{-1}) and simulated by the model (10.58 t ha^{-1}) was obtained in SD_1I_2.

Statistical evaluation of the treatments was given in Table 4. The higher R^2 and E values and lower NRMSE values indicated good model performance. According to grain and biomass yield results, R^2 is close to "1" for all applications, which means there is a very good relationship between simulated and measured values (Raes et al., 2015). NRMSE values for grain yield and biomass of SD_1 and SD_2 was found 5.70 and 9.43% which ranged from 6 to 15%, indicating good agreement.

According to EF values of grain yields for SD_1 and SD_2 and biomass yields for SD_2 a good performance was obtained between the predicted and measured values. The EF value of SD_1 biomass yield is in moderate agreement with the number 0.49.

Canopy cover (CC)

Canopy cover percentages of the treatments was determined by the GreenCrop Tracer program (Liu and Pattey, 2010). Observed canopy cover values for I_2 irrigation application and SD_1, SD_2 sowing date treatment in the date 19 and 27 April, 13 and 24 May, 02 and 13 June in 2016 was compared with model simulations (Figure 4).

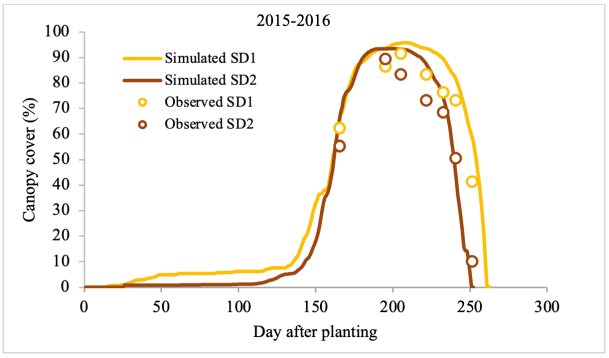

Figure 4. Observed and simulated canopy cover values of wheat grain yield for SD_1I_2 and SD_2I_2 treatments.

There was an important linear relationship and good agreement between the measured and simulated canopy cover for SD1 and SD2 respectively, with R^2=0.89, RMSE=5.87% and EF=0.92 for wheat under full irrigation and different planting dates in the cropping season 2015-2016.

Model validation

Grain and Biomass yield

Winter wheat was planted as a normal sowing date on October 12th (SD_1) and as late sowing date November 8th (SD_2) during 2016-2017 growing season. Wheat that was planted on SD_1 had greater grain and biomass yield than did those planted on SD_2 for all irrigation strategies (Table 5). The highest and lowest biomass and grain yield was obtained in SD_1I_2 (5.12 t ha^{-1}) and SD_2I_1 (1.76 t ha^{-1}) respectively. There was a significant decrease in crop yield of 65% between the two treatments.

As it shown in Table 5, R^2 for grain yield and biomass was found between 0.90 and 0.98 respectively. It can be said that the model prediction values were closer to the observed values. According to NRMSE (%) and EF values model simulations showed good agreement with

Table 4. Observed and simulated grain and biomass yields with statistical parameters in calibration stages (2015-2016)

Growing years	Treatments	SD_1				SD_2			
		Grain Yield (t ha^{-1})		Biomass (t ha^{-1})		Grain Yield (t ha^{-1})		Biomass (t ha^{-1})	
		Observed	Simulated	Observed	Simulated	Observed	Simulated	Observed	Simulated
2015-2016	I_1	3.81	4.11	7.02	8.37	1.84	2.07	4.56	5.51
	I_2	5.78	6.20	10.25	10.58	4.71	5.14	9.23	9.52
	I_3	5.18	5.36	9.09	9.85	3.54	3.70	7.45	8.16
	I_4	5.12	4.97	8.80	9.29	3.25	3.11	6.97	7.49
R^2		0.92		0.97		0.93		0.98	
RMSE (t ha^{-1})		0.28		0.83		0.27		0.66	
NRMSE (%)		5.70		9.43		7.97		9.38	
EF		0.84		0.49		0.99		0.95	

Table 5. Observed and simulated grain and biomass yields with statistical parameters in validation stages (2016-2017)

Growing years	Treatments	SD_1				SD_2			
		Grain Yield (t ha^{-1})		Biomass (t ha^{-1})		Grain Yield (t ha^{-1})		Biomass (t ha^{-1})	
		Observed	Simulated	Observed	Simulated	Observed	Simulated	Observed	Simulated
2016-2017	I_1	3.13	4.08	6.88	7.91	1.76	2.00	4.09	5.22
	I_2	5.12	5.89	8.99	9.38	4.17	4.89	7.91	8.37
	I_3	4.86	5.12	8.17	8.60	3.23	3.56	6.59	7.16
	I_4	3.98	4.58	7.72	8.01	2.35	2.97	5.88	6.14
R^2		0.90		0.96		0.98		0.96	
RMSE (t ha^{-1})		0.62		0.69		0.47		0.69	
NRMSE (%)		14.40		7.67		14.74		11.21	
EF		0.56		0.72		0.95		0.90	

field observed values. Similar results were also found by Araya et al. (2010), Zeleke et al. (2011), Iqbal et al. (2014), Kale Celik et al. (2018), Davarpanah and Ahmadi (2021). A significant correlation was found between observed and simulated grain and biomass yield for the SD1 and SD2 sowing period (Figure 5) (P value=0.000 <0.05 according to the F test).

The comparation between SD_1 and SD_2 grain yield and biomass values and yield reductions (%) were given Figure 6 and Figure 7. Higher grain yield and biomass were obtained when planting on October 12th compared to November 8th.

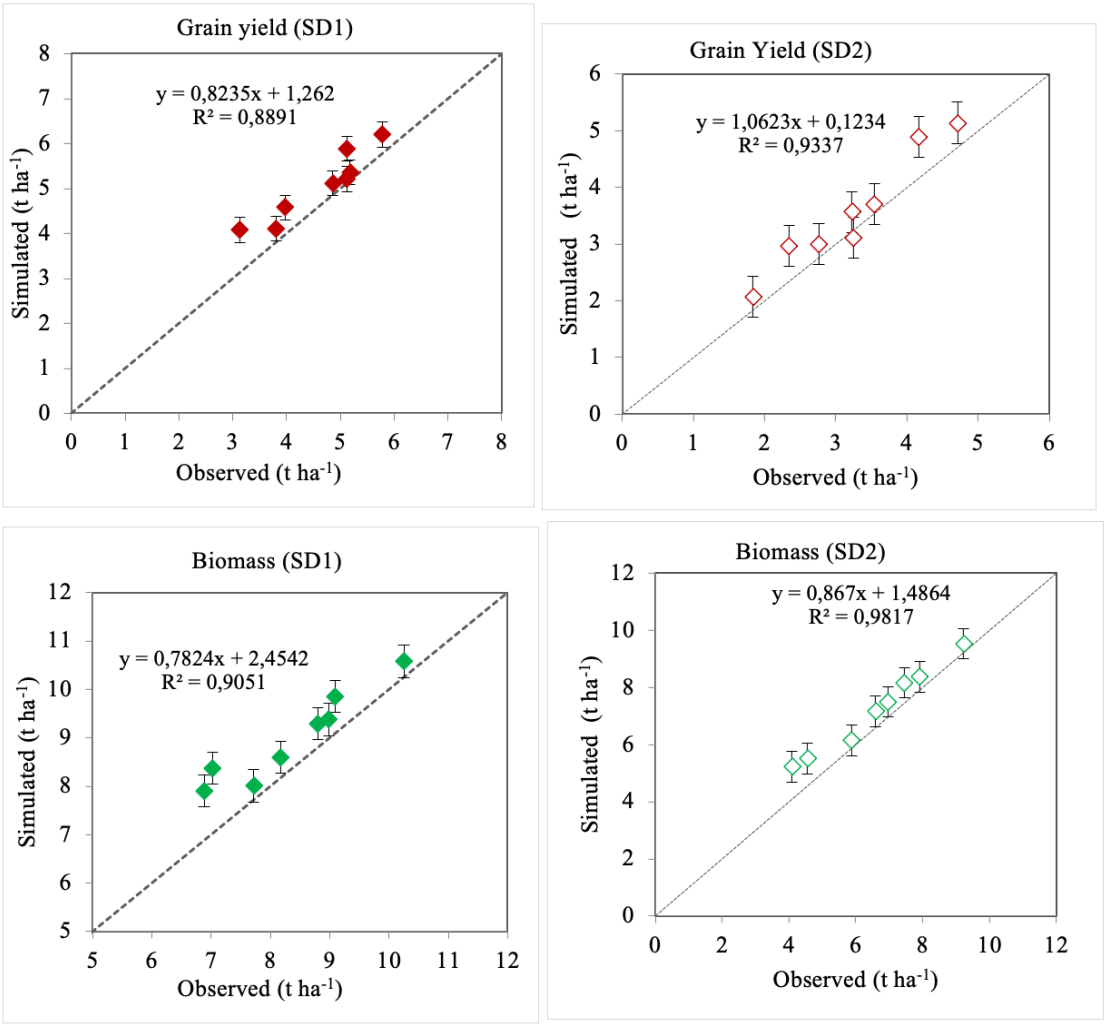

Figure 5. Relation between simulated and measured wheat grain yield and biomass.

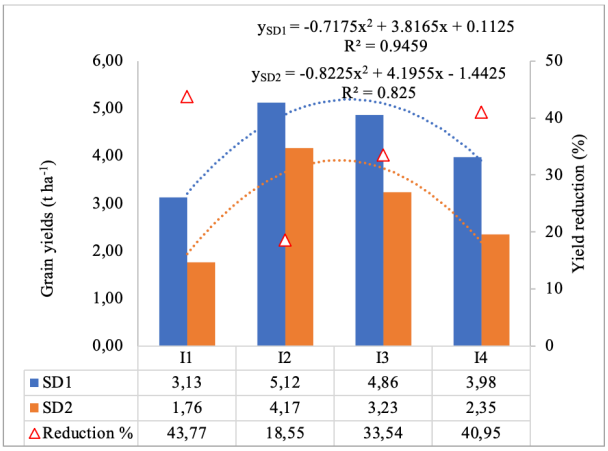

Figure 6. Observed winter wheat grain yield under irrigation strategies and different planting dates.

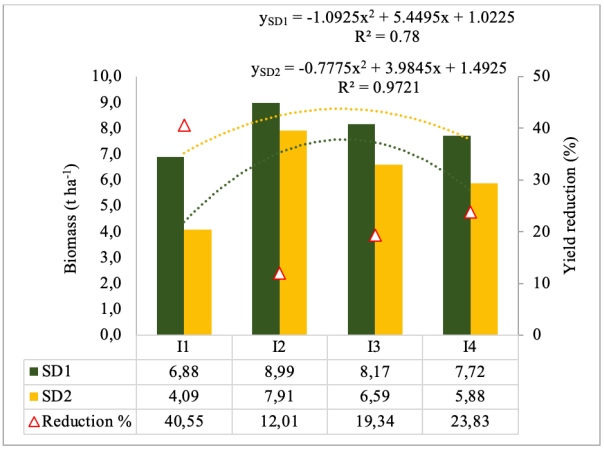

Figure 7. Observed winter wheat biomass under irrigation strategies and different planting dates.

The higher than 40% grain and biomass yield reduction occurred in the rainfed treatment. When I_3 and I_4 applications are compared, irrigation during the germination and emergence period gives more reasonable results than irrigation during the germination and tillering period.

Jin et al. (2014) state that the reason for this difference is "due to higher growing degree days (accumulated warmth) promoting canopy cover growth and grain and biomass yield accumulation at an earlier planting date". Presumably, the percent of canopy cover affected the transpiration rate and thus the accumulation of grain and biomass yields (Farahani et al., 2009).

Soil moisture content

The soil moisture content was determined by gravimetric method. Soil moisture content for I_1 and I_2 for SD_1 was given in Figure 8.

It was found that the model had overestimated soil water content compared to observation values. However, there was significant relation between observed and predicted soil moisture content. (Figure 9).

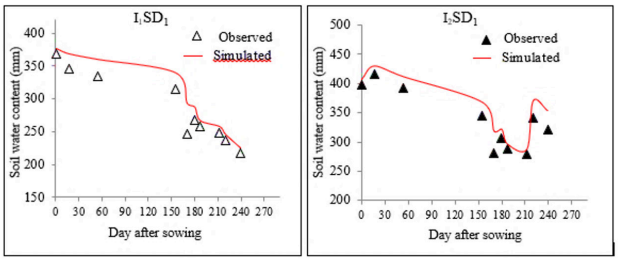

Figure 8. Soil moisture content for calibration period.

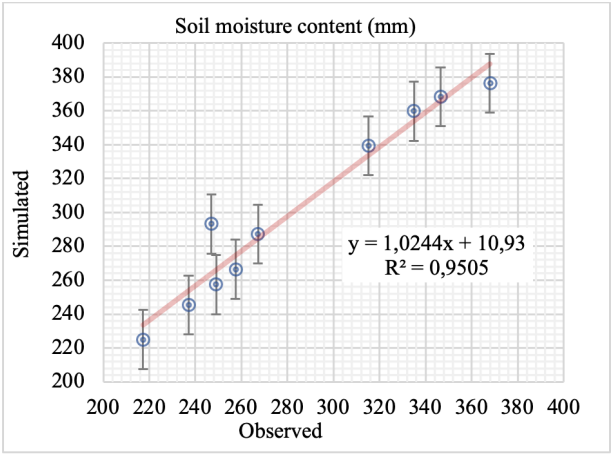

Figure 9. Relation between simulated and measured soil moisture content for I_1SD_2.

The R^2, RMSE and E showed good performance between the simulated and the measured values for soil water content of I_1 and I_2 treatments (R^2 = 0.91-071, RMSE= 21.35-21.44 and E= 0.97–0.96). Higher R^2 and E values and lower RMSE values indicated good model performance.

In general, the model can predict soil moisture content values with acceptable accuracy. Similar results have been found by various researchers (Farahani et al., 2009; Hussein et al. 2011; Mkhabela and Bullock, 2012; Kale and Tarı 2012; Igbal et al. 2014; Toumi et al., 2016).

Canopy cover

A high level of similarity was found between the canopy cover percentage predicted by the model and observed in the field. This similarity was presented as an example for SD1 and all irrigation treatments at Figure 10. While the coefficient of determination (R^2) for the treatments SD_1I_1, SD_1I_2, SD_1I_3 and SD_1I_4 were 0.88, 0.90, 0.89 and 0.90 respectively and were 0.93, 0.97, 0.98 and 0.88 for SD_2. It was found that the model predicted CC values correctly in winter wheat and various other crops at the several similar studies also (Heng et al., 2009, Hsiao et al., 2009, Farahani et al., 2009; Tavakoli et al., 2015).

When CC values of different planting dates were compared for the whole year for I_2, it was seen that the plant started to cover the soil surface earlier in SD1 than

in SD2 (Figure11).

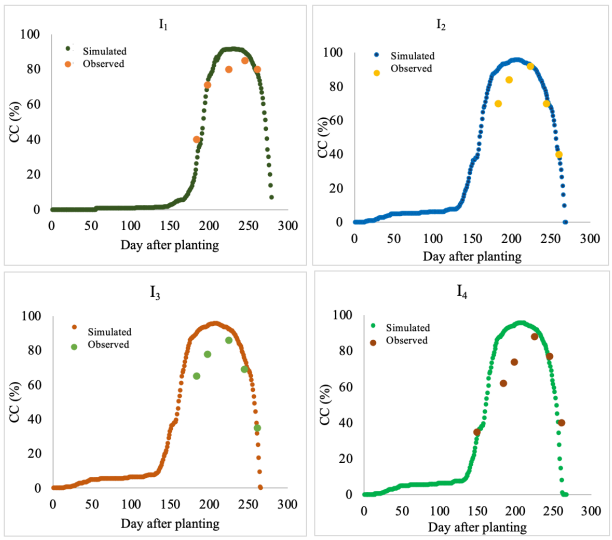

Figure 10. Comparation of observed and simulated CC values for SD1 and all irrigation treatments.

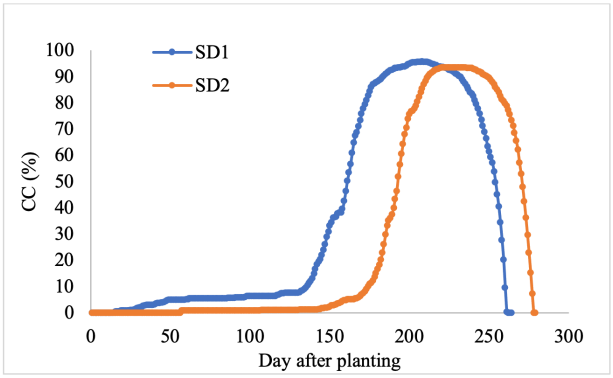

Figure 11. Comparation of simulated CC values for SD_1 and SD_2 treatments.

The model efficiency coefficient and RMSE values of SD1 and SD2 treatments are given in Table 6 for each irrigation application of CC.

Table 6. Statistical evaluations of CC simulated and observed values

Treatments		RMSE	EF
SD_1	I_1	10.20	0.74
	I_2	13.2	0.62
	I_3	8.20	0.81
	I_4	10.29	0.69
SD_2	I_1	3.31	0.97
	I_2	4.45	0.95
	I_3	4.48	0.98
	I_4	10.82	0.81

Accordingly, the model efficiency values were between 0.62 and 0.98 and was within acceptable limits (Raes et al. 2015). AquaCrop was able to accurately simulate the canopy development and senescence over the season. However, AquaCrop lightly overestimated the canopy development during the middle of the growing period. EF and R values close to "1 (one)" indicated the overall good agreement between the simulated and observed canopy cover.

CONCLUSION

In this study, the effect of different sowing scenarios and irrigation strategies in order to adapt to water scarcity conditions, which is an important problem due to climate change, and to achieve optimum wheat yield, was investigated using the AquaCrop model. The model was calibrated and validated under the conditions of the Central Anatolia region and field data were collected in the experimental area during the 2015–2017 growing season. The model was run under two different planting dates and four irrigation treatments water applied at different growth stages. In comparing sowing dates to determine the optimum date of winter wheat, it was concluded that the current sowing date (SD_1) did not have a negative effect on grain yield however late sowing dates (SD_2) would significantly reduce grain yield and biomass yield. Considering the biomass and grain yields in terms of irrigation, the highest yield was obtained in the irrigation water had been applied during the Germination+ Tillering+Heading stages. Yield reduction was 38.9% in rainfed, 5.1 % when irrigated during Germination + Heading stages and 22.3% when irrigated during Germination+Tillering stages. Among all the treatments, the SD_1I_2 treatment gave the best results. Simulation results were compared with observed the final biomass and yield, soil water content and canopy cover. These results showed that the AquaCrop model is useful for simulating winter wheat biomass, grain yield, soil water and canopy cover under different planting dates, and irrigation strategies.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, 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.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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The insecticidal effects of different plant extracts on confused flour beetle, *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae)

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Abstract
This study was carried out to determine the insecticidal effects of different plant extracts on confused flour beetle, *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) and time-varying LD50 and LD90 values. LD50 and LD90 of the formulations were determined by the probit analysis method. The studies were conducted under controlled conditions in a climate cabin and air conditioning room at 25 ± 2°C and 60% humidity 2019 to 2020 in Fırat University Bioengineering Department in Turkey. In this study, Grainpro AITC-ALPHA (Monoterpen, Allyisothiocyanate,Cocoate methyl esters), Grainpro AITC-GAMMA (Gamma terpene, Allyisothiocyanate, Cocoate methyl esters-Eugenol), Grainpro GAMMA-EUG (Gamma terpinene, Eugenol, Cocoate methyl esters), and Grainpro AITC-EUC (Eucalyptus oil, Allyisothiocyanate, Cocoate methyl esters) plant formulations were used in doses (In all doses, different µL drug doses prepared in 1 ml were mixed in 1 liter of water and applied by obtaining 0.2 µL, 0.4 µL, 0.6 µL, 0.8 µL, 1 µL doses for each formulation). The two upper and two lower doses of the most effective drug obtained were then tested and their effectiveness was determined. It was determined that the insecticidal efficacy of the pesticides increased as the pesticide dose increased and two lower and two upper doses of the most effective dose of 1 µL were subjected to efficacy trials. Among these formulations, 1 µL and 1.10 µL doses of Gainpro AITC-EUC formulation were the most effective on the pest.
Keywords: Insecticidal effect, LD50, LD90, Plant extracts, *Tribolium confusum*

INTRODUCTION

The adults and larvae of confused flour beetle *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) are a secondary pest and commonly found in houses, supermarket warehouses and granaries, mills and flour warehouses (Ebelling, 1992). This species does not directly damage whole grains, but feeds on grains damaged by other store pests. Furthermore, *T. confusum* provides a source of cracked kernels and powdered food for them from mechanical harvest damage when trying to consume other cereal crops. Gases released from the thorax of adults produce odor in products. This leads to the product getting moldy, the color of the flour turning grayish and its quality decreasing (Aitken, 1975; Hill, 1990). This pest causes significant losses in cereal crops in our country and the world (Trematerra et al., 2011). In the control of this pest, both chemical and organic preparations have been used from time to time in various studies.

In some studies conducted in our country: Determination of the fungicidal effect of essential oils of 32 different plants against all biological stages of *T. confusum* was carried out. Again, the effects of essential oils obtained from garlic and onion

and some active substances such as diallyl sulfide, diallyl disulfide and dipropyl disulfide and their mixtures against the pest were studied. Determination of the fumigant effect of the main compounds (allyl isothiocyanate and allyl disulfide) of *Rosmarinus officianalis* (Rosemary) L. (Lamiales: Lamicaea) and *Laurus nobilis* L. (Laurales: Lauracea) essential oils together with carbon dioxide (CO2) and nitrogen (N2) is considered among some important studies conducted in recent years (Erler 2005; Isikber et al, 2006; Karci, 2006; Gozek, 2007).

In previous studies, it was found that 0.1, 1 and 5 ppm doses of spinetoram insecticide had a fumigant effect on *T. confusum*, *Sitophilus oryzae* L., 1763 (Coleoptera: Curculionidae) and *Rhyzopertha dominica* Fabricius, 1792 (Coleoptera: Bostrichidae) feeding on wheat. In the same study, essential oils extracted from *Pagostemon heyneaus* Benth. (Lamiales: Lamiacea), *Ocimum basilicum* L. (Lamiales: Lamiaceae) were found to have contact effect on *T. confusum* (Shaaya et al., 1997). Essential oils were extracted from the leaves of *Curcuma longa* L. (Zingiberales: Zingiberacea) plant known as Indian saffron and its contact and insecticidal effects were studied against *R. dominica, S. oryzae* and *Tribolium casteneum* Herbst, 1797 (Coleoptera: Tenebrionidae) (Tripathi et al., 2002).

The use of plant-based insecticides has come to the forefront, especially due to the degradation problems and toxic effects of chemical pesticides. The use of plant-based insecticides has come to the forefront, especially due to the degradation problems and toxic effects of chemical pesticides. Especially the formation of resistance to chemicals also affects the success of the pest management (Tiryaki et al., 2010).

The main objective of this study was to develop alternative control methods to chemical control in terms of control of stored product pests and to determine the lethal doses of new generation plant-based insecticides. These insecticides are Grainpro AITC-Alpha, Grainpro AITC-Gamma, Grainpro Gamma-Eug and Grainpro AITC-Euc herbal insecticides developed by Biohaust company. These insecticides were developed by the aforementioned company for the control of storage and warehouse pests. The results of this study will be used both in the licensing studies of insecticides and in the control of warehouse and warehouse pests.

MATERIALS AND METHODS

Material

The studies were conducted under controlled conditions climate cabin and air conditioning room (25 ± 2°C and 60% humidity) to between 2019 to 2020 years in Bioengineering Department in Turkey. *Tribolium confusum* adults, flour-bran mixtures, Grainpro AITC-Alpha, Grainpro AITC-Gamma, Grainpro Gamma-Eug and Grainpro AITC-Euc pesticides contests are given below were the main materials of the study (Table 1, Figure 1). All formulations are developed by the company Biohaust. No excipients were used in the formulations. The formulations are designed as liquid formulations.

Table 1. Plant formulations and their ingredients used in the determination of LD50 and LD90 values on *Tribolium confusum*.

Pesticide Used	Ingredients
Grainpro AITC-Alpha	Monoterpen, Allyisothiocyanate, Cocoate methyl esters
Grainpro AITC-Gamma	Gamma terpene, Allyisothiocyanate, Cocoate methyl esters-Eugenol
Grainpro-Gamma-Eug	Gamma terpinene, Eugenol, Cocoate methy esters
Grainpro AITC-Euc	Eucalyptus oil, Allyisothiocyanate, Cocoate methyl esters

Figure 1. The test pesticides used in the study: "Grainpro AITC-Alpha, Grainpro AITC-Gamma, Grainpro Gamma-Eug and Grainpro AITC-Euc".

Method

Insect Rearing and Applications

The pest was placed on flour and bran in 15x30x5 cm container at a temperature of 25±1°C in laboratory conditions and kept until it reached the adult stage. The study was initiated with larvae hatched from the eggs of the new generation of adults produced under laboratory conditions. First of all, the broken grains that the pest would feed on were treated with pesticide for 10 seconds by dipping method.The grains were then dried on an absorbing paper (Figure 2). The dried wheat was taken into 10x5 cm tubes with 5 repetitions for each dose. Ten adult *T. confusum* pests were placed on 15 grams of cracked wheat (Figure 2) LD 50 and lD90 mortality rates of herbal insecticides (Biohaust Company, 789 Teviot Road, JimboombaQld 4280, Australia) were determined on the tested material.

For all botanical insecticides, 1 ml mixture was obtained by using 100 µL formulation + 900 µL pure water. Thus, 1 ml of mixture containing 100 µL pesticide at a concentration

of 0.1 was obtained. This mixture was diluted in 100 ml of water and a mixture with a concentration of 0.001 was obtained. For each pesticide, doses of 0.2 µL, 0.4 µL, 0.6 µL, 0.8 µL and 1 µL were used. To provide these doses, insecticidal activities were analyzed by taking 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml from the mixture, respectively. Studies were performed in 5 cuvettes for each dose. Adult individuals were examined at 24-hour intervals and the results were noted.

The Determination of The LD50 and LD90 values and Statistic Analyses

The LD_{50} and LD_{90} values of the dose causing the highest mortality rate of each pesticide were determined. Descriptive statistics of the data set were expressed as mean±standard deviation. Differences between means were compared with one-way ANOVA followed by Tukey's post-hoc test. The values of lethal doses (LD_{50} and LD_{90}) were determined for certain times using probit analysis. All statistical analyses were carried out using the IBM SPSS software version 21.0 statistical program. The doses determined for LD50 and LD90 were determined by us according to the experimental composition by taking the recommended doses that the company wants to be applied to warehouse pests and/or the precedent doses of similar plant-based drugs used against this pest as an example.

Figure 2. Cracked wheat to which the formulations were applied.

RESULTS AND DISCUSSION

The insecticidal effects of Gainpro plant extracts prepared at different concentrations on insects are shown in Table 2. According to the ANOVA test, the Gainpro AITC-Alpha viability ratio showed a significant difference according to doses ($p<0.05$) (Table 2). When the lethal effects of the application doses were compared according to the Tukey test, the 1 mL dose (23.61±14.48) was significantly more effective than the 0.2 mL (40.33±7.76) and 0.4 mL (33.48±11.28) doses, while the 0.8 mL (24.46±13.16) and 0.6 mL (27.76±15.96) doses were significantly more effective than the 0.2 mL dose (40.33±7.76) ($p<0.05$). The LD_{50} and LD_{90} values and time-dependent insecticidal effect of Gainpro AITC-Alpha plant extract on *T. confusum* are given in Tables 3&4 and Figures 3&4.

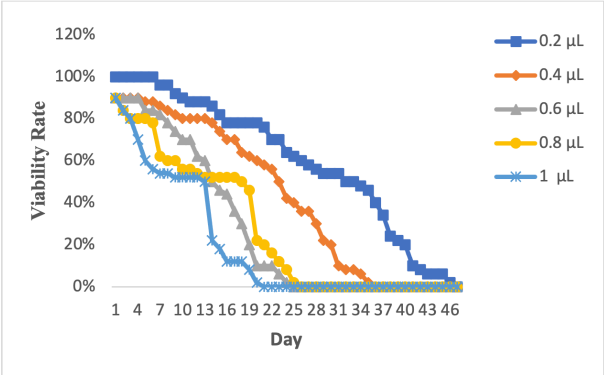

Figure 3. Effect of different doses of Gainpro AITC-Alpha formulation on *Tribolium confusum*.

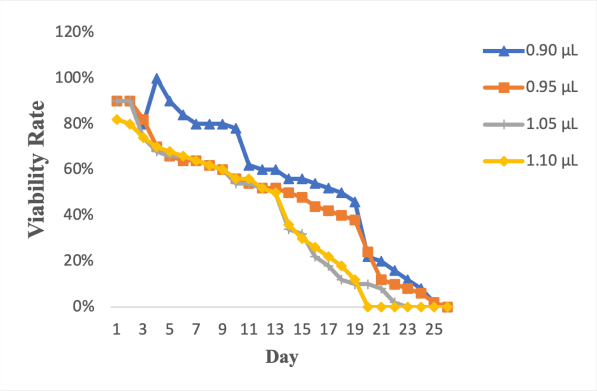

Figure 4. Effect of different doses of Gainpro AITC-Alpha formulation on *Tribolium confusum*.

The viability rate of Gainpro AITC-EUC insecticide showed a significant difference according to the doses ($p<0.05$) (Table 2). When the lethality effects of the application doses were compared according to the Tukey test, 1 mL (18.55±13.28), and 0.8 mL (18.65±13.01) doses were found to be statistically significantly more effective than the 0.2 ml dose (27.20±9.23) (Table 2). The LD_{50} and LD_{90} values and time-dependent insecticidal effect of Gainpro AITC-EUC plant extract on *T. confusum* are given in Tables 5&6 and Figures 5&6.

Table 2. The percentage mortality of Gainpro extracts on *Tribolium confusum*.

	0.2 mL		0.4 mL		0.6 mL		0.8 mL		1 mL		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	p value
Grainpro AITC-Alpha	40.33a	7.76	33.48ab	11.28	27.76bc	15.96	26.46bc	13.16	23.61c	14.48	**0.000***
Grainpro AITC-Gamma	27.20a	9.23	26.26ab	10.83	22.53ab	12.93	18.65b	13.01	18.55b	13.28	**0.015***
Grainpro-Gamma-Eug	39.03a	4.59	38.03a	5.97	32.36ab	10.42	26.56bc	11.54	21.68c	12.71	**0.000***
Grainpro AITC-Euc	23.96	6.98	21.20	9.45	22.83	8.25	21.06	9.58	20.32	11.62	0.565

* $p<0.05$

Table 3. LD_{50} and LD_{90} values of Gainpro AITC-Alpha (Doses 0.2, 0.4, 0.6, 0.8 and 1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
2	3.342 [a] (1.663–102.81)	19.236 [a] (4.828–21837.296)
3	2.346 [a] (1.424–13.43)	10.240 [a] (3.706–423.769)
4	1.668 [a] (1.203–3.939)	5.390 [a] (2.715–36.942)
5	1.360 [a] (1.059–2.357)	4.047 [a] (2.341–15.203)
6	1.242 [a] (1.003–1.923)	3.403 [a] (2.119–9.992)
7	1.146 [a] (0.921–1.730)	3.821 [a] (2.294–11.490)
8	1.101 [a] (0.888–1.636)	3.787 [a] (2.274–11.319)
9	1.115 [a] (0.869–1.819)	4.910 [a] (2.624–20.724)
10	1.059 [a] (0.823–1.733)	5.176 [a] (2.677–24.411)
11	1.088 [a] (0.828–1.915)	6.033 [a] (2.897–37.245)
12	0.990 [a] (0.772–1.598)	5.245 [a] (2.667–26.369)
13	0.924 [a] (0.736–1.385)	4.512 [a] (2.448–18.083)
14	0.652 [a] (0.559–0.781)	2.119 [a] (1.519–3.834)
15	0.599 [a] (0.246–3.807)	2.129 [a] (1.013–27859.61)
16	0.545 [a] (–)	2.033 [a] (–)
17	0.521 [a] (–)	1.898 [a] (–)
18	0.481 [a] (–)	1.758 [a] (–)
19	0.435 [a] (–)	1.402 [a] (–)
20	0.372 [a] (0.063–0.620)	0.868 [a] (0.542–33.541)
21	0.358 [a] (0.047–0.600)	0.823 [a] (0.513–40.640)
22	0.331 [a] (0.042–0.542)	0.793 [a] (0.494–26.616)
23	0.313 [a] (0.081–0.480)	0.707 [a] (0.463–4.866)
24	0.276 [a] (0.058–0.422)	0.619 [a] (0.406–3.874)
25	0.265 [a] (0.043–0.411)	0.538 [a] (0.352–5.338)
26	0.254 [a] (0.036–0.394)	0.501 [a] (0.329–6.291)
27	0.249 [a] (0.016–0.395)	0.500 [a] (0.322–14.476)
28	0.237 [a] (0.058–0.348)	0.473 [a] (0.323–2.267)
29	0.223 [a] (0.184–0.256)	0.436 [a] (0.379–0.530)
30	0.221 [a] (0.183–0.254)	0.426 [a] (0.371–0.519)
–: not calculated. a: µg/ml		

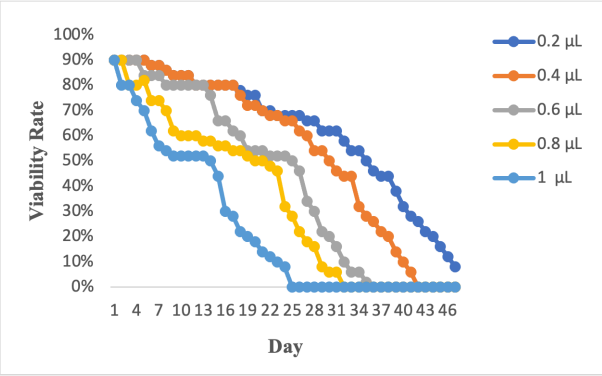

Figure 5. Effect of different doses of Grainpro AITC-EUC formulation on *Tribolium confusum*.

In the case of Gainpro AITC-Gamma insecticide, the viability rate differed significantly according to the doses ($p<0.05$) (Table 2). When the lethality effects of the application doses were compared according to the Tukey test, the 1 mL dose (21.68±12.71) was found to be statistically significantly more effective than the 0.2 mL (39.03±4.59), 0.4 mL (38.03±5.97) and 0.6 mL (32.36±10.42) doses, and the 0.8 mL (26.56±11.54) dose was found to be significantly more effective than the 0.2 mL (39.03±4.59) and 0.4 mL (38.03±5.97) doses (Table 2). The LD_{50} and LD_{90} values and time-dependent insecticidal effect of Gainpro AITC-Gamma plant extract on *T. confusum* are given in Tables 7&8 and Figures 7&8.

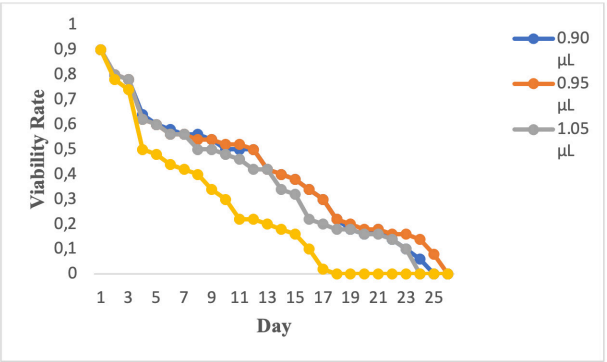

Figure 6. Effect of different doses of Grainpro AITC-EUC formulation on *Tribolium confusum*.

Table 4. LD_{50} and LD_{90} values of Gainpro AITC-Alpha (Doses 0.90 0.95 1.05 1.1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1	2.122 [a] (–)	4.804 [a] (–)
2	1.843 [a] (–)	3.617 [a] (–)
3	1.766 [a] (–)	4.635 [a] (–)
4	1.185 [a] (–)	1.569 [a] (–)
5	1.241 [a] (–)	1.961 [a] (–)
6	1.293 [a] (–)	2.423 [a] (–)
7	1.287 [a] (–)	2.574 [a] (–)
8	1.231 [a] (–)	2.304 [a] (–)
9	1.188 [a] (–)	2.105 [a] (–)
10	1.118 [a] (1.035–3.468)	1.872 [a] (1.383–970.380)
11	1.294 [a] (–)	9.937 [a] (–)
12	1.127 [a] (–)	5.371 [a] (–)
13	1.066 [a] (–)	3.346 [a] (–)
14	0.945 [a] (0.760–1.005)	1.476 [a] (1.235–5.755)
15	0.936 [a] (0.821–0.985)	1.344 [a] (1.187–2.287)
16	0.915 [a] (0.818–0.958)	1.237 [a] (1.136–1.619)
17	0.906 [a] (0.820–0.947)	1.186 [a] (1.107–1.428)
18	0.899 [a] (0.827–0.936)	1.132 [a] (1.075–1.276)
19	0.891 [a] (0.826–0.926)	1.092 [a] (1.047–1.191)
20	0.815 [a] (0.664–0.871)	1.010 [a] (0.972–1.091)
21	0.780 [a] (0.555–0.850)	0.973 [a] (0.928–1.039)
22	0.780 [a] (0.533–0.848)	0.937 [a] (0.880–0.984)
23	0.678 [a] (0.000–0.809)	0.894 [a] (0.013–0.957)
24	0.539 [a] (–)	0.833 [a] (–)
25	0.552 [a] (–)	0.740 [a] (–)
–: not calculated. a: µg/ml		

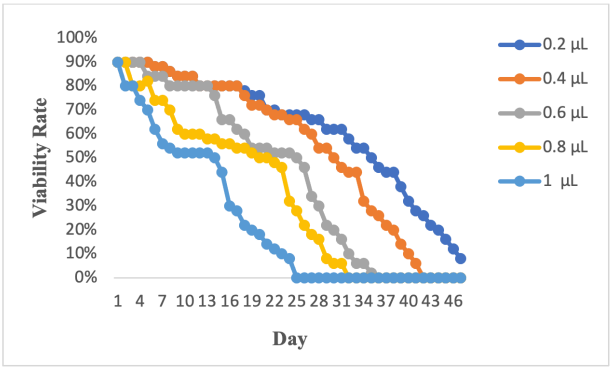

Figure 7. Effect of different doses of Gainpro AITC-Gamma formulation on *Tribolium confusum*.

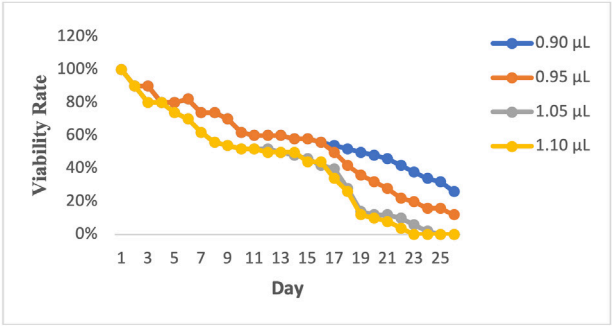

Figure 8. Effect of different doses of Gainpro AITC-Gamma formulation on *Tribolium confusum*.

The viability rate of Gainpro Gamma-EUG did not differ significantly between doses ($p>0.05$) (Table 2). The LD_{50} and LD_{90} values and time-dependent insecticidal effect of Gainpro Gamma-EUG plant extract on *T. confusum* are given in Tables 9&10 and Figures 9&10.

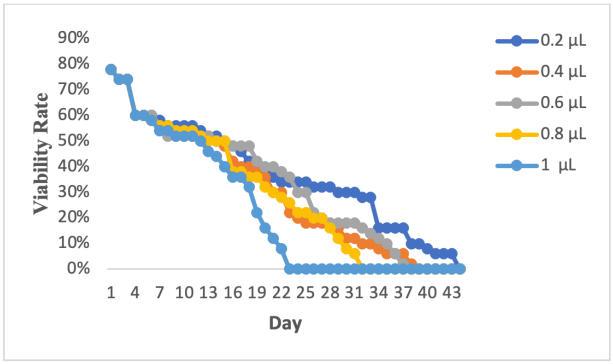

Figure 9. Effect of different doses of Grainpro Gamma EUG formulation on *Tribolium confusum*.

Figures 3-10 show that the viability rate decreased as the dose increased. At 1 ml dose, the viability of insects decreased significantly after the 13th day. Similarly, at a dose of 0.8 ml, the viability of the insects decreased drastically after day 19 (Figure 3).

Table 5. LD_{50} and LD_{90} values of Grainpro AITC-EUC (Doses 0.2 0.4 0.6 0.8 1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-4	–	–
5	2.653 [a] (–)	12.355 [a] (–)
6	1.927 [a] (–)	117.193 [a] (–)
7	1.621 [a] (–)	113.752 [a] (–)
8	1.501 [a] (–)	140.394 [a] (–)
9	1.136 [a] (0.686–40.846)	46.602 [a] (–)
10	0.936 [a] (0.604–6.791)	29.807 [a] (–)
11	0.841 [a] (0.532–8.121)	35.051 [a] (–)
12	0.712 [a] (0.390–195.75)	56.391 [a] (–)
13	0.498 [a] (0.306–0.763)	8.906 [a] (2.879–1102.948)
14	0.450 [a] (–)	5.870 [a] (–)
15	0.403 [a] (0.263–0.532)	3.720 [a] (1.866–25.825)
16	0.378 [a] (–)	2.300 [a] (–)
17	0.342 [a] (–)	1.619 [a] (–)
18	0.316 [a] (–)	1.303 [a] (–)
19	0.293 [a] (–)	1.131 [a] (–)
20	0.274 [a] (–)	1.089 [a] (–)
21	0.226 [a] (–)	0.998 [a] (–)
22	0.197 [a] (–)	0.939 [a] (–)
23	0.178 [a] (0.093–0.245)	0.840 [a] (0.644–1.377)
24	0.158 [a] (0.078–0.222)	0.703 [a] (0.551–1.075)
25	0.145 [a] (0.067–0.209)	0.648 [a] (0.509–0.971)
26	0.141 [a] (0.067–0.202)	0.579 [a] (0.460–0.827)
27	0.131 [a] (0.058–0.190)	0.527 [a] (0.418–0.738)
28	0.088 [a] (0.018–0.154)	0.472 [a] (0.351–0.702)
29	0.082 [a] (0.015–0.147)	0.427 [a] (0.311–0.619)
30	0.085 [a] (0.018–0.149)	0.411 [a] (0.328–0.646)
–: not calculated. a: µg/ml		

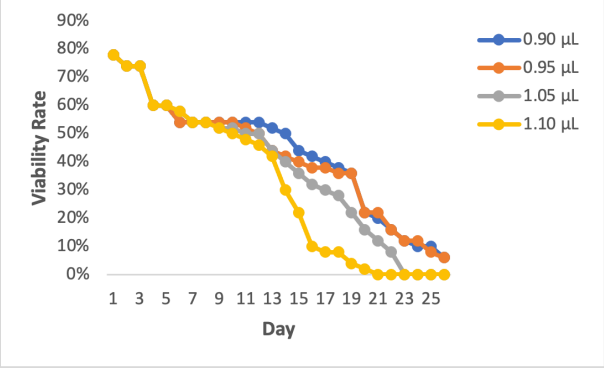

Figure 10. Effect of different doses of Grainpro Gamma EUG formulation on *Tribolium confusum*.

A concentration of 1 µg/ml of Gainpro AITC-Alpha extract showed a very strong insecticidal effect and by day 21 there were no living insects and all individuals were dead (Figure 3). The LD_{50} value of the plant formulation observed on day 21 was 0.358 µg/ml and the LD_{90} value was 0.823 µg/ml (Table 3). The 1.10 µg/ml concentration of Gainpro AITC-Alpha extract showed a very strong insecticidal effect and no live insects were observed on the 20th day of the application (Figure 4). The LD_{50} value of the plant formulation observed on day 20 was 0.815 µg/ml and the LD_{90} value was 1.010 µg/ml (Table 4).

A concentration of 1 µg/ml of Gainpro AITC-EUC extract showed a very strong insecticidal effect and no live insects were observed on day 19 (Figure 5). The LD_{50} value of the plant observed on the 19th day was 0.293 µg/ml and the LD_{90} value was 1.131 µg/ml (Table 5). A concentration of 1.10 µg/ml of Gainpro AITC-EUC extract showed a very strong insecticidal effect and no live insects were observed on day 18 (Figure 6). The LD_{50} value of the plant formulation observed on day 18 was 0.778 µg/ml and the LD_{90} value was 1.044 µg/ml (Table 6).

A concentration of 1 µg/ml of Gainpro AITC-Gamma extract showed a very strong insecticidal effect and no live insects were observed on day 25 (Figure 7). The LD_{50} value of the plant formulation observed on day 25 was 0.426 µg/ml and the LD_{90} value was 1.359 µg/ml (Table 7). The 1.10 µg/ml concentration of Gainpro AITC-Gamma extract showed a very strong insecticidal effect and no live insects were observed on day 23 (Figure 8). The LD_{50} value of the plant formulation observed on day 23 was 0.872 µg/ml and the LD_{90} value was 0.997 µg/ml (Table 8).

Table 6. LD_{50} and LD_{90} values of Grainpro AITC-EUC (Doses 0.90 0.95 1.05 1.1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-2	–	–
3	4.198 [a] (–)	50.718 [a] (–)
4	1.182 [a] (–)	2.907 [a] (–)
5	1.156 [a] (–)	3.374 [a] (–)
6	1.062 [a] (–)	2.672 [a] (–)
7	1.043 [a] (–)	2.643 [a] (–)
8	0.997 [a] (–)	2.037 [a] (–)
9	0.974 [a] (–)	1.749 [a] (–)
10	0.941 [a] (–)	1.684 [a] (–)
11	0.937 [a] (0.787–0.991)	1.404 [a] (1.209–3.291)
12	0.928 [a] (0.782–0.980)	1.367 [a] (1.193–2.724)
13	0.857 [a] (0.03–0.941)	1.486 [a] (–)
14	0.847 [a] (0.383–0.925)	1.345 [a] (1.161–6.192)
15	0.834 [a] (0.379–0.914)	1.306 [a] (1.143–4.613)
16	0.831 [a] (0.611–0.898)	1.155 [a] (1.073–1.549)
17	0.834 [a] (–)	1.078 [a] (–)
18	0.778 [a] (–)	1.044 [a] (–)
19	0.709 [a] (–)	1.039 [a] (–)
20	0.687 [a] (–)	1.017 [a] (–)
21	0.734 [a] (–)	1.023 [a] (–)
22	0.665 [a] (–)	0.993 [a] (–)
23	0.616 [a] (–)	0.955 [a] (–)
24	0.669 [a] (–)	0.885 [a] (–)
25	0.466 [a] (–)	0.731 [a] (–)

–: not calculated. a: µg/ml

Table 7. LD_{50} and LD_{90} values of Gainpro AITC-Gamma (Doses 0.2 0.4 0.6 0.8 1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-2	–	–
3	16.597 [a] (–)	–
4	6.635 [a] (2.036–3843.36)	145.497 [a] (–)
5	4.447 [a] (1.750–3310.741)	70.368 [a] (–)
6	2.424 [a] (1.331–24.327)	24.379 [a] (5.776–9001.231)
7	1.903 [a] (1.189–7.907)	14.346 [a] (4.592–600.628)
8	1.699 [a] (1.091–6.308)	13.837 [a] (4.463–556.365)
9	1.444 [a] (0.979–4.129)	11.390 [a] (4.031–274.216)
10	1.387 [a] (0.957–3.645)	10.445 [a] (3.864–199.409)
11	1.387 [a] (0.957–3.645)	10.445 [a] (3.864–199.409)
12	1.569 [a] (0.979–8.153)	19.698 [a] (5.033–4436.794)
13	1.492 [a] (0.954–6.512)	17.486 [a] (4.779–2330.720)
14	1.331 [a] (0.893–4.282)	13.740 [a] (4.274–757.059)
15	0.999 [a] (0.748–1.859)	7.309 [a] (3.123–74.317)
16	0.808 [a] (–)	3.999 [a] (–)
17	0.752 [a] (–)	3.433 [a] (–)
18	0.684 [a] (–)	3.118 [a] (–)
19	0.616 [a] (–)	2.908 [a] (–)
20	0.597 [a] (–)	2.631 [a] (–)
21	0.553 [a] (–)	2.850 [a] (–)
22	0.523 [a] (–)	2.522 [a] (–)
23	0.502 [a] (–)	2.409 [a] (–)
24	0.453 [a] (–)	1.753 [a] (–)
25	0.426 [a] (–)	1.359 [a] (–)
26	0.398 [a] (–)	1.198 [a] (–)
27	0.361 [a] (0.002–0.662)	1.034 [a] (–)
28	0.340 [a] (0.069–0.537)	0.953 [a] (0.590–20.663)
29	0.311 [a] (0.037–0.498)	0.806 [a] (0.502–23.218)
30	0.300 [a] (0.072–0.456)	0.754 [a] (0.491–5.580)

–: not calculated. a: µg/ml

Table 8. LD_{50} and LD_{90} values of Gainpro AITC-Gamma (Doses 0.90 0.95 1.05 1.1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-2	–	–
3	1.486 [a] (–)	2.407 [a] (–)
4	–	–
5	1.863 [a] (–)	5.489 [a] (–)
6	1.355 [a] (–)	2.342 [a] (–)
7	1.260 [a] (–)	2.375 [a] (–)
8	1.140 [a] (1.058–1.812)	1.761 [a] (1.371–12.397)
9	1.127 [a] (1.042–2.956)	1.867 [a] (1.386–259.598)
10	1.120 [a] (–)	2.592 [a] (–)
11	1.129 [a] (–)	3.243 [a] (–)
12	1.100 [a] (–)	2.726 [a] (–)
13	1.084 [a] (–)	2.525 [a] (–)
14	1.066 [a] (–)	2.836 [a] (–)
15	1.016 [a] (–)	1.924 [a] (–)
16	0.990 [a] (–)	1.937 [a] (–)
17	0.943 [a] (0.621–1.012)	1.561 [a] (1.258–35.221)
18	0.905 [a] (0.753–0.956)	1.297 [a] (1.159–2.111)
19	0.897 [a] (0.835–0.931)	1.103 [a] (1.055–1.207)
20	0.888 [a] (0.824–0.923)	1.083 [a] (1.040–1.174)
21	0.879 [a] (0.810–0.915)	1.070 [a] (1.030–1.156)
22	0.868 [a] (0.799–0.903)	1.036 [a] (1.002–1.102)
23	0.872 [a] (0.819–0.900)	0.997 [a] (0.971–1.043)
24	0.869 [a] (0.816–0.895)	0.973 [a] (0.950–1.014)
25	0.874 [a] (0.822–0.897)	0.960 [a] (0.940–1.003)
–: not calculated. a: µg/ml		

Table 9. LD_{50} and LD_{90} values of Grainpro Gamma EUG (Doses 0.2 0.4 0.6 0.8 1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-8	–	–
9	2.950 [a] (–)	–
10	2.950 [a] (–)	–
11	2.950 [a] (–)	–
12	1.420 [a] (–)	–
13	0.597 [a] (–)	–
14	0.417 [a] (–)	–
15	0.273 [a] (–)	–
16	0.164 [a] (–)	–
17	0.099 [a] (–)	–
18	0.062 [a] (–)	–
19	0.089 [a] (–)	39.457 [a] (–)
20	0.099 [a] (–)	9.918 [a] (–)
21	0.079 [a] (–)	6.732 [a] (–)
22	0.085 [a] (–)	3.959 [a] (–)
23	0.099 [a] (–)	1.736 [a] (–)
24	0.104 [a] (–)	1.241 [a] (–)
25	0.099 [a] (–)	1.223 [a] (–)
26	0.093 [a] (–)	0.968 [a] (–)
27	0.093 [a] (–)	0.924 [a] (–)
28	0.103 [a] (0.020–0.181)	0.782 [a] (0.565–1.632)
29	0.098 [a] (0.019–0.172)	0.687 [a] (0.505–1.287)
30	0.101 [a] (0.025–0.170)	0.589 [a] (0.444–0.952)

–: not calculated. a: µg/ml

Table 10. LD_{50} and LD_{90} values of Grainpro Gamma EUG (Doses 0.90 0.95 1.05 1.1).

Day	Lethal Concentration Doses	
	LD_{50} (95% confidence limit)	**LD_{90} (95% confidence limit)**
1-8	–	–
9	1.281 [a] (–)	91.031 [a] (–)
10	1.131 [a] (–)	14.908 [a] (–)
11	1.033 [a] (–)	6.441 [a] (–)
12	0.997 [a] (–)	4.941 [a] (–)
13	0.892 [a] (–)	3.139 [a] (–)
14	0.891 [a] (–)	1.607 [a] (–)
15	0.861 [a] (–)	1.412 [a] (–)
16	0.875 [a] (0.730–0.927)	1.194 [a] (1.103–1.572)
17	0.871 [a] (0.737–0.922)	1.169 [a] (1.089–1.464)
18	0.858 [a] (0.701–0.914)	1.162 [a] (1.082–1.478)
19	0.865 [a] (0.761–0.910)	1.101 [a] (1.047–1.247)
20	0.769 [a] (0.444–0.855)	1.049 [a] (0.994–1.266)
21	0.789 [a] (0.569–0.859)	1.014 [a] (0.970–1.119)
22	0.763 [a] (0.472–0.844)	0.976 [a] (0.924–1.054)
23	0.766 [a] (0.405–0.842)	0.920 [a] (0.825–0.965)
24	0.684 [a] (0.000–0.811)	0.903 [a] (0.222–0.964)
25	0.665 [a] (–)	0.878 [a] (–)
–: not calculated. a: µg/ml		

The 1 µg/ml concentration of Gainpro AITC-Gamma-EUG extract showed a very strong insecticidal effect and no live insects were observed on day 23 (Figure 9). The LD_{50} value of the plant formulation observed on day 23 was 0.099 µg/ml and the LD_{90} value was 1.736 µg/ml (Table 9). The 1.10 µg/ml concentration of Gainpro AITC-Gamma-EUG plant formulation showed a very strong insecticidal effect and no live insects were observed on day 21 (Figure 10). The LD_{50} value of the plant formulation observed on day 21 was 0.789 µg/ml and the LD_{90} value was 1.014 µg/ml (Table 10).

In terms of study results, the effectiveness of these extracts used against this pest or similar pests has not been determined in previous studies. In this study, the LD_{50} and LD_{90} values of these formulations were determined. However, different results were obtained in studies with some conventional and plant extracts against this pest and similar pests. Yiğit et al. (2021) determined the efficacy, LD_{90}, and LT_{90} values of three different doses (0.1%; 0.5% and 1.0%) of thyme essential oil (*Thymbra spicata var. spicata, Origanum majarona* and *O. saccatum*). In the study, it was stated that the LD_{90} values of three different thyme oils in 0.1% dose were 8.36, 4.81, 8.99, and 6.82, 1.90, 4.33 in 1% dose, respectively. Also, almost 100% death occurred on the 4th day of *O. majarona*. In our study, however, the 1.10 µg/ml dose of Gainpro AITC-Gamma-EUG showed the highest effect, but 100% mortality occurred at the end of the 21st day. This difference is thought to be due to the fact that the dose used in our study was approximately 99% lower than the study on thyme. In our next study, it is thought that this effect will be achieved in a very short time with a dose increase.

In our study, % lethal dose values and viability rates of the extracts were determined depending on time. In addition, the LD_{50} value of 1.10 µg/ml dose of Gainpro AITC-gamma-EUG was found to be 1.281 on the 9th day, while this value was found to be 0.10 on the 4th day in *Origanum majorana*, which is the most effective among thyme. This difference is due to the dose difference, as we mentioned above.

Mahmoud & Sabbour (2020) studied the insecticidal effects of four different natural essential oils on *T. confusum* and *T. castaneum*. They determined that coriander and caraway oils were highly effective against both pests, and that 2% dose in nano-formulations caused 70%-85% mortality on the 7th day of application. Gokturk et al. (2020) determined the effects of essential oils of *Ocimum basilicum L*., *Rosmarinus officinalis L*. and *Artemisia dracunculus* on *T. confusum*. At the end of 96 hours, it was determined that *O. basilicum* had 98.3%, *R. officinalis* 98.3%, *A. dracunculus* 93.3% lethal effects. The dose of plant oils used in the study was 20 µL, which is about 20 times the highest dose used in our study. In addition, while spraying method and petri dish application were used for pests in this study, a larger application plastic container was used compared to the food supply and petri dish in our study. In this study, the LD_{50} and LD_{90} values determined in our study were not studied.

Memon et al. (2020) studied the effects of 2%, 1.5%, 1%, and 0.5% doses of *Mentha longifolia* against *T. confusum*. It was determined that the 2% dose reduced the population the most and caused the least weight loss after feeding on wheat grain. In addition, it was determined that the 2% dose caused 96.70% mortality within 7 days. However, the dose rate in this study was investigated higher than in our study and no lethal dose study was performed.

Işıkber et al. (2019) studied the effects of mustard oil alone and with modified atmosphere applications against *Tribolium confusum*. They stated that the mustard essential oil and the 92% CO_2 concentration were more toxic to the larvae, pupae and adults of the pest. They determined that 10 µl/l mustard oil caused nearly 100% mortality in the pest. It was determined that LC_{50} value was 2.25 and LC_{90} value was 3.67 at the 24th hour of application of mustard oil against pest adults. In our study, the LD_{50} value of 1.10 µg/ml dose of Gainpro AITC-Gamma-EUG as the most effective dose was found to be 1.281 on the 9th day. This difference is thought to be due to the dose difference of the plant extract used.

As a result, it was observed that the insecticidal effectiveness of the plant extracts used in this study increased in parallel with the increase in dose, and the LD_{50} and LD_{90} values decreased depending on time due to the low concentration and dose. Diversification of the compounds with new studies including dose increase depending on the perspective of stored pests and organic pest control will provide an effective approach in the fight against warehouse pests. Among these formulations, 1 µL and 1.10 µL doses of Gainpro AITC-EUC formulation were the most effective on the pest.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, 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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RESEARCH ARTICLE

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Determination of the growth and survival characteristics of the kids of damascus goat x kilis goat F1 under breeding conditions

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Abstract

This study aimed to investigate the survival rate and growth performance of the kids of Damascus x Kilis goat F1 bred under intensive conditions. 37 male and 33 female kids of 54 dams (Damascus x Kilis F1 goats) in an enterprise of intensive dairy goat breeding in the Bismil district of Diyarbakır province were used in the study. The birth weight of kids and their monthly weights up to 7 months of age were determined. In the study analyzing the growth and development characteristics of kids of Damascus x Kilis F1 goats up to 210 days of age, it was determined that their mean live weights on the 0th (birth), 30th, 60th, 90th, 120th, 150th, 180th, and 210th days were 3.96±0.124, 7.47±0.306, 10.99±0.506, 13.71±0.592, 15.54±0.643, 24.08±1.019, 22.22±1.033, and 27.51±1.168 kg for males, respectively; whereas, the mean live weights of females were 3.40±0.128, 6.46±0.312, 9.61±0.490, 12.29±0.584, 15.79±0.772, 22.40±0.856, 22.60±0.809, and 24.11±0.937 kg, respectively. Given the type of birth, the mean live weights were found to be 4.12±0.124, 7.42±0.338, 11.29±0.549, 13.89±0.691, 16.51±0.764, 24.08±1.132, 24. 31±1.146 and 26.92±1.269 kg in singleton kids, and 3.28±0.103, 6.58±0.289, 9.42±0.418, 12.20±0.459, 14.83±0.608, 22.51±0.745, 22.74±0.753, and 24.90±0.915 kg in twins, respectively. Also, in the study the effect of sex and birth type on various period weights was analyzed and it was found that birth type had a significant effect ($P<0.005$) on birth weight, and live weights on the 60th and 90th days, while the effect of sex was insignificant. When the survival capacity of the kids was analyzed, the kids were observed to die at the time of birth, but there was no death in the later periods and the survival capacity was found to be 100% from birth until the 210th day.

Keywords: Intensive Breeding, Damascus Goat, Kilis Goat, Growth, Survival Rate

INTRODUCTION

Goat breeding is an important animal husbandry activity for people living in the rural areas both economically and socially (Kaymakçı and Engindeniz, 2010). In Turkey, goat breeding is carried out especially in high-altitude bush pastures where extensive breeding system is applied. However, intensive breeding systems and genotypes suitable for such system have become widespread in recent years (Ertuğrul, 1996). Especially in recent years, intensive or semi-intensive enterprises producing cheese and milk have been operating in Western Anatolia (Taşkın et al., 2013; Taşkın et al., 2017). Damascus goat originates from Nubian goat and is a breed with high milk yield and high fertility which is mostly bred in the provinces

along the Syrian border in Turkey. In studies conducted on these goats, lactation milk yield was reported as 300-600 liters, lactation duration as 250-280 days, and the rate of the number of kids litter size as 150-200% (Keskin et al., 2004; Keskin et al., 2016; Gül et al., 2016). Kilis breed, on the other hand, is the goat breed with the highest fertility and milk yield in Turkey, which is mostly bred in the provinces of Urfa, Gaziantep, Kilis and Hatay on the Syrian border of Turkey and mated with Syrian Damascus goats and Hair goats under natural conditions. Damascus goat is a significant breed that can be utilized in the improvement of our domestic goat breeds with low fertility and therefore Hair goats for milk yield and fertility (Kaymakçı, 2013). Kilis goat is a dairy breed with the highest milk yield in Turkey. Lactation period can be between 210-260 days, and lactation milk yield can be between 200-300 kg in public herds and 400-500 kg in elite herds. As a result of the studies, it was reported that the lactation milk yield of Kilis goats was 294-408 kg and the twinning rate was 10-34% (Keskin et al., 2004; Atac and Burcu, 2014; Gül et al., 2016). Additionally, Keskin and Tüney (2015) found the twinning rate to be 22% in their study.

One of the issues frequently discussed in livestock raising is the gains in live weight of animals. Growth refers to the weight gains in animals due to biological events that generally take place directly proportional to age. Growth is a process that begins in the embryonic stage and continues until the mature age (Tozlu Çelik and Olfaz, 2018). Different mathematical methods have been used to define growth.

Monitoring the growth and development of living organisms during some periods in the growth process would provide significant benefits to breeders for the organization of herd management, care and feeding. This would allow them to easily respond to the problems identified during such periods (Şahin et al., 2014).

The effect of birth weight on both survival rate and growth in living organisms is known. It is very important to control the birth weight and weaning weight continuously in order to increase their survival rate and to provide growth (Savaş, 2007). Therefore, monitoring growth and development in livestock raising and its utilization for improvement are also very important for the business economy (Kozaklı et al., 2022).

Damascus breed is preferred by breeders because the Damascus breed has high milk and fertility productivity, good adaptation abilities, and sufficient fattening properties. The reason why Kilis goats are hybridized with Damascus goats instead of pure breeding is that they are preferred by breeders to further increase the existing milk and reproductive productivity of Kilis goats. This study aimed to investigate the survival rate and growth performance of the kids of Damascus x Kilis F_1 goats bred under intensive conditions.

MATERIALS AND METHODS

Materials

In this study, 37 male and 33 female kids of 54 dams (Damascus x Kilis F_1 goats) in an enterprise of intensive dairy goat breeding in the Bismil district of Diyarbakır province were used.

Methods

The goat breeds available in the farm where the study was carried out are Kilis and Aleppo breeds. Flushing was applied in the farm during the mating period, one month before the matings. Matings were carried out by hand crossing method. For this purpose, estrus was detected by releasing search goats into the herd twice a day, early in the morning and late in the evening. Goats detected to be in heat were mated twice in a controlled manner with the same goats, in the morning and in the evening. Selenium and vitamin E supplementation were performed after umbilical cord care following birth, and birth weight, date of birth, type of birth, sex, and the number of dams were recorded by attaching a plastic ear tag after the birth weights were determined with a 10-gram precise scale within the first 24 hours. After birth, kids were provided with colostrum. The kids were kept with their mothers for the first week and then detached from their mothers. Since there was one milking practice daily in the enterprise, the kids were allowed to suck their mothers after milking, and after the suckling process, the kids were detached from their mothers, and dry alfalfa grass and kids' growth feed were supplied in the kid pens. The kids were weaned on the age of 90 days and began to be fed according to the standard feeding (dry vetch grass and goat feed prepared within the enterprise).

When the kids reached the age of 4 months, males and females began to be raised in separate paddocks. The live weights of the kids were weighed every month from birth to 7 months of age with a 10-gr precision scale in order to determine their growth and survival rate. The day before the weighing, the kids were fasted in the evening and weighed early in the morning. A ration prepared with barley, wheat, cottonseed meal, corn and vetch grass was regularly given to the animals in the enterprise, and marble powder and vitamin solids were added to the ration. The ration contains an average of 2700 kcal and 15% protein.

Fertility criteria according to the mating and lambing outcomes reported below were used in the study. Accordingly, the criteria were calculated as follows (Kaymakçı, 2006).

Pregnancy rate (%) = Pregnant goat / Goat under a billy-goat

Infertility rate (%) = Infertile goats/ Goat under a billy-goat

Fertility rate (%) = Breeding goat / Goat under a billy-goat

Twinning rate (%) = Twinning goat / Kidding goat

Fecundity = Born kids / Goats under a billy-goat

Litter size= Born kids/Kidding goat

The growth performances of the kids at various periods were analyzed using the GLM (General Linear Model) procedure, the significance of the differences between the group means was checked by the Duncan multiple comparison test, and the survival rates were determined by the Chi-Square (c^2) method (SAS 1995).

RESULTS AND DISCUSSION

Table 1 shows the fertility criteria of the crossbred Damascus x Kilis F_1 goats in the study. Accordingly, the obtained fertility criteria were as follows: pregnancy rate of 92.6%, infertility rate of 7.4%, fertility rate of 92.6%, twinning rate of 37%, fecundity rate of 1.29%, and litter size of 1.12%.

Table 1. Fertility Measures of Damascus x Kilis F_1 Crossbred Goats

Parameters	n	% rate
Number of Goats Giving Birth	54	-
Number of Kids Born	70	-
Number of Kids Died	9	-
Number of Goats Giving Birth to Twins	20	-
Number of Kids Alive	61	-
Number of Kids Born Twin	31	-
Number of twin Kids	40	-
Number of Singleton Kids	30	-
Pregnancy rate	-	92.6
Infertility Rate	-	7.4
Fertility	-	92.6
Twinning Rate	-	37
Fecundity	-	1.29
Litter size	-	1.12

Table 2. Descriptive Values of Sex and Birth Types of Damascus x Kilis F_1 Kids

Sex			Birth Type		
	n	%		n	%
Male	32	52.5	**Single**	37	49.2
Female	29	47.5	**Twin**	33	50.8
General	61	100.0	**General**	70	100.0

When the sex factor was taken into consideration in the kids of crossbred Damascus x Kilis Goat F_1 in Table 2, it was found that 52.5% of the kids were male and 47.5% were female. When the birth type factor was analyzed, it was reported that 49.2% of the kids were singletons and 50.8% were twins. Özdemir and Keskin (2018) reported the twin rates as 35.9% and 27.1%, respectively, in their study in Kilis and Gaziantep provinces. Gül et al., (2016) reported twinning rates as 10.56, 11.11 and 33.90, respectively, in their studies conducted with Kilis goats in 2012, 2013 and 2014. Keskin et al., (2017), in their studies conducted with Kilis goats in 2013, 2014 and 2015, reported the twinning rates as 30.4, 31.4 and 29.7, respectively.

Table 3 shows the analyses of the period weights of the kids of Damascus x Kilis F_1 by birth type and sex factors. It was reported that the difference between the period weights in terms of the sex factor was insignificant ($P<0.05$); the birth weights of singletons and twins were 4.12±0.124 and 3.28±0.103 kg, respectively; the weights on the 60th day were 11.29±0.549 and 9.42±0.418 kg, respectively; and the weights on the 90th day were 13.89±0.691 and 12.20±0.459 kg, respectively, for the birth type factor; and the difference between the weight means on the 30th, 120th, 150th, 180th, and 210th days was significant ($P<0.05$), but the difference between the mean weights of the 30th, 120th, 150th, 180th, and 210th days were insignificant ($P<0.05$).

Table 4 shows the live weight gains of the kids of Damascus x Kilis F_1 goats in different periods by birth type and sex factors. The differences between the weights of male and female kids in different periods were insignificant ($P<0.05$) in terms of sex factor. Given the birth type, the live weight gains on the 120th and 210th days (0.093±0.153; 0.073±0.143 and 0.090±0.01; 0.074±0.01) were statistically significant ($P<0.01$) and the live weight gains on the 150th and 180th days were statistically significant (0.222±0.177; 0.225±0.014 and 0.008±0.0005; 0.006±0.0005) ($P<0.05$). The differences between the live weight gains on the 30th, 60th, and 90th days were insignificant. The high gain in live weight on the 150th day can be associated with the fact that it coincides with the mating season (September–October) and exposure to supplementary feeding (flushing), as well as the active sexual cells during this period.

The survival rate of the kids was examined and showed that most of the existing mortality took place at the time of birth. The survival rate from birth to the 210th day was 100%.

The birth weights of males and females were found to be 3.96±0.124 and 3.40±0.128 kg, respectively when taking sex into consideration in this study. When the results from the studies by different researchers were analyzed, it was found that the values were higher than those by Erten and Yılmaz (2013), Gökdal et al., (2013), Tekin and Öğeç (2017), and Şimşek and Bayraktar (2006) and lower than those by Gök et al., (2015). In the study of Gül et al., (2022) it was stated that the birth weight of Kilis male kids was 3.6± 0.01 kg, while the female birth weight was 3.5±0.01 kg. Gül et al., (2016) reported that there was no significant difference in terms of birth weight and gender in their study. Given the birth type, it was found that the mean birth weights of kids were 4.12±0.124 kg and 3.28±0.103 kg for singletons and twins, respectively. These results were higher than the values of Şimşek et al., (2007), Erten and Yılmaz (2013), Gökdal et al., (2013) and

Table 3. Live Weight Averages (kg) of Halep x Kilis F_1 Kids at Various Periods

Factors	n	Birth	30th days	60th days	90th days	120th days	150th days	180th days	210th days
Single	37	4.12±0.124 [a]	7.42±0.338	11.29±0.549[a]	13.89±0.691[a]	16.51±0.764	24.08±1.132	24.31±1.14 6	26.92±1.269
Twin	33	3.28±0.103[b]	6.58±0.289	9.42±0.418[b]	12.20±0.459[b]	14.83±0.608	22.51±0.745	22.74±0.753	24.90±0.915
Sex									
Male	32	3.96±0.124	7.47±0.306	10.99±0.506	13.71±0.592	15.54±0.643	24.08±1.019	22.22±1.033	27.51±1.168
Female	29	3.40±0.128	6.46±0.312	9.61±0.490	12.29±0.584	15.79±0.772	22.40±0.856	22.60±0.809	24.11±0.937

*The difference between group means with different letters is significant (P<0.05).

Table 4. Live Weight Increases of Damascus x Kilis F_1 Kids at Various Periods for Birth Type and Sex

Factors	n	30th days $\bar{X}\pm S_{\bar{x}}$	60th days $\bar{X}\pm S_{\bar{x}}$	90th days $\bar{X}\pm S_{\bar{x}}$	120th days $\bar{X}\pm S_{\bar{x}}$	150th days $\bar{X}\pm S_{\bar{x}}$	180th days $\bar{X}\pm S_{\bar{x}}$	210th days $\bar{X}\pm S_{\bar{x}}$
Birth Type								
Single	37	0.169**±0.**113	0.077**±0.00**7	0.061±0.075	0.093±0.153[a]	0.222±0.177[a]	0.008**±0.00**05[a]	0.090**±0.01**[a]
Twin	33	0.143**±0.00**8	0.056**±0.00**4	0.066**±0.0**49	0.073**±0.**143[b]	0.225±0.014[b]	0.006**±0.00**05[b]	0.074**±0.01**[b]
General	**70**				**0.095±0.005** **0.089±0.003**			
Sex								
Male	32	0.161**±0.**104	0.070**±0.00**6	0.064**±0.00**6	0.065**±0.00**8	0.251±0.017	0.007**±0.00**4	0.109**±0.**144
Female	29	0.150**±0.**100	0.063**±0.00**6	0.063**±0.00**6	0.125±0.181	0.194±0.125	0.005**±0.00**3	0.051**±0.**145
General	**61**				**0.098±0.004** **0.086±0.003**			

*The difference between the group means with different letters is significant (P<0.05).

*The difference between the group means with different letters is significant (P<0.01).

Tekin and Öğeç (2017). The data obtained in the study were higher than the birth weights of the Kilis single kids but lower than the twin birth weights of Gül et al., (2022). Keskin et al. (2017), semi-intensive birth weights of Kilis goat kids reared under these conditions were reported as 3.56± 0.02 kg. It can be seen that the values obtained in this study are similar to the values obtained in our study.

The mean live weights of the kids of Damascus x Kilis F_1 goats on 30th day were found to be 7.47±0.306 kg and 6.46±0.312 kg for males and females, respectively in the study. The mean live weight on the 30th day in this study was reported to be higher than those obtained by Şimşek et al. (2007) and Erten and Yılmaz (2013) for males and similar to those obtained by Erten and Yılmaz (2013) for females. The values in this study were higher than those obtained by Gökdal et al., (2013) for both males and females. Given the birth type, the mean live weights

on the 30th day were reported to be 7.42±0.338 kg and 6.58±0.289 kg for singletons and twins, respectively, and it was reported that the mean live weights on the 30th day for singletons and twins in this study were lower than the values obtained by Şimşek et al. (2007) for male kids and higher than the values obtained by Şimşek et al. (2007) for female kids. It was also reported to be higher than the values obtained by Erten and Yılmaz (2013) for males and similar to the values obtained for females. Also, the values in this study were lower than those obtained by Gökdal et al., (2013), Tüfekçi and Olfaz (2016), and Şimşek and Bayraktar (2006).

It was reported in the study that the mean live weights of the kids of Damascus x Kilis F_1 goats on the 60th day were 10.99±0.506 kg and 9.61±0.490 kg for males and females, respectively. Accordingly, it was reported that the mean live weights on the 60th day were similar to the values obtained by Şimşek et al., (2007) for male kids and lower than the values obtained by Şimşek et al. (2007) for female kids; the values obtained by Erten and Yılmaz (2013) for male kids were higher, and the values obtained by Erten and Yılmaz (2013) for female kids were similar. Given the birth type, the mean live weights of the kids of Damascus x Kilis F_1 goats on the 60th day were 11.29±0.549 and 9.42±0.418 kg for singletons and twins, respectively. The mean live weights of singletons and twins on the 60th day in this study were lower than the values obtained by Şimşek et al., (2007) and Şimşek and Bayraktar (2006) but higher than the values obtained by Erten and Yılmaz (2013). Gul et al., (2016) in their studies conducted with Kilis goats in 2012 and 2013, found that the birth rates were 16.8±0.22, 16.5±0.21 in single-born males, 15.7±0.16, 15.4±0.17, respectively, in single-born females, and 15.0±0.19, 14.9 ± 0.28, respectively, in twin-born males. In females born with twins, they reported 14.4 ± 0.21, 14.5 ±0.35, respectively. Keskin et al., (2017) reported the 60th day live weight as 11.9±0.08 in 2013, 12.8±0.08 in 2014 and 12.1 ±0.06 in 2015. In another study, Keskin et al., (2022) reported in their study with Kilis goats in 2016, 2017, 2018, 2019 and 2020, 60th day live weights in males and females, respectively; 12.9±0.05; 12.4±0.04; 12.1± 0.04; 11.5±0.03; 11.5±0.04; 11.1±0.04 and 12.4±0.06; 11.7±0.05; 12.1±0.04; 10.8±0.05 in terms of birth type, again 13.2±0.05; 12.3±0.04; 12.6±0.04;

11.4±0.03; 11.5±0.03 and 12.2±0.07; 11.6±0.06; 12.2±0.07; 10.8±0.04; 10.8±0.05. It is seen that the values obtained in other studies are higher than the values obtained in our study (Gül et al., 2022).

In the study, it was reported that the mean live weights of the kids of Damascus x Kilis F_1 goats on the 90th day were 13.71±0.592 kg and 12.29±0.584 kg for males and females, respectively, and the mean live weights on the 90th day were lower than the values obtained by Gökdal et al., (2013) for males and similar to the values obtained by Gökdal et al., (2013) for females. Furthermore, the values for males and females in this study were lower than the values obtained by Şimşek et al., (2007), Şimşek and Bayraktar (2006); Gök et al., (2015), but higher than the values obtained by Erten and Yılmaz (2013) for males and similar to their values for females. Given the birth type, the mean live weights on the 90th day were 13.89±0.691 kg and 12.20±0.459 kg for singletons and twins, respectively, and the mean live weights on the 90th day for singletons and twins obtained in this study were lower than the values obtained by Şimşek et al., (2007) and Gökdal et al., (2013), Erdem et al., (2022) but higher than the values obtained by Erten and Yılmaz (2013) for singletons, and similar to their values for twins.

In the study, it was reported that the mean live weights on the 120th day were 15.54±0.643 kg and 15.79±0.772 kg for males and females, respectively. The mean live weight on the 120th day was higher than the values obtained by Erten and Yılmaz (2013) for both sexes, but lower than the values obtained by Tekin and Öğeç (2017) for both sexes. Given the birth type, the mean live weights on the 120th day were 16.51±0.764 kg and 14.83±0.608 kg for singletons and twins, respectively in this study, and the mean live weights on the 120th day for singletons and twins were higher than the values obtained by Erten and Yılmaz (2013) for males and females, but lower than the values obtained by Tekin and Öğeç (2017) for both sexes. In addition, Erdem et al., (2022) determined the 120th day live weight in males as 19.91±0.54 in males and 15.50±0.58 in females in their study on Damascus goats.

In the study, it was reported that the mean live weights on the 150th day were 24.08±0.019 kg and 22.40±0.856 kg for males and females, respectively. The mean live weight on the 150th day in this study was higher than the values obtained by Erten and Yılmaz (2013) and Gökdal et al. (2013) for both sexes. Given the birth type, the mean live weights on the 150th day were reported to be 24.08±1.132 kg and 22.51±0.745 kg for the kids born as singletons and twins, respectively. The mean live weights on the 150th day in this study were higher than the values obtained by Erten and Yılmaz (2013) for singletons and twins, similar to the values obtained by Gökdal et al., (2013) for singletons but higher than the values obtained by Gökdal et al. (2013) for twins.

In the present study it was reported that the mean live weights on the 180th day for males and females were 22.22±1.033 kg and 22.60±0.809 kg, respectively, based on the sex factor. The mean live weights of males and females on the 180th day in this study were higher than the values obtained by Erten and Yılmaz (2013) but lower than the values obtained by Gök et al. (2015). Regarding the birth type, the mean live weights on the 180th day were 24.31±1.146 kg and 22.74±0.753 kg for singletons and twins, respectively. Erdem et al., (2022) in their study on Damascus goats, determined the 180th day live weight as 21.73±0.44 in males and 20.39±0.48 in females. Given the birth type, the mean live weight on the 180th day was higher than the values obtained by Erten and Yılmaz (2013), Şimşek and Bayraktar (2006) but lower than the values obtained by Tüfekçi and Olfaz (2016) for both birth types. In this study, the main reasons why the live weight gain is not at the desired level between the 150th days and 180th days are; as this period coincides with August, it can be expressed as an increase in temperatures in the region and a corresponding decrease in feed consumption in animals.

In the study it was reported that the mean live weights of the kids of Damascus x Kilis F_1 goats on the 210th day were 27.51±1.168 kg and 24.11±0.937 kg for males and females, respectively. The mean live weight on the 210th day in this study was higher than the values obtained by Gökdal et al. (2013) for male kids but lower than the values obtained for female kids. Given the birth type, the mean live weights on the 210th day were 26.92±1.269 kg and 24.90±0.915 kg for the singletons and twins, respectively. The mean live weights on the 210th day in this study for singletons and twins were lower than the values obtained by Gökdal et al., (2013) for both birth types. The values of the present study were compared with those obtained in studies by different researchers, and some differences were found. This may be accounted for by using different genotypes in the studies, management, and breeding conditions.

CONCLUSION

Live weight is an important characteristic that should be focused on throughout the life of animals and is one of the major criteria used to identify breed characteristics, early selection, and the determination of growth and fattening studies. The birth weights and weaning weights of the kids significantly affect their live weights in the following periods. Poor birth weights and weaning weights affect their live weights in the following periods and, consequently, their survival rate negatively. Therefore, higher birth and weaning weights are required. This can be achieved by paying attention to feeding in the last period of pregnancy, especially in goats. The differences between the live weights of kids at birth and on the 60th and 90th days in the study were significant ($P<0.05$) when the sex factor was considered, and males were significantly ($P<0.05$) higher than females. When analyzing the survival rates of the kids, it was determined that all of the deaths took place at

the time of birth, there was no death in the following periods, and the survival rate of the kids was 100% from birth until the 210th day.

Consequently, the results obtained in this study, which aimed to determine the growth and survival rate of the Damascus x Kilis F_1 kids in an enterprise of intensive dairy goat breeding, were found to be compatible with the results obtained in many other studies. The study indicated that especially the survival rate performance of the kids was very satisfactory, and it was concluded that this was closely correlated with the breeding methods adopted on the enterprises. In this study, the live weight gain of kids was compatible with the other studies in the literature. Based on the results of this study, it was concluded that breeding selection based on live weights at 2nd and 3rd months of age could produce very accurate results and live weight could be an early selection criterion.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

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

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

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RESEARCH ARTICLE

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Effects of different explant and growth regulator combinations on in vitro propagation of Poinsettia (*Euphorbia pulcherrima* Willd.)

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Abstract
The aim of the research is to develop a suitable micropropagation protocol for the Poinsettia (*Euphorbia pulcherrima* Willd.) plant, which is used as an ornamental plant. In the sterilization step, mercuric chloride together with sodium hypochlorite is approved as the best option. Thus, contamination of explants is eliminated. At the shoot propagation stage, BA is absolutely necessary as all doses of BA increased shoot propagation compared to the control. The highest number of shoots per explant (2.62 shoots/explant) and the longest shoots (2.16 cm) were obtained from 1.0 mg.L^{-1} BA medium. The highest number of leaves (20.41 leaves/explant) was obtained from 2.0 mg.L^{-1} BA medium. Kinetin was also effective when adding different concentrations to the culture medium compared to the control. In general, BA is more effective than Kinetin in these parameters. Kinetin, on the other hand, performed much better than BA in terms of shoot number. On the other hand, the highest rooting percentage (58.2%) was obtained from the addition of 0.3 mg.L^{-1} NAA. The highest root number (5.10 roots/explant) was obtained by adding 0.1 mg.L^{-1} NAA. Good performance was found in the acclimatization phase with plantlets transferred to the soil with a high survival rate reaching 100%. Most of the plantlets started growing well. The plantlets grew well and did not show morphological abnormalities. In addition, a successful plant regeneration was achieved by adding 1.0 mg.L^{-1} BA and 0.5 mg.L^{-1} NAA on the callus produced in leaf disc explants and a very good organogenesis was determined in terms of roots and shoots.
Keywords: Poinsettia, *Euphorbia pulcherrima* Willd., *in vitro*, Micropropagation, Regeneration

INTRODUCTION

The Euphorbiaceae family is a family of taxa with high economic value (Mwine et al. 2011). Poinsettia (*Euphorbia pulcherrima* Willd) belongs to this family and belongs to the genus Euphorbia, which consists of about 2000 species. Poinsettia plant is the most popular ornamental plant in North America, Australia and various European countries (Castellanos, 2010). Their colors range from red to white, from yellow to orange-red (Bidarigh and Azarpour, 2013). Increasing its popularity as an interesting plant, these magnificent flowers can stay fresh and intact for 3-4 months. Its origin is Central America. It is considered one of the fastest growing plants (Toma and Almizory, 2012).

Poinsettia plant is one of the widely grown indoor ornamental plants (Clarke et al., 2008). In Iraqi ecological conditions, poinsettia is considered as an outdoor landscape plant. Euphorbia species are usually propagated by cuttings. Propagation by seeds is difficult, as the seeds lose their viability during storage. Ordinary reproduction is often done with cuts that focus on a period before the

most extensive sale time. The development of new *in vitro* regeneration procedures is likely to play a decisive role in successful production systems (Pickens et al., 2005; Trejo, 2020). In this context, it is of great importance to develop an effective micropropagation protocol *in vitro* and in sterile conditions. İn *vitro* micropropagation is a culture technique that has gained great popularity today. Tissue culture is also very important for the preservation of genetic material. With this technique, clone plants can be reproduced, endangered plants can be protected, gene sources can be protected and virus-free plants can be obtained (Babaoğlu et al., 2001).

In the research, it is aimed to determine the micropropagation methods of poinsettia in detail. With this study, it was aimed to develop a suitable sterilization and environment protocol for this species, to facilitate mass production by applying various concentrations of different growth regulators, to create a market for important landscape plants, and to test the adaptation of *in vitro* plants to external conditions. In addition, it is thought that the data obtained will be a basis for researches to quickly switch to ornamental plant production and to ensure success in production.

MATERIALS AND METHODS

This study was carried out in the plant tissue culture laboratory of the Department of Horticulture, Faculty of Agricultural Engineering Sciences, Duhok University, Northern Iraq Region, in 2021 and 2022.

The plant material was collected from healthy plants grown in local nurseries in the city of Duhok. Different explants were tested, including apical buds, axillary buds and leaves (1-2 cm). In this study, regeneration shoot tip and nodal explants of Poinsettia plant, which is an important ornamental plant, were examined, the effect of different plant growth regulators and concentrations on obtaining adventitious shoots from different explants was investigated, thus an effective protocol for *in vitro* was created. Shoots about 1.5 cm long were washed under tap water with the addition of detergent every 10 minutes for about 1 hour and then rinsed. In addition, ascorbic acid and citric acid (100 and 150 mg) solutions were prepared and then the explants were kept in this solution for 30 minutes.

Explants were immersed in 1.5%, 2.0% and 2.5% v/v sodium hypochlorite (NaOCl) solution for surface disinfection under a laminar air flow cabinet for fifteen minutes and then washed three times with sterile distilled water. Another sterile material was used to disinfect at 0.15% for 7 and 10 minutes using mercury chloride ($HgCL_2$). In this way, the sterilization protocol was determined. MS (Murashige and Skoog, 1962) was used as the basic medium. Microshoots that responded well at this stage were tested at BA 0, 0.5, 1, 1.5, and 2 $mg.L^{-1}$ and kinetin 0, 0.5, 1, 1.5, and 2 $mg.L^{-1}$ and transferred to the propagation stage. For shoot propagation experiments, three explants were subcultured in each culture dish and five vials were used for each treatment. The cultures were then incubated in the growth chamber at below 25+2°C and exposed to 1000 lux lighting for 16 hours per day. The number of leaves per explant, the number of shoots per explant, and the mean shoot length were recorded 4 weeks after the first culture (Ogras et al., 2022).

In the rooting step, auxins (IBA and NAA) were used at 0, 0.1, 0.2, and 0.3 $mg.L^{-1}$ using half MS medium. For root promotion, the bottles were sealed with aluminum foil. After six weeks in subculture, rooting parameters such as percentage rooting, number of roots per explant, and mean root length were recorded. Rooted plantlets were transferred to outdoor conditions and sprayed with Benlate fungicide (0.1%). It was then transferred to pots containing sterilized peat moss. The plants were sprayed with a nutrient solution containing ¼ MS salts. Plants were transferred to normal greenhouse conditions after 8-10 days. For the callus induction experiment, leaf discs of about 1 cm^2 were taken from the plants grown *in vitro* from the initial stage. Three explants were taken into each culture dish and five petri dishes were used for each replicate. After 8 weeks in culture, parameters such as percent callus induction, callus weight and callus consistency were recorded.

Planting was done in nutrient media in culture tubes, with 3 explants in each jar. The experiment was set up in 3 replicates for each explant type and nutrient medium, with 10 jars per replicate. It is designed as Completely Random Design (CRD). Comparison was made according to Duncan's multiple range test ($P < 0.05$) using a computerized SPSS and JMP program.

RESULTS AND DISCUSSION

Micro shoots consisting of apical bud explants were selected for the determination of developmental stages. This is because in the initial stage apical buds are much better than axillary buds. The results showed that the addition of BA was highly beneficial for raising the values of the amplification parameters compared to the control treatment. However, no critical difference was noted between the different concentrations of added BA. The highest number of shoots per explant (2.62 shoots/explant) and the longest shoots (2.16 cm) were obtained from the addition of 1.0 $mg.L^{-1}$ BA. The highest number of leaves (20.41 leaves/explant) was recorded from the addition of 2.0 $mg.L^{-1}$ BA (Table 1).

On the other hand, Table 2 shows that Kinetin is also effective when adding different concentrations to the culture medium compared to the control. Addition of 1.5 $mg.L^{-1}$ Kinetin produced the highest number of shoots and leaves (3.63 shoots/explant and 18.03 leaves/explants), respectively. Whereas, control treatment gave the longest shoots reaching 2.03 cm compared to 0.58 cm recorded for 1.5 $mg.L^{-1}$ kinetin. In general, BA was more effective than kinetin in terms of leaf number and

Table 1. Effect of BA Concentrations on poinsettia multiplication stage after four weeks in culture MS medium

Treatments (BA mgL^{-1})	Number of Shoots/Explant	Number of Leaves/Explant	Length of Shoots (cm)
0.0	1.05 ± 0.12 b	7.66 ± 0.48 c	2.03 ± 0.24 ab
0.5	2.35 ± 0.28 a	18.86 ± 0.80 b	2.13 ± 0.24 a
1.0	2.62 ± 0.25 a	20.30 ± 0.66 a	2.16 ± 0.16 a
1.5	2.36 ± 0.28 a	18.25 ± 0.17 b	1.66 ± 0.15 b
2.0	2.35 ± 0.24 a	20.41 ± 0.76 a	2.13 ± 0.33 a
LSD≤.05	0.13	0.49	0.14

Table 2. Effect of Kinetin Concentrations on poinsettia multiplication stage after four weeks in culture MS medium

Treatments (Kinetin mgL^{-1})	Number of Shoots/Explant	Number of Leaves/Explant	Length of Shoots (cm)
0.0	1.05 ± 0.12 d	7.66 ± 0.48 d	2.03 ± 0.24 a
0.5	2.13 ± 0.21 c	15.08 ± 0.60 c	1.18 ± 0.24 bc
1.0	2.96 ± 0.22 b	16.40 ± 0.66 b	0.86 ± 0.14 cd
1.5	3.63 ± 0.38 a	18.05 ± 0.19 a	0.58 ± 0.25 d
2.0	3.08 ± 0.27 b	15.28 ± 0.36 c	1.63 ± 0.37 ab
LSD≤.05	0.18	0.36	0.15

average shoot length. However, kinetin performed much better than BA in terms of shoot number (Figure 1).

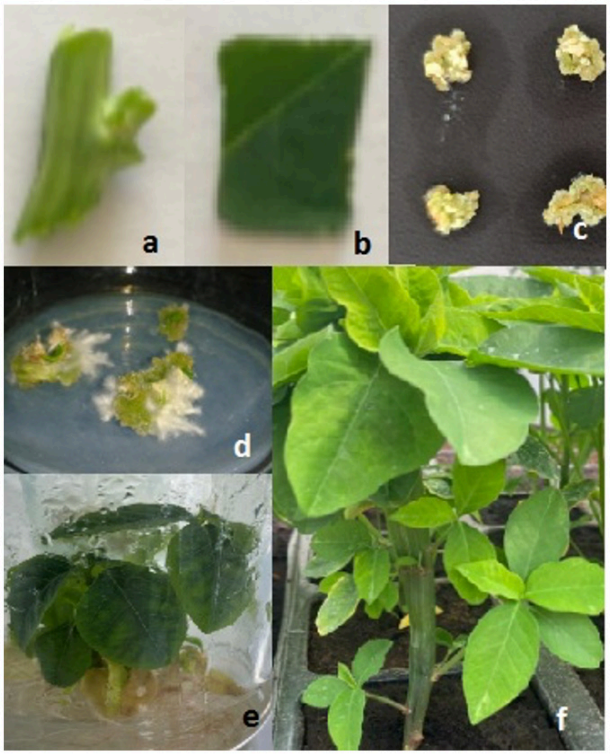

Figure 1. Tissue culture stages (a; auxiliary bud, b;Leaf discs for callus indication, c; callus, d; indirect organogenesis of roots, e; plants grown *in vitro*, f; after one week from transplating in greenhouse).

The best poinsettia microshoots produced in the shoot propagation stage were transported to the root formation stage by testing different concentrations of IBA and NAA at 0.0, 0.1, 0.2 and 0.3 mg.L^{-1}. (Table 3 and Figure 2). Addition of 0.2 mg.L^{-1} IBA was higher than the remaining treatments. It was critically higher than the control, which produced only 2.18 cm, as it reached the highest rooting percentage (63.1%), the highest root number (2.20 roots/explant) and the longest roots reaching 5.50 cm.

Figure 2. Root formation stage (A; effect of different IBA concentrations, B; rooted microshoots treated with 0.2 mgL^{-1} IBA, C; effect of different NAA concentrations, D; rooted microshoots treated with 0.3 mgL^{-1} NAA)

Explants were followed for root, callus and shoot formation. The shoot-forming explants were subcultured and grown. The shoots that reached a sufficient level were cleaned and taken into the rooting medium. Plantlets that grew and developed in the rooting medium were potted and new plants were obtained (a high percentage of plant survival reaching 90%). These plants performed very well under greenhouse conditions and showed no growth abnormalities or any physiological disturbances.

Table 3. Effect of different IBA concentrations on rooting parameters

Treatments (IBA ppm)	Percentage (%)	Number of Roots (pcs)	Length of Roots (mm)
0.0	58.50 ± 2.12 b	1.20 ± 0.12 c	2.18 ± 0.44 c
0.1	44.30 ± 1.44 d	1.80 ± 0.17 b	3.39 ± 0.12 b
0.2	63.10 ± 1.65 a	2.20 ± 0.32 a	5.50 ± 0.32 a
0.3	53.40 ± 2.12 c	1.55 ± 0.14 bc	3.61 ± 0.32 b
LSD≤.05	0.69	0.18	0.26

Table 4. Effect of different NAA concentrations on rooting parameters

Treatments (NAA ppm)	Percentage (%)	Number of Roots (pcs)	Length of Roots (mm)
0.0	58.50 ± 2.12 a	1.20 ± 0.12 b	2.18 ± 0.44 b
0.1	44.20 ± 2.44 b	5.10 ± 1.15 a	4.55 ± 0.70 a
0.2	33.00 ± 0.92 c	4.42 ± 1.05 a	4.53 ± 0.52 a
0.3	58.20 ± 2.25 a	4.43 ± 0.42 a	5.20 ± 1.12 a
LSD≤.05	1.37	0.99	0.61

Table 5. Callus induction in leaf disc explants affected by combinations of BA and NAA

BA+ NAA (mg.L^{-1})	Callus Induction (%)	Weight of Callus (g)	Remarks
0.0+ 0.0	0	0 c	-
0.0+ 0.5	0	0 c	-
0.0+ 1.0	0	0 c	-
0.0+ 2.0	0	0 c	-
0.0+ 4.0	5.6 c	0.58 b	Green, compact
1.0+ 0.0	0	0 c	-
1.0+ 0.5	6.6 c	1.16 b	Green, compact
1.0+ 1.0	6.0 c	0.65 b	Yellow, friable
1.0+ 2.0	6.6 c	0.5 b	Yellow, friable
1.0+ 4.0	6.6 c	1.2 b	Yellow, friable
2.0+ 0.0	0	0 c	-
2.0+ 0.5	93.33 a	2.6 a	Yellow, friable
2.0+ 1.0	60 b	1.49 b	Green, yellow, compact
2.0+ 2.0	66.66 b	1.4 b	Yellow, friable
2.0+ 4.0	93.33 a	2.57 a	Yellow, green, compact
3.0+ 0.0	0	0 c	-
3.0+ 0.5	60 b	2.31 a	Green, compact
3.0+ 1.0	80 ab	2.56 a	Yellow, compact
3.0+ 2.0	40 bc	2.52 a	Green, compact
3.0+ 4.0	60 b	2.63 a	Yellow, friable

In general, good callus induction was obtained from culture of leaf discs. Table 5 shows that the addition of BA and NAA at 2.0 mg.L^{-1} + 0.5 mg.L^{-1} NAA and 2.0 mg.L^{-1} + 4.0 mg.L^{-1} are the best combined treatments, creating and giving 93.33% callus induction. The consistency of the callus was very good, it had a brown to green color and a compact texture. This formed callus was selected for the next regeneration experiment. As mentioned above, the best callus produced from 2.0 mg.L^{-1} BA+ 4.0 mg.L^{-1} NAA was transferred to culture medium containing 1.0 mg.L^{-1} BA and 0.5 mg.L^{-1} NAA.

Toma and Al-Mizory (2012) have been very successful in acclimatizing poinsettia plantlets with a high survival rate of up to 90%. After several months of acclimatization, their plants did not show any abnormal features or any physiological problems.

Perera and Trader (2008) produced red poinsettia callus by grafting their explants into culture medium containing only BA and a combination of BA and IAA. No callus was formed from the culture medium without PGR. The combination between BA and IAA produced better red calluses than the use of BA alone. The best callus size (1.27 mm) and the highest number of bud calluses (1.29) were recorded with the half stem cut application. Many important points should be considered when sterilizing explants.

Sodium hypochlorite is very widely used in tissue culture for various herbaceous and woody plants (Perera and Trader 2010), but a high rate of contamination (90%) was incurred when using different concentrations of NaOCl (1.5, 2.0 and 2.5%) This problem forced to use mercuric chloride (HgCl2) at 0.15% for 10 minutes. This

sterilant was very effective in preventing contamination by obtaining 100% healthy and clean cultures. These results are consistent with similar literature (Toma and Al-Mizory, 2012; Rangel-Estrada et al., 2015).

At the shoot propagation stage, both BA and kinetin were tested as cytokinins, and BA was more effective than kinetin in terms of leaf number and average shoot length. However, kinetin performed much better than BA in terms of shoot number. The results for callus induction in leaf disc explants showed that callus could be successfully induced while finding the best combination between auxins and cytokinins. Plant growth regulators are the key solution for callus induction in plant tissue culture, especially for moderate levels of auxins and cytokinins (George, 2008). Finally, for plant regeneration from the produced corn, it is very important to find the best combination between cytokinins and auxins to ensure organogenesis. In the present study, the corn produced was of the embryogenic callus type, which was able to regenerate roots and shoots after treatment with 1.0 $mg.L^{-1}$ BA and 0.5 $mg.L^{-1}$ NAA. This phenomenon proves the main role of auxins and cytokinins in cell division and differentiation (Pickens et al., 2005).

CONCLUSION

In conclusion, although the response of shoot propagation parameters varies according to cytokinin types and levels, the addition of cytokinins is essential for better shoot propagation of the poinsettia plant. Indole butyric acid (IBA) proved to be a better choice than naphthalene acetic acid (NAA) to initiate the rooting process in poinsettia microshoots. Although the acclimatization step is considered the most laborious step during micropropagation protocols, a high survival rate of poinsettia plantlets can be achieved if the grower follows the acclimatization steps very carefully. Browning of the plant material occurred as the proportions of sterilants used and the residence time of the plant material in the sterilant increased. Finding a better combination of auxins and cytokinins as well as a suitable explant is still key to inducing embryogenic callus in poinsettia explants. Indirect organogenesis on poinsettia callus can be achieved after obtaining an embryogenic callus by appropriate exogenous addition of plant growth regulators to the culture medium.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, 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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RESEARCH ARTICLE

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Effect of irrigation water salinity on morphological and physiological characteristics of celery

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Abstract

The objective of this study was to investigate the effects of salinity levels of irrigation water on the morphological and physiological characteristics of celery (*Apium graveolens* L.) during early seedling development. Celery seedlings of Balena cultivar were grown with saline irrigation water consisting of different NaCl levels (0, 50, 100, 150, 200, 250, and 300 mM). The results showed that increasing salt stress inhibited plant growth by destroying physiological parameters. Each increase in NaCl resulted in a decrease in the length, diameter, number, and fresh and dry weight of leaves. Dry matter, chlorophyll content, leaf temperature, and turgor loss improved when NaCl levels were increased; however, salinity caused a reduction in leaf relative water content. Leaf fresh and dry weights were lower under salt stress, even at 50 mM NaCl. Leaf temperature was higher in plants grown under salinity and reached the maximum level at 100 mM NaCl. The stomata on the abaxial side of the leaves were smaller but more numerous under salinity than in the control plants. It was concluded that celery's plant growth was significantly influenced by salinity and that it could endure salinity lower than 100 mM NaCl.

Keywords: *Apium graveolens* L., NaCl, Plant growth, Chlorophyll content, Stomata density

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INTRODUCTION

Celery (*Apium graveolens* L.) is a cool-season vegetable that belongs to the family *Umbelliferae* and is widely cultivated and consumed on a global scale. It is grown under irrigated conditions for both its leaves and roots, which are the edible parts (Ma et al. 2019). In Turkey, it is mainly cultivated for its roots, but its leaves are used to prepare cooked vegetables or raw in salads. It has high nutritional value and is rich in calcium, phosphorus, iron, carotene, vitamins, and other nutrients (Hedayati et al. 2019).

Several abiotic stress factors adversely affect plant growth from germination to harvest. Of these factors, salinity is the most hazardous stress that destroys the life cycle of crops (Munns 2005). It causes osmotic stress leading to physiological drought and/or ion toxicity due to excessive Na^+ and Cl^- ions (Eisa et al. 2012). Because low water potential in saline soils or irrigation water limits water uptake by plant roots, plants close stomata to prevent water evaporation (Flexas et al. 2007), which reduces photosynthetic activity (Huchzermeyer and Koyro 2005) and causes an increase in leaf temperature (Orzechowska et al. 2021).

Salinization results mainly from soil in arid and semi-arid regions or from the accumulation of salt ions in irrigation water (Rasool et al. 2013). About 1/3 of the irrigated land in the world suffers from salinity (Taiz and Zeiger 2002, FAO

2011). Celery is classified as a salt-tolerant species by Francois and Maas (1994); however, its production is limited by salinity in irrigation water (Gao et al. 2023). In this study, the effects of irrigation salinity in the form of different NaCl levels on plant growth, root morphology, physiological characteristics, stomata density and size of celery were investigated.

MATERIAL AND METHODS

This study employed seedlings of the celery hybrid cultivar Balena purchased from a local seedling supplier in a greenhouse in Eskişehir, Turkey. They were transferred to pots (0.5 L) containing a total of 160 g of the mixture of peat, perlite, and vermiculite (3:1:1), and then irrigated with distilled water. The plants were grown in a growth chamber with a temperature of 20°C/10°C during the day and night, and a photoperiod of 18/6 hours with a relative humidity of 65-70%. The pots were irrigated with the same amount of distilled water to stabilize moisture content and sustained until the start of salinity applications after four days of transplantation.

Irrigation water salinities were created with NaCl of 50, 100, 150, 200, 250, and 300 mM, and their electrical conductivities were read with EC meter WTW 3.15i as 5.4, 10.5, 15.3, 20.1, 24.8, and 29.4 dS m^{-1}, respectively. Distilled water was used as a control. Each pot was weighed on alternate days to complete the deficient water by adding respective NaCl solutions. Also, liquid N-P-K (8-8-8) was applied to the plants two times, 1 and 2 weeks after the transplantation. Thirty days after the salt treatments, when visual separation between salt treatments appeared, all measurements were taken.

Measurement of morphological characteristics

To determine leaf length, weight, diameter, and fresh and dry weights, above-ground parts of the plants were separated from the roots and the measurements were performed on these leaves. Root images were taken by a camera.

Measurement of physiological characteristics

The chlorophyll content was obtained by the portable chlorophyll meter SPAD-502 (Konica Minolta Corporation, Osaka, Japan) as the SPAD index. Leaf relative water content (LRWC) was determined with the use of the following formula (Eq. 1)

$$LRWC\ (\%) = (LFW - LDW)/(LTW - LDW) \times 100$$

Where, LFW= leaf fresh weight, LDW= leaf dry weight, and LTW= turgid weight. Dry weight was determined after drying at 80°C for 24 h and turgid weight was weighted after the leaf samples soaked in distilled water in a falcon tube for 24 h in the dark at 20°C (Kaya et al. 2003).

Measurement of stomatal characteristics

The impression technique for the stomata density (the number of stomata per mm^2 leaf area) on each plant was performed. The abaxial part of 3rd leaf from the top was carefully coated with transparent nail varnish in the middle between the main veins. The stomata number per unit area was counted at 400× magnification under the light microscope (Kaya 2023).

Statistical analysis

The data were analyzed by a completely randomized design with four replicates, and differences between means were compared by the Least Significant Differences (LSD) test at a 5% level.

RESULTS AND DISCUSSION

There were significant differences between salinity levels for morphological characteristics of celery (Table 1). Leaf number, height, diameter, and fresh and dry weights were significantly decreased by increasing NaCl levels. Each increase in salinity resulted in a decrease in these characteristics. Leaf length shortened with each increase in salinity, while no significant reduction occurred at 250 and 300 mM NaCl. Celery plants grown at increasing salinity had fewer leaves, with control plants having the highest leaf number with 10.25. Similar leaf numbers were obtained at 200, 250, and 300 mM NaCl. Leaf diameter was measured using a digital caliper to evaluate leaf thickness, and a significant reduction was observed at 50 mM NaCl compared to the control. However, celery plants exhibited similar leaf diameters at NaCl levels higher than 100 mM NaCl. Celery's leaf fresh weight declined dramatically as salinity increased, reaching a maximum value of 38.8 g per plant. Even the lowest salinity level of 50 mM NaCl had a hazardous impact on fresh weight. Similarly, a 50 mM NaCl concentration caused a decrease in leaf dry weight. As reported by Munns and Tester (2008), the main effects of salinity are the reduction in biomass growth by restricting mainly water uptake and accumulation of excessive Na^+ and Cl^- in tissues. Pardossi et al. (1999a) found an enhancement in the accumulation of Na^+ and Cl^- in mature leaves of celery under increasing salinity. In the present study, a significant reduction in leaf number, length, diameter, and fresh and dry weight of celery was determined and similar findings were reported by Soliman and El-Shaieny (2014), Ashmawi (2019), and Gao et al. (2023). This result agrees with the findings of Pardossi et al. (1999b) who found that fresh and dry weights of celery reduced as NaCl was increased. Although root properties were not measured, a reduction in root growth due to increasing salinity was displayed in Figure 1.

For the celery leaf temperature, chlorophyll content, relative water content, turgor loss, and dry matter, there were significant differences between salinity levels (Table 2). The highest leaf temperature was recorded in 100 mM NaCl with 26.2 °C, while the lowest was 24.6 °C in control plants. Chlorophyll content increased as salinity rose, although no significant variations in chlorophyll content

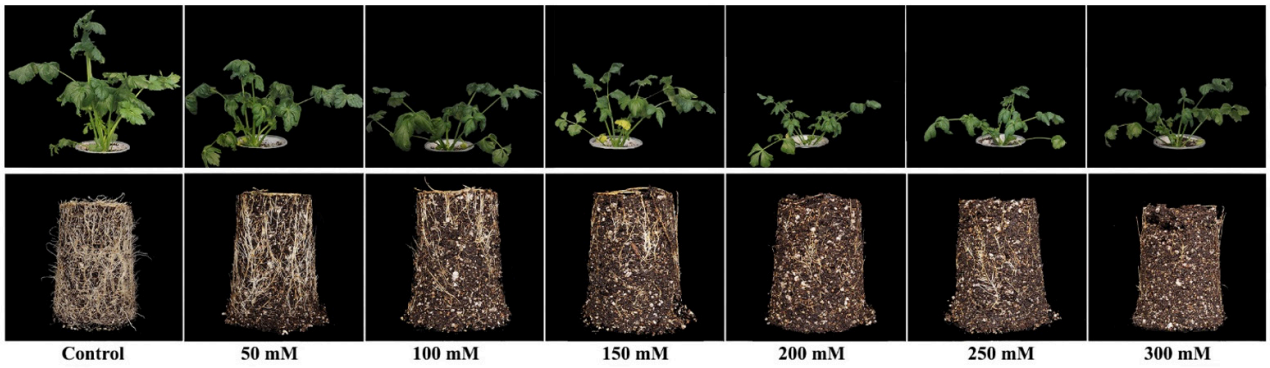

Figure 1. Visual inspection of celery roots and leaves exposed to different salinity (NaCl) levels.

Table 1. Effects of salinity on leaf length, number, diameter, and fresh and dry weights of celery.

Salinity (mM)	Leaf length (cm)	Leaf number	Leaf diameter (mm)	Leaf fresh weight (g plant^{-1})	Leaf dry weight (g plant^{-1})
Control	24.8^{a}	10.25^{a}	18.1^{a}	38.8^{a}	4.00^{a}†
50	21.0^{b}	8.00^{b}	13.8^{b}	22.2^{b}	2.45^{b}
100	19.4^{c}	7.50^{bc}	11.9^{c}	17.4^{c}	2.19^{b}
150	18.1^{cd}	6.75^{cd}	10.4^{d}	13.1^{d}	1.80^{c}
200	16.5^{de}	6.25^{de}	10.5^{d}	11.2^{de}	1.67^{cd}
250	16.1^{e}	6.00^{de}	10.3^{d}	9.2^{ef}	1.40^{d}
300	15.7^{e}	5.75^{e}	9.3^{d}	8.5^{f}	1.38^{d}
Significance	**	**	**	**	**

†: Letters connected with means in each column show significance levels at 5%. **: significant at 1%.

Table 2. Effects of salinity on leaf temperature, chlorophyll content, relative water content, turgor loss, and dry matter of celery.

Salinity (mM)	Leaf temperature (°C)	Chlorophyll content (SPAD)	Relative water content (%)	Turgor loss (%)	Dry matter (%)
Control	24.6^{d}	45.7^{c}	86.3^{a}	13.4^{e}	10.3^{f}†
50	25.5^{bc}	50.6^{b}	78.4^{b}	23.9^{d}	11.0^{d}
100	26.2^{a}	55.9^{a}	74.0^{c}	26.6^{c}	12.6^{d}
150	25.1^{c}	57.7^{a}	71.1^{d}	32.9^{b}	13.7^{c}
200	25.4^{bc}	57.0^{a}	70.9^{d}	33.3^{b}	14.8^{b}
250	25.7^{b}	58.7^{a}	70.7^{d}	34.4^{b}	15.2^{b}
300	25.4^{bc}	59.2^{a}	59.7^{e}	50.5^{a}	16.1^{a}
Significance	**	**	**	**	**

†: Letters connected with means in each column show significance levels at 5%. **: significant at 1%.

were recorded between 100 and 300 mM NaCl. Contrarily, Gao et al. (2023) determined lower chlorophyll content under salt-stressed celery plants. Increased salinity resulted in a decrease in relative water content, with the lowest value being 300 mM NaCl. Koyro et al. (2011) and Gao et al. (2023) recorded a similar trend in relative water content against salinity. There was an obvious increase in turgor loss due to salinity and the lowest salinity enhanced it two-fold. The dry matter content of leaves increased when salinity was increased. The control plants had the lowest dry matter, while the plants grown at 300 mM NaCl produced the highest dry matter.

Stomata density of celery was 90 per square millimeter (Figure 2). Stomata density was higher in the plants subjected to salt stresses than in the control; however, it reached the peak value (156 mm^{2}) at 150 mM NaCl and decreased at higher NaCl concentrations. A similar result was found by Kaya (2023) in lettuce. The size of the stomata decreased with increasing salinity, but no significant differences were observed between 100 mM and 300 mM NaCl. Salinities up to 100 mM NaCl caused an increase in stomata density and a decrease in stomata size. Increased salinity resulted in smaller size and more frequent stomata on the lower surface of celery leaves.

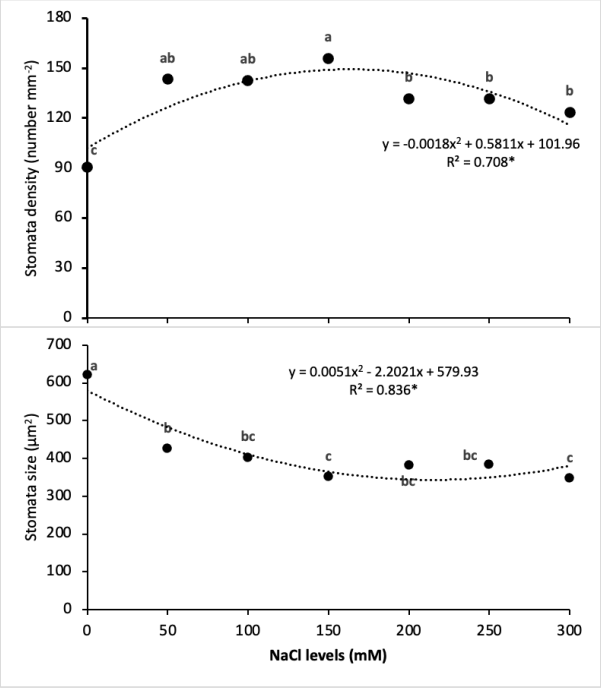

Figure 2. Stomata density (number per mm^2) and size (µm^2) of celery under different NaCl levels. Letters connected with each point show a significance level at 5%.

CONCLUSION

In the study conducted to determine the effects of salinity on celery plant growth, irrigation salinity inhibited remarkably aboveground and root growth of celery by reducing physiological activity. Smaller and denser stomata were observed under salt stress, although they were not significantly affected at NaCl levels higher than 100 mM NaCl. The results indicate that celery can be classified as moderately tolerant to irrigation salinity in the early growth stage and should be irrigated with water containing less than 100 mM NaCl.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

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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RESEARCH ARTICLE

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Novel methylation specific bisulfite primer pairs for epigenetic studies of *Capsicum* spp.

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Abstract
Over the past ten years, interest in epigenetic has rapidly increased. Heritable and stable changes in gene expression without any change in DNA sequence is in the field of epigenetics. Plants have a well-preserved epigenetic signature called DNA methylation. It is an essential epigenetic mark that protects genomic stability, silences harmful transposon insertions, and controls global gene expression in all developmental stages and environmental circumstances. All three sequence contexts, the asymmetric CpHpH context and the symmetric CpG and CpHpG contexts (where H is C, A, or T), are among DNA methylation sites in plants. Particularly, DNA cytosine methylation affects a wide range of biological processes, such as gene expression, chromatin structure, DNA packing, recombination, genomic imprinting, and DNA replication. The choice of primer pairs that flank cytosine methylation contexts is critical when designing for the detection of DNA cytosine methylation using bisulfite sequencing. We have developed and synthesized 26 bisulfite specific primer pairs suitable for DNA cytosine methylation investigations in peppers. These primers are specific to certain promoters, intergenic regions, and gene bodies (exons, introns, and UTRs). DNA samples taken from various tissues and developmental stages of *Capsicum annuum* L. Demre Sivrisi were analyzed by these primer pairs to confirm their utilization.
Keywords: Cytosine methylation, Epigenetics, Exon, Intron, Promoter

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INTRODUCTION

The study of heritable and physiological phenotypic trait variations in gene expression not regulated by alterations in the genetic code sequence of DNA is known as epigenetics. The word "epigenetics" describes the covalent modification of DNA, protein, chromatin or RNA not governed by the rules of central dogma of molecular biology. The location of DNA within the nucleus, adenine deamination at the RNA level, cytosine and adenine methylation of DNA at the sequence level, and remodeling the chromatin, which is impacted by acetylation, deacetylation, methylation, phosphorylation, SUMOylation, ubiquitination, ADPribosylation, proline isomerization, and deimination of histon and non-histon proteins, are some of the major enzyme-related epigenetic modifications (Smulders and Klerk, 2011; Karaca et al., 2016a; Araz et al., 2022; Cai et al., 2022).

DNA cytosine methylation of nucleotides is the most extensively researched epigenetic alteration in plants and is a fundamental mechanism for epigenetics in eukaryotic genomes. Nuclear DNA (nDNA) methylation is a unique characteristic of plant genomes. DNA methylation is a specific property of the plant genome that is known to control all genetic functions, including DNA replication and repair, gene transposition and transcription, cell differentiation and gene

silencing, imprinting and biodefense, and the expression of transgenes and foreign DNA in cells (Karaca et al., 2016a). It is known that methylation in nDNA can be species-specific, tissue-specific, organ-specific, and development stage-specific. Methylation of the nDNA can occur at either the adenine or cytosine nucleotide. Cytosine nucleotide is regarded as the fifth base in plant genomes and is known to be the most often methylated base (Smulders and Klerk, 2011). Numerous organisms have had their cytosine methylation investigated in relation to biotic and abiotic stressors, hormone control, cancer, bacterial host defense, embryonic and postnatal development, heterosis, imprinting and evolution (Peng and Zhang, 2009; Araz et al., 2022; Cai et al., 2022).

There are already several widely used methods for testing and identifying both global and gene-specific cytosine methylation. The gold standard for determining whole genome DNA methylation is bisulfite-mediated deamination since it can be done in large batches using massively parallel sequencing techniques and shows the methylation state of each cytosine nucleotide in a genome. The selective and total conversion of unmethylated cytosine to uracil by sodium bisulfite is exploited by bisulfite sequencing. After being chemically changed, cytosine nucleotides are amplified as thymine nucleotides using the polymerase chain reaction (PCR) (Figure 1) (Jin et al., 2013). Although using PCR has a technical benefit for bisulfite sequencing, this step is frequently the most challenging one overall. Using primer pairs that flank cytosine methylation contexts is essential for accurate bisulfite sequencing investigation of DNA cytosine methylation (Warnecke et al., 1997; Rand et al., 2006; Dhringa et al., 2014; Araz et al., 2022; Cai et al., 2022).

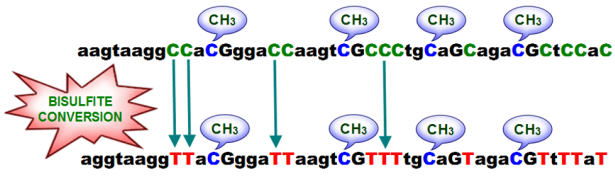

Figure 1. Alteration of Cytosine to Thymine with Bisulfite Conversion and Conseving of 5mC.

Numerous biological processes, such as chromatin structure, DNA packing, gene expression, genomic imprinting, recombination, and DNA replication are influenced by DNA cytosine methylation. In the 5′ regulatory regions of genes, methylation of CpHpG leads to transcriptional silencing. According to some research, transcribed sequences frequently have lower levels of methylations than silent gene promoters and specific coding regions. Significant variations exist in the pattern or amounts of cytosine methylation within the same tissue in different tissues or under different functional conditions. Certain degrees of DNA methylations may influence tissue-specific transcription, according to certain research. Furthermore, understanding the relationship between tissue-specific gene expression and tissue-specific methylation requires the measurement and analysis of levels and patterns of genome-wide and tissue-specific methylation in various tissues (Araz et al., 2022; Cai et al., 2022).

Epigenetic studies in pepper (*Capsicum* spp.) lags behind many other important crop species. The *Capsicum*, closely related to other members of the Solanaceae family such as potato, tomato, and tobacco, that originated in the New World, is a diploid and self-pollinating plant. Pepper is a crop of considerable economic significance that is also used as a raw material in industry (Ince et al., 2010a; Liu et al., 2017). The *Capsicum* genus contains 39 species and only *C. annuum* L., *C. baccatum*, *C. frutescence*, *C. chinense*, *C. pubescens*, and *C. assamicum* species are cultivated. Based on the presence and absence of capsaicinoid compounds, *Capsicum* species are grouped as pungent (hot/spicy) and nonpungent (sweet) pepper. The pepper fruits consist beneficial metabolites such as carotenoids (provitamin A), vitamins C and E, flavonoids, and capsaicinoids. Due to its vast variation in fruit form and the biochemical actions of unique metabolites like capsanthin and capsaicin, pepper is also a valuable model plant for fruit development (Paran and van der Knaap, 2007; Mazourek et al., 2009). Many studies were targeted toward various aspects, including the development of genetic and genomic resources for crop improvement in pepper. Recent studies on pepper genome sequencing have established a crucial basis for understanding the function of pepper genes (Kim et al., 2014, 2017; Qin et al., 2014; Hulse-Kemp et al., 2018). Additionally, the molecular mechanisms behind a number of significant pepper phenotypes have been clarified. A comparative analysis of the genomes of various *Capsicum* species revealed that the large appearance of leucine-rich domain protein (NLR) genes by retroduplication could give rise to functional nucleotide-binding (Kim et al., 2017). Up to date, methylation-sensitive amplified polymorphism (MSAP), gas chromatographic method, high-performance liquid chromatography (HPLC), and bisulfite sequencing have been used in pepper (Potris et al., 2004; Xu et al., 2015; Shams et al., 2020; Ince and Karaca, 2021; Araz et al., 2022; Cai et al., 2022).

The most popular methodology for studying DNA methylation is bisulfite sequencing, which provides details on the methylation profiles of each CpG, CpHpG, and CpHpH. Nevertheless, research on pepper's bisulfite-mediated cytosine methylation is scant. Pepper requires the construction of primer pairs to use bisulfite sequencing to determine epigenetic information, specifically cytosine methylation. In current study, primer pairs for bisulfite sequencing targeting specific gene bodies and promoters have been devised and established for several key pepper genes.

MATERIALS AND METHODS

Target DNA sequences

Pepper (*Capsicum annuum* L.) genomic DNA sequences were downloaded from NCBI GenBank databases (ftp://ftp.ncbi.nih.gov/). EpiOne software (Karaca and Ince, 2016) was utilized for mining the gene body entities such exons, introns, untranslated regions (5'-UTR and 3'-UTR), and promoters. Reversibly Knolle protein kn gene, disease-related protein-1 (PR-1) gene, capsanthin/capsorubin synthase gene, lipid transfer protein gene, pathogenesis-related protein 10 (PR10) gene, bHLH transcription factor Upa20 gene, SAR82A gene, defensin gene, SP gene, snakin (Sn) gene, hydroxycinnamoyl transferase gene, acyltransferase (Pun1) gene, and 3-oxoacyl-(acyl-carrier-protein) synthase gene were among the gene sequences examined in this study. These primer pairs are a set of 40 primer pairs derived from promoters and gene body components. Using the Primer 3 program (Untergasser et al., 2012), degenerate primer pairs were created depending on the following primary parameters: The predicted amplified product size was defined as 400–800 bp, the annealing temperature (Ta) as 58°C–62°C, and the GC content value as 40%–80%. Following design, Y (C/T) was used to replace the cytosine bases (C) in the forward primers and R (A/G) was used to replace the guanine bases (G) in the reverse primers (Ince et al., 2010b; Ince and Karaca, 2021).

Plant materials and genomic DNA extraction

Studies on the extraction of genomic DNA were conducted using mature seeds of *Capsicum annuum* cv. Demre sivrisi. Using a mortar and pestle, mature seeds were ground into a powder in order to extract DNA. The following adjustments were made to a DNA extraction process that was previously published in Karaca et al. (2005) and the modified protocol in Ince et al. (2011). Before incubating for two hours at 65°C, powdered 0.5–1.0 g tissues were mixed vigorously with a vortex using 2.48 mL of the preheated (65°C) extraction solution [0.4 mL 2 M tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl), pH 8.0, 0.4 mL 0.5 M ethylenediaminetetraacetic acid (EDTA), pH 8.0, 1.6 mL 5 M NaCl, 0.08 mL Triton-X 100], 1.42 mL 5.6% cetyltrimethylammoniumbromide (CTAB). The samples were incubated at 65°C in a water bath for 45 minutes with vortex intervals of 15 minutes. After centrifugation steps. DNA was revealed using 0.9 volume isopropanol and 0.1 volume NaCl buffer. Then, ethanol washing was performed. Finally, it was dissolved in 100 µL of TE and stored at 4°C before use. Genomic DNA's quantity, integrity, purity, and accessibility to enzymes were all verified (Ince et al., 2010b). Additionally, primer pairs screened on mature seed genomic DNA were tested from different tissue sources of pepper including pericarps and flowers before bisulfite treatment to confirm the integrity of extracted DNAs (Ince and Karaca, 2017; Ince and Karaca, 2021).

Bisulfite conversion

Using a bisulfite conversion kit from Invitrogen Corp. in Carlsbad, California, USA, genomic DNA samples of mature pepper seeds were bisulfite treated. 900 µL of ddH_2O, 50 µL of M-dissolving buffer, and 300 µL of M-dilution buffer were used to apply the C-T conversion buffer. After one minute vortexing of the C-T conversion buffer and five minutes of room temperature incubation, 130 µL of bisulfite-containing C-T conversion reagent was added to 0.5 µg of genomic DNA in 20 µL, thoroughly mixed, and centrifuged for a short while. The samples were incubated with a cycling type of conversion profile, which consisted of eight cycles of incubation at 53°C for 30 min and 37°C for 6 min after an initial denaturation at 98°C for 10 min. The GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) was used to incubate the conversion reactions for 10 minutes at 4°C. Samples of transformed DNA were purified and diluted in 22 µL of sterile water as soon as the reactions were completed (Ince and Karaca, 2017; Ince and Karaca, 2021).

Touchdown polymerase chain reactions (Td-PCRs)

A 25 µL reaction volume was used for a touchdown PCR (Td-PCR), which included a template of 3 µL bisulfite converted or control genomic DNA, 0.5 µM forward and reverse primers (Table 1), 80 mM Tris-HCl (pH 8.8), 19 mM $(NH_4)_2SO_4$, 0.009% Tween-20 (w/v), 0.28 mM of each dNTP, 3 mM MgCl2, and 1 unit of Taq DNA polymerase (Invitrogen Corp. Carlsbad, CA, USA). Using a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA), the Td-PCR amplification profile was performed as follows: initial denaturation at 94°C for 3 min, ten cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec in the first cycle, diminishing by 0.5°C each cycle, and extension reactions at 72°C for 1 min. The same cycling conditions as previously described were used for an additional 40 PCR cycles, with continuous annealing at 55°C. The conditions for denaturation and extension were the same as previously mentioned. Final extension reactions were conducted after the amplification reactions, completing reactions at 72°C for 10 minutes (Ince and Karaca, 2017; Ince and Karaca, 2021).

Purification of amplified products

PCR products were loaded onto 3% (w/v) high-resolution agarose gels (SERVA Electrophoresis GmbH, Heidelberg, Germany) with the presence of 1x DNA loading buffer. The gels were then electrophoresed at 5 V cm-1 at a constant voltage for 4-6 hours. Following electrophoresis, the PCR products were purified using a PureLink Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen Corp. Carlsbad, CA, USA) by cutting slits just ahead and behind with a clean razor blade. Samples of purified DNA were eluted in 13 µL of sterile water.

Ligation, transformation, cloning and sequencing reactions

Two microliters of 10x ligation buffer [400 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM DTT, 5 mM ATP (pH 7.8 at 25 °C)] and two microliters of 50% (w/v) polyethylene glycol 4000 were added to tubes holding 13 µL of purified PCR products. At least two hours were spent at 22°C for the ligation processes after adding 2 µL of pTZ57R/T and 1 µL of T4 DNA ligase enzyme (5 u/µL) and gently mixing them. Next, each PCR product was treated to 2.5–4 µL of ligation mixture. By using a Transform Aid Bacterial Transformation Kit (Thermo Scientific, Waltham, MA USA), vectors containing PCR products were then transformed into *E. coli* bacteria strain JM109. GeneJET Plasmid Miniprep Kit (Thermo Scientific) was used to extract plasmid DNA samples following colony selection and subculturing. The M13R sequencing primers (Macrogen Inc., Amsterdam, The Netherlands) were used to commercially sequence a total of 12 plasmids comprising PCR fragments from bisulfite treated genomic DNA and 4 plasmids containing PCR fragments from untreated DNA (Ince and Karaca, 2021).

Detection and statistical analysis of methylation

Using the software Sequencher, sequences were put together into contigs according to the contig assembly settings, which were set to a minimum overlap of 50 bases and a 90% identity match. From each clone sequence, the forward and reverse primer sequences were identified and cut off together with the vector sequences. The default KisMeth program setting, which used alignment lengths equal to or greater than 50% of the reference sequence length and alignment lengths equal to or greater than 80% positive match in the alignment, was used to analyze all data sets containing bisulfite treated sequences and the reference sequences (Ince and Karaca, 2017).

For every cytosine sequence context (CpG, CpHpG, and CpHpH), the methylation percentage values were computed using the percentage methylation (%), which was determined by dividing 100×C by (C+T). Using the nonparametric Mann-Whitney U test, values the three methylation sets of CpG, CpHpG, or CpHpH were determined and statistical significance was assessed within and between samples. The threshold of 0.05 for two-tailed P values was deemed statistically significant. The methylation percentage was utilized as the response, while the methylation context (CpG, CpHpG, or CpHpH) was employed as the factor (Ince and Karaca, 2017; Ince and Karaca, 2021).

RESULTS AND DISCUSSION

A total of 26 primers, named CA primer pairs, were chosen to amplify genomic DNA samples that had been bisulfite transformed. On genomic DNA samples, the amplified products' sizes varied from 402 bp to 1500 bp. But just 26 of the 48 primer pairs were able to amplify genomic DNA samples that had undergone bisulfite conversion (Figure 2). The amplified products had an average size of 556.7 bp per primer pair and ranged in size from 402 bp to 757 bp. As we observed, the primer pairs that amplified bands longer than 757 bp were unable to amplify genomic DNA samples that had undergone bisulfite conversion.

A total of 21 primer pairs were unsuccessful in amplifying bisulfite-converted DNA samples. This is a result of difficulties in the binding of primers during the amplification of genomic DNA that has been treated with bisulfite. One of the challenges encountered in this study was the presence of repetitive sequences in the target sequence, which is caused by the high frequency of cytosine residues. This leads to the formation of extended regions of uracil, which might potentially cause fragmentation of the DNA during the bisulfite-treatment process. It was postulated that this phenomenon was observed mostly in the aforementioned targets, particularly those exceeding a length of 757 base pairs. Additionally, it was shown that the utilization of degenerate primers, specifically generated as Y (C/T) in the sense strand and R (G/A) in the antisense strand (Teyssier et al., 2008; Gallusci et al., 2016; Xiao et al., 2020; Ince and Karaca, 2021), has the potential to enhance the efficiency of amplification reactions.

The findings of this study clearly indicated that the optimization of bisulfite conversion and PCR, as well as the selection of polymerase enzyme and buffers, exhibited lower efficacy compared to the utilization of primer pairs that specifically target the amplification process. It was observed that primers, namely reverse and forward primers, utilized for bisulfite PCR should consist of a length ranging from 24 to 29 bases. This conclusion was drawn based on our findings, which indicated that employing longer primer pairs at elevated annealing temperatures resulted in successful amplification. While

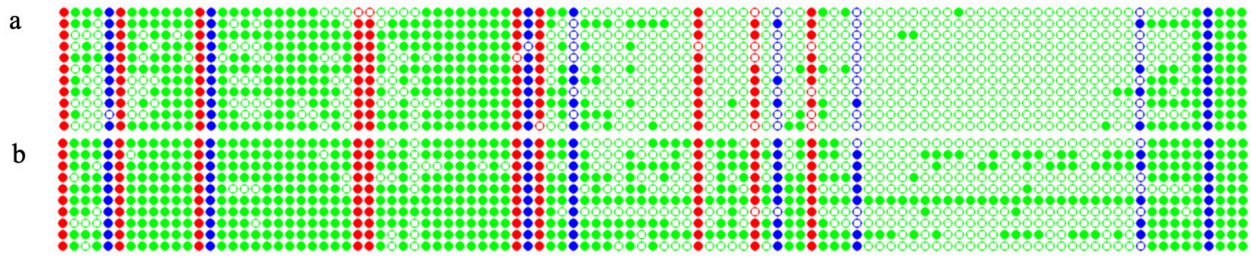

Figure 2. Some representative images for the outcomes of the bisulfite conversion of CA44 primer pairs are from the Kismeth program. Panel a: 15 (Days Post Anthesis) DPA pericarp, panel b: 60 DPA seed samples.

Table 1. Primer Pairs Suitable for DNA Methylation in Pepper.

ID	Acc. #	5′ ➔ 3′ Forward and Reverse sequences*	Genes	Region	Tar.	St./Fi.	TM
CA01	AJ276631	F: TGYTAAAYAATTAAGGGGTAATAATYA	kn gene	Promoter	1	76	60.3
		R: CAAATACATCCAAATACATATRRTACA			800	764	59.2
CA02	AJ276631	F: ATCGATYATAAGAGYAATAAAAATYAT	kn gene	Promoter	800	804	59.7
		R: GATAARCRAGAAAATCAARTAAARAA			1375	1352	58.7
CA05	DQ201633	F: GGCGTYTAGTAGTATTTTYAGTTTTT	PR-1 gene	Promoter	1	159	57.8
		R: CATARACCATAARCATAATCTACCATC			850	820	58.5
CA06	DQ201633	F: GCAGATATYYGTAYAAAAAYTTAAAAT	PR-1 gene	Promoter	850	870	59.3
		R: CATCAACTTTTCCAACTTAACAATTTC			1500	1390	60.4
CA12	DQ907615	F: TTGGYTTTATATAGYAAAAGAAAGTATT	capsanthin/ capsorubin synthase	Promoter	1	130	58.6
		R: ARRAATACTACAARRCCTCCAAC			1000	761	59.9
CA13	DQ907615	F: GGAAATTTTYATYGGGGTTYAAA	capsanthin/ capsorubin synthase	Promoter	1500	1657	60.5
		R: GAATTCTTCCAACARTTCRTTTTT			2309	2309	59.9
CA14	DQ907615	F: GTGAGTAAATAAYTTTGTTGGATGGAT	PR10 gene	Promoter	1	104	59.9
		R: GTTTGACRTTCAAATTTTCTATTTARC			750	607	59.8
CA17	DQ907615	F: CGTTTGAYGTTYAAATTTTYTATTTAG	PR10 gene	Promoter	750	893	60.5
		R: TACTAGRACCTRCTTCTTAAATRTRTT			1500	1486	60.0
CA19	AY804337	F: TAATATGAGTTGTAGTGGGATGATTGA	lipid transfer protein gene	Promoter	1100	1116	60.0
		R: ARAATRATGTTCCCTTTAATTTTTCTT			1700	1603	60.0
CA21	AY804337	F: TATATATGGATGTTTTGGGTYATAYAA	lipid transfer protein gene	Exon 2	2890	2723	59.7
		R: TTRATRATCATTCCATARAACAAATTA			2970	3190	59.9
CA22	AY804337	F: ATTAATTGTATAGYYAAAAGGAAAAYA	lipid transfer protein gene	Exon 3-Intron 3	3585	3406	59.6
		R: AGAAACAAATRRTAAAAARTAACATRC			3694	3856	60.1
CA39	EU046276	F: ATGGAATTGATATYGGAYAYTTTT	Upa20 gene	Exon 7-Intron 7	2609	2609	59.9
		R: GTACTCTTTTRRCTTCCACTRATT			3266	3266	60.0
CA44	X95730	F: TTACAYATAAGGYAYAYAAGTTTTTAG	defensin gene	Intron	2500	2506	59.9
		R: CTTGTTTCRTAAATTCACTTARACCTC			3100	3207	60.0
CA49	AY775331	F: GTGATTAAATTCTYGAGAYAAAGTAYA	SAR82A gene	3 UTR	2245	1831	59.2
		R: TTGCAARTTGARTTTACAATATRAAAR			2498	2377	60.0
CA54	AJ871130	F: CGTACAATGTTTTYGYATGTAATAAT	SP gene	Exon 4	2266	2008	60.4
		R: CTTCTGARAATTTTCRARTACARAATC			2487	2413	60.1
CA56	FJ570809	F: CCACTTATAATGTYYTGTTTYAYTTT	snakin (Sn) gene	Promoter	600	631	59.0
		R: CGGCGTATATAARTTAAATCTTCCTTT			1295	1094	60.4
CA59	EU616565	F: AAGTYYAAAGAAGATGGAAATAYAGT	hydroxycinnamoyl transferase	Exon	750	727	59.8
		R: GTCRRACTTRRCAATATAAAACTTA			1308	1226	59.8
CA60	AY819029	F: GGTCATTAGAAGGTYATAYYGCTY	acyltransferase (Pun1) gene	Promoter	1	1	58.3
		R: ATGATTRTTAAAATARTRARAATTRAAA			650	604	57.9
CA61	AY819029	F: CGTYTGAAAATTGAAATATATYTAGGG	acyltransferase (Pun1) gene	Promoter	650	624	59.7
		R: CCAAAGAARRAACCCTCCAAAATTA			1300	1207	60.7
CA62	AY819029	F: GAAAGAGAATTGGATTTYTAYATTTTT	acyltransferase (Pun1) gene	Promoter Exon 1	1300	1408	59.7
		R: AATRCAAAARCCATAATTAATTTAACA			2000	1912	60.3
CA64	AY819029	F: CTAGGYTATTTAGTYTATTTGTAGAAGYTA	acyltransferase (Pun1) gene	Intron 1	2700	2732	57.3
		R: CTTCTTATARCCATCCATATTTTCA			3400	3349	57.0
CA65	AY819029	F: GTAGTAGAATYAATGAGAGAAGGGAAA	acyltransferase (Pun1) gene	Exon 2 Intron 2	3200	3291	59.6
		R: CAAAARTATTCCTACCTTTRTTTCRTA			3752	3699	60.2
CA66	HQ229922	F: AYTGAAGAAGAAAGAATYAAGAATYAA	3-oxoacyl-synthase gene	Exon 1 Intron 1	1	41	60.1
		R: ATCAAARTATCAAATTCCCACATTTT			500	442	60.2
CA67	HQ229922	F: ACAYAAGGTAAAATTAAGGTTTGTGAG	3-oxoacyl-synthase gene	Intron 1	700	702	60.1
		R: ATAAAATCAAARAACATRRAAACAAAC			1200	1188	59.9
CA68	HQ229922	F: CAGTYTTTGGAAGTGATATYGATAAAT	3-oxoacyl-synthase gene	Exon 2 Intron 2	1300	1335	61.1
		R: ATAATATCARCTTCRCCCCTTCTAAT			2000	1953	62.5
CA70	HQ229922	F: ATTTTAAGCTAGAATGAAAATGTGTYY	3-oxoacyl-synthase gene	Intron 6 Exon 7	2700	2683	59.4
		R: GCTRTATCTTCAARAAATTATRARCTT			3456	3439	59.7

*Acc. #: accession number, Tar.: target, St./Fi.: start and finish sequences of primers, TM: melting temperature of primers

it is worth noting that a few products exceeded the length of 700 base pairs, amplicons within the range of 400 to 600 bases were found to be more suitable for transformation and cloning investigations. Based on empirical observations, it is recommended that researchers employ annealing temperature gradient spanning studies as a means to ascertain the ideal annealing temperature for primer pairs. Additionally, the utilization of a touchdown PCR profile, as proposed by Ince et al. (2010b), may prove beneficial in this regard.

The current investigation involved the selection of 26 primer pairs, as detailed in Table 1. These primer pairs were chosen based on their ability to amplify bisulfite-treated genomic DNA samples, their consistent product size between control DNA (unconverted bisulfite samples) and bisulfite converted samples, and their reliable amplification of various tissue sources of pepper, such as pericarps and flowers. In this study, a set of 26 primer pairs was employed to amplify a 14,473 base pair segment of genomic DNA from pepper plants, which includes 13 distinct genes. These genes include kn gene, pathogenesis related protein-1 (PR-1) gene, capsanthin/capsorubin synthase gene, lipid transfer protein gene, pathogenesis related protein 10 (PR10) gene, bHLH transcription factor Upa20 gene, SAR82A gene, defensin gene, SP gene, snakin (Sn) gene, hydroxycinnamoyl transferase gene, acyltransferase (Pun1) gene, and 3-oxoacyl-(acyl-carrier-protein) synthase gene (Figure 3).

The analysis conducted indicated that the pepper genomic DNA, spanning 14,473 base pairs, included a cumulative count of 2375 cytosine residues across the 13 genes encompassed within it. The dataset consisted of 264 CpGs, 335 CpHpGs, and 1926 CpHpHs, as illustrated in Figure 2. The highest and highly variable CpHpH concentrations were observed among the 26 DNA regions investigated.

Differential amounts and patterns of cytosine methylation were seen in the CpG, CpHpG, and CpHpH contexts, as determined through computational analysis using KisMeth (Gruntman et al., 2008). The CpG sites exhibited the highest levels of methylation, followed by CpHpG sites and CpHpH sites. The findings of the study revealed that genes exhibiting comparable biological functions, such as housekeeping or tissue-specific genes, as well as DNA sequences situated within similar genomic areas, such as chloroplast or mitochondrial DNA, exhibit similar levels of cytosine methylation.

The examination of 14,473 base pair DNA sequences indicated that the CpHpH contents exhibited the highest levels within the promoters, exons, and introns of the 13 genes under investigation. The cytosine methylation contents of CpHpG were found to be the second highest, while CpG contents exhibited the lowest levels among the various cytosine methylation contents that were examined (Figure 4). The disparities in CpG and CpHpG concentrations inside promoters exhibited somewhat smaller magnitudes when contrasted with those observed in introns and exons. Subsequent investigations have provided compelling evidence to support the notion that methylation of cytosine nucleotides within promoters holds greater significance when compared to methylation patterns observed in exons and introns. This finding suggests that methylation processes are significant in the control of gene expressions (Karaca et al., 2016a; Xiao et al., 2020; Cai et al., 2022; Jaiswal et al., 2022).

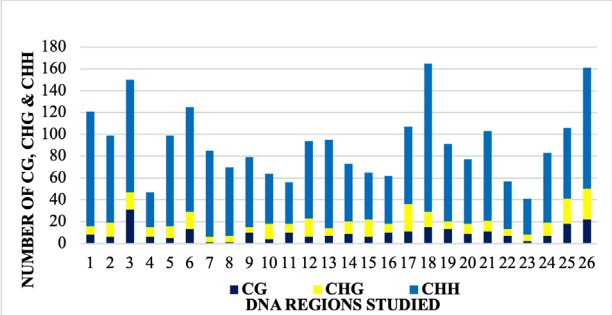

Figure 3. Numbers of CG, CHG and CHH Contents Among 26 Genomic Regions of Pepper

The current work demonstrates that there are variations in DNA cytosine methylation levels among genes, gene locations, promoters, and gene body components such as introns and exons. These findings provide unambiguous evidence of the presence of DNA cytosine methylation. The variations in CpG, CpHpG, and CpHpH contents observed in various genes and gene body entities may be associated with biological processes such as gene expression, genomic imprinting, recombination, and DNA replication. Our results are consistent with prior studies that have shown a lower level of methylation in transcribed regions compared to promoters (Teyssier et al., 2008; Gallusci et al., 2016; Karaca et al., 2016b; Xiao et al., 2020; Cai et al., 2022).

The majority of the genomic areas that were amplified using the proposed primer pairs exhibited variations in the methylation status of cytosine nucleotides. Bisulfite primer pairs have the potential to be employed for the identification and quantification of genomic DNA sequences that are unmethylated, methylated, or differentially methylated. Additionally, they can serve as internal or validation controls in high throughput investigations conducted at the genome-wide level (Xiao et al., 2020; Araz et al., 2022; Cai et al., 2022).

Epigenetic regulations arise as a consequence of differences in the degree of DNA cytosine methylation. The occurrence and variability of methylations have been associated with various genetic phenomena, such as DNA recombination, gene expression, and transposon silencing. While the epigenetic concept was experimentally discovered in pepper as the second crop, the level of DNA methylation in this crop is comparatively lower than that observed in numerous other crop species.

In this study, we presented the amplification efficacy of 26 bisulfite-specific primer pairs for both bisulfite-converted and unconverted genomic DNA samples. The primer pairs utilized in this study consisted of nucleotide sequences ranging from 23 to 30 base pairs in length. These primer pairs were shown to generate amplified products that were both singular and well-defined when subjected to elevated annealing temperatures. While certain amplified products exceeded a length of 700 base pairs, the sizes of the amplicons varied between 400 and 550 bases.

In the genes whose methylation levels were investigated in this study, differences were found in methylation levels according to gene regions. There were a total of 937 cytosine contents in the promoter regions, of which 91 CpG, 97 CpHpG and 749 CpHpH. On the other hand, there were 1270 cytosine contents in the exons, including 141 CpG, 195 CpHpG and 934 CpHpH. Introns contained 245 cytosines, 23 of which are CpG, 32 were CpHpG and 190 are CpHpH. There were only 73 total cytosine contents in the 3UTRs; of these, 9 were CpG, 11 were CpHpG, and 53 were CpHpH. Primer pairs possess the capability to not only facilitate the identification and quantification of unmethylated, methylated, and differentially methylated DNA sequences, but also serve as internal or validation controls in next generation sequencing technologies employed for DNA methylation investigations (Ince and Karaca, 2021).

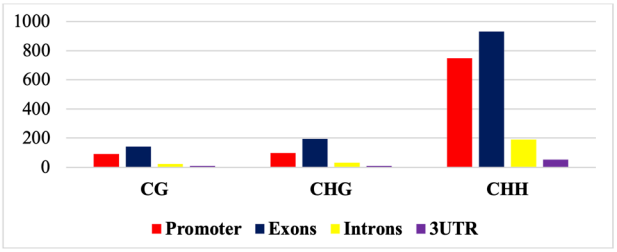

Figure 4. Number of CG, CHG and CHH Contents Presented in Promoters, Exons and Introns

CONCLUSION

The field of epigenetic research in pepper lags behind that of numerous other significant crops. In this study, it was presented 26 bisulfite specific primer pairs that demonstrate successful amplification of both bisulfite converted and unconverted DNA. Based on the empirical evidence and study outcomes, it is recommended that researchers should employ annealing temperature gradient spanning studies as a means to ascertain the best annealing temperature for primer pairs. Additionally, the utilization of a touchdown PCR profile is proposed as a viable approach in this regard. Using these suggestions one can get effective of amplifications, as can be seen by band intensity. The use of these primer pairs target cytosine nucleotides that were differently methylated. The utilization of bisulfite primer pairs may enable the acquisition of data pertaining to allele-specific methylation in pepper. The primer pairs disclosed in this paper have the potential to serve as internal or validation controls in genome-wide high throughput investigations, as they can target unmethylated, completely methylated, and differentially methylated genomic 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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RESEARCH ARTICLE

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Renovation of natural grass football fields using carpet-based hybrid method: The case of Atatürk Olympic Stadium

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Abstract
Since the playability and performance of hybrid turf pitches reflect natural grass pitch standards, they have been used in Europe for more than 20 years and have been frequently preferred in Turkey recently. Although hybrid turf systems are installed on football fields by various methods, the most popular one is the carpet-based hybrid method. These types of fields can be built from scratch or by renewing natural grass fields. When renovating natural grass fields, it should be considered that the processes and applications have a complex structure and the works should be carried out within a certain system. In this study, the renovation of the playing surface of the Atatürk Olympic Stadium, which is expected to host the UEFA Champions League Final in 2020, from natural grass to carpet-based hybrid grass has been examined. All renovation works were meticulously observed on site and the experiences gained were tried to be conveyed. In this way, it is aimed that the study will serve as a guide for future renovation work. Due to the complex nature of the renovation process, it is important to have the necessary knowledge when carrying out these operations. Since the renovation of these areas is expensive, it is important to ensure the necessary conditions are provided. The correct materials, essential technical equipment, and skilled personnel must be provided for the operations to be carried out, and measures must be taken to prevent any potential problems that may arise during the renovation phase.
Keywords: Atatürk Olympic Stadium, Football pitches, Hybrid grass, Renovation

INTRODUCTION

Football, which is extremely popular in Turkey, is the most popular sport worldwide (Hocaoglu & Bingol, 2022). It is played on every continent, in every country, and on many different levels (IFAB, 2021). Compliance of the football field surface with standards is key for both football players and spectators. The playing surfaces must comply with these standards in terms of functionality and broadcasting corporation visuals (Bingol, 2004).

The surface of football fields on which competitions are held can be completely natural or composed of a combination of natural and artificial materials (also known as the hybrid system), which is allowed for competitions; in some cases, the surface is allowed to be completely artificial (IFAB, 2021; Hocaoglu & Bingol, 2022).

According to the International Football Association Board's (IFAB) Dictionary of Football Terms (2018), the hybrid system is defined as 'a combination of artificial and natural materials to create a playing surface that requires sunlight, water,

air circulation and mowing' (IFAB, 2018). In the Union of European Football Associations's (UEFA) Field Quality Guide (2018), hybrid systems are mentioned in the 'Turf Support/Reinforcement Systems' section (UEFA, 2018), where they are described as 'attempts to combine the benefits of natural grass in terms of playing quality with the practical reinforcing and engineering advantages of artificial materials' (UEFA, 2018). In summary, a hybrid turf is a product composed of both natural and artificial turf that strengthens natural turf, increases its durability, and ensures its green appearance throughout the year (Hocaoglu & Bingol, 2022).

Hybrid grasses can be prepared via different methods, including the carpet-based hybrid method. This method can be applied in a new field facility as well as in an existing field after the soil surface is removed and subsoil is prepared and levelled. In addition, the carpet-based method can be used on sod farms, where thick and long rolls of grass carpets can be placed in the field (lay and play) (Hocaoglu & Bingol, 2022). Polypropylene synthetic material is placed on a specific backing material that is partially biodegradable and developed to ensure the deep and rooted growth of natural grass via knitting or weaving. This backing is in the base cloth (pad/cotton fibre/polypropylene) and appears in the form of a grid. The grid material and the base cloth are knitted together. Furthermore, this backing allows an easy and high-quality installation. Biodegradable fibres completely degrade within two months, thus creating a matrix of large uniform spaces through which plant roots can grow at locations where moisture and nutrients flow (Hocaoglu & Bingol, 2022; *Hybrid Turf System*, 2022).

Carpet-based hybrid systems offer several benefits. By providing permeability and oxygen flow to the surface layer, it provides the optimum conditions for natural grass and creates an ideal air–water soil balance. This increases the reliability of the field in terms of evenness and stability in areas with significant game traffic; furthermore, owing to the strong grass structure afforded, deep gaps do not occur. The carpet base, which serves as a geotextile, prevents the formation of deep crampon divots during undesirable weather conditions and heavy use, provides rapid regeneration in the lower root zone layer, and protects the grass root system. Furthermore, it improves the aesthetics of the field with its green appearance of synthetic fibers (Hocaoglu & Bingol, 2022).

The transformation of the Atatürk Olympic Stadium from a natural grass field to a carpet-based hybrid system is discussed and analyzed herein. This study may serve as a reference for similar renovations in the future.

MATERIALS AND METHODS

The main focus of this study is the 2019 renovation of the playing field at the Atatürk Olympic Stadium. The construction of this stadium began in 1998 and was completed in 2002, with natural grass used for the match field. The stadium was scheduled to host the UEFA Champions League Final in 2020, and renovation began before the event. During the renovation, the existing athletics track was removed, elevations were rearranged, and the natural grass field was converted to a hybrid carpet-based system. However, despite these renovation efforts, the UEFA decided to switch the competition venue from Istanbul to Lisbon because of the COVID-19 pandemic.

Material

The Atatürk Olympic Stadium, which is the area investigated in this study, is located in the Başakşehir District of Istanbul, Turkey (Figure 1). The facility, named after the country's founder Mustafa Kemal Atatürk, is the largest stadium in Turkey in terms. The stadium was built for Turkish athletes and football as preparation for the Olympic Games (*Atatürk Olimpiyat Stadyumu*, 2022).

The Atatürk Olympic Stadium, which opened on 31 July 2002 with a match between Galatasaray and Olympiacos attended by 77,087 people, is a stadium that satisfies the requirements of the International Association of Athletics Federations (IAAF), the Fédération Internationale de Football Association (FIFA), and the International Olympic Committee (IOC) for international football and world athletics championships.

The infrastructure of the stadium not only facilitates sports, social, and cultural activities, but also enables the training of athletes, coaches, and trainers. The Atatürk Olympic Stadium comprises two illuminated training

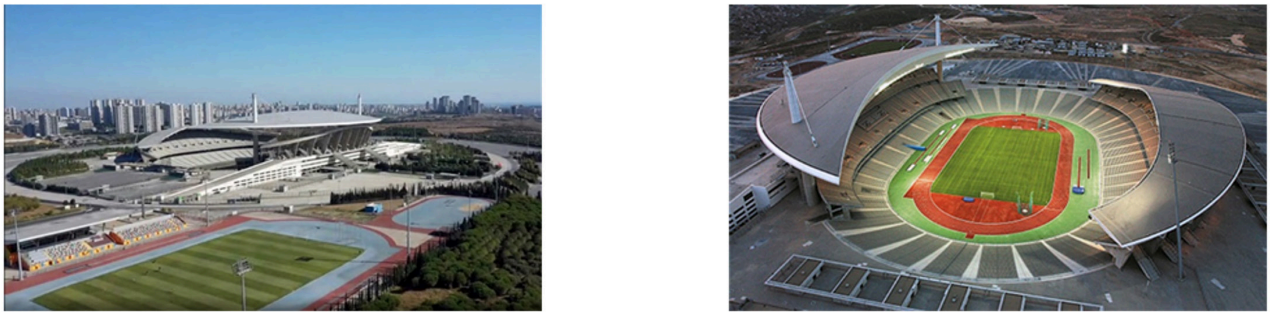

Figure 1. The Atatürk Olympic Stadium general appearance (*Atatürk Olimpiyat Stadı*, 2022; *Istanbul Atatürk Olimpiyat Stadyumu,* 2022)

and athletic fields (*Stadyum*, 2022) and is one of the few stadiums worldwide that has hosted large organizations (*Atatürk Olimpiyat Stadyumu*, 2022).

Method

During the renovation of the Atatürk Olympic Stadium, the athletics track was removed and replaced with tarmac, and the levels on the sides of the field were reduced by 90 centimeter. Additionally, the natural grass surface of the Atatürk Olympic Stadium was replaced with a carpet-based hybrid system. For a carpet-based hybrid system, a coverage distribution of 95% natural grass and 5% synthetic fibre is desired. The hybrid turf to be used depends on the carpet weight or filament density. Hatko Hybridgrass 55DS10, which contained 66,000 filaments per square meter, was used in the Atatürk Olympic Stadium. The material comprises a patented base cloth with more than 50% gap, thus allowing it to pass through high-quality OMEGA fibres after the germination of the seed's roots.

During the transformation to the carpet-based hybrid system, 10 centimeter of stripping (on average) was performed on the existing natural grass surface area to comply with the specifications, and the plant growth medium was renewed. After installing the carpet-based hybrid system, the filling was laid. The renovation was completed after the sowing of grass seeds.

On-site observations were performed during the renovation. The carpet-based hybrid system was examined based on six main criteria, and the renovation process is explained comprehensively herein. This study may serve as a reference for similar renovations in the future. The data and findings obtained during the renovation process were evaluated to provide suggestions for future renovations.

RESULTS AND DISCUSSION

The ground renovations completed at the Atatürk Olympic Stadium were as follows:

- Stripping of the athletics track and adjustment of the levels
- Preparation of the lower root zone of a carpet-based hybrid system
- Installation of a carpet-based 'hybrid carpet'
- Infilling of the upper root zone
- Sowing of grass seed
- Finishing works

Stripping of the athletics track and adjustment of the levels

According to the criterion stipulated by the UEFA, a stadium hosting the Champions League Final must have a seating capacity of 70,000 people with a full view of the stadium. Because of this requirement, the athletic track of the Atatürk Olympic Stadium was removed and then paved with asphalt during the renovation, and the elevation on the sides of the pitch was reduced by 90 centimeter to improve the view from seats with an unsatisfactory view (Figure 2).

Consequently, the seating capacity was 74,753, which satisfies the UEFA criteria. Furthermore, 250 of these seats were reserved for disabled spectators (*Atatürk Olimpiyat Stadı, Avrupa'yı kucaklamaya hazır*, 2020).

Preparation of the lower root zone of a carpet-based hybrid

In the field renovation, the existing natural grass surface was first removed. Fraise mowing was performed on the ground using a dedicated machinery to strip the natural grass surface (Figure 3). The existing plants and root zones were completely removed from the surface.

Subsequently, the existing infrastructure system in the field was inspected for any damage or deficiencies, which are to be eliminated. During this inspection, no renovations were performed on the infrastructure. A new root zone was established using a top dressing. At this stage, the ground was prepared for the carpet-based hybrid system by compacting and readjusting the ground levels (Figure 4). Subsequently, the levels were reverified to prevent undulation.

During the levelling, the undulating values in the longitudinal and lateral directions between 3 meter straight edge beams with 3 meter intervals were targeted to be less than 10 millimeter. Soil stability was measured using a penetrometer that can measure more than 1.5

Figure 2. Removing the athletic track and paving with asphalt (*Atatürk Olimpiyat Stadı'nın son hali*, 2020; *İstanbul Atatürk Olimpiyat Stadı Yenileme Projesi*, 2019)

Figure 3. Removing The Existing Natural Grass Surface (*Hatko Sport*, 2019)

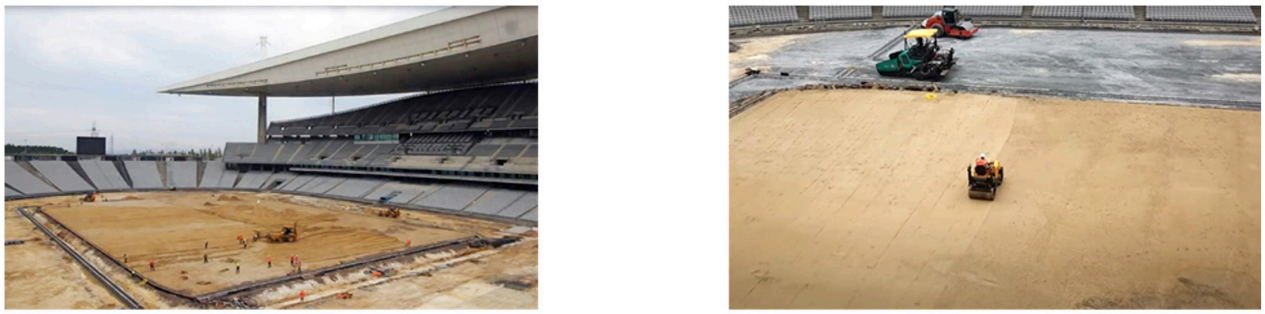

Figure 4. Establishment of the new root zone (*Hatko Sport*, 2019)

Figure 5. The process of laying the carpet-based 'hybrid carpet'

N/mm^2 (cone size = 1 cm^2). The irrigation springs in the infrastructure system were adjusted to 20 millimeter above the level of the completed lower root zone.

Installation of carpet-based 'hybrid carpet'

Before installing a carpet-based 'hybrid carpet,' the ground where the hybrid grass with a carpet base was laid, in which the lower root zone was prepared, was re-inspected for inaccurate field levels. During the laying process, 4 meter carpet-based hybrid grass rolls were laid on a field using a pedestrian-type self-propelled equipment.

Additionally, the corner points of the carpet-based hybrid area were determined. In the area where the carpet was to be laid, a stake was placed at each corner, which resulted in an excess of 15 centimeter on all edges, and a yellow or white string line was placed between each stake. The first roll was laid by moving it to one side such that it coincided with the string line marking the outer edge of the carpet at +15 centimeter. The hybrid carpet was laid meticulously such that it was stretched in both longitudinal and lateral directions.

The rolls were folded from 40 centimeter on the side where the first roll (and each subsequent roll) was installed and cut cleanly from the excess backing until the first tuft line without requiring the stitching of artificial fibres. This strip of clear backing served as the carrier tape for the carpet adhesive. A second roll was unrolled, and the carpet was positioned manually such that the outer tuft line was shielded by the previously cleared adhesive carrier strip (Figure 5).

The carpet was manually folded from 40 centimeter on the side where the first roll (and each following roll) was installed and cut cleanly from the excess backing until the first tuft line without requiring the stitching of artificial fibres. This strip of clear backing served as the carrier tape for the carpet adhesive.

A PU carpet adhesive was prepared for bonding the rolls. The adhesive, which was mixed slowly in a tube,

Figure 6. a) Application of the adhesive b) Bonding the rolls

was transferred to a glue gun for use (Figure 6a). The adhesive was applied to the carrier strip using a glue gun. The viscosity of an adhesive depends on the environmental temperature; therefore, an appropriate amount of adhesive must be used. Depending on the weather conditions, the adhesive and its carrier edge strip may require water spray to improve the hardening of the adhesive.

The adhesive-applied carpet was carefully unrolled and transferred onto the adhesive such that the fibre-woven strips matched perfectly with the other carpet (Figure 6b). After sealing the seam, rolling was performed using a specific hybrid turf carpet roller (HCR).

The timing and intensity of the rolling process of the adhesive-applied joints of the hybrid carpet were adjusted based on the weather conditions. After the carpets were adhered together, the adhesive was inspected every 10 minute by slightly lifting the edge of the upper carpet. After ensuring that the upper carpet did not adhere to the carrier strip, the rolling process was commenced. The seam was rolled twice at a moderate speed while ensuring that no wrinkles were visible in front of the HCR.

A sufficient hardening duration (6–12 h) was allowed before the carpet was infilled. The joints were re-inspected, and any loose joints detected were repaired using HATKO repair glue. These steps were repeated until the entire area was shielded with a carpet-based hybrid turf. A total of 5 day was required to lay the carpet-based hybrid system.

The root zone was filled after the bonding process was completed and the entire hybrid carpet was transformed into a single piece. However, a preliminary preparation was performed to ensure that the carpet and alignment would not slip and that the surface would not wrinkle/fold/undulate during the filling process. Based on the outer perimeter of the carpet-laid area, the hybrid grass carpet was folded back by 1 meter. A shallow channel (100 mm wide and 150 mm deep) was created on one goal side (short side) and one longitudinal side (long side) of the pitch. A trench was created along the inside of the string line, and the excavated material was uniformly preserved outside the string line. The folded carpet was opened, placed in the duct, and backfilled next to the trench using the preserved material. The trench was compacted using a tractor tire (a tractor with a turf-type tire), and the soil was relevelled without allowing undulations and/or damage to the elevations.

Infilling of the root zone

The preparation for the upper root zone involved filling the football field with carpet-based hybrid turf rolls. Prior to performing the topdressing process, the material to be used was stored on a clean surface to ensure dryness.

During the renovation of the field, a time-efficient spinner-type top-dressed equipment with an adjustable spreading width (3.5–13 m) was used to perform infilling (Figure 7a). A load weight not exceeding 1,500 kilogram was used to avoid damage to the field surface during topdressing. In addition, tractors, which were used to protect the surface, were equipped with weight-distributing turf-type tires. In the backfilling process, sand was dispersed on the field in layers with a maximum thickness of 6 millimeter.

Figure 7. (a) Topdressing process (b) Brushing and levelling with drag-mat

Figure 8. (a) Raking the field (b) Pumice application

Any loose material remaining on the surface after topdressing with the sand was cleaned by brushing, and the distribution of sand on the surface was ensured to be homogeneous using a levelling mat (Figure 7b). Consequently, the synthetic fibres in the hybrid system remained upright, and the buried fibres reached the surface. During this process, the longitudinal fibres of the carpet were brushed in the opposite direction. These processes were repeated until the filling reached a loose depth of 35 millimeter (32 mm compressed).

Additionally, the irrigation is to be considered during the laying and filling processes. To avoid the loss of springs in the irrigation system, a mark was created on the carpet at a distance of approximately 100 millimeter from the centre of the springs, and the hybrid grass carpet was folded down to reveal the springs. These points were marked with a flag and inspected by an irrigation manager to ensure that no springs were overlooked.

One of the main concerns pertaining to hybrid-system football fields is the formation of organic materials. Organic materials typically grow on the fibres, causing the latter to be embedded below the surface. After performing scanning, all organic residue accumulated on the field surface was cleaned, and synthetic fibres embedded in the organic layer were removed and rendered vertical (Bingol & Hocaoglu, 2022).

During the renovation, the field was groomed using spring-loaded rakes. The field was regularly raked up to the root zone surface. Consequently, the formation of organic materials was prevented, and any embedded synthetic fibres were removed and rendered vertical (Figure 8a).

During topdressing with sand, pumice was added to the mixture and applied to the area to maintain the strengthened ground and to improve its water-retention ability (Figure 8b).

Sowing of the turf seed

Football fields with hybrid systems are sowed the most effectively using 'dimple seeder' equipment with wide castings. Because field seeding can cut or destroy synthetic fibres, cutter/disc seeding equipment should not be used to prolong the life and efficiency of the fibres (Bingol & Hocaoglu, 2022).

Hence, during the renovation, a poking cylinder-type seeding (dimple seeder) equipment was used to sow grass seeds in the field (Figure 9). For seed planting, a grass seed mixture comprising 80% Lollium prenne and 20% Poa pratensis is desirable for the regional climate. A 22+05+06+2Mgo+Te fertilizer was used during seed sowing. Irrigation was initiated after seeding.

Finishing works

Finishing works were performed to develop the playing field, which resulted in form and maintenance phases. A pedestrian-type cylinder mowing equipment was used for mowing (Figure 10a). This is because heavy mowing equipment causes the fibres in the hybrid system to break and fold flat.

Performing daily mowing using a pedestrian-type cylinder mowing equipment weighing less than 200 kilogram reduces the risk of fibres folding in the hybrid system (Bingol & Hocaoglu, 2022). The preferred mowing height in the football field was 25–30 millimeter. To ease

Figure 9. Sowing of the turf seed


Figure 10. (a) Mowing the grass (b) Final appearance of the field (*Atatürk Olimpiyat Stadı'nın son hali*, 2020)

the management of the football game and create an aesthetically pleasing stadium, shaping was performed in opposite directions to form parallel patterns (Bingol & Hocaoglu, 2022).

Maintenance practices were commenced after the renovation was completed. An image of the completed stadium is shown in Figure 10b.

CONCLUSIONS

Reinforced turf establishment methods (hybrid turf) are typically preferred for the construction of football fields in Turkey, similar to the worldwide preference. The pitch required can be created or easily obtained by renewing natural grass-surfaced pitches. Herein, the renovation processes performed on the grounds of the Atatürk Olympic Stadium, which was expected to host the UEFA Champions League Final in 2020, was discussed. The most important processes were the removal of the existing natural grass surface and the application of the carpet-based hybrid system to the area.

The following aspects are noteworthy during the processes:

- The stripping process must be performed by experts, and the infrastructure system in the area should not be damaged.
- After top dressing with sand, field elevations should be inspected, and uneven levels should be prevented.
- Carpet-based hybrid grass rolls must be laid meticulously to ensure that they are tight and taut.
- The bonding of the rolls should be performed meticulously, and any loose joints must be identified and tightened.
- After laying the rolls, sandblasting should be performed using an appropriate distribution of sand.
- The material remaining on the surface should be cleaned by brushing, and a homogeneous distribution of sand should be ensured using a drag mat.
- During the laying process, the irrigation system should be monitored, and spring loss must be prevented by adopting the necessary precautions.
- Embedded synthetic fibres should be removed via raking and rendered vertical.
- Cylinder-type seeding (dimple seeder) equipment should be used for sowing grass seeds, and grass seed species suitable for the regional climate should be selected for seeding.
- Pedestrian-type rotary mowers or cylinder mowers are preferred for mowing.

Because carpet-based hybrid turf pitches exhibit a more complex structure than natural grass pitches, the renovation processes must be performed within a certain retrospective control system. An appropriate number of personnel must be trained to renew natural grass surface areas using a carpet-based hybrid system. Necessary equipment should be provided to ensure a well-functioning and problem-free field. The appropriate regeneration processes will contribute to the sustainability of the field, visual aesthetics, game quality, and health conditions of athletes.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, 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.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

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RESEARCH ARTICLE

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The nutritional composition of key apricot varieties cultivated in Türkiye with a focus on health-related compounds

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Abstract
Türkiye's favorable geographical location and diverse climatic conditions provide it an ideal host for a wide range of fruit species. In this content, apricots have garnered significant global attention. In this study, local varieties such as Hasanbey, Hacıhaliloğlu, Kabaaşı, and Şalak were examined, alongside foreign varieties commonly cultivated in coastal regions, specifically Mikado and Mogador. It was found that domestic varieties are notably different from foreign varieties in terms of their health-related components. Fruits from various varieties sourced from significant apricot-producing provinces were investigated. The study revealed a range in total carotenoid content from 5.59 to 10.3 mg/kg, antioxidant activity spanning 478.5 to 1969 mgTE/kg, and total phenol content fluctuating between 122 and 771 mgTE/kg. The 'Şalak' distinguishes itself significantly from others due to its elevated phenolic content, leading to a correspondingly higher level of antioxidant activity. In conclusion, it has been observed that the antioxidant content in this variety is significantly higher (approximately 4 times higher). The study results unequivocally demonstrate that, beyond the significance of cultivation location, genotype plays a pivotal role as an essential determinant in relation to the evaluated quality features.
Keywords: Antioxidant activity, Apricot, Fruit quality, Phenolic content, Total carotenoid

INTRODUCTION

Based on 2021 data, global apricot production amounted to 3,578,412 tons. Türkiye contributed significantly to this total, producing 800,000 tons, which accounts for nearly 22% of the world's apricot production (FAOSTAT, 2023).

Incorporating fruits into one's diet has been related to a decreased risk of cancer and cardiovascular, stroke disease (Ness and Powles, 1997; Block et al., 1992; Gazino et al., 2010). The myriad phenolic compounds present in apricot fruits are widely acknowledged for their advantageous impact on human health, attributed to their antioxidative, anti-inflammatory features, and immune system-enhancing capabilities (Madrau et al., 2009). Antioxidant capacity and concentrations of individual antioxidant components in fruits are influenced by various factors (Papp et al., 2010). Multiple researchers have documented these factors, encompassing geographical origin, ripening period, and the duration of the fruit development stage (Dragovic-Uzelac et al., 2007; Leccese et al., 2008; Drogoudi et al., 2008). In this highly diverse species, the genotypic structure plays a pivotal role in determining fruit quality parameters. In Türkiye, the selection of genotypes for apricot cultivation is influenced by climatic circumstances. High chilling-requiring genotypes are aptly suited for high-altitude regions, while low chilling-requiring genotypes flourish in the Mediterranean basin, characterized

by lower altitudes. Concurrently, indigenous varieties have traditionally been the preference for producing dried apricots, whereas foreign cultivars have been favored for fresh consumption.

In this comprehensive study, a range of widely cultivated apricot varieties, namely Hasanbey, Hacıhaliloğlu, Kabaaşı, Şalak, Magador, and Mikado, were selected based on their prevalence in specific provinces, namely Mersin, Hatay, Elazığ, Malatya, and Iğdır. These provinces were selected due to Türkiye's global leadership in apricot production. The study focused on assessing health-related compounds, namely total carotenoid content, total phenolic content and antioxidant activity.

MATERIALS AND METHODS

Materials

In this study, the materials under investigation comprised apricot varieties that are extensively cultivated in various regions of Türkiye. These included the Hacihaliloğlu, Kabaaşı and Hasanbey varieties, which are commonly grown in Malatya and Elazığ. Additionally, fruits from the Mikado and Mogador varieties sourced from the Hatay and Mersin (Mut) provinces, as well as apricots from Şalak trees in the Iğdır province, were also included in the study (Fig 1).

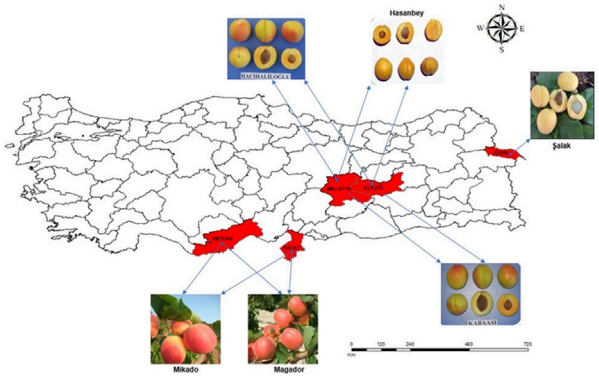

Figure 1. Information on varieties and provinces on the Türkiye map

Methods

In the study, apricot fruits harvested from different provinces during the 2020 growing season were utilized. Two kilograms of fruit from each variety were harvested from trees cultivated under typical farmer conditions and this collection was meticulously timed to align with the appropriate commercial harvest periods. These freshly harvested fruits were expedited to Çukurova University using a cold chain system to maintain their freshness. Upon arrival at the university, the fruit samples underwent biochemical analyses, which were conducted by the Food Engineering Departments. The fruit samples intended for biochemical analysis were stored at -80°C to preserve their integrity until testing.

Biochemical Analysis

Antioxidant Activity (AA) Analysis: In the analysis of AA, Klimczak et al. (2007) made some modifications to the spectrophotometric method proposed by the apricot extract. 5 mL of 80% methanol was added to 5 g of apricots and then centrifugation was performed (4 °C, 6000 rpm for 10 min). In this absorbance analysis, 100 µL of the extract was utilized, and 3000 µL of 1,1-diphenyl-2-picrylhydrazyl (DPPH*; 0.05 g/L in 80% methanol) was introduced. Subsequently, the samples were thoroughly mixed and allowed to incubate in the dark for one hour to achieve reaction equilibrium. Upon completion of this period, the absorbance of the samples was measured against an 80% methanol solution using a spectrophotometer (Perkin Elmer Lambda 25 UV/VIS, Massachusetts, USA, 2005) set to a wavelength of 515 nm. The AA values of the samples were expressed as % inhibition of DPPH* using the following equation.

$$AA\ (\%) = \frac{A_K - A_{\ddot{O}}}{A_K} x100$$

AA (%)=(A_K- A_Ö)/A_K x100

AK: The absorbance value of the control

AÖ: The absorbance value of the sample

After determining the inhibition percentages of DPPH*, the DPPH* inhibition percentages of the samples were converted to equivalent gallic acid values using the gallic acid equivalent graph (50-1000 mg/L) created by the same method.

Total Phenolic Content Analysis

Following the crushing of the apricots in a high-speed shredder, 5 g of the resulting mixture was transferred to a 50 mL centrifuge tube, and 45 mL of an 80% MeOH solution was added. Subsequently, the centrifuge tube was vortex-mixed for 30 seconds before initiating centrifugation (6000 rpm, 4 °C, 10 minutes). Following this procedure, the main stock extract for the samples was obtained. Subsequently, 100 µL was drawn from the clear portion, and 200 µL of Folin-Ciocalteau reagent, along with 3000 µL of distilled water, were added, left to stand for 10 minutes. Upon completion of the designated period, 100 µL of 20% Na2CO3 was introduced into the solution, which was then placed in a dark environment for 2 hours. Subsequently, the solution was read against the blank at a wavelength of 765 nm using the Perkin Elmer Lambda 25 UV/VIS spectrophotometer (Massachusetts, USA, 2005). The quantity of phenolic compounds, corresponding to the measured absorbance value in gallic acid within the samples, was determined using the standard curve equation prepared with gallic acid. The total amount of phenolic substances in the samples was expressed as "mg gallic acid/kg." (Abdulkasim et al., 2007).

Total Carotenoid Analysis

The total carotenoid content of apricot samples was assessed using the method developed by Lee et al. (2001). Five grams of apricot pulp was transferred to a Teflon tube, and 10 mL of the extraction solution (hexane: acetone: methanol/50:25:25, with 0.1% BHT content) was added. Subsequent to this procedure, centrifugation (4000 rpm, 10 min, 4 °C) commenced immediately after the application of mixing. Following centrifugation, absorbance was promptly measured at 450 nm without delay. The total carotenoid content is expressed in terms of β-carotene, and the extinction coefficient (E1/2) was considered as 2505 in the calculation.

$$Total\ carotenoid\ \left(\frac{mg}{100\ g}\right) = \frac{Absorbans * DF}{E^{\frac{1}{2}}} * 1000$$

DF: Dilution Factor $E^{1/2}$= Extinction Coefficient (2505)

Statistical Analysis

Fruit quality parameters were analyzed with three replications, each comprising 30 fruits. The data obtained at the conclusion of the study underwent analysis of variance using the randomized plot design in the JMP statistical package program. Subsequently, averages were compared using the LSD test at significance thresholds of 5%, 1%, and 0.1%.

RESULTS AND DISCUSSION

In this study, the health-related compound contents of select apricot cultivars were investigated. The cultivars were sourced from the top five provinces, renowned for intensive and extensive apricot cultivation. (Fig 1).

Some important health components of apricot varieties harvested from different provinces

Today's consumers are interested not only in the eaten quality of fruits but also in their health composition and levels of these components. In this context, values for total carotenoid content, antioxidant activity and total phenol content are presented in Table 1. In varieties harvested from different provinces, statistically significant differences have been identified in terms of these compounds. In our investigation, the total carotenoid content exhibited a range from 5.59 to 10.3 mg/kg. Notably, the Magador, harvested in Mersin, demonstrated the highest carotenoid content at 10.3 mg/kg. Following this, within the same statistical group, the Hacıhaliloğlu fruit obtained from Malatya province exhibited a carotenoid content of 9.39 mg/kg, while the Hasanbey and Kabaaşı varieties grown in Elazığ province had carotenoid contents of 9.51 mg/kg. The Hasanbey, cultivated in Malatya, displayed the lowest carotenoid content at 5.59 mg/kg. It is evident that the carotenoid levels in varieties, excluding Mikado, are notably impacted by the prevailing cultivation conditions. In this regard, it is necessary to select an appropriate location based on the variety for high carotenoid content. It has been observed that during the ripening of apricots, there is a significant increase in all carotenoid compounds, with a notable surge in β-carotene levels. In commercially ripe fruits, β-carotene levels can escalate up to 10 times compared to their unripe counterparts (Dragovic-Uzelac et al., 2007). In the same study, it was determined that apricots generally cultivated in the Mediterranean region (Neretva Valley) have higher levels of all carotenoids compared to those grown in the continental region (Baranja). As observed in this study as well, it has been partially confirmed that cultivation conditions and the ripeness level of fruits are the key factors determining carotenoid content. In the Şalak variety, where inter-provincial comparison is not possible, the carotenoid content has been determined to be 7.16 mg/kg. In addition to this, it has been determined that apricots with orange-colored flesh have higher carotenoid content compared to apricots with white flesh (Ruiz et al., 2005). Researchers have found carotenoid levels ranging from 1512 to 16500 µg 100 g-1 in the edible parts of 37 apricot varieties in their studies. Rodriguez-Amay (2010), has been reported that these variations in carotenoid levels are associated with genetic, environmental and agronomic factors.

Fruits are rich sources of plant-based nutrients that contribute positively to our health, including phytonutrients, antioxidants, and various flavonoids such as anthocyanins, flavonols, and polyphenols, as well as proanthocyanidins (Reed, 2002; Sun et al., 2002). The antioxidant capacity of fruits, or the levels of individual antioxidants, is influenced by diverse factors such as variety, ripeness stage (Hegedüs et al., 2010), geographic region, fruit position on the tree (Dragovic-Uzelac et al., 2007), storage conditions, harvest year (Leccese et al., 2012; Hegedüs et al., 2010), and the fruit growing period (Leccese et al., 2008).

In the major apricot production centers of Türkiye, the antioxidant activity values in the cultivated varieties have shown differences of up to fourfold, ranging from 476.9 to 1969.7 mgTE/kg. In this sense, the Şalak stands out significantly with an antioxidant activity value of 1969.7 mgTE/kg, being notably different from others. The Hasanbey, harvested in the Elazığ province and belonging to a different statistical group, was observed to have an antioxidant activity value of 1042.3 mgTE/kg. When comparing local varieties within themselves, it has been found that the antioxidant value of apricots grown in Elazığ province is significantly higher than those grown in Malatya. On the other hand, in foreign-origin varieties, apricots harvested in Hatay province have a higher antioxidant content than others. The lowest antioxidant activity was found in the Hacıhaliloğlu, cultivated in Malatya as 476.9 mgTE/kg.

Similarly, Su et al. (2020), local apricot varieties showed significant differences in antioxidant activity, ranging

Table 1. The compound contents related to health in apricot varieties harvested from various provinces of Türkiye.

Province	Varieties	Total Carotenoid (mg/kg)	Antioxidant Activity (mgTE/kg)	Total Phenolic (mgTE/kg)
Elâzığ	Hasanbey	9.51 a[1]	1042.3 b	243.0 bc
	Kabaaşı	9.51 a	581.1 f	122.2 g
	Hacıhaliloğlu	7.44 b	612.9 ef	173.8 ef
Malatya	Hasanbey	5.59 b	705.1 d	222.5 cd
	Kabaaşı	7.44 b	478.5 g	159.8 fg
	Hacıhaliloğlu	9.39 a	476.9 g	188.3 def
Hatay	Mikado	6.19 b	981.8 b	280.7 b
	Magador	6.57 b	785.4 c	194.5 def
Mersin	Mikado	6.61 b	681.7 de	210.5 cde
	Magador	10.3 a	668.5 de	180.8 def
Iğdır	Şalak	7.16 b	1969.7 a	771.6 a
LSD		1.91***[2]	78.3***	45.9***

(1): Differences between means are shown by separate letters

(2): ***: $p<0.001$

from 61.72 to 135.52 mg TEs 100 g^{-1}. This genotypic variation in antioxidant activity has been reported in numerous previous studies (Alajil et al., 2021; Karatas et al., 2021; Karatas, 2022). Moreover, Leccese et al. (2008) identified substantial variations in total phenol content and antioxidant capacity parameters associated with ripening time. Additionally, Bartolini et al. (2014) conducted a study investigating the impact of grafting the 'Pisana' variety onto two commercial Prunus rootstocks ('Apricot Seedling' and 'Myrabolan 29/C') on fruit quality and formation. The research was carried out over a period of two years. In the study, plants grafted onto Myrabolan 29/C rootstock exhibited the highest levels of total antioxidants and total phenolic compounds. Additionally, similar to this study, they have determined that climatic factors play a significant role in the antioxidant content. It has been determined that regardless of the rootstock, fruits that experience a dry ripening period exhibit an enhancement in their antioxidant potential. Similarly, Hegedűs et al. (2010), observed differences in antioxidant activity of up to 21 times among 27 apricot varieties and hybrids originating from different sources. As seen in the previous studies, it has been determined that various factors, primarily the genetic structure, can have significant effects on the antioxidant content of apricot fruits. In this sense, the Şalak, with its considerably high antioxidant content, has been identified as a significant resource for important breeding studies and the food industry.

Phenolics are compounds with high antioxidant potential. They achieve these properties through different mechanisms, such as scavenging free radicals like ROS, suppressing ROS formation, chelating pro-oxidant metal ions, inhibiting enzymes and preserving or enhancing the antioxidant defense. In this study, it was determined that total phenol values ranged from 122.2 to 771.6 mgTE/kg. Similar to the total antioxidant content, the Şalak variety stood out once again with the highest total phenol value. When the cultivation locations were evaluated individually, it was found that in Elazığ province, the Hasanbey variety contained higher levels of phenolic compounds, while other local varieties performed better in terms of phenolic content in Malatya province. In foreign-origin varieties, it is observed that Hatay province is more suitable in terms of phenolic compounds. Among the varieties, differences of up to six times in total phenolic content have been identified. Similarly, Hegedűs et al. (2010) found significant differences of up to 35 times in total phenolic content among 27 apricot varieties and hybrids from different origins. In addition, Su et al. (2022), determined the total phenolic content in 18 apricot varieties ranging from 0.29 to 0.69 g GAE kg-1. Researchers reported that besides the genotypic structure, the variation in climate factors between years also contributed to such significant differences in total phenolic content. The study conducted by Tarantino et al. (2018) found that although the total phenolic amount was low in the first year of the experiment, the total antioxidant capacity was significantly higher in the year 2015 compared to 2016.

As it seen in the previous studies, the examined compounds in fruits are influenced not only by the genotypic structure but also by various factors such as ripeness level, harvest year, climatic conditions. According to obtained results, it is believed that in foreign-origin varieties, the partial decrease in carotenoid content is related to the ripeness level of the fruits, while the partial increase in antioxidant activity is also attributed to the ripeness level. Overall, it is concluded that the Şalak, with its significantly high antioxidant and phenolic content, not only serves as an important source of nutrients for consumers but also holds significant potential as a valuable material for apricot breeding. If the Şalak variety exists, its lacking characteristics should be improved and its cultivation should be promoted in Türkiye to the same extent as Hasanbey, Hacıhaliloğlu and Kabaaşı.

CONCLUSION

This study has generated valuable information about the quality parameters specific to significant apricot varieties and production regions in Türkiye, which is a key apricot producer. It has been determined that, in addition to the variety, the production location also has a significant impact on fruit quality parametersIn relation to essential quality components influencing health, the 'Şalak' variety notably distinguishes itself from others, displaying a significantly elevated phenolic content that contributes to heightened antioxidant activity. This variety with outstanding fruit qualities can be used as a significant genitor in hybridization studies.

COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, 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.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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